Los siguientes resúmenes han sido presentados durante el evento y serán publicados oportunamente con formato proceeding por la revista AAPS Pharm Sci Tech
PRACTICAL UTILITY OF DIFFERENT SYSTEMS FOR CLASSIFYING DRUG-RELATED PROBLEMS (DRPs): ASSESSMENT BY HOSPITAL PHARMACISTS.

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Introduction
There are different classifications of DRPs with different focus (1). Both documenting and classifying DRPs are essential to improve the process of medication’s use. In practice, professionals need a patient-oriented base to implement pharmaceutical interventions (1-6).

Hospital Pharmacy Specialization (HPS) is a postgraduate career at the School of Chemical Sciences, National University of Córdoba (FCQBUNC). Most of the HPS students are working in health-system pharmacies. One of the modules included the concept of DRPs and different classification systems.

The objectives of this work are:
• To assess the different DRPs classification systems through 4 clinical cases/examples resolved/classified by pharmacists taking the HPS (FCQBUNC).
• To analyze the opinions of participating pharmacists in relation to the focus and relevance of the different DRPs classification systems.

Materials and methods
In the frame of module “Pharmaceutical Care I” (HPS), a workshop intended to identify and classify DRPs was planned. Four clinical cases with one DRP each (or DRP risk) were presented to six working groups of four pharmacists each. As few classifications have an official name, brief titles have been assigned, usually referring to the originating organization or researcher(s).

The groups resolved the cases by consensus using 4 classification systems: ASHP (7), 2nd Granada Consensus (8), PCNE V 5.01 (9) and Cipolle/Morley/Strand (10). Ideally, one type of DRP was expected by case according to each system (1,2). An average number of categories assigned by case was obtained.

In addition, the groups were requested to assess the focus and practical utility of the DRPs classification systems (1,2). A 5 points Likert scale was used from “totally agree” to “completely disagree”. For focus assessment “completely disagree” was related to technical aspects while “totally agree” was associated to a patient-oriented process.

For statistical analysis SPSS 15.0 software was used.

Results
Twenty four pharmacists discussed the DRPs classification systems during the workshop. Main outcomes are shown in Table 1.

Conclusions
A comparison among different DRPs classification systems was carried out (1,2,3). Whereas an ideal classification system should identify uniquely a given DRP (1), Cipolle/Morley/Strand and 2nd Granada Consensus seem to be better than the other systems (11). Moreover, both of them obtain high scores in the focus and practical utility assessments.

Acknowledgements
To Dr. María Eugenia Olivera (HPS director) and to all pharmacists from working groups.

References

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<th>DRPs classification system</th>
<th>Average number of categories assigned</th>
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<th>Practical utility*</th>
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<td>ASHP</td>
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<tr>
<td>Cipolle/Morley/Strand</td>
<td>1.1</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
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<td>2nd Granada Consensus</td>
<td>1.0</td>
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<td>4.0 ± 0.0</td>
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<tr>
<td>PCNE V 5.01</td>
<td>1.6</td>
<td>1.8 ± 0.5</td>
<td>2.0 ± 0.8</td>
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</table>

* 5 points Likert scale ± SD
CONSUMPTION OF TRANQUILIZERS AND STIMULANTS AMONG COLLEGE STUDENTS ATTENDING THE SCHOOL OF CHEMICAL SCIENCES AT CORDOBA (ARGENTINA).

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Introduction
Both licit and illicit drug intake is a public health problem worldwide that affects the entire population, with an early onset, usually at the adolescence and young adulthood (1,2). College students do not escape to this problem, which transcends from the individual to the collective space, affecting both, the society and its structures (3-7). Therefore, the epidemiological study of the student population is a first step to design or improve future prevention and/or intervention programs about drug use (8). The aim of this study is to estimate the magnitude of the consumption of tranquilizers and stimulants in the student population attending the School of Chemical Sciences (UNC) during 2009.

Materials and methods
A descriptive cross-sectional study, using a self-administered questionnaire. A random sample of 400 students was obtained, stratified by sex and college grades. An online standardized and previously evaluated questionnaire, designed by the Inter-American Observatory on Drugs (OID) for university students was applied (8). It included several modules, one of them aimed to the drugs under study. In accordance to the test, drug intake was measured as the lifetime prevalence (the number consumption across the lifetime), and the annual and monthly prevalence (amount and frequency of consumption within the month). Data analysis was performed using SPSS 15.0.

Results
A total of 203 students answered the questionnaire (50.1% of the total), 70.9% were female, with an average age of 23.1 ± 4.6 year-old (range 18-46), and 92.1% of the students were single.
Regarding the use of tranquilizers, 21.7% reported having consumed them at some time in their lives. From this percentage, 54.5% answered they consumed only by prescription, while 38.6% did so on their own. In addition, a 1.5% reported recent use (sometime during the past year), while current use was reported of 0.5%, in both cases without a medical prescription.
In relation to stimulants, 3.0% answered having consumed them sometime in their life. Also, while 66.7% said the consumption was on their own, a 33.3% did so under medical prescription. In this case, both the recent and the current use was zero (0.0%).

Conclusions
The results of this study provide interesting data about the problem of tranquilizers and stimulant drugs consumption in an undergraduate student population. Given the lack of studies of this nature, and although this research has limitations, the results obtained in terms of prevalence are lower than those of other countries in the region (8,9). This could be the result of a greater awareness of the harm and risk associated to the consumption of these drugs among students, which would act as a persuasive element, explaining in part the low intake (3,5,6). However, it is crucial to implement prevention strategies, as well as to provide information related to use of psychoactive substances, strengthening the formal education about the risks given that risk perception is one of the most important protective factors to prevent drug abuse (6).

Acknowledgments
To the School of Chemical Sciences authorities who provide the resources to carry out this study and to Dr Ruth A Fernández for her collaboration in the development of this project.

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References.

POTENTIAL DRUG TO DRUG INTERACTIONS DETECTED IN PRESCRIPTIONS DISPENSED AT A COMMUNITY PHARMACY IN CóRDOBA, ARGENTINA.

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Introduction

Drug to drug interactions (DDIs) are defined as the modifications in the action of one or more concurrently administered medications (1-5). DDIs may result in either increased or decreased efficacy, in treatment failure, or in an increased toxicity of medications (1-5). Pharmacists play an important role in protecting the patients from potential DDIs, especially in the case of drugs with a narrow therapeutic index (1-3,6).

While there is a lack of an effective screening system for detecting DDIs at dispensing in community pharmacies in Argentina, potential DDIs causing serious risks to patients’ health has not been studied extensively.

Consequently, the objectives of this study were to evaluate the nature, type and frequency of potential DDIs in prescriptions dispensed at a community pharmacy in Córdoba (Argentina), over a 3-month period.

Materials and methods

A database was developed with all the dispensations covered by the State Administration of Health Insurance (APROSS) between October and December 2009, yielding to a random population of 400 patients, excluding those who only used a medication at the time of the study. The information collected included date of the prescription, age, gender, prescribing physician’s ID, and the medications dispensed (name, quantity and composition). Potential DDIs were detected using the Drug Interaction Checker within www.medscape.com database and classified as severe, moderate or minor, depending on their severity of clinical significance, and according to their production mechanism in pharmacodynamic or pharmacokinetic. Data analysis was performed using SPSS 15.0.

Results

A total of 36,441 medications were dispensed to 7,798 patients during the study. From a total of 400 patients, 93 DDIs (23.3%) were detected in 67 different patients, 15 were severe (16,1% of all interactions) and the remaining 78 DDIs (83,9%) were moderate while a 59,6% of cases involved pharmacokinetic interaction.

Finally, 48.4% of the total DDIs detected involved different prescribers.

Conclusions

The present study revealed that the overall rate of potential DDIs in prescriptions dispensed was 23,3%, a number that should raise some concern and awareness in both, the medical and pharmaceutical community (1,7). A putative limitation of this study may be related to a certain degree of underreporting of potential DDIs, given that data was analyzed considering only the APROSS prescription, which did not include neither over-the-counter medications nor herbal preparations (1,2,5). A larger study including other community or hospital pharmacies may give more reliable results (1,3,5,7).

These results provide a panoramic view of the DDIs problem, and demonstrate the need of the implementation of a reliable screening system for DDIs at the time of dispensing (1,4,7-9). Identifying and preventing potentially harmful DDIs is a critical component of a pharmacist’s mission, shifting the pharmacist’s role from drug-oriented to patient-oriented (1,7).

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Acknowledgments

To the pharmacists of the Pharmacy Department (UEPC). To the Drug Information Center (CIME), School of Chemical Sciences, National University of Córdoba, for their cooperation in the realization of this study. To Dr. Miriam Virgolini for the revision of this abstract.

References

CALCULATING OF INDICATORS OF PHARMACEUTICAL INTERVENTIONS
MEANS OF Unit Dose Drug Distribution System (UDDDS)

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Introduction
The Pharmacy Service is responsible for the accurate safe effective utilization of the medicines in the hospital. This implies assuming the responsibility of drug selection, acquisition, conservation, preparation for administering, distribution and dispensing to patients when they are correctly prescribed.(1,2) The European model is based on the self evaluation as key process for the constant services improvement (2). To carry out this process, measuring with adequate specific quality indicators is required from time to time. Within the Pharmacy Service functions, the Resolution 641/2000 MS (3) defines establishing an effective safe drug distribution system. Among them, the UDDDS is the only one that allows obtaining the aims previously exposed, and it is the most suitable recognized.

Objective
To evaluate Pharmacist interventions (PI) done with specific indicators developed from UDDDS’s pharmacotherapeutic patient charts at Internal Medicine Service, in a public hospital of the province of Córdoba with posteriory to value the acceptance the PI for health team members.

Material and method
UDDDS’s pharmacotherapeutic patient charts completed for Internal Medicine Service in the Hospital Arturo Illia (Alta Gracia City, Córdoba) were analyzed during October and November in 2009. Analysis criterion: PI required by prescription, dosing and other errors detected in patient charts at Internal Medicine Service. Indicator: percentage of PI divided by total of analyzed charts. Standard: less than 5 % and numbers the PI total accept for realized PI.

Results
The total of analyzed charts in the study period was 66. The number of PI was 16 (24.2 %). The types of PI for detecting errors were classified (4) according to: lack of generic name 8 (12 %), dose problems 5 (7.6 %), and lack of medication or therapeutic substitution requirement 3 (4.5 %). In addition, with these results, the degree of PI acceptance by the health team of Internal Medicine was assessed, obtaining 87.5 %. Indicator (5): number of accepted PI divided by number of PI done during the study period multiplied by 100. Standard: higher than 90 %.

Discussion and conclusions
Evidently, procedures’ documentation is required for evaluating the quality of care. Pharmacy Service has a key position in the drug distribution and dispensing processes. With the PI, accurate administration of medicines is guaranteed for inpatients as well as prescription compliance and cost containment. Furthermore, the drug related problems are diminished, drug distribution and dispensing are rational based, relationships with nurses and other health team members are improved by PI in UDDDS, and they allow the obtainance of results in quality assessment to inform. Against who the degree lower of standar, bright the repercussion of prescriptors doctors, after the IF, there were obvious changes that will meet reflected in later works., The pharmacist’s responsibility in rational use of medicines and drug dispensing are the principal legitimization source of the pharmacy profession.

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4-Promoción de la utilización de medicamentos por su nombre genérico, Ley 25.649, Congreso de la Nación Argentina, República Argentina (Sept 19, 2002).
Introduction
Older Adults are characterized by multiple chronic diseases. An important part of them, are under complex treatment plans. In some cases, prescriptions include high risk for older persons and a safer alternative is available, reason why they are included in the criteria established by Beer. It is believed that the medication is inappropriate when the risk outweighs the benefit, making this more often in the third age, because of changes in the pharmacokinetics and pharmacodynamics related to aging process.

The objectives of this study are to determine the rate of drug consumption on the population studied, identify potentially unsafe prescription for elderly patients, according to Beer’s criteria and to identify misuse drugs most commonly used in drug treatments.

Materials and methods
Observational longitudinal multicenter study, 104 outpatients and housed in nursing homes were included, being all over 65 years old, randomly selected, under pharmacological treatments and regular assistance to the care and dispense centers between January and March this year. Information on the therapeutic drug plan was recorded when the patients took contact with the pharmacist.

Gathered data consisted on:
-Age,
-Sex
-Drugs used by patients,
-Dose/regimen used in each case

Treatment plans were evaluated using Explicit Criteria of inappropriate medication in older adults by Beer.

Results
Of the 104 patients, 67 were women and 37 were men with an average age of 74.80 years. To determine the rate of drug consumption in this population, ranges of drugs prescribed were established. These data can be seen on Table 1.

It should be considered that in those patients who have being prescribed lorazepam in a daily dose of 2.5mg (under Beer’s criteria > 3mg) reported open doses according to anxiety, for this reason they are considered of inappropriate use.

Making use of Beer’s criteria, it was determinated that 35.6% of the patients have potentially unsafe prescriptions, accounting for 8.92% of total drugs prescribed. Misuse drugs most commonly used in drug treatments were for occasional use (Hyoscine and Ergotamine). Table 2, show the frequency of use of drugs clustered according to if they are considered inappropriate, independently of dose or diagnosis, according to the condition of limitation.

Discussion
From the results obtained, and to sharpen the research of drugs responsible for adverse reactions in elderly patients, it is suggested to take into account the use of other tools to synergize this search (STOPP-START).

Conclusions
Studied population is similar to other author’s ones in potentially risky prescriptions. The most consumed drugs were the occasional-use ones.
References
2 Teodoro J. Oscanoa. Anales de la Facultad de Medicina. Universidad Nacional Mayor de San Marcos, Lima, 2005; 66(1) 43-52

Table 1
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<th>Number of drug Taken</th>
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<tr>
<td>Between 3 y 5</td>
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<td>Between 6 y 8</td>
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<td>Between 9 y 11</td>
<td>8</td>
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<tr>
<td>More than 12</td>
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Table 2
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<th>Inappropriate use sometimes</th>
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<td>Muscle relaxants</td>
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<td>Stimulant laxatives</td>
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<tr>
<td>CNS Depressants</td>
<td>6</td>
<td>Amiodarone</td>
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<td>GI Antispasmodics</td>
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<td>Naproxen</td>
</tr>
<tr>
<td>Metyldopa</td>
<td>4</td>
<td>Fluoxetine</td>
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<tr>
<td>Antihistamines</td>
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<td></td>
</tr>
<tr>
<td>Ergot</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Digoxin</td>
<td>5</td>
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USE OF INJECTABLE ANTIBIOTICS FOR INPATIENTS AT THE HOSPITAL NACIONAL DE CLÍNICAS

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Introduction
The Hospital Nacional de Clínicas (HNC) is a high complexity teaching hospital which includes with all medical specialties, except neonatology and maternity. It has a capacity of 160 beds and in the last 2 years the average occupancy has been 90%.

The Central Pharmacy is found within the premises of the hospital and it provides medications and other pharmaceutical products for the hospitalized patients and clinical services for outpatients under treatment. From the health point of view is of outmost importance an adequate use of antibiotics, because its widespread use promotes the emergence of resistant strains which implies the necessity of new drugs (1-2).

The consumption of injectable antibiotics (ATBi) by inpatients has increased in the last few years, with a large impact on the associated costs, which represent the 19.7% of the total drug costs.

General Objective: To analyze the inpatients consumption of ATBi during 1 month in the HNC.

Specific Objectives:
- To establish the most demanded ATBi in the HNC.
- To determine the number of inpatients with ATB indication.
- To quantify the ATBi treatments and its combinations.
- To determine the incidence of in the ATBi use by gender (sex) and age.

Materials and Methods
- A list with all the ATBi and its abbreviations and a register form for ATBi indication were designed.
- Research period: September 15 to October 15 of 2009.
- Inclusion Criteria: All inpatients in the HNC.
- The pharmacists were distributed in all clinical services of the HNC.
- The ATBi were classified according to its costs, by unit, into (3-4):
  - High: $5,50 AR or more
  - Middle: range $3,40-$5,50 AR
  - Low: not exceeding $3,40 AR

Results
We have obtained the following information in the HNC:
- Total of inpatients in this period: 760
  - with ATBi: 483 (63%)
  - without ATB: 233
  - with oral ATB: 44
  - ATBi consumption relative to its cost
    - 6.02% high cost
    - 84.61% middle cost
    - 9.37% low cost
- Besides, in several cases, 30 combinations of two or more ATBi were found in the treatment of the different pathologies (24% Metronidazol + Ceftriaxona and 17% Ciprofloxacin + Clindamicina).
- ATBi consumption (most frequently used): 21.4% Clindamicina, 18.6% Cefazolina, 15.5% Ceftriaxona, 12.2% Metronidazol, 8.8% Ciprofloxacina and 8.2% Cefazidima (middle cost ATBi).
- ATBi consumption by gender: 53% female.
- ATBi consumption by age group: 53% of inpatients are between 60 – 80 years old.
Conclusions
During the reviewed period the following was observed:
- A large amount of inpatients with ATBi treatment.
- A considerable consumption of the middle cost group of ATBi.
- No significant difference in the use of ATBi by gender.
- The age group between 60-80 years old was the most important in terms of ATBi consumption.

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Professors of the Hospital Pharmacy Specialization and pharmacy students of the School of Chemical Sciences – UNC.

References.

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CUSTOMERS/PATIENTS’ PERCEPTIONS AND EXPECTATIONS WITH PROFESSIONAL SERVICES IN COMMUNITY PHARMACIES: PILOT STUDY

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Introduction
Pharmaceutical care is the responsible provision of drug therapy for the purpose of achieving definite outcomes that improve a patient's quality of life. This concept was born in response to the increase of the life’s expectancy, the mayor prevalence of diseases (that goes together with longevity increase) and the growing number of drugs for those pathology’s treatments (among other factors). (1) Pharmaceutical care implies that pharmacists develop cognitive services which are patient-oriented benefits carried out by the professional for improving the drug use process or the pharmacotherapy’s result through specific knowledge application. (2) However, to decide what cognitive services should or could be implemented, the pharmacist must be able to understand which kind of care and services patients demand. Besides, offering a non required service is not feasible. Moreover, patients should know about the skills and aptitudes of pharmacist, because confidence and expertise in professional activities are required. (3-4) Surveys are a traditional way of conducting research on primary care setting, and they are frequently used to collect information on attitudes and behavior. They often employ a questionnaire as a tool for data collection. (5-6)

Objectives
To design and validate a questionnaire for assessing the customers/patients’ perceptions and expectations with professional services in community pharmacies.

Material and Method
A semi-structured and self-administered questionnaire was designed for this study. Questionnaires were distributed at different pharmacies during April 2010. A cross-sectional descriptive study was carried out. (6) The questionnaire started with an introduction where the purpose of the survey was stated and a thank you message was given to the participants. After the introduction, 5 questions were displayed mostly in a check list format. Two options were related to the perception of the patients about the professional role. Finally, demographic data (age, sex, activity, educational level, etc) was requested. Setting: ten community pharmacies in three provinces of Argentina (Santa Fe, Córdoba and San Juan).

Results
At present, 270 completed questionnaires were collected. The valid questionnaires were 266. The mean age of respondents was 45 years old and 62% of them were women. Thirty five percent of respondents were workers, 40.6% had high school studies, and 79.3% had health insurance. An equilibrium between business and profession oriented activities was marked by the 64.7% of the surveyed people. With regard to the activities, most people thought the pharmacist is capable of advising on medicines and/or medical conditions (62.8%) and counselling about how to use medicines (59.8%). The lowest ranked item was “To sell other products” (4.1%) different than medicines. Concerning to the kind of care, 81.6% of patients expected that the pharmacist spends the necessary time for proper counselling.

Conclusions
A questionnaire was designed to measure the customers/patients perceptions and expectations with the professional role of the pharmacist. The pilot study showed some confusing items for respondents.

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Most of people perceived that the pharmacist has many skills to maintain equilibrium between commercial and professional profile. Customers expectations were focused on the necessary time to receive proper advise from pharmacist.

Acknowledgements
To Dr. Santiago Palma, professor of Community Pharmacy. To Dr. Pedro Armando and Mgter Sonia Uema for helping in methodological aspects.

References
PUBLIC POLICIES FOR PROVISION OF DRUGS FOR DIABETES MELLITUS TYPE II. A CASE STUDY: BAHÍA BLANCA CITY

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Introduction
The epidemiological transition has determined that some chronic diseases are nowadays the main causes of morbidity and mortality. Diabetes Mellitus Type 2 (DMII) affects 80% of diabetic population. Pharmacological treatment in general involves oral hypoglycemicants, and glibenclamide and metformin are the most commonly used (1). In Argentina, public policies from different levels of government guaranteed public provision of these drugs to the people without any type of health coverage: national law 23.753 (1989), provincial law 11.620 (1994) and REMEDIAR, a national program for access to generic drugs (2002).

In 1996 PRODIABA, a provincial program which provides free oral hypoglycemicants for diabetic patients, was created. While both programs, REMEDIAR and PRODIABA should deliver glibenclamide 5 mg and metformin 500 mg, delays in the delivery of metformin determined the intervention of the municipality of Bahía Blanca (MBB). Local authorities had to provide this drug to ensure the treatment of type 2 diabetic population without health coverage.

The objective of this paper is to quantify monetarily (expenditure) the current municipal provision of metformin and the amount of this drug that should be allocated to potential type 2 diabetic patients if they had pharmacological treatment.

Materials and methods
The glibenclamide and metformin tablets delivered by PRODIABA and MBB to the population treated in some primary health care centers of public sector (CAPS) were quantified from October 2008 to September 2009. Potential type 2 diabetic population of MBB was estimated based on the prevalence in the adult population of the central area of Argentina (2) and using projected population statistics for 2008 (based on Censo 2001) were used.

Results
In the review period 625 type 2 diabetic patients were treated at primary level. The municipal provision of metformin tablets was 118210 (3940 treatments), more than half of the amount provided by PRODIABA and it was 34% of the total public provision. The municipal expenditure was 25000 ($ 0,21 per tablet).

According to prevalence data and projections of adult population without any type of health coverage of MBB, there may be 4940 type 2 diabetic patients. However, only 625 received public treatment (12,6% of potential patients). If the municipality provided the drugs to guarantee access for all patients with DMII, the total annual provision would be 2.751.580 tablets (91.719 treatment) with a total cost of 577.832 (prices according to the municipal public procurement).

Conclusions
Although access to treatment for DMII would be guaranteed by provincial and national public policies, it was showed that local levels must manage additional provisions, which questions the effectiveness of centralized policies on the provision of drugs. Municipal government, through the CAPS, seems to be the appropriate level to ensure and promote chronic disease treatments.

References

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NON AVAILABLE MEDICINES FOR PEDIATRIC PATIENTS. DRUG UTILIZATION STUDY AT THE HOSPITAL INFANTIL MUNICIPAL. CÓRDOBA, ARGENTINA.

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Introduction
Once all R&D steps are overcome, pharmaceutical industry must apply for governmental authorization approval. The information included must specify the indication(s) with the age range and dosage form. In some situations, physicians are allowed to prescribe for any other indication or dosage form, if there is reasonable scientific evidence. The use of a drug in any age range different of that labeled is frequent in pediatrics and geriatrics, and this circumstance represents a matter of several investigations (1B3). The methods used in Pharmacoepidemiology offer tools to study the use of medicines in the population, among them Drugs Utilization Studies (DUE) are one of the most useful (4).

In this context, we wanted to describe the use of medicines in hospitalized patients at the Hospital Infantil Municipal (HIM) and assess whether medications prescribed are marketed in Argentina in therapeutic indications and on appropriate dosage form.

Materials and methods
Between September and November 2009, a pilot observational, descriptive, cross-sectional study was carried out.

Prescriptions made to hospitalized patients at HIM were registered. Information was supplemented by reviewing the medical records and verbally checked with doctors and nurses. Drugs were classified according to the ATC Code (5).

All prescriptions were compared with dosage forms approved by ANMAT (6) to determine the lack of availability. Also, they were analyzed with sources related to Evidence Based Medicine (7). A drug existing in more than one situation was selected for analysis. The information was processed using the statistical program SAS 9.2

Results
A total of 5757 medical indications were sorted out (105 different drugs; 327 children). Among them, 312 received at least one prescription and patients received a mean of 5 medicines (1B21). The average period of hospitalization was 4 days (1B24). Six ATC groups included the 90% of the prescriptions drugs (A: 28%; N: 24%; J: 16%; R: 11%; H: 6% and B: 4%).

In the 16% (n=165) of prescriptions, dosage form adaptation and off-label use were needed. They were necessary in 12% (n=39) of children.

The 71% (n=117) of prescriptions was related to tablets and capsules for oral use.

Scientific evidence related to safety and efficacy in pediatrics could not find for the 18% (n=19) of the drugs used, even more some of them were not recommended for use in this age group. The 65% (n=213) of patients received at least one prescription of these drugs.

Sildenafil was selected as an example. It is marketed as oral tablets of 25, 50, 100mg. For a patient with pulmonary arterial hypertension was prescribed 6mg three times a day. It is considered orphan drug by EMA. Safety and effectiveness in pediatrics is not well-known. The pharmaceutical formulation should be manipulated to be administered.

Conclusions
A relevant proportion of prescribed medicines were not marketed in the suitable dosage form. For this situation was necessary its manipulation to administer them.

A important group of patients were exposed to use of drugs without sufficient data about safety and efficacy or not recommended for use in children.

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Acknowledgments:
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- Secretaría de Salud de la Municipalidad de Córdoba
- Hospital Infantil Municipal de la ciudad de Córdoba (HIM)

References:
NON AVAILABLE MEDICINES (NAM), ANALYSIS OF DRUG INFORMATION REQUESTS (2008-2010)

Fontana D, Lascano VM, Vega EM, Mazzieri MR#


Introduction
The lack of availability of a medicine, at the market or at the health system, is a limiting factor to achieve a therapeutic goal (1). The classical approach focused the problem on orphan drugs (OD) for rare diseases. However, the present pharmacotherapeutic scene includes other situations that lead to unavailability, such as medicines for neglected diseases, inappropriate dosage form, off-label use of a drug product, among others (2,3).

Our research group proposed to study that problem from a different point of view. So, we have carried out pharmacoepidemiological studies to identify needs of non available medicines (NAM), to understand the causes and to propose interventions.

Furthermore, our experience working at the Drug Information Center (CIME-UNC), has demonstrated that pharmaceutics and therapeutic information about medicines is, generally, limited, biased and outdated. To overcome these difficulties, specially detected at health institutes (HI), a NAM Database (NAM-DB) was developed in collaboration with the CIME-UNC (4,5).

Objective
To identify the lack of drug products and to analyze the causes of their non-availability by using the requests received at CIME-UNC during 2008-2010.

Materials and methods
Type of study: Observational, cross-sectional descriptive.
Period: April 2008 to April 2010
Setting: CIME-UNC and HI at Córdoba.
Data collection: Requests for drug information obtained from the NAM-DB.
Variables: Drugs classified by ATC code (7) and situations related to non-availability.
The availability of medicines was confirmed by consulting the electronic database of approved drugs by ANMAT (6) and also by direct contact with the authorized laboratory.
NAMs were compared with drug requested to ANMAT for Compassionate Use in the same period and OD list of FDA and EMA.
Data processing: Microsoft Excel 2007

Results
134 information requests about NAM were received, which met 48 drugs. From those, 87 were confirmed as strictly NAM.

About the consultants, 39 (54.9% of requests) were physicians, 22 were pharmacists (31.0%), 9 were patients (1.4%) and 1 nurse made the 12.7% of requests. Same requests were made by more than one professional.

According to the ATC code, the main groups involved were:
-A: 18.4% (n=16)
-C: 18.4% (n=16)
-J: 13.8% (n=12)
-N: 9.2% (n=8)

Furthermore, the following situations of non-availability were detected:
-Non marketed drugs: 58.6% (n=51).

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- Adjusting dosage form: 29.9% (n=26).
- Off-label use: 11.5% (n=10).

The 22% (n=19) of NAMs identified coincided with a requested drug to ANMAT for Compassionate Use. The 28% (n=24) and 7% (n=6) are included in OD list of FDA and EMA, respectively.

**Conclusions**

Drug information requests were useful to identifying NAM and non-availability situations. The types of situations were similar to those found in previous studies. Group A (alimentary tract and metabolism) was the most frequently involved. The collaborative approach allowed the resolution of requests with evidence based information in time. The importance and benefits of interacting between HI and the University was demonstrated.

**Acknowledgments**


**References:**

MIDAZOLAM PRESCRIBING PATTERN IN THE INTENSIVE CARE UNIT OF THE ITURRASPE HOSPITAL OF SAN FRANCISCO, CORDOBA. A RETROSPECTIVE SURVEY

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Introduction
Midazolam is a benzodiazepine available as a sterile, non pyrogenic parenteral dosage form for intravenous or intramuscular injection. It is a potent sedative, anxiolytic and hypnotic agent that requires slow administration and individualization of dosage. Parenteral Midazolam is used therapeutically in the management of agitation in mechanically ventilated patients in intensive care units (ICU). There is a wide intra and inter individual variability in sedative dose requirements in this patients. Patient’s heterogeneity, the frequent and variable organic dysfunctions, the drug interactions and the possibility of metabolite accumulation could explain this variability \(^{(1-2)}\). However this fact must not justify the use of excessive doses to achieve the goals of sedations.

The goal of this study is to analyze retrospectively the Midazolam prescription habits in terms of appropriateness of dose to reach a sedation level compatible with mechanical ventilation (Ramsey Scale > 4)\(^{(3)}\), in the ICU of the previously mentioned hospital.

Methods
A retrospective analysis during from May to October 2006 and from May to October 2009 was performed.

Data about patients, hospitalization days, days with sedation, and dose by patient were collected retrospectively from the individual charts at the ICU service, which are kept in the Pharmacy Service. Average of Midazolam (15 mg/ampoule) by patient was calculated from the number of ampoules and converted to mg/kg/h.

Results and discussion
Literature describes recommended dose of Midazolam to achieve sedation from 0.03-0.2 mg/kg/h (3.4-22.4 ampoules of 15 mg/day) to 0.18-0.3 mg/kg/h (22.5-33.6 ampoules of 15 mg/day)\(^{(1-4,6-7)}\). The use of doses above these is not recommended.

356 patients (adults 17-85 years, both sexes) were surveyed (173 in 2006 and 183 in 2009). From the observed population, just 29% (45 patients) in 2006 and 24.6% (50 patients) in 2009 needed sedation for mechanical ventilation and they were prescribed Midazolam (15 mg/ampoule) associated with Fentanyl (0.05 mg/ml ampoule). The patients were assigned to one of the following categories:

**Category 1**: patients having a dose of 3.4 y 22.4 ampoules of 15 mg/day\(^{(5,6-7)}\).

**Category 2**: patients having a dose of 22.5-33.6 ampoules of 15 mg/day\(^{(1-4)}\) and

**Category 3**: patients administered with doses above recommended.

The 92% (2006) and 82.2% (2009) of the patients were assigned to category 1 and 3.9% (2006) y el 15.6% (2009) to category 2. Only 3.9% (2006) and 2.2% (2009) of the patients received doses higher than recommended. These patients were in a very critical condition and were hospitalized in the UCI for a longer period of time. Midazolam as other benzodiazepines can develop tolerance and then an increase in the doses is required. However due to the risks associated to such high levels, a change in the drug is recommended.

Conclusions
The dose appropriateness was 96% (2006) and 97.8% (2009). These findings are in agreement with reports from other authors. This study generates up-to date information on the Midazolam prescription habits in the hospital, and suggested that the treatment in this ICU appears to be appropriated. Drug use evaluations are useful indicators for following trends of drug prescription, optimizing drug use and controlling expenditure.
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SANITARY EDUCATION FOR THE PREVENTION AND CONTROL OF HUMAN INTESTINAL PARASITOSIS ON THE BANKS OF PINTO RIVER, CÓRDOBA

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Introduction
In marginal populations with poor access to drinking water, limited sewage treatment, deficient participation of the state on the healthcare of the population and poor nutrition, intestinal parasitoses are one of the most prevalent diseases; they are one of the most prevalent infections worldwide (1). Sanitary education is one of the essential cornerstones for health promotion and a main tool to modify the factors that determine the prevalence of intestinal parasitoses in these populations (2).

The goal of this work was to know the microbiological quality of drinking water, the epidemiologic, social and sanitary habits of the people of the area under study in order to determine the factors that condition the prevalence of human intestinal parasitoses and design and elaborate in this way the educational tools to improve hygiene behaviors to prevent the transmission of these pathologies.

Materials and methods
Epidemiologic survey
An observational, descriptive and transversal study was carried out in Costa del Río Pinto (Córdoba, Argentina) tackling the socio-epidemiologic dimensions that condition the prevalence of intestinal parasitosis (housing, water, excreta, sewage, pets, presence of symptoms, habits, nutrition, access to the health system).

Population studied: people that accepted to be surveyed (100º).
Instrument: after validation, a study of complete numbering or population census was carried out.
INFOSTAT processing.
Laboratory analysis
Microbiologic analysis of drinking water on 35 samples: recount of aerobic mesophilic bacteria and multiple tube technique.

Results
The population census showed the following relevant data as to the risk factors for the prevalence of intestinal parasitoses: excreta deposition is carried on by means of drainage and a 100º of homes have brick bathrooms of which 54% are outside the home and shared with other people; a 100% of families consume water from the river and 80% have symptoms compatible with parasitoses as well as a history of parasitoses. There are dogs and chickens in a 100% of homes. There is no sewage collection system, sewage is burnt in every home. According to a medical report, there is no undernourishment in the area. The nearest health care center is 30 kilometers away.

The microbiologic analysis of drinking water included samples from the river, irrigations ditches, filter and homes. The analyzed samples did not comply with what is established by the Argentine Alimentary Code to classify as drinking water.

A sanitary education program designed by the career of Pharmacy was carried on for two years. It consisted in three health campaigns that included educational workshops with techniques of incentivation, recovery of popular consciousness, explanation of concepts, prevention measures, simulation activities and delivery of pedagogical-didactic materials.

Conclusions
The interdisciplinary work with the integration of curricular contents allowed us to understand the situation in an integral way and to propose solutions for intestinal parasitoses affecting urban marginal populations.

References
PHARMACOVIGILANCE: ANTIBACTERIALS FOR SYSTEMIC USE REPORTED TO CHACO’S PERIPHERAL EFFECTOR

Gruszycki MR, Tauguinas AL, Alba DA, Baez M, Gruszycki AE, Osicka RM


Introduction
Antibacterials for systemic use are one of the groups of drugs widely used by the population. Very often these drugs are misused or an abuse is made, so caution must be exercised when its administration is required, otherwise we run the risk of transforming a simple benign disease that disappears spontaneously, in other which can be serious and even deadly. This paper aims to analyze the reports involving the antibacterials for systemic use that were reported to Chaco’s peripheral effector, during 2001-2009 period.

Materials and methods
We performed a retrospective analysis of filed reports using the Pharmacovigilance database during the mentioned period and the Official Yellow Card of the National Pharmacovigilance System (ANMAT). For its evaluation we used the Dictionary of Adverse Drug Reactions (ADR-WHO) (1) and the Anatomical Therapeutic Chemical Classification of Drugs by the WHO (ATC). According to the severity of the adverse reaction, we established three categories: mild, moderate and severe. Causality was assessed using the Naranjo y Colab. algorithm, codifying the RAM as definite, probable, possible, not probable, conditional/unclassified (2-3). For the statistical analysis Microsoft Office Excel 2007 was used.

Results
From 1,248 spontaneous reports, 21% corresponded to antibacterials for systemic use, J01 according to the ATC classification. In the gender distribution, 50% were females, 35% male and 15% unspecified. The age groups most commonly reported were from 15 to 29 years 30%, 0 to 14 years 28%, 30 to 44 years 20%, 45 to 59 years 13%, 60 to 74 years 7% and 75 years and over 2%. In accordance with the intensity of ADRs were mild 43%, moderate 43% and 14% severe. In relation to causality of the ADRs we found that were: probable 67%, possible 23%, defined 9% and not probable 1%. According to the ATC code, drugs involved corresponded to the beta-lactam antibacterials, penicillins (J01C) 53%, other beta-lactam antibacterials (J01D) 12%, quinolone antibacterials (J01M) 12%, macrolides, lincosamides and streptogramins (J01F) 8%, sulfonamides and trimethoprim (J01E) 7%, other antibacterials (J01X) 4%, aminoglycoside antibacterials (J01G) 3% and finally tetracyclines (J01A) 1%. The most affected organs and systems were: gastrointestinal 33%, skin and appendages 29%, general of the whole organism 17%, central and peripheral nervous system 7%, respiratory 4%, cardiovascular 3%, psychiatric 2% and others in less proportion.

Conclusions
Among the reports received that involved antibacterial for systemic use, the most frequently reported RAM were those related to gastrointestinal disorders, followed by the ones of the skin, being the beta-lactam antibacterials, penicillins the predominant subgroup, with more than half of the reports.

References

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DETECTION AND DEVELOPMENT OF NON AVAILABLE MEDICINES (NAM) IN ARGENTINA

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Introduction
Non available medicines (NAM) are defined as those which are not produced and marketed in Argentina (1,2). Our research group studies the NAM from different points of view. The lack of availability is addressed from a pharmacoepidemiological perspective and the design and development are approached from medicinal chemistry and pharmaceutical technology. Finally, the results are intended to transfer to the productive sector.

In order to get available some drugs in Argentina, and provide specific responses to patients, we initiated the development of different medicines. This work described the actions, in relation to NAMs, that were carried out in our laboratory originated from that interrelation between academy and society.

Materials and methods
Pharmacoepidemiological studies about perception of health professionals were done to identify NAM in our country. The unavailability was confirmed by checking in the Regulatory Agency (ANMAT) database of approved medicines (3) and calling to authorized manufactures. Once the unavailability of those drugs was confirmed, different solutions from the perspective of the medicinal chemistry and pharmaceutical technology were proposed. Therefore, hemin and sodium p-aminosalicylate (PASS) were selected to develop the products.

Hemin was obtained from defibrinated and washed erythrocytes, developing a medicine similar to Normosang® (Orphan Europe) (4). In relation to PASS, a new dosage form was proposed, using as reference the PASS tablets, USP (5). The analytical techniques and the stability studies were carried out according to international standards (6).

Results
A preliminary survey from interviews with key informants at national levels allowed the detection of several NAM. Hemin is the only pharmacotherapeutical option currently existing for the treatment of acute intermittent porphyria (AIP) (7). The PASS is used in combination therapy for multi-drug resistant tuberculosis (MDR-TB) (8). Hemin and PASS for the indications above mentioned were confirmed as NAM in Argentina.

Hemina-UNC as a stable pharmaceutical formulation of hemin arginate for intravenous use, and PASS as a new pharmaceutical equivalent were developed. The PASS-EXT was formulated as a powder for oral solution of extemporaneous reconstitution. Both developments were made at lab-scale production. Analytical techniques for quality control of active ingredient, products, process and stability studies were developed and validated. Working standards for analytical assays were developed and prepared. Because the PASS-EXT is a pharmaceutical equivalent, a new HPLC analytical technique to evaluate the pharmacokinetic behavior in human beings was developed and validated.

In the context of technology transfer, instructional and standard operational procedures for the preparation and quality control of both products were written.

Links with Regulatory Agencies and pharmaceutical industries for the registration and commercialization of both drugs were established.

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Conclusion
Pharmacoepidemiological studies were useful for identifying NAM and related situations. Hemina-UNC and PASS-EXT were developed under GMP standards at lab-scale production. At present, there is a batch to be used as pharmaceutical compounding.

We are carrying out actions to transfer the results to national production laboratories to market Hemin and PASS in Argentina.

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Secretaría de Extensión Universitaria de la Universidad Nacional de Córdoba, Argentina (SEU-UNC).
Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET).

References
CONSUMPTION OF METHYLPHENIDATE IN THE PROVINCE OF SAN JUAN

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Introduction
Methylphenidate is used for the treatment of Attention-Deficit Hyperactivity Disorder (ADHD)\(^1\). The wrong use of Methylphenidate, due to an inappropriate diagnosis, or a treatment without the appropriate medical monitoring, exposes children to the hazards of this drug, which safety profile has not been fully investigated. There is evidence that the use of Methylphenidate has spread beyond the strictly recommended limits. The main reasons are its probable beneficial effect, without considering the necessary precautions as well as its hazards and negative effects. Besides, some social groups use it as a “practical alternative” to “strengthen” the intellectual capacity in their children and improve their behavior, so that they can reach a high intellectual performance\(^2\).

The objective of this research is the characterization of the population treated with methylphenidate, and according to the results obtained from research, propose activities that promote the rational use of this medicine.

Materials and Methods
A retrospective statistical study was carried out using the program Statgraphics. The raw data was obtained from medical prescription recipes of Class II Psychopharmaceuticals, from October of 2008 to October of 2009, provided by the Pharmacy Division of the Ministry of Public Health of the Province of San Juan. The information was gathered through tables specifically designed for that purpose.

The variables analyzed were: diagnosis, sex, domiciles, dates of prescription and minimum data demanded by law 19,303 of Psychopharmaceuticals and Stupefacients.

Results
From the 1500 recipes surveyed, 90% of the cases belong to the diagnosis of ADHD, 5% for autism and the remaining 5% is distributed in unspecified diagnoses. Male sex prevails with the 75% over the female sex, with 25%. It was also observed that the departments with higher consumption are: Rawson, Rivadavia and the Capital city, all of which belong to the urban area. It was observed a consumption increase of 15% in the month of June, followed by 14% in May, 13% in March, 11% in August and 10% in October. Said months coincide with an increase in the school demands. Regarding the minimum data declared in the recipes, 23% of the recipes surveyed present incomplete or absent information.

Conclusions:
Due to the results of the investigations, multiprofessional works will be intensified with physicians, psychologists and pharmaceuticals, in order to have a more strict control on the use of this drug. In addition, together with the Pharmacy Division, we will address the compliance requisites demanded by the law regarding the information declared in the recipes, and take corrective and promotion measures for the rational use of this drug. Apart from that, said information will be provided to the System of Pharmacovigilance of the ANMAT.

Acknowledgments
We thank the Pharmacy Division, Ministry of Public Health of the Province of San Juan.

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USE OF INSULIN AND DRUG INTERACTIONS IN DIABETIC OUTPATIENTS WITH CHRONIC RENAL FAILURE

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Introduction
The economic burden of diabetes mellitus and its complications is increasing so rapidly in the world that drug use research in this disease has important public health benefits. The general aim of this work is to value the use of insulin and the drug interactions in a group of diabetic outpatients insulin-dependent exclusively in relation to morbid conditions associated such as arterial hypertension and renal failure.

Materials and methods
Cross-sectional study performed in the Hospital Provincial del Centenario, located in Rosario city. All medical prescriptions of hypoglycemic drugs, which led to their dispensations, were collected from the hospital pharmacy corresponding to outpatients during 2008. There were selected prescriptions of patients who got insulin as the only hypoglycemic drug. These patients were defined as exclusively insulin-dependent (EID). In addition, it was collected from these prescriptions all the included drugs and diagnosis. Insulin consumption was calculated as defined daily doses (DDDs) and prescribed daily dose (PDD). Potential interactions of drugs to diabetic outpatients with chronic kidney disease (CKD) were analyzed using Interdrugs software (Medicamentos Rothlin) The statistical analysis was performed using Epi Info 3.3.2 and Excel 5.0 versions.

Results
A total of 853 prescriptions were collected throughout the year 2008 corresponding to 146 outpatients. The age range was from 5 to 80, being 25% less than 37 years old, 50% between 37 and 60 and the remaining 25% over 60 years old. The mean age for hypertensive patient group was 57.7 (95% CI=55.0 - 60.3) and for patients with CKD was 53.6 (49.7 - 57.6). The table 1 shows the prevalence of arterial hypertension (AHT) and CKD according to the age ranges among EID patients. In the studied year the total consumption of NPH insulin rose to 42,600.0 DDDs and the DDP: 68.0 (66.1 - 69.9) UI. In turn, regular insulin consumption was 4,912.5 DDDs and DDP: 44.0 (41.0 – 47.0) UI. From the total of outpatients that acquired insulin from pharmacy office, all of them used NPH insulin and only the 28.1% (41/146) used both insulin types. Of the total EID patients, 18.5 % (27/146) has a diagnosis of severe chronic renal failure. The table 2 shows the main results of the drug interactions analysis.

Conclusions
The low consumption of regular insulin compared to NPH insulin could be justified at least in part by the lack of available strips in hospital pharmacy necessary for glycemic self-control. The prevalent age range for EID patients is less than those reported (1), as mean age of patients with end-stage renal disease (2). The drug interactions analysis shows that more than half of the patients with CKD had interaction among prescribed drugs, although they were of moderate to mild risk.

References

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### Table 1: Prevalence of AHT and CKD according to the age ranges among EID patients. Data were obtained from prescription collected of hospital pharmacy.

<table>
<thead>
<tr>
<th>age</th>
<th>total</th>
<th>with AHT</th>
<th>with CKD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;13</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13-25</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25-37</td>
<td>10</td>
<td>1 (10.0%)</td>
<td>2 (20.0%)</td>
</tr>
<tr>
<td>37-46</td>
<td>20</td>
<td>11 (55.0%)</td>
<td>5 (25.0%)</td>
</tr>
<tr>
<td>49-61</td>
<td>48</td>
<td>27 (56.3%)</td>
<td>13 (27.1%)</td>
</tr>
<tr>
<td>61-73</td>
<td>27</td>
<td>19 (70.4%)</td>
<td>7 (25.9%)</td>
</tr>
<tr>
<td>73-83</td>
<td>6</td>
<td>4 (66.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>62 (42.5%)</td>
<td>27 (18.5%)</td>
</tr>
</tbody>
</table>

### Table 2: Main results of the drug interactions analysis

<table>
<thead>
<tr>
<th>Number of patients with more than one drug plus the insulin</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of different drugs prescribed plus insulin</td>
<td>29</td>
</tr>
<tr>
<td>Number of patients with relevant interactions among prescribed drugs</td>
<td>16</td>
</tr>
<tr>
<td>Number of patients with more than one interaction</td>
<td>12</td>
</tr>
<tr>
<td>More frequent interactions</td>
<td>Fucoxanide / Enalapril</td>
</tr>
<tr>
<td></td>
<td>Ibuprofen / Enalapril</td>
</tr>
<tr>
<td>Number of patients with more frequent interactions</td>
<td>6  in each case</td>
</tr>
</tbody>
</table>
INFORMATION TO THE PATIENT IN THE PACKAGE INSERTS OF OVER THE COUNTER MEDICATIONS

Fulfillment of the Dispositions No 7572/06 and 7573/06 of the ANMAT

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Introduction
The National Administration for Drugs, Food and Technology (ANMAT), in bulletin of December 2006 reports two dispositions. In both it binds laboratories to adapt the package inserts of various over-the-counter (OTC) presentations for ACETAMINOPHEN, NAPROXEN, DICLOFENAC, IBUPROFEN and ASPIRIN, within the next 60 days.

The OTC status of these drugs has generated many debates about the health problems of self-medication. The great majority is consumed without medical prescription, for what we consider it to be very important that package inserts should contain information to the patient, in non-scientific language that a lay person can easily understand, which is the propose of the above mentioned regulations. We have analyzed package inserts in order to assess the current level of accomplishment of present legislation.

The work was carried out by teachers and students of the career of Pharmacy, as well as the personnel belonging to the medications control area responsible of Buenos Aires Province.

Materials and methods
The analysis on the package inserts was performed, from all the products containing the drugs listed in ANMAT’s norms 7572/06 and 7573/06, accessible to the general patient (i.e. available in community pharmacies) in our region, considering only monodrug presentations. Each of the items regulated in these normative were analyzed (see Table 1).

A general form to register frequency of fulfillment of these items for each product was elaborated, and average degree of fulfillment of each item was assessed. Potential risks for the patient associated to lack of information or misinformation in any of these items were taken into account.

Results
For the general profile, a fulfillment index of less than 50% was verified for the items of Qualitative Formula, Use of the medicine, How to use it, Contraindications, Warnings and Precautions, Adverse Reactions, Interactions and Toxicology Services (Table 1). It is worth noticing that clarity in these items is essential to promote safe use of OTC medications, since physician advice is absent.

We found the fulfillment of the Items Active and Dose, Overdose and Review to be in the range between 53 and 75%. The rest were fulfilled in a percentage higher than 85%.

We also found that some laboratories have a registered product under the "Free-sale" condition and another one by prescription, although both products have the same active and dose.

Discussion
The results reflect the volume of information accessible to the patient available in the region, being able to differ from an analysis of the totality of products registered commercialized in Argentina.

The items in which less frequency of accomplishment was detected are precisely those related to assure the safety of the patient when consuming OTC medications contrasting it with the towering fulfillment in legal aspects and of commercialization.

A remarkable disparity in the recommended dose in bulletin, was also observed, which constitutes a fundamental topic for the protection of the patient.

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We consider that the fulfillment (especially in the case of OTC medication) of so tied norms to the sanitary problem of the self-medication in Argentina, an only message would have to be demanded without exception.

References
(2) Disposition ANMAT N ° 7572/06, and Annexes
(3) Disposition ANMAT N ° 7573/06 and Annexes
(5) Magazine K@iros digital version http://www.kairosweb.com.ar

Table 1

<table>
<thead>
<tr>
<th>Analyzed items</th>
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<tr>
<td>Trade Name</td>
<td>96,4</td>
</tr>
<tr>
<td>Active and Dose</td>
<td>60,7</td>
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<tr>
<td>Sale Condition</td>
<td>92,9</td>
</tr>
<tr>
<td>Qual-quantitative formula</td>
<td>32,1</td>
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<tr>
<td>Therapeutic action</td>
<td>85,7</td>
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<tr>
<td>Use of medicine</td>
<td>50,0</td>
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<tr>
<td>How to use it</td>
<td>39,3</td>
</tr>
<tr>
<td>Contraindications</td>
<td>35,7</td>
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<tr>
<td>Warnings and Precautions</td>
<td>42,9</td>
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<td>Adverse Reactions</td>
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<tr>
<td>Interactions</td>
<td>39,3</td>
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<td>Overdose</td>
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<td>Toxicology Service Reference</td>
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<tr>
<td>Presentations</td>
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<td>Conservation</td>
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<td>Legend</td>
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<td>Certificate Nº</td>
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<td>Review</td>
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VARIATIONS IN THE MONO-DRUG CONSUMPTION IN HEALTH CARE AREA OF GODOY CRUZ (MENDOZA)

Palomo VB1; Manucha WAF2; Calderón CP3


Introduction
The Health Care Area of Godoy Cruz has 16 Centers of Health and a Distribution Center of Pharmaceutical Supplies(CDPS) who centralizes the pharmaceutical management. Our objective was to analyze the variations of the mono-drug (medicine with a single agent) consumption, to detect the most prescribed and to relate them to the consultations and prescriptions during four years in this Area.

Materials and Methods
An observational, descriptive and retrospective study was carried out from 01/01/2006 to 31/12/2009. The drug formulary contains 23 mono-drugs groups, 17 of Cardiovascular System(CS), 20 of Nervous System(NS) and 2 of Oral Hypoglycemic agents(OH). The numbers of dispensed units(u), pharmaceutical specialties(PS), mono-drugs of the drug formulary, consultations, prescriptions and chronic patients were obtained. The Principle of Pareto or Analysis ABC was applied by drug group and single agent, identifying the groups of drugs and the single drugs with important impact in the global consumption. The percentage of the total of each group and variations between 2006-2009(V2006/2009) were calculated.

Results
The results are expressed in the Tables 1, 2 and 3

Conclusions
During the course of this study the following was detected:

- An increase in the variety of specialties and prescribed mono-drugs.
- The most consumed group was the CS, followed by NS and HO, with a greater increase in the NS and HO.
- For cardiovascular pathologies mainly enalapril, then amlodipine and atenolol were dispensed, but V2006/2009 was in the inverse order.
- Alprazolam was the most consumed drug of NS until 2008; followed by clonazepam, which registered the major increase in V2006/2009 among the analyzed drugs.
- The metformin was the most consumed of OH and the one which registered more increase.
- The consultations and prescriptions amount diminished, but their proportion was stable.
- Some changes are indicators of a more rational use of medicines: the major use of clonazepam that is less addictive than alprazolam, and metformin because it has been demonstrated that metformin decreases both morbidity and mortality.
- It is necessary to deepen the studies of drug consumption in chronic diseases like arterial hypertension, diabetes type 2 and those of the NS not only due to its prevalence, but also because of the important changes registered in the consumption profile. This would contribute to understand the motivations that led to this significant change.

Table 1. Consumption

<table>
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<tr>
<td>PS</td>
<td>154</td>
<td>189</td>
<td>180</td>
<td>168</td>
<td>+9</td>
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<tr>
<td>Mono-drugs</td>
<td>114</td>
<td>134</td>
<td>127</td>
<td>122</td>
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<tr>
<td>u</td>
<td>2415045</td>
<td>262382</td>
<td>2878510</td>
<td>2420879</td>
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### Table 2. Consultations/Prescriptions

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<th>2009</th>
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<td>239486</td>
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<td>183161</td>
<td>184349</td>
<td>-23</td>
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<tr>
<td>Prescriptions</td>
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<td>176565</td>
<td>166396</td>
<td>173616</td>
<td>-22</td>
</tr>
<tr>
<td>Indicating it prescribes/consults x100</td>
<td>92.66</td>
<td>90.70</td>
<td>90.85</td>
<td>94.18</td>
<td>+1.6</td>
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### Table 3. Chronic patients

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<tr>
<td></td>
<td>3146(56%)</td>
<td>1383(25%)</td>
<td>821(15%)</td>
</tr>
</tbody>
</table>

* Corresponding author. Tel: +54 2652 424689, int. 162; e-mail: cpcalderon2000@gmail.com
COMSUMPTION OF NSAIDS IN A PHARMACY IN SAN LUIS, ARGENTINA

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Chacabuco y Pedernera. 5700 San Luis, Argentina

Introduction

The NSAIDs are non-steroidal anti-inflammatory drugs that include frequently drugs that only have analgesic and antipyretic properties. These drugs are very often self-administered for relieving pain and the flu syndrome, either as a single drug or in combination with others. The consumers are very conscious of the drugs’s high abilities to cause adverse reactions of varying intensity and severity. Their acute and chronic toxicity is epidemiologically important and it is a cause for concern. The aim of this study was to analyze the consumption of NSAIDs in a pharmacy in San Luis city during two months.

Materials and methods

An observational, descriptive, cross-sectional and retrospective study was carried out during October and November 2009. The following data were obtained from a pharmacy in San Luis city: sex (F: feminine, M: masculine), consumed drugs and problems of health. The drugs and the diagnoses were classified according to classification ATC (The Anatomical Therapeutic Chemical Classification System) and ICD10 classification (International Classification of Diseases, 10° Edition), respectively. The medicines that belong to groups N and M of the ATC classification were analyzed in this study. Excessive consumption, acquisition form, and the health problems for which they were acquired, were registered and analyzed. The results were expressed as percentage.

Results

Distribución de Consumption by sex: Nervous System drugs (N): F 43.86%; M 56.14% and Skeletal Muscle System drugs (M): F 68%; M 32%.

Groups of Medicines: N 7.67% and M 12.80% respect of the total of used medicines. Within of total N, the 41.30% was represented by NSAIDS, and within of total M, the 100%.

The drugs more used of N group were: AAS (acetylsalycilic acid) 29.82%, Acetaminophen in combinations to fixed-dose (CFD) 28.07%, and AAS in CFD 12.28%; and these medicines were used to treat the following prevalent health problems: flu 31.58%, and the moderate and mild pains 29.82%.

The drugs more used of M group were: ibuprofen 33%, diclofenac 23%, and diclofenac in CDF 7%; and these medicines were used to treat the following prevalent health problems: moderate and mild pains 59%, arthritis and arthrosis 25%, and fever 7%.

Combinations to fixed-dose (CFD): N 52.63%, and M 24%.

Form of Acquisition: N: 12.28% with prescription, and 87.72% without prescription; M 44% with prescription, and 56% without prescription.

Conclusions

According to the results of this study, we can conclude that the prevalent health problems were flu and moderate and mild pains. The NSAIDS of the M group were the most used. The M group was mainly acquired by patients of the feminine sex. Ibuprofen and diclofenac were the more prescribed drugs within the M group, and AAS and acetaminophen within N group. A high self-medication with NSAIDS was registered, but principally with medicines of N group. Moreover, a high percentage of CFD with low therapeutic value was used. Therefore, it is necessary to implement educational measures to alter these practices that can have many negative consequences for the population.
INCIDENCE OF INFECTIONS CAUSED BY MULTIDRUG-RESISTANT ACINETOBACTER SPP IN THE INTENSIVE CARE UNIT OF A PUBLIC HOSPITAL IN CÓRDOBA

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²National Hospital of Clinics, National University of Córdoba
³Hospital Pharmacy Specialization Students

Introduction
The Acinetobacter genus is distributed as parasitic or free-form on animate or inanimate objects in nature and hospital environment. It is one of the microorganisms causing nosocomial infections with a pattern of multidrug-resistance. It occurs mainly in patients admitted to Intensive Care Units and the associated mortality is high (26% - 68%). The infection control can be achieved through proper hygiene and disinfection technique.

The general objective was to investigate the incidence of infections caused by multidrug-resistant Acinetobacter spp in the Intensive Care Unit (ICU) at the Regional Hospital José Bernardo Iturraspe, during the years 2002-2006. Specific objectives were: analyzing biological materials infected by multidrug-resistant Acinetobacter spp, determining the pattern of resistance, proposing measures to control the spread of bacteria in the institution and to prevent the emergence of endemic strains.

Materials and methods
A retrospective study, from January 2002 to November 2006, was carried out, and 1616 inpatients from Intensive Care Unit were included. Nosocomial infections caused by Acinetobacter spp were identified and antimicrobial susceptibility test was conducted to study the resistance patterns by measuring minimum inhibitory concentration (plate dilution technique).

Samples from different inanimated elements were taken to culture and confirm the pathogen presence.

Results
During the study period, 55 cases of infection caused by multidrug-resistant Acinetobacter spp were identified, representing 3.4% of the population study. The outbreaks took place in 2004 with 23 cases and 2006 with 17 cases (see Table 1). The resistance pattern obtained is similar to that reported in other studies: imipenem 0%, piperacillin-tazobactam 40%, amikacin 48%, ciprofloxacin 96%, ceftazidime 97%, and gentamicin 100% (see Table 2).

Resistant strains were detected most frequently in endotracheal secretions (n=27), and there were 22 missing data (see Table 3). Besides, they were isolated from other services than ICU: Internal Medicine 8%, Outpatients Wards 8%, Surgery 4 % and Maternity 1% (see Table 4).

Conclusions
The study demonstrated a nosocomial infection of 3.4% by multidrug-resistant Acinetobacter spp in ICU. The strains were susceptible to imipenem. About 50% of the infected biological materials were endotracheal secretions.

The suggested measures for preventing the emergence of endemic strains were the following: decontamination of ICU, staff training, updating of standard operating protocols, new cases surveillance and environmental monitoring.

Acknowledgements
To Dr. Elena Vega and Mgter. Sonia Uema for checking the abstract.

References

² Parisia L, teléfono 03564-430634 ó 15648079, E-mail: lucilisdero@hotmail.com

<table>
<thead>
<tr>
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<td><strong>Resistance (%)</strong></td>
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<td>48</td>
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<tr>
<td>Ceftazidime</td>
<td>97</td>
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<tr>
<td>Ciprofloxicin</td>
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<td>Gentamicin</td>
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<td>Piperalicina-Tazobactam</td>
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<td><strong>N° of cases</strong></td>
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<tr>
<td>Eschar</td>
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<tr>
<td>Blood culture</td>
<td>1</td>
</tr>
<tr>
<td>Catheter tip</td>
<td>3</td>
</tr>
<tr>
<td>Discharge of abdominal surgical wounds</td>
<td>1</td>
</tr>
<tr>
<td>Endotracheal secretions</td>
<td>27</td>
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</table>

<table>
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<th>Medical Service</th>
<th>% of cases</th>
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<td>Medical Clinic</td>
<td>8</td>
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<tr>
<td>Surgical Clinic</td>
<td>4</td>
</tr>
<tr>
<td>Outpatients</td>
<td>8</td>
</tr>
<tr>
<td>Maternity</td>
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<td>Intensive Care Unit</td>
<td>34</td>
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USE OF CARDIOVASCULAR DRUG IN TYPE 2 DIABETIC OUTPATIENTS

Quaglia NB, Cuis, NG, Bertero J, Nuñez MH, Marzi MM

1-Área Farmacología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. 2-Cátedra de Farmacología, Facultad de Medicina, Universidad Nacional de Buenos Aires. Suipacha 531, Ciudad de Rosario, Argentina. CP: 2000

Introduction
Diabetes is a highly prevalent disease and its serious consequences of morbimortality leads to overload health system (1). Macro and microvascular complications have been described as morbid results directly associated to insufficient control of diabetes (2). Previously, we have found a very deficient glycemic control among diabetic outpatients from Hospital Provincial del Centenario in Rosario city. The aim of this work is to describe the prescribed drugs in connection with cardiovascular system disease in the same group of patients.

Material and Methods
Cross sectional study was performed from prescriptions collected that include some oral hypoglycemic drug (OHD) in the pharmacy office from Hospital Provincial del Centenario during 2008. All drugs that appear in these prescriptions taking action in cardiovascular system were also collected. These drugs were classified according to their effect. Results are shown as percentages with confidence intervals of 95% (CI95%) or means ± SEM. Inferential analysis was performed as required. Excel 5.0 and Epi Info 3.3.2 versions were used.

Results
A total of 2270 prescriptions that include oral hypoglycemic drugs (OHD) were collected. These were dispensed to 580 outpatients throughout the studied year. Among them, a group of 498 representing the 85,9% (82,7-88,5)% got metformin and/or glybenclamide (HOG), while another group of 82 remaining outpatients [14,1% (11,5-17,3)%] got OHD and insulin, at least, in one occasion (IG). Means of age 55,34 ± 0,60 years old to HOG and 58,04 ± 1,30 to IG, there is a slightly tendency to be significant (p=0,08). From the total of patients who took some drugs for cardiovascular system, that is 358, the 99,2% (97,4-99,8%) got some drugs belonging to antihypertensive (aHTA) and/or hypolipemic (HL) family. Table 1 describes the patient’s proportion that used aHTA or HL drugs between both outpatient groups.

Conclusions
Among diabetic outpatients, the higher cardiovascular system drug prevalence is connected to aHTA and HL drug family. A significant higher proportion of type 2 diabetic outpatient users of insulin required antihypertensive (aHTA) and hypolipemic (HL) compared to those who only used oral hypoglycemic drugs. The mains of age between both groups are particularly closed. It is understandable that type 2 diabetic patients using insulin request in a higher proportion of drugs associated to cardiovascular system, however, it would be expected that means of age are as far as possible. Improvement in disease control would make possible to delay insulin demands and also the major morbid condition of diabetic disease.

References
<table>
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<th></th>
<th>OHG</th>
<th>IG</th>
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<tr>
<td>a- HTA</td>
<td>55,00%</td>
<td>80,50%</td>
<td>0,0001</td>
</tr>
<tr>
<td>HL</td>
<td>15,10%</td>
<td>24,40%</td>
<td>0,05</td>
</tr>
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</table>

Table 1: Proportion of patients who got some anti-hypertensive (a-HTA) and hypolipemic (HL) drugs in the group who got some oral hypoglycemic drugs (HOG) and in the group who got oral hypoglycemic drugs plus insulin (IG). In the last column is shown p-value resulting from perform test to proportion comparison.
THE IMPORTANCE OF THE CORRECT INTERPRETATION AND EXECUTION OF THE DRUG INSTRUCTION LEAFLETS.

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Introduction
There is a wide range of drugs that in their administration form are very unstable, therefore they are presented as finished products that have greater stability formulations. At the moment of their use, such drugs required the participation of suitable personnel for their preparation and appropriate administration. For that purpose, the leaflet should provide correctly detailed instructions.

In our case, we work with clotting human FVIII concentrate (drug for the hemophilia treatment), high purity product, purified by chromatography, with double viral inactivation and thermal treatment, obtained from human plasma coming from total blood units and plasmapheresis. Such factor is presented in lyophilized form and is reconstituted with water for injection, for its use as intravenous solution. Our objective is to evaluate the impact on dosage when the procedures described by the manufacturer at the moment of the preparation and administration of a drug are not respected.

Materials and Methods
The product (lyophilized and solvent) should be brought to room temperature for its reconstitution, the dissolution of the lyophilized product should be completed and can present particles in suspension, therefore it should be filtered using the provided material. The filtered solution should be clear, limpid or slightly opalescent, colorless or slightly yellowish, without visible particles.

In this case, the leaflet presents a defined procedure for its reconstitution and administration. In this work, we evaluate what happens, if the described steps, as detailed in the leaflet, are not specifically followed. For the determination of factor VIII activity in the finished product, the chromogenic plate method, as described in the European Pharmacopoeia, is employed, and it is performed on the filtered product.

For this work, the determination of FVIII activity in the samples was carried out, a) prior to the filtration, b) employing the filter incorrectly according to the specifications detailed in the leaflet and c) using the filter correctly.

Results
The results obtained from the tested samples are evaluated, and the statistical analysis of this data shows a loss of FVIII activity which is up to 20% when the filter is used correctly, and near 40% when it is utilized incorrectly. This indicates that there is sharp decrease of the FVIII activity (UI/ml) when the leaflet instructions are not followed.

Conclusions
During the development of our research, we arrived at the conclusion that when the instructions given by the manufacturer (in this case the correct filtration) at the moment of the drug administration are not respected, the drug’s therapeutic action can be drastically modified and consequently it does not cover the patient's needs.

The person who makes this step is a very important link in the “FINAL CONDITIONS” of the drug, and for this reason, it is extremely critical and relevant his or her training for the correct interpretation and execution of the leaflet, which implies a better use of each formulation property.
AN ASSESSMENT OF DRUG COSTS IN CARDIOVASCULAR SURGERIES

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CONICET - Universidad Nacional de Sur
12 de octubre, Ciudad de Bahía Blanca, CP: 8000

Introduction
Different estimates of incidence of drug costs in cardiovascular surgeries and related treatments emerge depending on whether indirect costs are included or not. Leah (1) estimated that drugs absorb 22% of direct and indirect costs of cardiovascular diseases in Europe. Russell (2) states that drugs represent 4-5% of direct costs of heart diseases in United States.
This paper analyzes the relative importance of drugs in total direct cost of twenty cardiovascular surgeries performed between 2006 - 2007 at the Penna Hospital (Bahía Blanca) and correlates these costs with other patient-specific variables.

Methods
Drug spending were regressed in terms of days of hospitalization (total and by type of care unit). Also analysis of variance was applied in order to identify significant differences in drug requirements by groups of patients according to age, sex, pathology and previous diseases. Statistical tests were performed in SPSS 15.0.

Results
Drugs absorbs 4.2% of total direct costs of cardiosurgeries. Dipyrone (1g), morphine (4mg) and cepazolin (1g) represent 58% of total medication spending.
Inpatient days account for 69% of the variability in drug expenditures of cardiovascular surgeries. The goodness of fit improves substantially (up to 78%) if inpatient days are disaggregated into common, intermediate and intensive care.
The observed large variability in drug spending among patients is related with their biological characteristics, health status and also with sample size.
ANOVA tests indicate that male patients require more inpatient days (p < 0.058) and respiratory drugs (p < 0.091) than women.
Patients over 50 years old demanded more hospitalization days (p < 0.019) and cefazolin (p < 0.073). The younger group required, in turn, more antibiotics (p < 0.041).
Interventions on ischemic heart disease and myocardial revascularization required more inpatient days (p < 0.012), consumed more cardiac drugs (p < 0.03) and heparin (p < 0.099) than other cardiosurgeries.
The existence of previous pathologies does not affect inpatient days, affecting only spending amount in anxiolytics (p < 0.088).

Conclusions
Although this work is not enough to generalize results, it represents a breakthrough in cost estimates of cardiovascular diseases in Argentina. These results are useful for the design of economic evaluations (cost-utility and cost-effectiveness studies) that promote the efficiency in public resource allocations.
Furthermore, the incidence of sex, age and type of cardiosurgery performed on hospital care and drug spending offers tools to improve budgetary planning mechanisms in hospitals.

References

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PARTICIPATIVE EDUCATION FOR ENHANCING SANITARY CONDITIONS
AFTER IMPROVING THE QUALITY OF DRINKING WATER IN AN URBAN
MARGINAL POPULATION

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rosariorollan@arnet.com.ar

Introduction
Water makes a healthy environment possible; yet, it can also be the first carrier of disease. The availability of suitable water for cooking as well as for personal and domestic hygiene is essential to guarantee the health and well being of people (1). As part of an Interdisciplinary Project of Sanitary Education in marginal populations, this work assessed the bacteriologic and physicochemical quality of drinking water in a population settled on the banks of Pinto River – also a recreational river-, Province of Córdoba, and determined the chlorine dose necessary for disinfection in order to elaborate strategies for educating the population as to the safe usage of this resource, applying a constructivist approach (2).

Materials and methods
A total of 50 samples of water from the river, pre-filter and from population homes were analyzed, determining in each sample: total mesophilic bacteria (PCA, 35º, 24 hs); total coliforms (NMP, 35º, 48 hs); fecal coliforms (NMP 44º, 24 hs); pH, temperature, total hardness, sulphates and arsenic. By trial and error method with microbiological test, the efficacy of 0,05, 0,1 and 0,4 ml of sodium hypochlorite (55 gr/l) for disinfection was proved. Education strategy: identification of risky practices and criteria of prioritization based on a scale of analysis of behavior, definition of goals of the education practice and design of interventions.

Results
The analyzed samples showed the following results: total mesophiles from 200 and more than 500 UFC/ml; total coliforms more than 3 NMP/100 ml; presence of fecal coliforms / 100 ml. Total sulphates and hardness below 400 mg/ml, arsenic 0,05 mg/L, pH from 8,10 to 8,5, and temperature from 10ºC to 16,5ºC. By trial and error method, it was found that with a solution of 0,05 ml of sodium hypochlorite per liter of water with a contact time of 30 minutes the studied bacteria are completely eliminated. Education strategy: the punctuation of the scale of behavior was 20, the selected strategy was a participative methodology (PHAST) intended to improve the health and quality of life of the dwellers in order to decrease the diseases transmitted by water.

Conclusions
The water samples analyzed do not comply either physicochemically or microbiologically with what is established by the Argentine Alimentary Code to classify as drinking water making its consumption a risky practice for the health of the population. The participative methodology for sanitary education enables us to perform a significant work of awareness for the disinfection of water in order to achieve a behavioral change related to the characteristics of the population under study.

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PHARMACOTHERAPY OF THE TUBERCULOSIS (TB). ASSESSMENT OF THE QUALITY OF ANTI-TB DRUGS PROVIDED BY THE NATIONAL TB CONTROL PROGRAM

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Corresponding author: meoliver@fcq.unc.edu.ar

Introduction
Although there are potentially curative treatments since more than half a century, the TB is even today the most important cause of avoidable deaths and is a very important problem in developing countries. One of the biggest problems facing the TB is that the established set of rules is not fulfilled. In Argentina, anti-TB treatments supplied by the National TB Control Program are dispensed by pharmacists in public hospitals. There is no information easily available neither about the quality of the anti-TB products, nor if it is maintained after the long supply chains.

Objective
To determine the quality of formulations of anti-TB agents supplied in public hospitals for use in national TB control program. In vitro assays of solid dosage forms containing isoniazid (ISO), rifampicin (RIF), pyrazinamide (PIR) and ethambutol (ETA) as single units and also ISO/RIF Fixed Dose Combination were performed.

Materials and methods
Rifampicin (capsules 300 mg), isoniazid (tablets 300 mg), pirazinamide (tablets 250 mg), ethambutol (tablets 400 mg) and RIF/ISO Fixed Dose Combination (tablets 300/150 mg). Twelve units of each product were tested according to their individual USP monographs, with in vitro dissolution test and quantification by HPLC and UV-Vis methods. Dissolution profiles were constructed.

Results and discussion
The quality of products containing antibiotics is one of the basic elements to guarantee the treatment success. In the case of anti-TB treatment, the lack of quality can result in both effectiveness decrease and side effects occurrence. The tablets containing RIF, ISO, and PIR greatly exceeded the established values of Q. However, those containing ETA presented a dissolved percentage at the lower limit. In contrast, Q times for products containing RIF and ISO in the fixed drug combination do not meet the codified requirements. This could result in drug unloading which would seriously compromise the treatment effectiveness in in vivo situations. It is known that RIF exhibits variable bioavailability from solid oral dosage forms and this problem is more apparent when it is formulated as fixed dose combination, in presence of other first-line anti-TB drugs. Pharmacist must be aware of the patient’s risks. Clinical follow-up is encouraged.

Conclusions
Formulations of anti-TB agents supplied as single units successfully passed in vitro evaluation. However, the RIF/ISO fixed drug combinations do not. This non compliance can compromise drug effectiveness. The obtained results will be reported to the National Health Authority.

Acknowledgements
We acknowledge financial support received from SACyT/Ministerio de Salud de la Nación, Argentina (Argentine National Ministry of Health).

References
ADVERSE DRUG REACTION (ADR) AS CAUSE OF HOSPITAL ADMISSION IN A TEACHING HOSPITAL

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Background
Adverse Drug Reaction (ADR) is an important public health concern, because it can difficult the diagnosis, delay the treatment [1], reduce the patients’ quality of life [2] and raises the unnecessary expenditure for the hospitals.

Objectives
The purpose of this study was to: 1) assess the prevalence of ADR-related hospital admission; 2) identify the demographic characteristics of patients with possible ADR; 3) identify the therapeutic classes often involved with ADR; 4) identify, per system, the clinical manifestations of ADR; 5) identify the risk factors for hospitalizations due to ADR and 6) assess the causal relationship [3] between the use of drug and the development of ADR.

Methods
A cross-sectional observational study was performed. It was conducted in an internal medicine ward in a teaching hospital. Data was collected from August 2008 to December 2008. All patients aged 18 years old or over, with hospitalization period over 24 hours, who agreed to participate in the study signed an informed written consent were interviewed about their symptoms, complaints and causes of admission, as well as what were the drugs used 15 days prior the hospitalization.

Results
During the period of the study, 115 (46.4%) patients were hospitalized due to ADR; the majority was woman [76 (66.1%)] and no-elderly people [66 (57.4%)]. According to the ATC code, the main therapeutic classes commonly involved with hospitalization were drugs that act in cardiovascular (48.7%), digestive (22.9%) central nervous systems (14.1%). Regarding the type of drug prescriptions, 90.6% were prescription drugs, 9.4% were psychotropic drugs, and 9.1% were non-prescription drugs. 58.0% of medicines ADR-related admission belonged to national list of essential medicines. The clinical manifestations of ADR, according to ICD-10, most commonly observed in the study were: disorders in digestive (23.0%), respiratory (20.2%) and circulatory systems (14.6%), as well as symptoms, signs, abnormal clinical and laboratory findings (20.2%) (Table 1). Only polypharmacy was detected as a risk factor for ADR-related admission (p = 0.006). The assessment of causal relationship showed that of the 178 ADR identified, six were definite, 54 probable and 118 possible (Table 1). The five drugs most used prior to hospitalization were: omeprazole, captopril, insulin, acid acetylsalicylic and furosemide (Table 2).

Conclusion
This pharmacoepidemiological study shows the need of patient’s therapeutic monitoring in the first and secondary health levels, to promote the right use of the drugs and to drop hospital admission in the third health level due to drug-related problems.

Acknowledgements
The authors would like to thank the Program of Support to Scientific Development of the School of Pharmaceutical Sciences at the Universidade Estadual Paulista (PADC-FCF/UNESP) for the financial and institutional support.

References

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Table 1. Frequency of symptoms, complaints and causes of hospital admission, per systems (ICD-10), according to the ADR Probability Scale, reported by hospitalized patients in an internal medicine ward in a teaching hospital - Ribeirão Preto, SP, 2008 (n=115)

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>Total ADR N (%)</th>
<th>Classification according to the ADR Probability Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definite N (%)</td>
<td>Probable N (%)</td>
</tr>
<tr>
<td>Digestive system</td>
<td>41 (23,0)</td>
<td>0</td>
</tr>
<tr>
<td>Symptoms, signs and abnormal clinical and laboratory findings</td>
<td>36 (20,2)</td>
<td>0</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>36 (20,2)</td>
<td>2 (33,3)</td>
</tr>
<tr>
<td>Circulatory system</td>
<td>26 (14,6)</td>
<td>0</td>
</tr>
<tr>
<td>Endocrine, nutritional and metabolic</td>
<td>11 (6,2)</td>
<td>1 (16,7)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue</td>
<td>9 (5,1)</td>
<td>2 (33,3)</td>
</tr>
<tr>
<td>Musculoskeletal system and connective tissue</td>
<td>9 (5,1)</td>
<td>0</td>
</tr>
<tr>
<td>Genitourinary system</td>
<td>3 (1,7)</td>
<td>0</td>
</tr>
<tr>
<td>Nervous system</td>
<td>3 (1,7)</td>
<td>0</td>
</tr>
<tr>
<td>Blood and blood-forming organs</td>
<td>2 (1,1)</td>
<td>1 (16,7)</td>
</tr>
<tr>
<td>External causes</td>
<td>2 (1,1)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>178</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 2. Frequency of the 5 drugs most used prior to hospitalization for patients admitted at the internal medicine in a teaching hospital, whose Adverse Drug Reactions (ADRs) were considered admission’s reason, Ribeirão Preto, São Paulo, 2008 (n = 115)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Freq. N</th>
<th>ADR-related admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole</td>
<td>23</td>
<td>Fatigue (7), abdominal pain (6), bronchospasm (4), chest pain (4), cough (4), diarrhea (2), edema (2), back pain (2), vomiting (2), hepatotoxicity (1), anaemia (1), leg pain (1), fever (1) tachycardia (1)</td>
</tr>
<tr>
<td>Captopril</td>
<td>20</td>
<td>Bronchospasm (8), angina (6), cough (4), diarrhea (3), tachycardia (2), gastrointestinal ulcer (1), fever (1), abdominal pain (1), vomiting (1), hypoglycemia (1), somnolence (1), hypotension (1)</td>
</tr>
<tr>
<td>Insulin</td>
<td>20</td>
<td>Insulin resistance (8), bronchospasm (5), chest pain (5), diarrhea (2), anemia (1), hypoglycemia (1), edema (1)</td>
</tr>
<tr>
<td>acid acetylsalicylic</td>
<td>17</td>
<td>Bronchospasm (14), angina (3), hypertension (1), gastrointestinal ulcer (1)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>14</td>
<td>Abdominal pain (4), dizziness (3), diarrhea (3), nausea (1), anemia (1), erythema (1), hyperglycemia (1), fever (1)</td>
</tr>
</tbody>
</table>
SENSORY ACCEPTABILITY STUDY OF FLUORIDE GEL USE FLAVORED ORAL

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Introduction
Sensory evaluation is the analysis of materials through the senses\textsuperscript{1}, it is used in various industries (food, cosmetics, pharmaceuticals).
In the Faculty of Chemical Sciences of the Catholic University of Córdoba, it has been developed a low-cost gel with anticariogenic, therapeutic and preventive properties\textsuperscript{2,3,4} (new product), to be used in populations at caries risk. The product must possess good organoleptic characteristics that allow sensory acceptability, which facilitates the therapeutic goal.
The purpose of this study is to assess the acceptability of a gel formulation in a population of untrained judges randomly selected to voluntarily agree to test and survey.

Material and methods
Population:
The test was conducted by a panel of 200 judges untrained adults, healthy, both sexes, aged between 15 and 75.

Materials:
Two gels, which differ in the flavouring used. Group A (original formula), and group B (new flavor). The vials were intercropped delivered one per week, listed with four digits random numbers.

Methods
Survey Structure
a) Introduction and instructions for use.
b) Evaluated attributes: smell, appearance and consistency, colour, taste and feeling after use, for each attribute, a 9-point hedonic scale was used, from 1 - dislike very much, up to 9 - I love it. Finally the respondent can express their comments on the product.
Procedure used to evaluate the gel:
Each judge was given 5 grams of every product, the survey and a measuring spoon, indicating that the test had to be done on faste.

Statistical analysis:
We obtained absolute and relative frequencies for each category of the hedonic scale. We calculated means, medians, standard deviations of acceptability and normality test for each sample (Shapiro-Wilks). Significant differences were analyzed using a nonparametric median test with a significance level of 5%.
The software used was Infostat 2009p version.

Results
It was noted that data was not normally distributed (p <0.05). In this case, hypothesis testing for nonparametric statistics should be performed.
There were significant differences in all attributes except appearance and consistency of sample A being the highest average acceptability for all attributes (Table 1).

Conclusion
Selected sensory attributes allowed to establish significant differences between samples A and B. Sample A (original) had greater acceptability with respect to sample B (proposed). In appearance and consistency, where there is no significant difference, the result is consistent evidence of acceptability as it may be the...
result of subjective association between color, smell and taste, because preparation remained constant.

References:
3 - Fluoride and Fluoridation, American Dental Association, Disponible en http://www.medlineplus.gov/spanish

Table 1: Results of sensory evaluation of fluoride gels A and B

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Test Medium (α=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>Odor</td>
<td>6,71</td>
<td>1,35</td>
<td>6,24</td>
</tr>
<tr>
<td>Appearance and consistency</td>
<td>6,60</td>
<td>1,37</td>
<td>6,30</td>
</tr>
<tr>
<td>Color</td>
<td>7,21</td>
<td>1,11</td>
<td>6,85</td>
</tr>
<tr>
<td>Flavor</td>
<td>6,38</td>
<td>1,45</td>
<td>5,71</td>
</tr>
<tr>
<td>Sensation after use</td>
<td>6,00</td>
<td>1,18</td>
<td>5,48</td>
</tr>
</tbody>
</table>

REF: SD: Standard Deviation

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INTRODUCTION

Aging is closely linked to a broad array of risk factors that are associated with chronic disease and declining health. Appropriate drug therapy can bring benefits and better quality of life to elderly patients, but the use of potentially inappropriate medications can also be harmful to these patients. Several factors increase the risk of adverse consequences of drug therapy, including overuse of medications, medication errors, patient nonadherence, and inappropriate drug use. This last issue has been documented to be frequent in older persons and is thought to be the major contributor to drug-related morbidity. Such inappropriate use can be expected to affect both personal well-being and use of health care services.

Normally, a panel of experts is consulted to prepare a list of minimum medicine needs for a basic health care system or population. In the lists, the most efficacious, safe and cost-effective medicines for priority conditions are recommended. Therefore, they could be assumed as guidelines provided but the last prescription decision concerns to the physician and/or the health team. The objective of this work is to examine drug utilization at a public nursing home according to three panels of consensus criteria.

MATERIALS AND METHODS

Type of study: Observational, descriptive, cross-sectional.
Setting: Hogar de Ancianos Padre Lamónaca (Córdoba, Argentina). Public dwelling for older adults with low incomes.
Data collection: prescribed drugs, age and gender. Data was elicited from medical charts at random, one day a month. Medicines were classified according Anatomical Therapeutics Chemical (ATC) Code (1).
Data process: Microsoft Excel 2003.
The most prescribed drugs were compared with three different lists: WHO Model list of Essential Medicines (2), National Formulary (NF) (3) and Beers criteria (4).

RESULTS

In 2006, at the institution were 140 residents distributed in 5 houses. The average of prescriptions by day was 4 (range = 0-15), and 120 older adults received at least one medicine. During the study, 161 different drugs were used. However, only 32 represented the 80% of the prescriptions. From the 20 medicines most prescribed, 8 were not included in the WHO list, three were not in the NF and two were recommended to avoid according to Beers criteria.

DISCUSSION AND CONCLUSIONS

The medicines included in each list are different, therefore, a direct comparison is not possible. WHO Model List is oriented to adult patients and use the most restrictive criteria. Although Argentina NF is based on WHO list, the experts considered the inclusion of more medicines. Both are lists of essential medicines adapted to different settings. Conversely, by using Beers criteria results a list of drugs to be avoided in persons older than 65 years of age, regardless of their level of frailty. The three lists are population-based designed and they are not intended to substitute professional judgment regarding the individualized needs of a patient. However, they are useful tools to minimize drug-related problems in elderly people.

ACKNOWLEDGMENTS

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References.

EDUCATIONAL INNOVATION IN THE SUBJECT PUBLIC HEALTH OF THE SCHOOL OF CHEMICAL SCIENCES, UNIVERSIDAD NACIONAL DE CÓRDOBA

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Introduction
Public Health mission is to satisfy the society concern of guarantee those conditions that allow the people to be healthy (1-2).

For that it is fundamental to account with properly educated and qualified professionals.
In this context and given the impact that some illnesses have in our region, Dengue and Yellow Fiber (both epidemical in Argentina last year), Influenza A H1N1 (declared pandemic by the OMS), and Leishmaniasis (considered an emergent problem by the Health Ministry of the Nation) (3-5) it was elaborated a new practical activity (PA) of the subject Public Health being its objectives the following:
To integrate and to study in depth the acquired concepts during the subject education in relation to transmissible illnesses, epidemic, endemic, Health Promotion strategies, Illness Prevention and Health Education.
To develop abilities in the pharmacist professional that allow him to give answers to new sanitary situations, actively participating in primary prevention activities.

Materials and methods
Throughout virtual classroom of the subject, related material to each one of the pathologies was given.
After that, students were divided by groups randomly assigning them one of the previously mentioned pathologies.
During the PA the students had a short time for comments among them about the read material.
Then, it was discussed about the population to which the Project would be directed.
Device of each of Primary Prevention Project (brochures, leaflets, or others) was discussed for 1 h by each group.
Later, they presented its project in front of the professors and their classmates.
Finally, the prepared material was set at the virtual classroom in order that all the students had access independently that the topic they prepared.

Results
In 2009 the subject had 96 students, the 84,37% of them carried out this practical activity divided in 23 groups.
The students learnt and could apply the acquired knowledge of 4 new topics, their impact and ways of prevention.
The diffusion strategy selected by the students was brochures in a 95,65%. They justified their option because in their opinion brochures are considered of easy reading, manipulation and distribution. The remaining 4,35% adopted the poster modality because they considered them more easily watchable by all.
All the groups manage the elaboration of simple and easy interpretation material, according to the selected population of reference.

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Conclusions
The activity was well accepted by the students, which showed a big interest, contributing with additional material and actively participating in the elaboration. Besides the fact that this activity lead to incorporate new knowledge, it was an integrating activity doing easy the comprehension of the topics.

Acknowledgments
To all the participating students of Public Health subject.

References
5. Centros para el Control y la Prevención de Enfermedades de EE.UU. (CDC) [pagina Web]. Atlanta: Centros para el Control y la Prevención de Enfermedades de EE.UU.; 2009 – [actualizada el 11 de junio de 2009; acceso el 11 de junio de 2009]. Disponible en: http://www.cdc.gov/spanish/
“EDUCATION FOR THE RESPONSIBLE USE OF MEDICINAL PLANTS”
AN EXTENSION APPROACH: FROM THE UNIVERSITY TO THE COMMUNITY

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Introduction
The National University of La Plata (UNLP) is characterized as one of the Argentine National Universities who consider the extension, interaction of her teachers with the community, as a priority (1,2). Recently, the Chair of Pharmacognosy (Faculty of Exact Sciences, UNLP) presented an extension project, which was accredited and subsidized by the SEU-UNLP (Secretary of University Extension, UNLP) This project involves the training of the population group of older adults, who use traditional medicines with higher attendance, in the recognition of the advantages and disadvantages that implies the use of medicinal plants and other natural products as therapeutic resource associated with synthetic drugs.

Materials and methods
At a first stage, a series of tasks including:
- Data collection (ex ante, in via, post facto) previous identified variables, indicators and indices, using direct observation techniques, inquest, document analysis and qualitative techniques.
- Collection, by extension agents, of information materials (literature, informative publications, samples of medicinal plants) and preparation of teaching materials (PPS) to be used during the meetings. The methodology to be implemented with the people, basically consists of two or three meetings during which we developed:
  - Informative talks by the extension agents and / or experts
  - Workshops, working groups and discussion groups to analyze their own experiences, with the participation of assistants supervised by extension agents.
  - Display for teaching purposes of medicinal plants, herbal medicines, nutraceuticals and dietary supplements
  - Dissemination through the media, conferences and publications

Results
While the project is in its beginning, so far has worked on the development of diagnostic inquest and determination of the parameters to be evaluated, as well as in the preparation of teaching material adapted to the profile of the recipients of our proposal. As a future projection, we’ll expect that the attending these meetings:
  a) Achieve interpret the information contained on the label and can thus recognize the quality of a particular herb.
  b) Know evaluate the information contained in advertising or commercial publicity distinguishing that that is misleading.
  c) Know differentiates a dietary supplement of herbal medicines.
  d) Learn to recognize potential problems that might arise when using them simultaneously with the synthetic drug prescribed for the most frequent illnesses in this age group, managed to avoid and made aware about the need to inform your doctor, so that he has the data that lead to better health.

Discussion/conclusions
Perhaps the most notable aspects of this project are: a) The direct contact and dialogue between extension agents and the people, becoming fixtures in an educational activity focused on the recipient, through their active participation. b) Training of students and young graduates, for one of the main roles to play in their professional lives, as is the task of education in the subject area related health pharmaceutical care or monitoring the patient.
Acknowledgments
SEU-UNLP (Secretaría de Extensión Universitaria, Universidad Nacional de La Plata)

References

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EDUCATIONAL PROGRAM FOR THE APPROPRIATE USE OF MEDICINE


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Introduction
The main purpose of school education in the appropriate use of medicine is to provide students at school age with information about the use of medicine, in order to acquire confidence and good habits to influence appropriate behaviours and attitudes in the usage of medicaments.

Methods and materials
This workshop arises from the necessity of implementing a preventive task that was developed by the Professional Association of Pharmacists (Colegio de Farmacéuticos de Santa Fe, 1era. Circunscripción), and not knowing how to put it into action, it was crucial to ask for the disciplinary contribution of the Professional Association of Educational Psychologists (Colegio de Psicopedagogos). The simplicity of the workshop’s setting up and its creativity makes it entertaining for the children and at the same time it has a profound methodology; since it takes into account the process of reviewing new skills and ways to put them into practice to display different new modes of behaviour.

First stage
The workshop mode is applied to students from 10 to 11 years old.
✓ The workshop’s estimated time is about two hours and a half.
✓ A play or a film is used to exemplify different everyday situations in relation to the use of medicaments.
✓ Children are encouraged to establish a dialogue with the purpose of analyzing and giving their opinions about the performance.
✓ After that, they are asked to make all the questions they want.
✓ Then, all the students’ questions and concerns on the matter are answered.
✓ Children are invited to draw entitled pictures to recreate a brief summary of the apprehended experience and after they have finished their drawings, they have to explain it on their own.

Secondary stage
After concluding the first stage, schoolteachers of the institution agreed that we should hold another meeting in 3 months’ time to evaluate the course of the educational campaign at which students must be involved, adhering to the established norm which its main aim is the appropriate usage of medicine.
As a closure, a certificate is given to every child to prove the participation in the workshop and a book is gifted for the school library.

Conclusion
Bearing in mind the limitations of the Program, it was resolved that the main purpose for this activity would be only to raise awareness of the correct or appropriate use of medicine taking into account the range of age of the children and the implemented methodology (workshop).

Acknowledgments
Crearse, Theatre company.

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PEDAGOGICAL DESIGN FOR PHARMACEUTICAL TRAINING (PT) IMPLEMENTATION

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Introduction
The Pharmacy Career curriculum of the Facultad de Ciencias Exactas (UNLP), consists of a 10 semester formation cycle which includes a theoretical-practical teaching, and compulsory PTs (300 hs) during the last four-month period (1) (2).

The pedagogical proposal to be implemented for offering a homogeneous educational service had to take into account the application of pedagogical techniques within professional environments with structural differences (organization and resources) and such resources allocated by the Faculty.

This work aims to design and implement a service training pedagogical proposal with problem-based learning.

Materials and Methods

Theoretical content design: By relating such contents prescribed under the Curriculum and the professional practice development status, diagnosed through interviews with key referents, the need to include know how was taken into account, from an integrating strategy of them, enabling to solve problems more effectively within each working environment. (60)

Teaching structure design:
- **PTTU (Pharmaceutical Training Teaching Units) certification:** Performed by the professorship, according to regulation (3), within such bodies developing PT.
- **Signature of Agreements:** Operational and Frame Agreements, which stipulate operating conditions (instructor designed by the company, attendance schedule of students and number of vacant places)
- **Teachers-instructors Integration:** the professorship has three teachers of its own and teacher instructors (TI) within each PTTU. Given the number of PTTU and teaching simultaneity, a communication method based on electronic mail, phone communication, and interviews enabling the attendance supervision was designed, generating written documentation for its analysis, within a Continuous Improvement Cycle.

Evaluation method design of the PTTU operation: By analyzing documentation derived from monthly meetings with students and TI, from the final reports of each student and from the incidence record.

Design of the knowledge acquired Certification method: By integrating the theoretical issues with the practical experience achieved during the PTs, the students perform emerging products: applied research, cross-sectional study, monograph and management tool application. Through the evaluation of these products, every knowledge acquired as well as the use of tools for problem-based learning were certified.

The instructors carried out a student’s report based on the following dimensions: learning, communication and environmental relationship.

Results
Contents on the following were included: Company Management Tools, Human Factor Management, Scientific Work Making and Professional Development Context.

Between 2006-2009 21 PTTU were accredited, after evaluating 64 professional spaces (Table 1). PT seats are located at Office Pharmacies, Hospital Pharmacies and Pharmaceutical Industries. There are 58 vacant places yearly (Table 1). Total number of TI amounts to 24 (Table 1). All PTTUs maintained their certification status.

The certification of students’ knowledge was performed through the evaluation of 657 works for period 2006 – 2009 which implied the proof reading of 4500 pages, distributed as follows: 2006: 9 works (3 students), 2007: 71 works (13 students), 2008: 181 works (25 students) and 2009: 396 works (36 students). The students’ passing percentage is: 98.70 %.

Conclusions
The implementation of the pedagogical proposal designed enabled to create a mixed teaching environment faculty – private and public companies which enabled professionals to be invested with
competences achieved and closely related with their professional exercise and their development status within each environment. Further studies are required to improve the Educational Quality.

References


<table>
<thead>
<tr>
<th>Professional exercise</th>
<th>Companies evaluated</th>
<th>Agreements signed</th>
<th>Vacant places/year</th>
<th>Instructors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office pharmacy</td>
<td>30</td>
<td>12</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>Hospital pharmacy</td>
<td>13</td>
<td>4</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Industrial Pharmacy</td>
<td>21</td>
<td>5</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>21</td>
<td>58</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 1: Number of professional spaces which were accredited as Teachers’ Unit of Pharmaceutical Teaching, number of vacant places available and teachers instructors

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EVALUATION OF THE IMPACT OF NEW INFORMATION AND COMMUNICATION TECHNOLOGIES IN UNIVERSITY EDUCATION

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Introduction
The New Information and Communication Technologies (NICTs) can be defined as tools and processes used to store, organize, and share information electronically, allowing transmission, processing and dispersion of information in real time. In the subject “Prepracticanato Profesional de Farmacia”, belonging to the 8º semester of the Pharmacy career, a virtual classroom was implemented under the Moodle learning platform as a new teaching strategy. In this course, there are activities designed to train and develop students with the skills necessary to use different sources of information on drugs in relation to the proper use and resolution of problems related to these, thereby promoting their rational use. Our aim was to reinforce what students have learned in class and become familiar with the use of NICTs by providing them with all the necessary tools.

Objective
Assess the impact of the implementation of NICTs through a virtual classroom, in addition to traditional teaching in the subject “Prepracticanato Profesional de Farmacia”.

Materials and methods
An online semi-structured survey was drawn up and the students answered anonymously at the end of the course. 366 questionnaires were analyzed for the years 2008 (n = 99), 2009 (n = 125) and 2010 (n = 142).

Results
During the three years, most of the students visited the virtual classroom more than 10 times (2008 = 64.94%, 2009 = 66.38% and 2010 = 69.05%). The students felt that the virtual test exercises provided "much better" or "better" understanding and integration of the content of the subject (2008 = 41.95% and 50%, 2009 = 39.79% and 51.9 % and 2010 = 37.41% and 52.94%, respectively).

Over the three years, an average of 98.67% of students said they would like to continue the use of NICTs for Professional practice. After the course, 92.2% found useful the application in the pre-professional year of the tools learned in the “Prepracticanato”.

Conclusions
The use of NICTs is a very interesting and useful tool for the student during the development of the subject “Prepracticanato”, and this was reflected in the use of Moodle during this time. In turn, using these new methodologies of study, the teacher may permit better monitoring and other ways of working.

References

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“IMPROVEMENT OF ACTIVITIES PROGRAM OF THE SUBJECT “BASES FOR THE QUALITY CONTROL OF VEGETAL MEDICINAL SUBSTANCES”

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Introduction
The increased consumption of plants and their preparations with therapeutic purposes is a trend very widespread anywhere in the world. The pharmacist is the health professional that has in its duties the knowledge and handling of Medicinal Plants. Nevertheless, related concepts to natural drugs have been restricted in the Pharmacy curriculum to the Pharmacognosy subject, which teaches the vegetal and animal drugs that have been studied scientifically and have been established as official drugs in different Pharmacopeias from the world. Thus, the unofficial medicinal plants, including the native ones, are excluded even though they are commercialized and consumed with therapeutic intent. The National Institute of Medicaments from Argentine (INAME) established the nationwide legal frame for the Vegetal Drugs (VD) and their preparations, which do not fulfill with the requirements to be employed in the elaboration of medicines with high exigencies of quality, security and efficacy, under the name of Phytotherapeutic Medicines (PM). The objectives of “Bases for the quality control of vegetal medicinal substances” (BQCVMS, elective subject at the final year of the degree in Pharmacy, Fac. Cs. Químicas, UNC), are to introduce pharmacy students in statutory regulation of PM, provide the necessary tools to guarantee the quality and security of the VD and their preparations, and teach the concepts that allow them, in their professional exercise, to elaborate and dispense PM and to advise on the use of unofficial VD.

Materials and methods
With the aim of updating the activities program of the subject BQCVMS, we resorted to recommendations of the WHO, statutory regulations that regulate the different activities that can be made in our country with the PM and scientific bibliography mainly about unofficial VD.

Results
Theoretical topics were introduced in order to respond to the specific objectives proposed by the subject:
1) Quality assurance, security and efficacy of medicinal plants and their preparations.
2) Methodology to guarantee the quality and security of vegetal drugs and their preparations.
3) Phytotherapeutic Medicines (PM).
4) Admitted vegetal drugs for PM elaboration.
5) Vegetal toxic drugs.
6) Vegetal drugs and active principles in dietary supplements, nutraceuticals and functional foods.

The practical activities include three laboratory works that carry out different methodologies to guarantee the quality and security of the MP products that are commercialized in our country and other about publicity of products that are elaborated with Medicinal Plants.

Conclusions
BQCVMS studies in depth the concepts previously acquired in Pharmacognosy and introduces new important topics. It is the only subject that teaches the legal frame of the PM and confronts the problem of unofficial VD. In addition, it includes different methodologies to guarantee the quality and security of the MP products that are commercialized in our country. By this way, we offer a better formation to the students interested in this skill of the pharmaceutical professional exercise.

References

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EVALUATION OF COSMETIC AND PHARMACEUTICAL PRODUCTS MADE IN THE FRAMEWORK OF THE IMPLEMENTATION OF A SYSTEM OF PERSONALIZED EDUCATION

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Introduction
In order to evaluate the performance in the laboratory of advanced students of Pharmacy, teachers of Pharmaceutical Technology II, carried out controls of pharmacotechnical stability of formulations elaborated during the practical Design of a Pharmaceutical Formulation. Vegetal plants material was chosen between species with recognized therapeutic activity, and assigned to different groups of students. The aim was to develop a pharmaceutical or cosmetic product efficient, stable and secure. The preformulation study, implementation of good manufacturing practices (GMP), formulation and development of pharmaceutical products for topical use, was considered. The quality assurance thereof, following the norms established by Argentina Pharmacopoeia VII edition was evaluated. This work was developed within the framework of the implementation of the pedagogy method known as Personalized System of Instruction (PIS). These controls were conducted as a part of the evaluation process of the method implemented by the laboratory since 2007. In this paper the analysis of results as a positive conclusion about didactic resource implemented is well detailed.

Materials and Methods
The quality of pharmaceutical dosage forms and / or cosmetics designed and development for topical application by students was evaluated.

Stability assays: Organoleptic Characteristics (color and smell); Uniformity (macroscopic and microscopic), optical microscope and magnifying glass; pH: with a pHmeter Broadley James Corporation; Rheological Behavior: apparent viscosity Brookfield rotary viscometer RVF; Extensibility: extensometer; Heat Resistance: dry oven brand FAH (Industria Argentina); Water Content (Determination of loss on drying weight): dry oven brand FAH (Industria Argentina), Mechanical Stress: lab centrifuge; Short Term Stability assay or Accelerated Aging: dry oven brand FAH, freezer.

Microbiological assay: The count of total CFU was made and media culture for aerobials was used.

Individually analysis, physicochemical and microbiological test were performed according to the specific recommendations of Pharmacopeia.

Results
The sensory characteristics of all pharmaceutical dosage forms kept uniform. Most topical products showed pH values in the optimum range for skin (5 to 6). No significant change of pH was discovered during the test period. In terms of its rheological properties non-Newtonian pseudoplastic flow, with good degree of structural recovery, was found in all the products as well as stable against thermal and mechanical stress. No significant loss of weight in normal conditions of pressure and temperature was observed. The samples remained physically, chemically and microbiologically stable during the test period.

Conclusions
From the results analyzed can be concluded that students correctly applied the rules of GMP, worked applying and relating theoretical and practical knowledge’s acquired during their studies. This experience enriches and encourages to the future professionals to face the challenges of the pharmaceutical profession and solve them wisely. The self-assessment was made by the teaching team and determined

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that the present methodology is appropriated as a method of instruction, and will be adopted as a teaching method of choice from now on.
IMPLEMENTATION OF PERSONALIZED SYSTEM OF INSTRUCTION AT THE LABORATORY OF PHARMACEUTICAL TECHNOLOGY II OF THE NATIONAL UNIVERSITY OF TUCUMAN

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Introduction:
The aim of Teachers of Pharmaceutical Technology II Laboratory is the, constantly seeking of new tools and teaching resources for students of pharmacy (future professionals) to achieve optimum gain while training in their career and get a better relationship teacher - pupil. This will introduce significant changes in the roll of teachers and students assigning to the latter an active role beyond that, in practice, they behave as passive listener or scoring information. The teacher’s purpose is to give the student involvement in the design of novel formulations that stimulate inventiveness by giving security and promoting their knowledge. The proposal is to assign to the teacher an integrator role, giving up sole possession of knowledge to share with the group, and orient it to achieve its objectives. The teacher behaves like a cheerleader posing questions, creating problem situations, encouraging and showing alternatives. All this enriches their relationship with the group allowing you to achieve superior results quantitatively and qualitatively. The objective is to become student in an individual subject, this can be made possible with their practice, future development, with particular attention to the responsibilities contracted by the fact of studying something concrete, facilitating also the evolutionary development of self-control.

Materials and Methods
The challenge was to allocate groups, a vegetal species with popularly known medicinal properties, was assigned with the purpose to design and develop a pharmaceutical dosage form, containing an active principle on an extract, all accompanied by the relevant bibliographical support. This research and design process was followed by teachers on a permanent basis, not only guiding the student, but proposing solutions to various problems that arose along its cognitive evolution. Students should orally present their work and prepare a paper following the guidelines that required the local health authority for approval and subsequented commercialization of product.

Results
10 monographs where presented, which constituted a contribution to rescue the popular knowledge with scientific backing. These final papers were presented at scientific national conferences for students, winning awards and accolades. In parallel a continual self-assessment, through surveys, with the aim of improving the teaching-learning process is being performed. The slogan sparked interest in some of them, in such a measure that they request to continue with the work as students under training in the laboratory.

Conclusions
Analyzing the assessment instruments and the results it can be inferred that this methodology allowed students to feel encouraged themselves in their knowledge, to develop research and to design formulations. We suggest the starting point of a participatory integration of teachers and students in the planning, organization and development of all areas of the student, with stimulation of autonomy, creativity and critical analysis.

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SURVEY REPORT: POSTGRADUATES’ PERCEPTION OF HOSPITAL PHARMACY SPECIALIZATION CLASSES IN 2009

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Introduction
The Hospital Pharmacy Specialization (HPS) is a postgraduate career at School of Chemical Sciences, National University of Córdoba (1). The first cohort of pharmacists began in July 2009. Surveys are used to gather information from a group of people. The questionnaire approach permits anonymous replies, can be used with many respondents and generates mostly quantitative data (2-5). Thus, it is a useful tool for checking the postgraduates’ perspective.

Objective: to assess the perceptions of postgraduate students during the first semester of HPS (at FCQ-UNC) focused on the teaching performance, the adequacy and utility of themes, and the strategies utilized.

Materials and methods
The survey instrument was an anonymous self-administered semi-structured questionnaire with 3 subsets of items, and an open one for comments or suggestions. The subsets were: teaching performance (individual estimation), and usefulness of both virtual classroom (Moodle) and final test by module as a whole. A 5 points scale from bad to excellent was utilized. All pharmacists from the HPS were surveyed, between July and December 2009. Surveys responses were transferred to an Excel spreadsheet for analysis, and the scale was converted into percentages.

Results
All the postgraduate students (N=27) were surveyed about the 5 modules taken during the first semester of HPS:
1. Role of pharmacist in health system. Pharmaceutical legislation and policies.
2. Research methodology.
3. Drug selection and information.
4. Pharmaceutical care I.
5. Drug distribution and dispensing.

The average values in percentages and range obtained by module were the following.
Response rate: 61.5% (44.4%-77.8%)
Assessment of postgraduates related to:
a) Teaching performance subset: 79.1% (66.4%-85.3%).
   Theme expertise: 82.7% (69.9%-88.9%)
   Objectives’ explanation: 78.7% (64.8%-85.6%)
   Logical sequence: 77.8% (65.1%-84.6%)
   Encouraging participation: 79.1% (64.9%-85.0%)
   Educational resources quality: 78.4% (67.1%-85.6%)
b) Virtual classroom subset:
   General information: 79.4% (71.6%-83.8%)
   Available resources: 79.2% (72.6%-85.0%)
   Activities: 76.0% (65.3%-81.9%)
c) Module test subset:
   Content integration: 77.7% (70.6%-83.2%)
   Practical utility: 80.4% (73.5%-87.4%)
Comments/suggestions by module: 9.1% (0.0%-15.8%)

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This item ranged between 0 and 3 by module, with a mean of 1.4.

**Discussion and Conclusions**

Questionnaires were distributed and collected in a different way along the semester. Therefore, the response rate was under 50% in the first 2 modules.

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IDENTIFICATION OF CYCLE-STRETCH SPECIFIC COMPETENCIES OF PHARMACY CAREER OF UNIVERSIDAD CATÓLICA DE CÓRDOBA (UCC), ARGENTINA.

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Introduction:
Academic competencies are developed during training in the education system, which are classified in: ingress competencies, cycle-stretch competencies and graduate competencies. This classification is based on sequential, progressive and spiral nature of development in academic training. The cycle-stretch competencies refers to knowledge, abilities and attitudes required as key inputs, in order to course successfully the first years of career. They are the necessary resources for the sequential organization of knowledge and degrees of increasing complexity to ensure the cognitive development of learning subjects. Moreover, according to the degree of generality, academic competencies are classified into generic and specific (specifically related to the knowledge, abilities and practical skills of a subject area). In this work, cycle-stretch specific competencies (CSC) of Pharmacy Career of UCC were identified, understood such as specific of pharmaceutical disciplinary field, required to course, successfully, the first years of the career.

Material and Methods:
In this work, qualitative-descriptive research methods were used, based on documental analysis of teaching materials and subject programs of the early years of the career, in order to obtain the matrix "contents" vs. "CSC." Thus, the contents were considered independent variables, and the CSC, dependent variables. A semi-structured survey with CSC was developed to validate them by key informants. Descriptive statistics tools (univariate and multivariate) were used, being the variables of qualitative research and, within them, nominal (CSC) and ordinal (ranking of CSC according to their importance from the survey results).

Results:
25 CSC were identified, which, after multivariate cluster analysis, were grouped into three groups:
GI: 10 competencies related to basic training course.
GII: 10 competencies related to biomedical training cycle.
GIII: 5 competencies relates to pharmaceutical professional training cycle.
10 of the 25 competencies, were cognitive, 11 are technical and 4 technical and cognitive combination according to the UNESCO classification criteria.
CSC were validated by key informants, being those related to basic and biomedical training cycle, considered most important to stay in Pharmacy Career of UCC. Equal importance to cognitive and technical competencies was given.

Conclusions:
This methodology allowed to identify the competencies needed to enter and remain in Pharmacy career of UCC. Their identification can enable mechanisms to improve the transition from Middle School to University, because they are related to previous training required (entrant profile), which is important first step in the articulation between educative levels.

References:

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Introduction
The supervised professional practice on Chemistry is the last subject that pharmacy students of the Faculty of Chemical Sciences of the UNC should take. It is a theoretical and practical subject. The Laboratorio de Hemoderivados is one of the places where students can choose to do the practice.

Objectives
To assess student performance as regards: 1) Familiarization with the products produced in the Laboratory. 2) Acquaintance with the different departments of the Quality Control, characteristics and functions 3) to acquire knowledge and skill about the activities carried out by the Department of Physics-Physicochemical Tests.

Materials and Methods
The evaluation of the proposed objectives was made between the years 2004-2008. 26 students were tested. The activities done by the practitioners were divided into two parts:

A) Knowledge Acquisition about:
1) The Procedures and Instruction Sheet about the practical activities that will be carried out (1,2,3).
2) Biosecurity Regulations: Handling precautions and elimination of biological and non-biological material (solvents, solids, etc.). Handling and treatment of used materials.

B) Skill Acquisition:
1) Training provided by instructors in the practical activities that will be carried out in the area.
2) Carrying out the following activities supervised by the instructors:
   2.1) Training in the use, maintenance, and calibration of different equipments (pH-meter, spectrophotometer UV-vis., conductimeter, osmometer, HPLC, electrophoresis cell, etc.).
   2.2) Quality control of the water used in the different production areas: determination of pH, conductivity, oxidable substances.
   2.3) Sampling of: Raw materials, packaging, supplies, elaboration of labels, etc.
   2.4) Physicochemical control of raw materials and supplies.
   2.5) Determination of Total Proteins (Gornall Method) and determination of purity (electrophoresis on cellulose acetate) in raw material, (plasma) and fractionation processes (Fraction II+III and Fraction II+IIW) and semi-finished products (Fraction II; IV, V).
   2.6) Reagent preparation.
   2.7) Process control of injectable generic drugs in parallel with the instructor.
   2.8) Control of the Finished Product of injectable blood products and generic drugs in parallel with the instructor.

Results
From the 26 practitioner students’ assessment, we observed the following Table 1.

Conclusions

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From the assessment, strengths and weaknesses were observed.

*Weaknesses:* poor handling and interpretation of bibliography, and problems with the identification, and use of glass material.

*Strengths:* Good attitude to learn observed from: their participatory attitude, constant questioning, a permanent research spirit, awareness of the activities they do, and interest in continuing their professional work in the Laboratory.

**References**

1) Procedures and Instruction Sheet: SOP Technical Supervision, SOP Quality Assurance, SOP Quality Control, SOP Quality Control, Physicochemical, SOP Quality Control, Microbiology.


3) United Stated Pharmacopeia, USP XXXI

<table>
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<th>Grades</th>
<th>Passing</th>
<th>good</th>
<th>Very good</th>
<th>Outstanding</th>
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<td>Knowledge Acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Students</td>
<td>20,0%</td>
<td>50,0%</td>
<td>20,0%</td>
<td>10,0%</td>
</tr>
<tr>
<td>Skill Acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Students</td>
<td>19,0%</td>
<td>50,0%</td>
<td>16,0%</td>
<td>15,0%</td>
</tr>
</tbody>
</table>
**β-LACTAMS AND THEIR POTENTIAL USE AS ANTICANCER DRUGS**

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**Introduction**

Recent studies have demonstrated that some β-lactam antibiotics possess cytotoxic activity against a group of tumor cells. For this reason, and considering that β-lactam antibiotics have been used for many years to treat bacterial infections with limited or no toxicity, we evaluated the antiproliferative action of a 2β-(heterocyclyl)thiomethyl penicillins library.

**Materials and methods**

6β-aminopenicillinic acid (6-APA) obtained by deacylation of natural penicillins, have been used as starting material. We modified different positions of the 6-APA core, introducing several substituents, many of them known pharmacophores. To carry out these transformations, we have used homogeneous and solid-phase synthesis strategies.

**Results**

The antiproliferative activity of 25 synthetic penicillin derivatives was tested *in vitro*. Herein we report the results related to the most relevant group of compounds. Initially we employed a 20 µM concentration and two human tumor cell lines (cervix HeLa adenocarcinoma; breast MCF-7) and two murine cell lines (B16-F0 melanoma; LM3 mammary adenocarcinoma). Additionally, epithelial cells derived from normal mammary gland (NMuMG) were used as controls. The antiproliferative activities, expressed as IC₅₀ values, are summarized in Table 1. Among the tested compounds, 1a, 1b and 1d did not exhibit a considerable cytotoxic effect toward control NMuMG cells. In general terms, in tumor cell lines, compounds have shown the follow order of potency: 1f-1c > 1a > 1e-1g > 1b > 1d. Penicillin 1a, showed the greatest selectivity, being approximately eight times more active in HeLa cells than in NMuMG cells.

**Conclusions**

From the analysis of this penicillin library we can see that 2β-(benzothiazolo-2-yl)thiomethyl group (1a and 1e) and 2β-[(4,5-diphenyl-oxazolo-2-yl)thio)methyl group (1b, 1c, 1f, and 1g) are important to achieve activity in the compounds tested, while no significant change from varying the ester groups in position 3 of the penam nucleus. These preliminary results will help us to design and synthesize new analogues in order to obtain better activity and selectivity in antitumor therapy.

**Acknowledgements:** CONICET, ANPCYT and Universidad Nacional de Rosario.

**References**


Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} values (µM)^a</th>
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<tr>
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<td>HeLa</td>
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<tr>
<td>3-(Benzothiazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid benzyl ester (1a)</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>3-(4,5-Diphenyl-4,5-dihydro-oxazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid benzyl ester (1b)</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>3-(4,5-Diphenyl-4,5-dihydro-oxazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (1c)</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>3-(4,5-Dihydro-thiazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid benzyl ester (1d)</td>
<td>ND</td>
</tr>
<tr>
<td>3-(Benzooxazol-2-ylsulfanylmethyl)-6,6-dibromo-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (1e)</td>
<td>14±4</td>
</tr>
<tr>
<td>6,6-Dibromo-3-(4,5-diphenyl-4,5-dihydro-oxazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (1f)</td>
<td>7±2</td>
</tr>
<tr>
<td>6-Chloro-3-(4,5-diphenyl-4,5-dihydro-oxazol-2-ylsulfanylmethyl)-3-methyl-7-oxo-4-thia-1-aza-bicyclo[3.2.0]heptane-2-carboxylic acid methyl ester (1g)</td>
<td>13±4</td>
</tr>
</tbody>
</table>

^aThe molar concentration of a drug that is required for 50% inhibition, determined from dose-response curves. Results represent the average of, at least, three experiments. ND: not determined, corresponding to compounds that inhibit proliferation in less than 50% at 20 µM.
SINGLE CRYSTAL X-RAY DIFFRACTION STUDIES OF N-BENZENESULFONYL-1,2,3,4-TETRAHYDROQUINOLINE

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Introduction

Despite the fact that the intrinsic activity of a drug is determined by its chemical structure in solution and the mode of interaction with receptors, the ability to release the drug to patients safely and efficiently may depend on its solid-state properties.

Single-crystal X-ray diffraction is the most common experimental method to understand the structural characteristics of a compound. Although this structure does not necessarily resemble the molecule present in the biological medium, data coming from those studies are considered as useful information in Medicinal Chemistry.

As part of an ongoing research project (1-3), a library of N-benzenesulfonyl derivatives of heterocycles has been prepared, some of them have presented interesting antiparasitic (1, 2), and antibacterial (3) activities. In this study, we report the crystal structure of N-benzenesulfonyl-1,2,3,4-tetrahydroquinoline, BSTHQ, one of the compounds of the library. It was carried out to get information that will be used in rational drug design and virtual screening.

Materials and methods

Solution growth methods were used to obtain single crystals of BSTHQ. Therefore, different solvents, temperatures, evaporation rates, and diffusion of anti-solvents were tried. X-ray data were collected on a Nonius Kappa CCD diffractometer with the specimen cooled to 173(2) K in a constant stream of nitrogen to optimize diffraction quality.

Results

Single crystals of BSTHQ were obtained by slow evaporation of a dilute n-hexane solution. It was found that BSTHQ crystallizes in the orthorhombic space group P2₁2₁2₁ with 4 molecules in the unit cell (Z= 4).

Although there is no chiral centre in the molecule, the space group is non-centrosymmetric, requiring all the molecules in the crystal to have the same handedness.

In the structure of BSTHQ, the heterocyclic ring adopts a conformation with atoms N₁, C₉, C₁₀, C₁₄ practically coplanar and atoms C₂ and C₃ above and below the plane, respectively. Relevant torsion (dihedral) angles are: N₁-C₂-C₉-C₁₀ = 3.5° and N₁-C₂-C₃-C₄ = -59.4°. The overall molecular conformation can be described by two dihedral angles, namely C₂-N₁-S₁₁-C₁₄ = 65.8°, which defines the benzenesulfonyl moiety position relative to the heterocyclic ring and N₁-S₁₁-C₁₄-C₁₉ = 72.7° angle, defining the orientation of the benzene ring in relation to the sulfonyl group. This conformation is maintained by three intramolecular hydrogen bonds, namely C₂-H₂B⋯O₁₂, C₈-H₈B⋯O₁₃ and C₁₅-H₁₅⋯O₁₂ with C⋯O distance in the range of 2.825-2.930Å.

There are no significant π-stacking interactions. A single intermolecular bond, of type C-H⋯O, contributes to crystal stabilization. A lack of strong intermolecular interactions accounts for the relatively low melting point of the crystal (61.5-62.0°C).

Conclusions

The crystal structure of BSTHQ (crystallized by slow evaporation of n-hexane) was successfully resolved. Its overall molecular conformation can be described by two torsion angles, namely C₂-N₁-S₁₁-C₁₄ and N₁-S₁₁-C₁₄-C₁₉. This conformation is maintained by three intramolecular hydrogen bonds, namely C₂-H₂B⋯O₁₂, C₈-H₈B⋯O₁₃ and C₁₅-H₁₅⋯O₁₂.

References


DESIGN OF A LIBRARY OF N-BENZENESULFONYL DERIVATIVES OF HETEROCYCLES AS POTENTIAL ANTIPARASITIC DRUGS

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Introduction
The “fragment-based drug design” for library generation is a strategy for lead discovery. It makes the assumption that the combination of active structures could lead to molecules with better or new pharmacological response (1). Based on this methodology, we design a library of N-benzenesulfonyl derivatives of active heterocycles (BS-Heterocycles) that combine two biologically active moieties: the benzenesulfonyl (BS) and the heterocycles. Indeed, heterocycles are structures commonly found in most of the active natural products and in half of the drug currently used in medicine (2). On the other hand, BS is a substituent frequently present in molecules with biological activity. Perhaps the best known example is the sulfonamide with antibacterial activity. The great success of the BS could be attributed to its structural features: a polar S-O bond and the phenyl group that offers great chance to interact with biological targets. Moreover, a BS bound to different heterocycles has led to analogs with similar or better biological activities than their precursors (3-6). A good example are the BS derivatives of Arthemisine, that shown increased antimalarial activity compared with their parent molecule (5). Therefore, the combination of a BS and a biologically active heterocycle appears to be a very promising hypothesis for design of antiparasitic drug.

Materials and methods
The library was composed of 7 families, each one based on an active heterocycle. We select the 1,2,3,4-tetrahydroquinoline (A), 2-methyl-1,2,3,4-tetrahydroquinoline (B), 1,2,3,4-tetrahydroisoquinoline (C), benzotriazole (D) e indole (E), indoline (F) and 2-methylindoline (G). We include 16 compounds with different substitution on the BS, for each family. The substituents were chosen by applying Craig strategy (7) in order to get a proper variation of physicochemical properties. Compounds were obtained by classical synthesis (8, 9) and the library was enlarging by using parallel synthesis (10).

The activity against Plasmodium falciparum (P.f) was tested as a part of the WHO/TDR program. First, the percentage of inhibition at two concentrations was measurement. For the Hits, the IC_{50} was measured as well as the cytotoxicity.

Results
A library of 109 synthetic derivatives of BS-Heterocycles was prepared and all the compounds were assayed for anti-plasmodium activity. Some of them showed IC_{50} values around 10µM with good cytotoxicity and were selected as prototypes against P.f (11). We identified 6 promising structures for P.f activity. Two of them presented good IC_{50} of 1.5µM, the others showed IC_{50} between 8-12µM. From obtained results we could conclude that the BS group is favorable for the antiplasmodial activity. Also, the presence of electron withdrawing and lipophilic groups were favorable for the activity.

Conclusions
We have designed and synthesized 109 BS-Heterocycles searching for new anti-plasmodium drugs. From that library, six compounds met Jorgensen criteria and could be identify as prototypes. These findings demonstrated the quality of the criteria proposed in the fragment-based drug design approach. One of the most important observations was the positive impact of the BS incorporation to the heterocycle.

Acknowledgments
RJP acknowledge CONICET and Fulbright-Bunge Born fellowships. We also thank to Dr. Reto Brun (Screening Center of the Swiss Tropical Institute) for antiparasitic activity measurements.

References
ADVANTAGES OF A DESCRIPTORS SELECTION METHOD WITH MULTIPLE SOLUTIONS APPLIED TO QSPR MODELS FOR ADMET PROPERTIES PREDICTION

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Introduction
QSPR (Quantitative structure-property relationship) methods are widely used for ADMET (Absorption, Distribution, Metabolism, Elimination, Toxicology) properties prediction in drug discovery. However, there are some critical issues, which are difficult to address. The usual problems are the selection of the proper number of descriptors that model a desired property, redundant descriptors, chance correlation, overfitting, and getting complex models with low predictive capacity. A method that avoids these problems and in addition provides several models would allow to interpret them using physicochemical principles, and to choose the most suitable one. This work uses a technique based on evolutionary algorithms (1), which provides different predictive models as output. Validity is analyzed for estimating human intestinal absorption (HIA).

Materials and methods
From the best reported models for HIA prediction in (1) we selected 16 with cardinality ≤ 10 and MSE (mean square error) average over 50 runs < 0.135. Each model was assessed individually and, in addition, a global analysis of selected descriptors was performed.

Results
The models had an average cardinality of 6 descriptors, a low figure which quickens the analysis. Out of descriptors we found, 75% belong to 7 families which are related to physicochemical properties connected to HIA:

- Molecular properties: LAI (Lipinski Alert Index), ALOGP and TPSA(Tot) were the most chosen; the last one gives information about absorbed fraction that is an usual way to express intestinal absorption.
- 1-D Descriptors (functional group counts): nArCONHR (number of secondary amides-aromatic) was the most chosen in this group, in accordance with the capacity to donate and accept H bonds from the amides, thus contributing to the aqueous solubility (essential to HIA); in the same way the aromatic group contributes to lipophilicity. The nROH (number of hydroxyl groups) descriptor was also prominent, which shows OH group’s importance and its ability to form H bonds.
- Atom-centered fragments: the favourite one was O056 (alcohol), in line with was explained formerly. It is necessary to note that nROH was not chosen when O056 was selected, thus avoiding variable intercorrelation.
- 3D Descriptors: 3D-Morse, WHIM, RDF and GETAWAY, which provide data related to molecular size and shape, both of which are properties associated to lipophilicity and thus to HIA.

Depending of the needs of the study, the modeller will be able to choose among models with few descriptors and low prediction error (e.g. models #5 or #10 in (1)) or models with more descriptors which come from various families and optimal prediction error (e.g. model #9 in (1)). In all cases, these models improve those previously presented on literature.

Conclusions
Using a method which provides multiple statistically significant models allows the modeller to choose one based on different criteria, such as the quantity of descriptors used, the prediction error derived,

17 Supporting Information file sm008.xls - Worksheet Exp-HIA-NSGAI1.
and/or the type of descriptors in each model. In our particular study we demonstrate how to apply it for the prediction of HIA.

References.
DELPHOS: COMPUTATIONAL TOOL FOR SELECTION OF RELEVANT DESCRIPTOR SUBSETS IN ADMET PREDICTION

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Introduction
The design of QSAR (Quantitative Structure-Activity Relationship) methods constitutes an important research topic in modern drug discovery. One of the first and most important steps is the selection of the subset of descriptors that are relevant to the activity or property under study. In general, the descriptor selection task could not be manually achieved by experts, given the inherent complexity and non-linearity of the structure-activity relationships. Moreover, the number of molecular descriptors that may be calculated for a single compound is huge. Thereby, it is important to have a computational method for the selection of the subset of molecular descriptors to be used in a QSAR model.

Materials and methods
In this paper we present DELPHOS that is a computational tool that was recently made available to assist pharmacists who work with QSAR/QSPR prediction models. The first release of DELPHOS has been launched in May 2010 and may be found at “http://lidecc.cs.uns.edu.ar/”. The computational kernel of DELPHOS is based on a novel approach which has been recently published in a QSAR-related journal¹. DELPHOS makes use of a two-phase computational method where the first phase executes a multi-objective optimization, using evolutionary algorithms, and the second phase is a thorough validation of the results obtained in the first step. Currently the DELPHOS software has the following features:

GUI. A Graphic User Interface designed for using the software without the need to know specific details of the code or the applied methods.

Data handling. Input data can be fed to the method using the CSV file format or standard Matlab matrix files. Computation performed after any phase can be saved and later restored.

First Phase: Feature Searching and Evaluation. This phase is responsible of doing a coarse searching and a fast evaluation among all feasible subsets of descriptors. Several different parameters could be set for this phase.

Second Phase: Learning Method. Using the data computed in the first phase, a thorough evaluation is applied in order to determine which subsets of the coarse selection are the most relevant ones.

Post-processing. After the second phase has been executed, tables showing final results and several statistical metrics are presented.

Results
DELPHOS was successfully tested on three physicochemical and biological properties that are important for pharmacokinetic screening of drugs: hydrophobicity, blood-brain barrier penetration and human intestinal absorption. Moreover, our method has overcome prediction capacity of subsets of descriptors obtained by other competitive methods¹.

ON DESIGNING CONFIDENT STATISTICAL QSPR MODELS
Introduction

Modern drug discovery is differentiated by activity or property prediction techniques that allow virtual screening of leads. In this regard, QSPR (Quantitative Structure-Property Relationship) methods aim at modeling a biological or physicochemical property from its molecular structure. Although during last decade the number of papers in this subject was high, prediction capacity of QSAR models still remains to be improved\(^1\). In this paper we present a number of caveats for avoiding common errors on statistical models for QSPR methods. In addition, we also propose different alternatives to address these issues.

First of all, any prediction method must have a clear validation procedure. The best validation procedure consists in using an additional source of data that was separated from the very beginning from the training data used for constructing the model. This testing data should be used only one time, otherwise it might be misleading. When the number of available compounds is not large enough – this is the more typical situation – a cross-validation procedure should be carried out, in order to have an unbiased estimation of the prediction capacity. K-fold crossvalidation techniques are far better than the widespread leave-one-out (LOO) procedure, which is overoptimistic compared to the testing prediction capacity.

Many new statistical and machine learning methods are commonly applied for inferring structure-activity relationships. These methods might be a double-edged weapon, since they allow to fit any non-linear relationship. However, it is easy to fit noise or find chance correlations\(^2\).

Another crucial decision is the selection of the subset of descriptors that are relevant to the activity or property under study. This selection determines the prediction capacity and the degree of interpretability of the model.

Materials and methods

We propose three different models based on a neural network method. Model 1 has relevant descriptors and an appropriate training. Model 2 was overfitted. Model 3 was appended with 10 more random descriptors. Prediction capacities are evaluated using the training set, a testing set (with 30% of the initial data) and LOO. We used a database of 439 compounds with information of experimental logP values.

Results

The table shows prediction capacity using the abovementioned strategies and evaluation methods. MSE (Mean Square Error) and \(r^2\) (coefficient of determination) are considered as goodness of fit metrics.

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSE</td>
<td>(r^2)</td>
<td>MSE</td>
</tr>
<tr>
<td>Training</td>
<td>0.0666</td>
<td>0.9713</td>
<td>0.0624</td>
</tr>
<tr>
<td>Testing</td>
<td>0.2136</td>
<td>0.9088</td>
<td>0.2349</td>
</tr>
<tr>
<td>LOO</td>
<td>0.2042</td>
<td>---</td>
<td>0.0736</td>
</tr>
</tbody>
</table>

Conclusions

Rigorous design and comprehensive validation of QSPR models are essential for obtaining confident methods. Training or LOO evaluations are not representative enough for reporting true prediction capacity. Even though these caveats are not sufficient for a good QSPR method, we believe that they constitute necessary design decisions.

Acknowledgments

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References.


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QUALITY CONTROL OF *Melissa officinalis* COMMERCIALIZED MEDICINAL PRODUCTS BY CAPILLARY ELECTROPHORESIS

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**Introduction**

A large percentage of world population, consume regularly infusions and/or crude extracts derived from plant material for the primary health care.

The leaves of “lemon balm” (*Melissa officinalis* L.) are traditionally used in the folk medicine because of their sedative, aromatic, digestive and antispasmodic properties. The European and the British Pharmacopoeia assays for *Melissa* leaf quality control establish the quantification of total hydroxycinnamic derivatives expressed as rosmarinic acid (RA). This compound is a natural constituent in several herbs belonging Lamiaceae family, therefore was used as a marker compound.

By the other hand, “catmint” (*Nepeta cataria* L.), which is used in gastrointestinal and respiratory hyperactive disorders is a common adulterant of *M. officinalis*.

The aim of this work is to develop a capillary electrophoresis (CE) method for the characterization and quality control of Lamiaceae family herbs in a simple, fast and reliable way, as well as, an approach to detect rapidly *M. officinalis* adulteration or substitution, particularly by *N. cataria* in different commercial medicinal samples.

**Materials and methods**

The analyzed samples were: infusions (water extracts, 10% w/v) from ground plant material obtained from both *M. officinalis* cultivated and commercial herbs; tinctures (alcoholic extracts, 10% w/v) both elaborated in our laboratory and commercial. Spray-dried (SDE) and lyophilized extracts (LE) were obtained from the infusions; also, a based *Melissa* cosmetic product (commercial whitening toothpaste, WT) was investigated.

A Büchi 290 Mini-spray Dryer at 140 ºC, and a L-A-B5 Rificor equipments were used for drying and lyophilizing the water extracts, respectively. The analyses were performed with a capillary electrophoresis (CE) instrument, Beckman P/ACE MDQ. RA was acquired from Sigma-Aldrich Co. (St. Louis, MO).

**Results**

Melissa leaf consists of the dried leaves of *Melissa officinalis* L. It contains not less than 4.0 per cent of total hydroxycinnamic derivatives expressed as RA of the dried drug. The RA concentration was evaluated according to established in the official assays. A CE method was developed, the optimized experimental conditions were as follows: background electrolyte, 20 mM sodium tetraborate buffer, pH 9.2; applied voltage, 25 kV; hydrodynamic injection, 0.5 Psi during 5 s; capillary temperature 25 ºC; sample temperature, 25 ºC. The proposed methodology was validated and applied for the characterization and quality control of the Melissa based samples.

In several samples, was found that the RA value did not satisfy that specifications. In these cases, after the botanical analysis, the identity of the mentioned samples was confirmed as *N. cataria*, which permitted confirm the adulteration and/or substitution of *M. officinalis* by *N. cataria*.

On the other hand, it was observed that in SDE samples, the RA values were higher than in LE ones, for both *Melissa* and *Nepeta*.

Respect to tinctures, in those prepared by our group from the cultivated lemon balm the RA value was in accordance with the pharmacopoeias’ requirements. In the case of the commercial ones, the RA value was below than the established in the monographies.

With regard to WT sample based on *Melissa* extract (among others herbs), the presence of RA was not confirmed or was below of the detected by the methodology.

**Conclusions**

The present CE method represents a useful tool for determination of RA in commercial medicinal samples. The developed technique allows detect in a reliable and fast way the possible adulteration

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82
and/or substitution, by *Nepeta cataria*, of medicinal products labeled as *Melissa officinalis*. Apparently, the drying process is more favorable than the lyophilization considering RA yielding and operation cost.

### Table 1- Rosmarinic acid content in the analyzed samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cultivated <em>Melissa officinalis</em> grounded leaves <em>(a)</em></td>
<td>6.706 g/100 g dry weight</td>
</tr>
<tr>
<td>2. Commercial labeled <em>Melissa officinalis</em> grounded leaves <em>(a)</em></td>
<td>0.208 g/100 g dry weight</td>
</tr>
<tr>
<td>3. Commercial labeled <em>Melissa officinalis</em> grounded leaves <em>Piper Pool™</em> <em>(a)</em></td>
<td>0.126 g/100 g dry weight</td>
</tr>
<tr>
<td>4. Commercial tea bag <em>Taragui™</em> <em>(b,d)</em></td>
<td>12.082 g/100 g dry weight</td>
</tr>
<tr>
<td>5. Commercial tea bag <em>De mi campo™</em> <em>(b,e)</em></td>
<td>8.832 g/100 g dry weight</td>
</tr>
<tr>
<td>6. Commercial tea bag <em>Ensueños Taragui™</em> <em>(c,d)</em></td>
<td>7.533 g/100 g dry weight</td>
</tr>
<tr>
<td>7. Commercial tea bag <em>Patagonia™</em> <em>(c,d)</em></td>
<td>0.225 g/100 g dry weight</td>
</tr>
<tr>
<td>8. Spray dried extract from sample 1</td>
<td>0.412 g/g SDE</td>
</tr>
<tr>
<td>9. Spray dried extract from sample 2</td>
<td>0.016 g/g SDE</td>
</tr>
<tr>
<td>10. Lyophilized extract from sample 1</td>
<td>0.280 g/g LE</td>
</tr>
<tr>
<td>11. Lyophilized extract from sample 2</td>
<td>0.001 g/g LE</td>
</tr>
<tr>
<td>12. Commercial labeled <em>Melissa officinalis</em> tincture <em>Parafarm™</em></td>
<td>0.308 mg/ml</td>
</tr>
<tr>
<td>13. Tincture elaborated from sample 1</td>
<td>0.754 mg/ml</td>
</tr>
<tr>
<td>14. Homeopathic tincture elaborated from sample 1</td>
<td>0.571 mg/ml</td>
</tr>
<tr>
<td>15. Commercial labeled <em>Melissa officinalis</em> homeopathic tincture <em>Cangallo™</em></td>
<td>0.677 mg/ml</td>
</tr>
<tr>
<td>16. Commercial whitening toothpaste based on <em>Melissa</em> extract</td>
<td>ND <em>(f)</em></td>
</tr>
</tbody>
</table>

*(a) Infusion (10%)*  
*(b) Labeled *Melissa officinalis*  
*(c) Infusion (tea bag/100 ml)*  
*(d) Herbal blend containing *M. officinalis* (15%)*  
*(e) Herbal blend containing *M. officinalis* (not declared)*  
*(f) ND: not detected*
CONTROL OF A SOLUTION’S DOSAGE PROCESS USING THE VARIABLE DATA CONTROL CHARTS

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Introduction
Statistical process control (SPC) involves using statistical techniques to measure and analyze the variation in processes.
The intent of SPC is to monitor product quality and maintain processes to fixed targets. SPC procedures can help you monitor process behavior. (1)
A phenomenon will be said to be controlled, when through the use of past experience, you can predict, at least within limits, how the phenomenon may be expected to behave in the future.
The aim of this work is to evaluate a dosage process of 10, 20 ml of a buffer solution, using the Variable Data Control Charts.
If it is proved that the process is under statistical control, the calculated limits to control the future production reducing the number of necessary samples can be adopted.

Materials and methods
The dosage of the buffer solution has been done using a liquid dispenser (5-25 ml)
25 samples of 5 results each have been taken every 30 minutes.
The dosage solution has been weighed using a scale considering the 2nd decimal place and the volume has been calculated taking into account the solution’s density.
The average and the standard variation have been estimated and X and R calculated.
It has been verified if the process is under statistical control.
The graphics for monitoring the dosage process have been used. Samples have been taken every 10 minutes for 3 hours of production.
The temperature was fixed in 20ºC

Results
The results of the buffer`s dosage process are showed in table A.

X Chart

X (center line): 10,20
Upper Control Line (UCL): 10,22
Lower Control Line (LCL): 10,18

R Chart

R : 0,034
UCL: 0,07
LCL: 0,00

All values were within the control limits for both X and R.

All values of the process monitoring were within the control limits.

Conclusions

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Analyzing the obtained data, the process is under statistical control. There is no result outside the control limits and there are no random patterns; therefore, it is possible to use the graphics for the monitoring of the data.

The calculated limits have been adopted to control the future production.

References.

<table>
<thead>
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<th>No</th>
<th>X</th>
<th>R</th>
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<tr>
<td>1</td>
<td>10.20</td>
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</tr>
<tr>
<td>2</td>
<td>10.19</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>10.21</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>10.21</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>10.20</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>10.19</td>
<td>0.04</td>
</tr>
<tr>
<td>7</td>
<td>10.19</td>
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</tr>
<tr>
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<td>10.21</td>
<td>0.04</td>
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<td>10.19</td>
<td>0.03</td>
</tr>
<tr>
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<td>10.21</td>
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<td>10.20</td>
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<td>24</td>
<td>10.19</td>
<td>0.03</td>
</tr>
<tr>
<td>25</td>
<td>10.21</td>
<td>0.04</td>
</tr>
</tbody>
</table>
WATER PURIFICATION WITH CHLORINE DIOXIDE

Arroyo P.1, Damino C.2

Laboratorios Ethicus1, Joaquin V. Gonzalez 673, Godoy Cruz, Mendoza
DROFA S.A2, 25 de Mayo 1943, Ciudad, Mendoza

Introduction
Chlorine and its derivatives are highly oxidizing and corrosive, from State elemental chlorine, hypochlorite, chlorite, chlorate and perchlorate, their species reduced the most stable and found greater proportion in nature as salt "Sodium chloride". Chlorine dioxide is an abnormal where its valence is + 4, stabilized, because the electron in the third period is not known where is located the atomic orbitals 3px, 3py, 3pz and the 3d atomic orbitals are 5, appear in this layer, so the location of this electron will be orbital 8, this makes greater stability and low reactivity as oxidizing chlorinated compound. To react rusting will do so according to the following:

\[
\text{ClO}_2 + 2\text{H}_2\text{O} + \text{O}_3 + 5\text{e}^- \rightarrow \text{ClO}_2^- + 2\text{H}_2\text{O} + 5\text{e}^-
\]

Where oxygen is oxidizing agent and not the chlorine, as it will be at its most stable form in nature as the chlorine dioxide (ClO2) chloride is a disinfectant with a capacity greater than the chlorine biocidal product and its derivatives. As with selective oxidative qualities, your application will be in places in that apart from disinfection requires the organoleptic quality of water. Also has large effect on the control of taste, odour, and destruction of organic substances that provide color or which are precursors of trihalomethanes (e.g. Chloroform) which may react with ozone. Therefore applies when the raw waters contain high concentrations of precursors, that with the traditional colour would result in the disinfection by-products. It is used in its watery form, stabilized at a buffer carbonate, owns the property to be more effective as algicida, antimicrobial agent, biocidal product, disinfectant, Sanitizer, born and fungicide. Concentrations to fluctuate in 1: 10,000 to 1: 50,000 L of water for purifying the microbiological load and water quality. and 1: 1000 L of water as Sanitizer solution.

Equipaments and methods
Take of simple
Taking into account patterns of sampling in the C.A.A. Samples were taken, in sterile and labeled, packaging two 100 ml water each of the areas to look (well, pool of irrigation and irrigation canal) by applying the solution of chlorine dioxide (1: 10000) only to one of the samples.

Method
Melt the APC to bath, leave to cool up to thirty-seventh. Take special 1 ml of each sample and place it in properly labeled Petri dish plates. Dump the APC on boards that contain samples and mix with circular movements. Incubated at 37 ° with Board reversed during 48 hours. Saw the appearance of UFC (colony- forming units).

Results
(corresponding to the total number of samples)

Water without ClO2 > 200 UFC/ml
Water with ClO2:< 1 ufc/ml 1

Conclusions
It was noted that with low concentrations of Clorhine Dioxide can purification of water with a high microbial load.

References
Código Alimentario Argentino
www.cepis.ops-oms
www.greenfacts.org
Table No 1: Clorhine Dioxide compared to the chlorine derivatives

<table>
<thead>
<tr>
<th>Sodium Chloride/Hypochlorite</th>
<th>Clorhine Dioxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosive – oxidating</td>
<td>Not corrosive – not oxidating</td>
</tr>
<tr>
<td>Vacancy action virucida</td>
<td>Virucida</td>
</tr>
<tr>
<td>150 ppm is disinfectant</td>
<td>5 ppm is disinfectant</td>
</tr>
<tr>
<td>Consumed with organic matter</td>
<td>Cash in the presence of organic matter</td>
</tr>
<tr>
<td>Alters the smell and taste</td>
<td>50 Ppm is insipid</td>
</tr>
<tr>
<td>Form cloraminas (toxin)</td>
<td>There is no reaction with amines</td>
</tr>
<tr>
<td>Cash in limited range of pH</td>
<td>Cash in wide range of pH</td>
</tr>
</tbody>
</table>

Table No 2: The aqueous Clorhine Dioxide in concentrations used for purifying features

<table>
<thead>
<tr>
<th>Characteristic of ClO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insipid and inodorous</td>
</tr>
<tr>
<td>Thermodynamics stable</td>
</tr>
<tr>
<td>Fungicide-Bactericide-Virucida-Esporica</td>
</tr>
<tr>
<td>150 times more effective than the chlorine</td>
</tr>
<tr>
<td>Does not need to be activated</td>
</tr>
<tr>
<td>Cheap</td>
</tr>
<tr>
<td>Not inflamable</td>
</tr>
</tbody>
</table>

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Damino, Carina: 0261-156544557, tel/fax 0261-4380600, e-mail: carinadamino@hotmail.com
VALIDATION STRATEGY APPLIED TO A POTentiometRIC METHOD DEVELOPED FOR DETERMINATION OF TOTAL FLUORIDE IN TOOTHPASTES.

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Introduction
The validation of new analytical procedures, i.e., the proof of its suitability for the intended purpose, is a requirement that have been clearly documented by regulatory authorities. However the approach to validation is varied and opened to analyst interpretation (1). Several methodologies for chromatographic methods validation are available in literature, but not many for potentiometric procedures. Using the ICH guidelines as a basis we have selected the relevant parameters and acceptance criteria for designing experimental studies needed to validate a novel method for determination of total fluoride in toothpastes (2).

Materials and methods
Samples containing sodium fluoride (0.10%p/p) and monofluorphosphate (0.76%p/p) as active ingredients were hydrolyzed with perchloric acid at room temperature and total fluoride was determined using an ion selective electrode (ISE) by a potentiometric method employing TISAB solution. In this procedure 1.4 g of toothpaste were suspended in 20.0 mL of deionized water and 25.0 mL of 70% p/p perchloric acid were added. Solution was stirred 15 min and diluted to 250.0 mL in volumetric flask after neutralization with 25.0 mL of NaOH 10 mol L\(^{-1}\). For potentiometric measures 2.00 mL of sample solution were diluted to 20.0 mL with TISAB and solution potential was established with a fluoride ISE provided by OAKTON. TISAB solution was obtained by dissolving 58.5 g of NaCl, 61.4 g of sodium acetate and 0.30 g of sodium citrate in 1.0L deionized water and adjusting pH to 5.5.

The whole analytical procedure, including all the steps of the sample preparation, was applied in the determination of specificity, linearity, range, accuracy and precision. Spiking experiments for recovery and linearity investigations were performed in the whole working range using a synthetic mixture of matrix components as placebo and standards of sodium fluoride and monofluorphosphate.

Results
No matrix components interferences were detected during specificity assays performed by comparing average results of the response of reagents and placebo through a statistical t-test. Linearity was established in the range 4.0 to 12.0 mg L\(^{-1}\) by an F-test for residuals homoscedasticity and a lack of fit test. No statistical difference was obtained between the calibration set performed with pure standard solutions and matrix based calibration set, confirming the absence of “matrix effect”. Main recovery was not different from 100 % at a 95% confidence level, with values ranging from 98.0 to 101.3% in spiked validation samples obtained for lowest, medium and highest calibration levels. Repeatability and intermediate precision were evaluated by applying an analysis of variance that showed no statistical difference between series performed in sextuplicate for medium calibration level in difference days and by different analysts. Relative standard deviation of the method resulted in 0.96%.

Conclusions
An extended characterization and validation of the proposed method was carried out following international guidance obtaining excellent performance results. The procedure is simple, fast and efficient and may be applied in the routine quality control of toothpastes.

Acknowledgments

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The authors thank UNL for financial support from “Proyecto CAI+D 2009 Tipo II PI-12-62 and Laboratorio LAFORMED S.A. for providing drugs and pharmaceuticals.

References


DETERMINATION OF VOLATILE ORGANIC COMPOUNDS BY HS-SPME/GC-MS IN *Tagetes argentina* FROM CORDOBA (ARGENTINA)

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**Introduction:**

In the Central West of Argentina, there is a frequent use of native and exotic plants in folk medicine. However, many of them (especially natives) are still poorly known from the phytochemical and pharmacological point of view. *Tagetes Argentina* – "chilchil del campo", "chinchigua"- (Asteraceae), is used as a diuretic and digestive and the reports of their organic volatile compounds (VOC), has been described from studies in essential oils obtained by hydrodistillation. In this work, the determination of VOC in fresh inflorescences was performed by Headspace Solid Phase Microextraction/Gas Chromatography-Mass Spectrometry (HS-SPME/GC-MS) previously optimized in prior studies. This technique was selected because the results produced by HS-SPME are more representative of the biogenic volatile organic compounds (BVOC) released from vegetables, in addition to its high flexibility, less time required for analysis and high sensitivity to determination of terpenes.

**Materials and Methods:**

**Samples**: 100.0 mg of fresh inflorescences of *Tagetes argentina* collected between March and April, 2010, in the Sierras Grandes (Córdoba, Argentina).

**Extractant phase**: carboxene-divinylbenzene-polydimethylsiloxane (Supelco).

**Temperature**: 40 °C (Polyscience brand bath).

**Incubation time**: 5 min.

**Extraction time**: 30 min.

Determinations (triplicate) Gas Chromatograph HP 5890 Series II with Mass Detector HP 5970, column HP-5 30 m; injector: 225 °C, detector: 230 °C, oven: 40 °C (5 min) to 200 °C to 5 °C / min (5 min), He: 99.99% (5 psi).

The volatile organic compounds were identified by comparing of mass spectra with library (Wiley-NIST) and by determining of Kovats retention index (KI).

**Results:**

21 volatile compounds were identified (over 95 % of total): (E)-Tagetone (9.1 %); Tagetone (10.2 %); trans-Tagetenone (30.2 %); D-Verbenone (21.5 %); Limonene (0.5 %); cis-β-Ocimene (5.3 %); Dihidrotagetone (3.1 %); α-Terpinolene (0.5 %); 1,3,8-p-Mentatriene (0.7 %); allo-Ocimene (0.8 %); α-Piridinone (2.1 %); p-Cymen-8-ol (1.4 %); Piperitone (0.4 %); Isothymol (1.4 %); β-Caryophyllene (2.1 %); Aromadendrene (1.1 %); α-Humulene (0.9 %); Germacrene D (2.4 %); Bicyclogermacrene (2.9 %); trans-γ-Cadinene (0.3 %) y δ-Cadinene (0.5 %).

**Discussion and Conclusions:**

The results for major VOC from *T. argentina* are similar to reported for those essential oil, although in this case, components previously unreported were identified: D-Verbenone, Limonene, α-Terpinolene, 1,3,8-p-Mentatriene, allo-Ocimene, α-Piridinone, p-Cymen-8-ol, Piperitone, Isothymol, β-Caryophyllene, Aromadendrene, α-Humulene, Germacrene D, Bicyclogermacrene, trans-γ-Cadinene and δ-Cadinene, allowing a more complete characterization of the specimen. The smaller amount of material used in HS-SPME (100 mg of sample) and the minor analysis time are the major advantage for phytochemical studies, compared with the large quantities and time required to obtain the essential oil.

**References:**

4- Vázquez, A.M. *et all* (2009) XIX Congreso Farmacéutico Argentino y XIII Congreso de la Federación Farmacéutica Sudamericana.
STABILITY-INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR RELATED SUBSTANCES AND DEGRADATION PRODUCTS DETERMINATION IN CO-TRIMOXAZOLE TABLETS.

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Introduction
Current compendial (USP) methods for the analysis of trimethoprim (TMP) and sulfamethoxazole (SMX) in co-trimoxazole tablets and oral suspension, involve the use of liquid chromatography for the assay in both pharmaceuticals dosage forms and two TLC procedures for chromatographic purity in suspension. In one of them TMP degradation products are evaluated under short-wavelength UV light, whereas in the other plate sulfanilamide (SAM), sulfanilic acid (SA) and sulfamethoxazole-N₄ glucoside are visualized by modified Ehrlich’s reagent (1). In the present work a modification of the USP RP-HPLC assay method is proposed for the simultaneous determination of active pharmaceutical ingredients (APIs) and its degradation products or related substances in co-trimoxazole tablets.

Materials and methods
Following ICH stability test guideline Q1A (R2), tablets containing 400 mg SMX and 80 mg TMP were stressed under oxidative, reductive, acidic, alkaline, temperature and light exposure conditions to conduct forced degradation studies. The liquid chromatographic separation of degradation products was achieved on a ZORBAX Eclipse XDB – C18 (4.6mm×150mm, 5µm particle size) column, using a mobile phase consisting of 0.1% triethylamine, 100 mmol L⁻¹ sodium acetate pH 5.9 ±0.1 and acetonitrile, in a solvent gradient (Table 1). The flow rate was 1.0 mL min⁻¹, temperature of the column was 25 ºC and 20µL of solutions were injected. UV detection was performed at 254 (4) nm. Resolution between APIs and degradation products was studied, although employment of a diode array detector allowed selectivity confirmation by peak purity evaluation. A combined standard solution containing TMP, SMX, SAM and SA was used in the analysis of three naturally aged samples for the simultaneous determination of those analyte and evaluation of other degradation products.

Results
APIs were found to degrade significantly in reductive conditions. Mild degradation was occurred under acidic, alkaline and oxidative stress; whereas drugs were stable to light and heat exposure. Stressed samples showed 13 secondary peaks in oxidative treatment, 9 in reductive, 7 in acidic, 6 in alkaline, 3 in photolytic and 1 in thermolytic conditions, respectively. In all cases resolution of APIs from secondary peaks were ≥1.5 and peak purity of main peaks resulted ≥ 99.9 %. Total analysis time, avoiding stabilization of chromatographic gradient for next injection, was 25 min. Three primary batches of a pharmaceutical product provided by a local industry, packaged in the same container closure system as proposed for marketing and naturally aged for three years, showed, besides APIs main peaks, three secondary peaks. The first one corresponds to SA in concentration ranging 0.10-0.12%p/p in relation to SMX. The other two peaks, with high spectral correlation with SMX and around 0.2% of it area, were also detected in the oxidative and reductive degradation respectively.

Conclusions
Simultaneous determination of APIs, related substances and degradation products in co-trimoxazole tablets could be achieved using a modified USP chromatographic method. Selectivity of the method was proved allowing its utilization as stability-indicating methodology for the quality control of this pharmaceutical formulation during its shelf life.

Acknowledgments
Laboratorio LAFORMED S.A. Formosa, Argentina, for providing drugs and pharmaceuticals.

¹ María Mercedes De Zan  Tel/FAX: 54 342 4575205; e-mail: mmdezan@fbcb.unl.edu.ar
References.

Table 1: Gradient program used in the separation of degradation products.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Buffer (%)</th>
<th>Acetonitrile (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>23</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>
DETERMINATION OF ASCORBIC ACID USING PHOTOSTABILIZING AGENTS

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Introduction
Ascorbic acid, an important water soluble vitamin, is highly sensitive to heat, alkali, oxygen and light (1). Its rapid degradation in aqueous solutions, especially upon exposure to light, clearly complicates the assay studies. We have demonstrated that the formation of binary and multicomponent complexes with HP-β-CD and TEA strongly reduced the photodegradation process in solution (2). This fact appears to be of great interest for the assay of the vitamin, due to the exposure of the solutions to light over prolonged periods of time.

In this study, based on the significant enhancement of the stability and photostability of ascorbic acid in aqueous solutions due to the formation of complexes, an HPLC method for its determination was developed and validated.

Materials and methods
All chemicals used were of analytical grade and the solvents were HPLC grade. Analyses were performed using an Agilent 1100 series system.
The method was validated according to ICH guideline.
The results obtained by applying the developed method for the estimation of the drug in pharmaceutical formulations, are compared with those found using the reference method (3).

Results and discussion
Under the experimental conditions, good linearity of the calibrations was established. Recovery studies on intra-day and inter-day experiments showed satisfactory repeatability, intermediate precision and accuracy.
Ascorbic acid in the commercial formulations analyzed was found in the range of 81.0–100.2%, compared to the declared values. The reported relative standard deviation (R.S.D.) values reflected the high precision of the proposed method when is applied to the assay of commercial pharmaceuticals, in comparison with those obtained by the reference method.
Furthermore, these studies confirm that the formation of complexes allows the obtention of reliable analytical results, due to the increase in the stability and the photostability of ascorbic acid during the preparation of the samples and their later analytical assay.

Conclusions
A chromatographic method was developed in the presence of the ligands HP-β-CD and TEA, without loss of ascorbic acid during preparation of the analytical samples and the assay. All the validation parameters were found satisfactory, after which, the method was applied to the determination of drug in pharmaceutical formulations.
The developed methodology represents a great improvement over the reference method, which requires more preparation time and standardization of the titration solutions. In addition, this method requires a simple sample preparation procedure, decreasing the degree of uncertainty and the time of the assay. So, they provide high throughput solution for the determination of ascorbic acid, either in the pure form or in pharmaceutical formulations.

Acknowledgments
We thank Ferromet S.A. (agent of Roquette in Argentina) for their donation of hydroxypropyl-β-cyclodextrin.

References
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DETERMINATION OF IONOPHORE ANTIBIOTICS IN FOOD ANIMALS BY THIN-LAYER CHROMATOGRAPHY METHOD

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Introduction
Ionophore antibiotics are widely used in veterinary medicine as anticoccidial drugs for poultry and as growth promoters for farm animals. The ionophores, despite their utility, possess a narrow range of safety; several species (horses in particular) appear highly susceptible to the toxic effects of these compounds, compared to other animal species. Thin layer chromatography method is more sensitive, economic and exact for monitoring low amounts of different chemical compounds.

The purpose of this study was to apply a thin layer chromatography (TLC) method to identify ionophore antibiotic residues in feeds according to harmonized protocols (FA VII).

Materials and methods
Feed sample extracts were obtained using acetone. Samples were homogenized with vortex and centrifuged. The clear supernatant obtained was transferred to a fresh glass tube (S1). The frame was extracted with acetone again, and the second supernatant (S2) was combined with the first one and evaporated. After full drying, the residue was redisolved in 1 ml of acetone for TLC analysis.

The standards provided by DSM Nutritional Products Argentina SA were dissolved in acetone. TLC method was carried out according to the FAVII Ed technique. About 20 µL of the extract were pointed on silica plates (Silica gel 60 FB254 0.2 mm). Treated plates were transferred to a TLC tank containing 99% ethyl acetate as mobile phase. Chromatograms were observed on UV light at 366 nm and with sulfuric anisaldehyde.

Results:
The table below shows the different retention factors (Rf), the fluorescence obtained with UV light and finally the results obtained after being revealed by sulfuric anisaldehyde.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Rf</th>
<th>Fluorescence UV&lt;sub&gt;366&lt;/sub&gt;</th>
<th>p-anisaldehyde sulfuric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monensin</td>
<td>0.77</td>
<td>blue</td>
<td>yellow</td>
</tr>
<tr>
<td>Virginiamycin</td>
<td>0.55</td>
<td>blue violet</td>
<td>yellow-brown</td>
</tr>
<tr>
<td>Maduramicin</td>
<td>0.88</td>
<td>blue</td>
<td>pink</td>
</tr>
<tr>
<td>Lasalocid</td>
<td>0.97</td>
<td>violet sky</td>
<td>black</td>
</tr>
</tbody>
</table>

Conclusions
Different authors reported some techniques for extracting the ionophore antibiotics (Tomassen MJH, et al, 2004) (CT Elliott et al, 1998) (D. Guglielmo et al, 1999). Acetone was selected because it had been the only solvent drawing all the antibiotics in the same method with high reproducibility for the different samples assayed.

Thin layer chromatography is a simple non expensive and exact technique which can be easily performed in most laboratories. Among chromatographic techniques HPLC is more accurate but it has some limitations. For direct investigation of residues in poultry feed TLC is a low cost, fast and accurate technique able to analyze at least 10 samples at the same time.

References
- FA VII ed. primer tomo.
**QUANTITATION OF ENROFLOXACIN AND CIPROFLOXACIN IN BROILER CHICKEN FEATHERS BY HPLC-FLUORESCENCE**

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**Introduction**

Enrofloxacin is a quinolone antimicrobial widely applied in the treatment and prevention of diseases in poultry. An HPLC-fluorescence method was developed to quantify enrofloxacin (ENR) and its metabolite ciprofloxacin (CIP) in broiler chicken feathers. Feathers are not used regularly as a matrix to detect and quantify veterinary drugs. However, are frequently incorporated as a protein source into the diets of other food animals (cattle, swine, rainbow trout, salmon). Direct extraction with acetone was performed. Samples were analyzed to validate this specific technique which has not been reported until now.

**Materials and methods**

- Extraction was performed following a technique developed by San Martín et al (1) modified. Finely cut feathers (blank: from untreated animals) were placed in centrifuge tubes and fortified with ENR and CIP. Acetone was added and samples were vigorously shaken during 20 minutes. Samples were centrifuged at 4000 rpm for 10 minutes. Supernatants were transferred into drying tubes. The extraction was repeated twice. Supernatants were combined and evaporated to dryness under nitrogen. Residues were dissolved in 75 µl methanol 0.1% tetrahydrofuran. 475 µl aqueous 0.1% tetrahydrofuran were added. Samples were centrifuged and supernatant was filtered and injected into the chromatographic system.

- Chromatographic conditions: mobile phase (water : acetonitrile : triethanolamine pH 3), flow rate 1,2 ml/min, column Phenomenex Luna C\(_{18}\), fluorescence excitation 278 nm and emission 446 nm.(2)

- The calibration curves were made with standards of ENR and CIP at the range of 0.1 – 2 µg/ml of mobile phase.

- The validation procedure was performed following Commission Decision 2002/657/CE of the EU (3).

**Results**

The calibration curve was linear over the range of concentration evaluated with a correlation coefficient of 0.99740721 for ENR and 0.99860554 for CIP. The detection limits were ENR 0.062 µg/g and CIP 0.040 µg/g. The quantitative limits were ENR 0.080 µg/g and CIP 0.050 µg/g. The specificity was demonstrated by analysis of blank samples and intra-day and inter-day precision as the coefficient of variation was 3.88 to 7.47 for ENR and 2.73 to 7.07 for CIP. Recoveries of 94.57 to 100 % and 93.33 to 100 % for ENR and CIP were calculated, respectively.

**Conclusions**

The analytical method developed to determine ENR and CIP in broiler chicken feathers demonstrated linearity, precision and accuracy under the analytical conditions which include acetone extraction and quantitative analysis by liquid chromatography with fluorescent detector. This technique might have important applications in residues studies of ENR and CIP in feathers.

**References**

DISC INTRINSIC AND IN VIVO DISSOLUTION RATES OF CRYSTALLINE AND AMORPHOUS DEFLAZACORT

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Introduction
Studies from our laboratory have demonstrated that deflazacort (DEF), a poorly water-soluble glucocorticoid, exhibits a crystalline thermodynamic stable form (DEF-1) and a metastable amorphous phase (DEF-a). Since amorphous forms usually display higher dissolution rates than their crystalline counterparts, their presence in raw powders and pharmaceutical products, by design or accident, can incorporate in them distinct properties; thereby, the importance of characterizing crystalline and amorphous phases. In this study, the disc intrinsic dissolution rates (DIDR) of DEF-1 and DEF-a were determined and compared with corresponding in vivo dissolution rates from compacted pellets in rats, in order to determine if DEF-a had improved the dissolution characteristics of DEF.

Materials and Methods
Dissolution studies were performed on a Hanson SR6 dissolution tester, using the USP rotating disc apparatus, 200 mL of deaerated water, 37 °C and 50 rpm. Compactled pellets (0.5 cm²) were produced at a compression force of 0.55 ton. XRPD was used to investigate possible phase changes after compression and dissolution. The in vivo dissolution rates were determined by the implantation technique [1] using male Wistar rats. The animals were divided in 4 groups (n = 6): 1) sham-operated control, 2) not operated control, 3) DEF-1 and 4) DEF-a. After 72 h, the pellets were removed cleaned and dried and mean dissolution rates per area were calculated. Additionally, the biological effects of the absorbed API were evaluated by following animal weight loss and adrenal gland atrophy. All animal experiments were performed according to an approved protocol (Facultad de Ciencias Químicas, UNC).

Results
XRPD data demonstrated that the compaction force applied to obtain the compacts causes no phase changes in DEF-1 but devitrifies DEF-a, which converted to DEF-1. The DIDR of DEF-a was slightly higher than that of DEF-1 (i.e. 9.00 and 7.76 µg/min.cm², respectively) but the values were not statistically different (P=0.31). Similar trends were observed for the in vivo dissolution rates, being the values not significantly different (P=0.07). Also, the animal weight loss was similar for both experimental groups; however, small differences for the glandular atrophy rate were detected. No phase changes were observed for both samples during dissolution, as indicated by XRPD measurements.

Conclusions
The in vitro and in vivo dissolution characteristics of two solid phases of DEF were analyzed. No statistically differences were observed for the in vitro as well as the in vivo dissolution rates of DEF-a and DEF-1, due to DEF-a devitrifies by effect of the applied compaction force. The DIDRs were well reflected in the in vivo dissolution rates of compacted implants.

References
DETECTION OF COCAINE, BENZODIAZEPINES, CANNABINOIDS AND AMPHETAMINES IN ORAL FLUID CONDUCTORS BY ELISA

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Introduction
The use of psychoactive substances (PAS) like cocaine (COC), amphetamines (CA), benzodiazepines (BZD) and Δ⁹-tetrahydrocannabinol (THC) has been growing in recent years, being used by millions of people around the world, usually with serious consequences for the user and society. Many fatalities associated with traffic accidents can be attributed to behavioral and psychomotor disturbance that these substances can induce, especially for professional drivers, with are submitted to high working hours and make use of these substances to avoid sleep and / or promote their improvement. In Brazil the number of traffic accidents has increased significantly, being the ninth most common cause of death in the country. However, despite the legal provision for the supervision of drivers suspected of being under the influence of SPA (1), is only possible to identify the estimated quantity of ethanol. Considering that the approach of transit drivers fits in the field of forensic toxicology (2), this study aimed to carry out tests on samples of oral fluid (OF) collected from various capitals of Brazilian drivers. Objective: Screening analysis for detect the presence of SPA (CA, COC, BZD and THC) in samples of OF by ELISA (enzyme linked immuno sorbent assay) as well as documentation of the use of psychoactive substances by professional drivers in order to provide subsidies for new public policies.

Material and methods
3251 samples were collected in 26 Brazilian states and federal district, using the collection device Quantisal®. The samples were brought to the laboratory where they were submitted to screening by enzyme immunoassay ELISA and sent for confirmatory analysis by SPME-GC-MS and / or LC-MS / MS because the technique of screening does not provide sufficient specificity to establish a criminal case. The confirmatory tests are being processed.

Results
We analyzed 3251 samples of OF collected from drivers of Brazilian state capitals, to identify the presence of COC, THC, CA and BZD. For the CA were analyzed only 1158 samples due to a problem of specificity of the kits used because did not detect much of CA marketed in Brazil as amfepramone and fenproporex. Of the total of samples analyzed, 2% had tested positive for COC, 1% for BZD, 1% for THC and 1,2% for CA.

Conclusion and discussion
The use of OF as biological matrix in police approach in traffic has many advantages over other conventional matrix, such as urine and blood, like noninvasive collection, difficult adulteration, besides indicating the recent use of the substances. The test was adequate for analysis of THC, COC and BZD, but without satisfactory results for CA. In specificity studies employing matrix-enriched with CA more usual in Brazil, as amfepramone and fenproporex we did not obtain positive results even in high concentrations. It is observed the importance of confirmatory testing, the relevance of research and the necessary investments in research and public policies for the effective control of these substances in the Brazilian traffic.

References

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CHARACTERIZATION OF DRUG SUBSTANCES POLYMORPHIC SYSTEMS USING THERMAL ANALYSIS

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Introduction
During the development of the drugs, studies are performed to identify and characterize the relevant polymorphic forms of the drug substance. These studies aim to develop a mechanistic understanding of how the physical and chemical properties of these polymorphic forms influence important performance aspects of the drug substance and the drug product. Formulation scientists then select appropriate polymorphic form(s) for development and determine what manufacturing controls are necessary to ensure the quality of drug substance and drug product; thus their bioavailability, safety and efficacy will be assured.\textsuperscript{1}

In this opportunity, we are going to show the results obtained on a drug substance A which has activity in the central nervous system and presents at least six different crystalline forms.

As is well known, several different analytical techniques are applied during solid phase screening studies. Among the performed studies (thermoanalytical analysis, microscopy, spectroscopy, X-ray powder diffraction) one of them showed to be very useful to understand the thermal behavior. Thus, attention was focused on thermal analysis and especially the hot stage microscopy allowed us to solve the different thermal events.

Materials and methods
The drug substances A crystalline samples were provide by Research & Development lab of GADOR S.A. API Division.

The instruments used for thermal analysis were Calibrated sub-ambient TA Instruments DSC (Differential Scanning Calorimetry) Q-100 with MDSC (Modulated Differential Scanning Calorimetry), calibrated TA Instruments TGA (Thermo gravimetric analysis) Q-500. The microscopes Nikon Eclipse E200 and Wagner & Munz Micro-Hot-Stage PolyTherm A are used. The FT-IR analysis was performed on Shimadzu IRAffinity/I. X-ray powder diffraction (XRPD) patterns were obtained on a X’Pert PW3020 diffractometer (Philips, The Netherlands).

Results
According to available patents’ information, the drug substance A presents six polymorphic forms. Two of them have shown discrepant results when the information obtained in our laboratories was compared with the bibliographic data.

The polymorph screening by conventional DSC technique\textsuperscript{2} of these two crystalline samples had showed profiles different from the expected according to the previously published on patented forms.

The conventional DSC profile for both samples shows two endotherms associated with the respective melting processes, without loss of mass according to TGA results. In both cases, the endotherms are within the same temperature range but have different intensities. However, one of the samples showed an exothermic process between the first and the second endotherm. On the other hand, the samples had a similar crystalline habit when they were observed by microscopy.

The challenge was to determine whether the two samples were pure polymorphic forms and the cause of exothermic process observed in one of the samples.

Conclusions

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Conventional DSC and complementary studies as FT-IR, X-ray powder diffraction and hot stage microscopy were employed to characterize the samples. So far, the obtained results demonstrate that the samples are mixtures of two solid phases. Also, MDSC studies will let us know the thermodynamics and kinetics events that occur during the samples thermal treatment.

References.
1 International Conference on Harmonization (ICH), Guidelines Specifications Q6A, 1999.
DEVELOPMENT AND VALIDATION OF A METHOD FOR DETERMINING ACETAZOLAMIDE IN RAT INTESTINAL FLUID BY HPLC.

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Introduction
Acetazolamide is a potent reversible carbonic anhydrase inhibitor widely used in the medical treatment of glaucoma. It works by reducing the rate of formation of aqueous humor which lowers intraocular pressure in patients treated with this drug. It is also used, either alone or in association with other drugs, in the treatment of various forms of epilepsy, or as a promoter of diuresis in instances of abnormal fluid retention (1-2). The two major problems presented by Acetazolamide are its low aqueous solubility, 0.7 mg/ml, and its low permeability coefficient, $4.1 \times 10^{-6}$ cm/s (3). The Single Pass Intestinal Perfusion model (SPIP) is commonly used to investigate intestinal drug permeation, and to predict in vivo absorption of drugs in humans. Thus, the aim of this work was developed a specific HPLC method for the determination of ACZ in intestinal perfusion experiments.

Materials and methods
The HPLC assays were carried out with a high pressure liquid chromatograph (Agilent 1100 Series) consisting of a standard automatic injector, an isocratic pump, thermostatted column compartment, and diodes and multi-wavelength detector. The chromatographic conditions were: ACN/MeOH/H$_2$O (3:2:95) mobile phase, in an isocratic mode, 25 ºC, flow 1.5 ml/min, wavelength 265 nm. We used a reverse phase column 250 x 4.6 mm GraceSmart RP 18 5u (GRACE). The injection volume was 50 µl. The analytical curve was built on rat intestinal fluid obtained by the technique of SPIP. The sample preparation involved protein precipitation with phosphoric acid. The stability of ACZ was tested by their incubation in intestinal fluid at 37ºC for 3 hours.

Results
The method developed was specific for Acetazolamide, none of the components of the infusion solution interfered with the peak of the drug. The calibration curve was linear in the concentration range from 0,192 to 2,112 µg/ml of Acetazolamide, with a correlation coefficient of 0,9981. The detection limit was 0,116 µg/ml and the limit of quantification was 0,351 µg/ml. Inter-day and intraday accuracy given by relative error (%RE) of quality control samples were ≤ 10% while inter-day and intra-day precision given by relative standard deviation (%RSD) of quality control samples were ≤ 10%. In stability studies under the test conditions the analyte was found to be stable. The percentage recoveries at low, medium and high concentrations for Acetazolamide were 108,6%.

Conclusion
This method is simple, reliable and can be routinely used to accurately determine the permeability of Acetazolamide in SPIP studies.

References

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ASSESSMENT OF THE CRITICAL CONTROL POINTS AT THE SELECTION AND CONDITIONING STAGE OF THE PLASMA UNITS USED IN THE PRODUCTION OF BLOOD PRODUCTS.


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Av Valparaíso S/N Ciudad Universitaria- Cba, Tel/Fax 0351-4334122/24, email: hnieres@hemo.unc.edu.ar

Introduction
The Quality Control of a drug covers different aspects, such as specifications, sampling, analysis and records, which allows to verify whether the components involved in the pharmaceutical production meet the established acceptance criteria.

In the Parenteral Pharmaceutical Products, the microbiological sterility and the depyrogenation constitute relevant security parameters, therefore the results of the Microbiological Quality Control of Raw Materials and Intermediate Products provide information that allows you to know the control conditions of the risks concerning to sterility and depyrogenation of the final product.

The Laboratorio de Hemoderivados UNC (Blood Products Laboratory UNC) uses plasma units to make a pool of initial plasma. This pool, processed by the Cohn method, gives rise to semi finished products and later to different blood products.

These serologically suitable units are selected in controlled environments and later externally washed using disinfectant solutions with the purpose of being employed in the Pool Formation process, that should meet certain pre established specifications.

Objective
To establish a monitoring strategy of high-risk points associated with the microbiological quality of the plasma pool, as support of activities of Microbiological Quality Control of said pool.

Materials and methods
Different quality tools are employed: HACCP, flow diagrams, dual intake matrix for risk evaluation, brainstorming, cause-effect diagrams, fish bone diagram, and analytical tools.

Environmental control by settlement plate of nutrient Agar and Glucose Agar Sabouraud.
Assessment of the antimicrobial activity of disinfectants: Agar plate technique by inhibition halo measurement against Bacillus subtilis subsp. spizizenii strains ATCC, and Staphylococci epidermidis.
Control of plasma unit surfaces: Plates (Rodac) with Agar Tripto Soya plus disinfectant inhibitors. (Tween 80, soy lecithin).

Results
The implementation of the HACCP plan allowed to identify the flow-chart of the process since the plasma units sanitation until the plasma pool formation. The risks were identified and evaluated systematically. They were also valued according to Gravity and Occurrence criteria (Table 1). The most appropriate preventive and corrective measures were considered, including the development of a microbiological control strategy and the identification of Control Critical Points (CCP), establishing control limit parameters.

The process of Plasma Pool formation was followed during six months, and consequently we obtained environmental control results within specification. In the control of plasma unit surfaces, a 90% showed a lack of microorganism and the remaining 10% gave counts lower than the action limit. The antimicrobial activity of disinfectants prepared according to SOP, was effective against the used microorganisms. The dilution percentage of said disinfectants correlates with the inhibition halos of the corresponding reference samples (Table 2).
Conclusion
The obtained results enabled us to prove that the implementation of this control strategy (Table 3) constitutes an efficient support for the Quality Control of Plasma Pool, which maintains the microorganism count in initial plasma on acceptable levels.

### Table 1. PCC Determination from risks analysis

<table>
<thead>
<tr>
<th>Stage</th>
<th>Danger</th>
<th>Prob.</th>
<th>Grav.</th>
<th>Risk</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>CCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Units Selection</td>
<td>1- Broken plasma units</td>
<td>Freq.</td>
<td>Mod.</td>
<td>Higher</td>
<td>YES</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>2- Polluted air</td>
<td>little.freq.</td>
<td>Low</td>
<td>Insign.</td>
<td>YES</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td>Plasma Units washing</td>
<td>3- Insufficient concentration</td>
<td>Little.freq.</td>
<td>High</td>
<td>Higher</td>
<td>NO</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>4- Solution does not reach all plasma units</td>
<td>Little freq</td>
<td>High</td>
<td>Higher</td>
<td>NO</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>5- Insufficient washing steps</td>
<td>little.freq.</td>
<td>low</td>
<td>Insig.</td>
<td>YES</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>6- Use of unsuitable water quality</td>
<td>Null</td>
<td>low</td>
<td>Insig.</td>
<td>NO</td>
<td>YES</td>
<td>---</td>
<td>---</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>7- Lack of drained baskets</td>
<td>Little.freq.</td>
<td>Mod.</td>
<td>Minor</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

S.1: Are there control measures in this process stage? S.2: Was this stage specifically designed to eliminate or to reduce the risk until it reaches an acceptable level? S.3: Can a danger exist, or increase the risk until it reaches an unacceptable level? S.4: Can a subsequent stage eliminate the danger or reduce the risk until it reaches an acceptable level?

From the obtained results of the environmental control, surfaces and antimicrobial activities, we observed that they tend to keep under control, therefore they met the specifications. However, some points related to the operational procedures should be adjusted.

### Table 2: Results of the antimicrobial activity assessment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Critical Limits</th>
<th>Monitoring System</th>
<th>Type</th>
<th>Reference Hypochlorite</th>
<th>Reference Virkon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma unit Integrity</td>
<td>No applicable</td>
<td>Visual</td>
<td>While processing</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Environmental control</td>
<td>≤50 CFU/ plate</td>
<td>Settlement plate</td>
<td>While processing</td>
<td>2.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Antimicrobial activity</td>
<td>Inh. Halo≥3.0</td>
<td>Method on plate</td>
<td>In the laboratory</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Sufficient disinfectant amount in container</td>
<td>150 L.</td>
<td>Measurement by weight</td>
<td>While processing</td>
<td>3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Cleaning control of plasma units, scissors and operators gloves</td>
<td>≤20 CFU/ plate</td>
<td>Surface plate (Rodac)</td>
<td>While processing</td>
<td>4.8</td>
<td>3.3</td>
</tr>
<tr>
<td>Microbiological control of water</td>
<td>≤10 CFU/100ml</td>
<td>Filtration through membrane</td>
<td>In the laboratory</td>
<td>7.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Results of the antimicrobial activity assessment

- **Sol.Work**
  - 5 %: 7.7*
  - 1 %: 3*

*Average of processes

### Table 3. Parameters taken for the monitoring system

<table>
<thead>
<tr>
<th>N°</th>
<th>Parameter</th>
<th>Critical Limits</th>
<th>Monitoring System</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plasma unit Integrity</td>
<td>No applicable</td>
<td>Visual</td>
<td>While processing</td>
</tr>
<tr>
<td>2</td>
<td>Environmental control</td>
<td>≤50 CFU/ plate</td>
<td>Settlement plate</td>
<td>While processing</td>
</tr>
<tr>
<td>3</td>
<td>Antimicrobial activity</td>
<td>Inh. Halo≥3.0</td>
<td>Method on plate</td>
<td>In the laboratory</td>
</tr>
<tr>
<td>4</td>
<td>Sufficient disinfectant amount in container</td>
<td>150 L.</td>
<td>Measurement by weight</td>
<td>While processing</td>
</tr>
<tr>
<td>5</td>
<td>Cleaning control of plasma units, scissors and operators gloves</td>
<td>≤20 CFU/ plate</td>
<td>Surface plate (Rodac)</td>
<td>While processing</td>
</tr>
<tr>
<td>6</td>
<td>Microbiological control of water</td>
<td>≤10 CFU/100ml</td>
<td>Filtration through membrane</td>
<td>In the laboratory</td>
</tr>
</tbody>
</table>
IMMUNOGLOBULINS FORMULATION: INFLUENCE OF THE SAMPLES PREPARATION ON THE ANALYSIS OF THE MOLECULAR DISTRIBUTION OF THE COHN FRACTION II (HPLC)

Novillo T., De la Iglesia G., Linares M., Vilches A. P., Canavesio L., Ahumada A.


Introduction
The fraction II (FII), obtained from human plasma by alcohol fractionation (Method 6 of Cohn) \(^{(1)}\), is used as raw material in the formulation of INJECTABLE INTRAVENOUS AND INTRAMUSCULAR IMMUNOGLOBULIN. The analysis of its molecular distribution (High Performance Liquid Chromatography) \(^{(2)}\) is part of the control to assess its quality, being the polymer percentage a constraining variable \(^{(3)}\) for its qualification as suitable.

Objectives
1) To determine the optimum dissolution conditions, 2) To evaluate statistically (Test t and F) the polymer stability depending on the time since the sample preparation until its quantification (pre-analysis time), 3) To define the dissolution temperature (DT) and pre-analysis time (pt) so that the molecular distribution be representative of the original sample.

Materials
5 batches of FII, phosphate buffer (3), BIO-RAD gel filtration standard, HPLC equipment (Hewlett Packard 1050), TSK gel 3000 SW column and guard column, water-ice bath, and magnetic stirrer.

Method
The Fractions II are kept at a temperature below -20ºC until their complete dissolution for their subsequent analysis. For each one of the 5 batches, we proceeded according to Table 1. Run Conditions: Injection volume: 20 µl, Flow: 0.5 ml/min, \(\lambda = 280\) nm, Stabilization time: 2 h, Dilution: 100 µl of FII in 1.9 ml of phosphate buffer \(^{(3)}\)

Results
Through the Test \(t\) and \(F\), statistically significant differences on average, corresponding to the quantification of polymers for the FII that were dissolved at room temperature were observed. On the other hand, it was observed that, regardless the manner of dissolution, the course of time does not affect the percentage of polymers. At initial time, it was seen a minor percentage of polymers in the samples dissolved by water-ice bath compared to those dissolved at room temperature.

Conclusion
The molecular distribution of FII is representative of the original sample when it is dissolved in refrigeration conditions. If the dissolution is done at room temperature, the sample should be processed immediately. The time that passes since the preparation until its analysis (tp=0, 4 and 8 h) does not affect the percentage of polymers.

References
(2) SOP LH-DT-CC-F-021 Determination of the Molecular Distribution using HPLC.
(3) European Pharmacopeia 6\(^{th}\) edition.
Table 1: Analysis Conditions

<table>
<thead>
<tr>
<th>Manner of dissolution of FV (Td)</th>
<th>Time since it is prepared until it is injected (tp)</th>
</tr>
</thead>
</table>
| FII dissolved in water-ice bath, shaking for 30 min.                                         | 0 h  
4 h  
8 h |
| FII dissolved at room temperature, shaking for 90 seconds.                                    | 0 h  
4 h  
8 h |
DETECTION OF COUNTERFEIT MEDICINES WITH THE VIDEO SPECTRAL COMPARATOR: THE EXAMPLES OF VIAGRA AND CIALIS.

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Introduction
Counterfeit medicines are drugs that have “been deliberately and fraudulently mislabeled with respect to identity and/or source” (1). It is estimated that 10% of the global pharmaceutical market is counterfeit (2-4). In Brazil, Viagra and Cialis, coated tablets of sildenafil and tadalafil, respectively, are the most counterfeited medicines (5). The counterfeit verification of such medicines is carried out by different advanced techniques (6-10). It is known that the interaction of light with matter is quantized, so a study of the radiation frequencies emitted or absorbed can characterize a material (11). The video spectral comparator – VSC – is equipped with light sources and filters of different wavelengths, being able to acquire and perform a comparison of spectra of electromagnetic radiation. This unit is used in the scientific examination of documents (12-19). Considering this, the present work proposed to develop a new analytical methodology to quickly distinguish fake and genuine coated tablets of Viagra and Cialis, using a VSC.

Materials and methods
Fake and authentic tablets (20) were used as sample. A VSC 5000 (Foster & Freeman Ltd., UK), employing optical amplification of 20 times was used. Scans were performed between 400 nm and 1000 nm, at one point for each tablet. Were employed the methods of lighting reflectance, absorption, transmittance, infrared fluorescence and visible fluorescence (after irradiation at 254 nm and 365 nm), in order to test their abilities to differentiate the fake from the authentic tablets. Images were obtained from samples analyzed.

Results
The absorption, reflectance and infrared fluorescence spectra exhibited greater ability to distinguish authentic and fake tablets of Viagra and Cialis. Fluorescence was found in counterfeit tablets and this was not observed in authentics. This divergence in the behavior of the coating opposite the radiation can be easily perceived in the visual comparison of images and spectra obtained under the conditions considered ideal for the detection of counterfeits.

Discussion and conclusion
The coating is responsible for homogeneity, color and brightness peculiar to each coated tablet. The pharmaceutical industry applies high technologies in the coating process plus a rigid quality control that discard any tablet with physical imperfections (21-23). When electromagnetic radiation is emitted or absorbed, there is a fixed transfer of energy between the object and the medium. Spectrochemical measurements of the phenomena of absorption or luminescence emission are dependent on the nature of sample and the concentration of the species involved (11). The uses of other substances, or concentrations or methods than those used as standard by industry in the production of batches of tablets originate coatings different from those in authentic tablets. Therefore, inauthentic coatings, yet visually mimics the true, will display differences with regard to interaction with electromagnetic radiation, enabling the differentiation between genuine and counterfeit medicines.

A rapid, reliable and without sample preparation method was employed in the detection of counterfeit Viagra and Cialis. This is the first study that used a VSC in medicine analyses. This technique can easily differentiate genuine from counterfeit tablets and showed promising to be used in routine analysis.

References
OPTIMIZED A METHODOLOGY FOR EPHEDRINE USING ACETIC ANHYDRIDE AS DERIVATIZING AGENT

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Introduction
Ephedrine is a sympathomimetic amine with β-feniletilamine structure, caracterizing amphetamine stimulants. Due to various adverse effects reported, its use were restricted by Food and Drug Administration in 2004, and, in Brazil, has its sale regulamented by Portaria 344/1998 (1). However, weight loss products containing ephedrine are still being sold in drugstore or online. Therefore, methods efficient to detect and quantify ephedrine are required. For Gas Chromatography analysis, the derivatizations is an essencial step to improve chromatographic profile, increase the thermal stability and volatility, improves the specificity, precision and sensitivity, a lower polarity, to prevent loss of analyte by adsorption column or by thermal decomposition (2). Thus, this study aimed to optimized a methodology for ephedrine in the Gas Chromatography Flame Ionization Detector (GC-FID) using acetic anhydride as derivatizing agent.

Materials and methods
From a stock solution (SM) at 1000mcg/mL of ephedrine in methanol:isopropanol:NH₄OH, 100mcL was taken to dryness at 40°C under N₂ flow, and derivatized with 20mcL of acetic anhydride. Different times (5, 10, 15, 30, 60, 70 or 80 minutes) and temperature (60 ºC, 80 °C or 100 ºC) reaction were tested. Subsequently, the sample was taken to dryness at 40 °C under N₂ flow and reconstituted with 50mcL methanol. An aliquot of 1mcL was injected into GC-FID under the following conditions: column temperature of 80 °C for 2 minutes, from 40 °C/min until reaching 250 °C, remaining for 1 minute, from 40 °C/min until reaching 290 °C and lasting for 2 minutes. Injector and detector temperature was 300 ºC and 250 ºC, respectively.

Results
To 60 °C were tested five times, 10, 15, 30 and 60 minutes for derivatization, though were observed ephedrine without derivatized. At 80 °C were tested 15, 30, 60 and 80 minutes. For 30 minutes of reaction, the largest area corresponds to pseudoephedrine, and in 60 minutes, corresponds to ephedrine. For 100 °C were tested 15, 30, 60 and 80 minutes. The largest area corresponds to ephedrine with 60 minutes of reaction was reached its largest area.

Conclusion
Due to varied temperatures and derivatization time described in literature for ephedrine, we conclude that due higher area, lower intensity peak of pseudoephedrine, and absent of non derivatizated substances, the best results was 100 ºC for 60 minutes.


A NOVEL APPLICATION OF IMMOBILIZATION ON MEMBRANES FOR THE SEPARATION AND SPECTROFLUORIMETRIC QUANTIFICATION OF AMILORIDE AND FUROSEMIDE IN PHARMACEUTICAL SAMPLES AND URINE.

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Introduction
Amiloride (AMI) and furosemide (FUR) are drugs widely used in different types of diuretics. These pharmaceutical formulations are supplied in numerous therapeutic indications, such as arterial hypertension, cardiac insufficiency, and hepatic cirrhosis since they rise the rate of urine formation, increasing the excretion of electrolytes, especially sodium, chloride and water (1,2).

Materials and methods
Polyamide membranes Millipore were placed in filter holder and 10mL of sample solutions at pH 11 were filtered through it using a positive pressure, keeping a flow rate of approximately 4 mL min⁻¹. Filtered solutions were reserved. After the membrane was dried, the disc was placed in a solid sample holder, and the AMI fluorescence spectrum was scanned. The excitation and emission wavelengths were adjusted at 365 and 406 nm, respectively. For FUR determination, the filtered solutions were adjusted at pH 2.7. Then, sample and standards solutions were introduced into the spectrofluorometer and the fluorescent emission was measured at λₑₘ 415 nm using λₑₓ 237 nm.

Results
FUR and AMI exhibit overlapped fluorescent spectra and urine produces background fluorescence that precludes the direct determination of these diuretics by conventional fluorimetry. This problem could be solved separating the analytes through a polyamide membrane. The optimum separation conditions were obtained filtering at pH 11, at which AMI keeps as neutral specie retained by the polyamide membrane, while the anionic form of FUR, remains in solution (3). The calibration graphs are linear in the range 3.20×10⁻⁴ to 0.8 µg mL⁻¹ and 1.33×10⁻³ to 4.0µg mL⁻¹ for AMI and FUR, respectively, with a detection limit of 9.62×10⁻⁵ and 4.01×10⁻⁴ µg mL⁻¹ (S/N= 3).

Conclusions
The present study demonstrates the feasibility of using a membrane as a novel support for solid-phase extraction procedures, focused on AMI and FUR separation and determination. Both sensitivity and selectivity are, then, substantially increased due to both the preconcentration on the support and the separation of the analyte from the matrix.

Acknowledgments
The authors gratefully appreciate the financial support from INQUISAL-CONICET (Instituto de Química de San Luis – Consejo Nacional de Investigaciones Científicas y Tecnológicas), FONCYT (Fondo Nacional de Ciencia y Tecnologia), and National University of San Luis (Project 22/Q828).

References
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DATA FUSION APPLIED TO THE PHOTODEGRADATION STUDY OF CIPROFLOXACIN USING A TANDEM DETECTION SYSTEM (UV-VIS AND FLUORESCENCE) AND MULTIVARIATE CURVE RESOLUTION

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Universidad Nacional del Sur, INQUISUR-CONICET Alem 1253 B8000CPB, Bahía Blanca, Argentina

Introduction
Ciprofloxacin is a synthetic fluoroquinolone derivate which has broad-spectrum activity against many gram-negative and gram-positive pathogenic bacteria, via the inhibition of the DNA gyrase responsible of DNA preservation. It is partially metabolized, but principally is eliminated unchanged via kidney. Consequently, a high amount of this compound is released into the environment through biological waste of humans and animals, which can cause environmental and food pollution (1). Moreover, these species may undergo changes in their chemical structure due to environmental factors, giving rise to products with different characteristics to the parent species, and whose polluting action must be taken into account. This paper presents an exploratory study of the photochemical degradation of ciprofloxacin in order to monitor the reaction throughout the time and understand the kinetic of the photodegradation under different conditions. The proposed method is a simulation of the effect of the environment as a time function, and a rapid information is obtained through Chemometric, avoiding the separation techniques commonly used to study this kind of reactions.

Materials and methods
The photodegradation of ciprofloxacin was studied at different pH using a continuous flow system under ultraviolet radiation. The UV source was a 15 W germicidal lamp (max. 254 nm). Photodegradation reaction (20 min) was monitored by UV-Vis and fluorescence in a tandem arrangement (diode array detectors). Spectra (200 to 500 nm for UV-Vis and 370 to 800 nm for fluorescence) were recorded at steps of 4 s and then analyzed by multivariate curve resolution-alternating least squares (MCR-ALS) algorithm (2).

Results
Data obtained by UV-Vis and fluorescence were processed by MCR-ALS using the so called “data fusion” approach, i.e. the data were simultaneously analyzed. MCR-ALS made it possible to obtain the concentration profiles of the species involved in the reaction and the corresponding pure spectra (both UV-Vis and fluorescent). From the results it can be seen the typical degradation profile of ciprofloxacin and also the presence of four photodegradation products. Three of them are intermediate products and the forth is the final product. All the species have different UV-Vis spectra. However, fluorescence spectra related to ciprofloxacin and the first two intermediate products are quite similar. The third intermediate product has a different fluorescence spectra and the final product is not fluorescent. From the results also it can be seen that the fotodegradation was more effective at higher pH. These preliminary results are promissory as a start point to study the kinetic degradation of the fluoroquinilones in several media.

Conclusions
The fusion of UV-Vis and fluorescence data by MCR-ALS makes it possible to study the kinetic of ciprofloxacin degradation. The proposed method constitutes a fast and reliable technique for obtaining both pure spectra and concentration profiles over time of ciprofloxacin and its photodegradation products, avoiding separation techniques.

Acknowledgements
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References
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DEVELOPMENT AND VALIDATION OF A NEW HPLC ANALYTICAL METHOD FOR DETERMINATION OF CINNARIZINE IN TABLETS

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Introduction
Cinnarizine, a cerebral blood flow improver, is widely used orally for treatment of cerebral apoplexy, cerebral arteriosclerosis and post traumatic cerebral symptoms, (1).
The target of this study is to develop a new, simple, and fast analytical method by HPLC to quantify cinnarizine and its degradation products in tablets, together with its latter validation study.

Materials and methods
The chromatographic system is a Gilson HPLC, with Diode Array Detector at 254 nm and a column Luna C8 (2) 5 µm 250 mm x 4.6 mm. The mobile phase consisted of 50% of acetonitrile HPLC grade (by Sintorgan) and 50% of a buffer solution pH 3.8 (0.05M), at a flow rate of 1.5 ml min⁻¹. The temperature of the column was held at 30 ºC. The injection volume was 20 µl. Each test required 10 min.
The standart reference of cinnarizine was suplied by USP. Cinnarizine standard stock solution of 0.4 mg ml⁻¹ was prepared in acetonitrile. Standard solutions were prepared from the stock solution by dilution with mobile phase.
Sample solutions of cinnarizine tablets were prepared from 20 tablets, which were finely powdered and well mixed. A equivalent to 75mg of cinnarizine was weighed and dissolved in 100ml of acetonitrile, shaken for 20 min and filtered. Further dilute 1.0 ml of the filtrate to 10 ml mobile phase.
The preparation of the degradation product was with cinnarizine Saporiti™ 20mg of cinnarizine was dissolved in 50ml 0.1M HCl , heated in water bath for 8 h. Diluted 2ml in 10ml with mobile phase.

Results
The analytical method development and validation for Cinnarizine tablets and its degradation products was done by submitting drug and tablets to acid degradation.

Specificity:
Test studies shows that the chromatographic peaks are applicable to Cinnarizine and its degradation products.

Linearity:
Linearity calibration curve shows linear response over the range of concentration used (between 0.064 mg/ml and 0.096 mg/ml).

Accuracy:
The accuracy of the method was determined by recovery studies. CV=0.99, Texp < Ttab.

Precision
The data shows that the reproducibility and repeatability of the assay procedure was satisfactory, showing no marked changes in the chromatogram when the method was determined for the same sample under different analyst in different operational conditions, the degree of reproducibility showed results within their limits. Further there was no interference due to excipients. The repeatability study was determined for repeated analyses (n=10) and resulted in a CV of 0.43%.

Conclusions
The analysis of the obtained results can demonstrate that the proposed method is very useful to the determination of this drug in pharmaceutical dosage forms and could be used for pharmaceutical analysis of Cinnarizine tablets.

Acknowledgments
We thank to Gabriela Beatriz Di Chiачcio for the technical assistance.
References:

### Accuracy

<table>
<thead>
<tr>
<th>% conc. Estud.</th>
<th>mg Teór.</th>
<th>mg Práct.</th>
<th>% Recup.</th>
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<td></td>
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<tr>
<td>ttab:</td>
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<td>GL: 16</td>
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### Linearity

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<tr>
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<td>Correlation Coefficient</td>
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<tr>
<td>CV of slope (&lt; 2%)</td>
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### Reproducibility

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<th>S2:</th>
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### Repeatability

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MULTIPLE RESPONSE OPTIMIZATION APPLIED TO THE DEVELOPMENT OF A ION PARING LIQUID CHROMATOGRAPHIC

Teglia CM, Cámara MS, Robles JC, De Zan MM

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Introduction
In developing a HPLC method, optimization may be applied to reduce the analysis time achieving desirable values for critical chromatographic parameters. The need of taking into account different aspects of the analysis at the same time calls for the use of multicriteria optimization. Experimental design, specifically surface response analysis, is a valuable tool in order to deal with this scope (1). In addition, when different objective functions have to be optimized, the so called “Derringer’s desirability function” is a powerful strategy (2).

Nicarbazin (NICA) is the generic name for the complex of 4,49-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP) in a 1:1 molar ratio, that is used as a feed additive against coccidiosis in poultry. No methodology was found in literature to the simultaneous assay of both components of NICA, possibly due to the bad retention of HDP in conventional RP-HPLC since it height polarity and basic properties.

In this work an ion paring- high performance liquid chromatographic (IP-HPLC) method was developed, optimized and validated for its application in the determination of DNC and HDP in bulk material and coccidiostatic formulations.

Materials and methods
Nicarbazin standard and sample solutions were prepared in dimethylformamide and diluted with mobile phase to appropriate concentration. Separation was achieved in an Inerstil ODS-3 4.6 x 150 mm column with 5µm particle size and monitored at 300 nm. Mobile phases consisting of mixtures of buffer solution at different pHs containing different concentrations of sodium 1-heptanesulfonate (SHS)/ sodium acetate (SA) and acetonitrile were tested. A central composite design was used to find the optimal combination of column temperature, buffer pH, SHS and SA concentration to obtain the more desirable responses for resolutions, retention times, peaks areas and peaks width. Optimized method was validated following ICH guidance.

Results
The response surface obtained for the global desirability function produced a maximum value (D=0.883) for a buffer concentration of 12.6 mM HS and 3.6 mM SA, a pH value of 2.8 and oven temperature of 22.5 ºC. The individual response values corresponding to the latter value of D are shown in Table 1.

The stress testing undertaken according ICH guidelines in conjunction with peak purity analysis revealed that method is selective and stability-indicating. The calibration curves constructed for DNC and HDP were linear over the concentration range of 0.64–0.96 mg mL⁻¹ for NICA (80.0–120.0% of the expected concentration in samples solutions). Precision of the assay, investigated with respect to both repeatability and intermediate precision was estimated from six replicates and are shown in Table 2. Recovery experiments of spiked matrix in three concentration levels of a commercial sample containing 8.0%p/p NICA were performed to evaluate accuracy, with results ranging 98.0-101.4%. No statistical difference from 100%, at a confidence level of 95%, was found.

Conclusions
The use of experimental design allows us to find the optimal chromatographic condition in the simultaneous determination of HDP and DNC in bulk and pharmaceutical dosage form. Developed method shows good results respect to precision, accuracy and selectivity in the studied concentration range. In addition its simplicity allows for application as routine analysis and pharmaceutical stability experiments in quality control assays.

Maria Mercedes De Zan Tel/FAX: 54 342 4575205; e-mail: mmdezan@fcb.unl.edu.ar
References.


Table 1: Response value corresponding to optimized factors

<table>
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<tr>
<th>Compound</th>
<th>$t_r$(min)</th>
<th>Width</th>
<th>Area</th>
<th>Resolution</th>
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<tr>
<td>DNC</td>
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<td>0.073</td>
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<td>2.87</td>
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Table 2: Intra-and inter-day precision of the method.

<table>
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<tr>
<th></th>
<th>HDP</th>
<th>DNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-day (n=6) %RSD</td>
<td>1.40</td>
<td>1.13</td>
</tr>
<tr>
<td>Inter-day (n=12) %RSD</td>
<td>1.52</td>
<td>1.16</td>
</tr>
</tbody>
</table>
Introduction

Ectomyelois ceratoniae, Ceratoniae (Zeller) (Lepidoptera: Pyralidae) or carob moth, is a polyphagous insect in larval state recognized as economically damaging pest in many regions around the world. This moth is commonly found in pomegranates, figs, nuts, almonds, pistachios, dates and macadamias (1). In La Rioja, Argentina, carob moth was recently introduced damaging nut. Insecticide use for its control is a not efficient strategy because its larval stage happens inside the fruit affecting quality. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control. Insecticidal activity of many plants against several insect pests has been demonstrated (2).

In the course of screening for new bioactive substances produced by plants for pest control in this work was assayed effect of Tibouchina paratropica (Melastomataceae) subextracts on Ectomyelois ceratoniae. Tibouchina is a genus belonging Melastomataceae, a pantropical family with over 185 genera that include about 5,000 species (3). Many species of Tibouchina have showed different biological activities such as antitumoral (4), antifungal (5-6), trypanocidal (7) and antioxidant (8).

Materials and methods

Tibouchina paratropica (Griseb.) Cogn. (Melastomataceae) was collected in El Nogalar (1,750 m ads), Tucumán. A voucher specimen has been deposited at the Herbarium of Miguel Lillo Foundation, Tucumán, Argentina.

Air-dried leaves (1575 g) of T. paratropica were homogenized with acetone–H\(_2\)O (7:3) at room temperature by maceration during seven days, filtered and concentrated in vacuo. The concentrated solution was subjected to liquid-liquid partition to give different subextracts: CH\(_2\)Cl\(_2\) (6.6g) (SED), EtOAc (13g), n-BuOH (30.4g) and water-soluble portions (4.2g) (SEA).

E. ceratoniae were collected at first time from Aicuña, La Rioja, Argentina on nuts and were reared under controlled conditions in laboratory of National University of Chilecito (La Rioja, Argentina) with a natural diet. This food was made with nuts previously disinfected with a 5 % NaClO, washed with distilled water, dried at 50°C during 1 hour, powered and conserved at -18°C. Concentrations of 1000; 750; 500 and 250 mg/L of (SED) and (SEA) were prepared using hidroalcoholic solution as dissolver. 2.5 mL subextracts were added at 5 g of food and solvents were evaporated at 35°C under reduced pressure. After 48 hours 0.250 mg of food was placed into fifteen glass containers at 2 cc of capacity. The negative control was food only impregnated with dissolver. Into each container was placed a larva of 3\(^{\text{rd}}\) instar and the vial was covered with cotton speck. Mortality was valued at five and ten days. All tests were performed by triplicate.

Results

On showed 100% of mortality to the five days for 1000; 750 y 500 mg/L doses of (SED) and (SEA) of T. paratropica. None of the subextracts had a significant effect at doses under 500 mg/L.

Conclusions

These results indicate that both subextracts have promising effect on control of E. ceratoniae. This actions could be evaluated like control alternative in integrated pest management programs.

Acknowledgments

We wish to thank Lic. Alberto Slanis of Miguel Lillo Foundation for identification of the plant material. Research Council of the National University of Tucumán (CIUNT) for financial support. Miss María José Mulet (UNdeC).
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COMPARATIVE STUDY OF VOLATILE ORGANIC COMPOUNDS (VOC) BY HS-SPME/GC-MS IN FRESH INFLORESCENCES OF THREE Tagetes SPECIES FROM CÓRDOBA, ARGENTINA.

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Universidad Católica de Córdoba
Camino a Alta Gracia Km 7,5 CP 5017 Córdoba

Introduction:
The genus Tagetes (Asteraceae) are plants used in folk medicine for a variety purposes. In Cordoba (Argentina) can be found three species: T. minuta, T. filifolia and T. argentina, whose reports on their volatile organic compounds (VOC) was based on studies of its essential oils obtained by hydrodistillation. In this work a comparative study of the composition of VOC in fresh inflorescences of Tagetes species by Headspace Solid Phase Microextraction/Gas Chromatography-Mass (HS-SPME/GC-MS) previously optimized was carried out.

Materials and Methods
Samples: 100.0 mg of fresh inflorescences from T. minuta, T. filifolia and T. argentina (collected in the Sierras Grandes, Córdoba, Argentina).
Extractant phase: carboxeno-divinylbenzene-polydimethylsiloxane (Supelco).
Temperature: 40 ° C (Polyscience brand bath).
Incubation time: 5 min.
Extraction time: 30 min.
Determination (triplicate) Gas Chromatograph HP 5890 Series II, Mass Detector HP 5970, in the same conditions of analysis in each case: column HP-5 30 m; injector: 225 ° C, detector: 230 ° C, oven: 40 ° C (5 min) to 200 ° C to 5 ° C / min (5 min), flow, He: 99.99% (5 psi).
The volatile organic compounds were identified by comparing of mass spectra with library (Wiley-NIST) and by determining of Kovats retention index (KI).
Descriptive statistical analysis was performed, significant differences were analyzed using analysis of variance (ANOVA, α = 0.05) with Tukey test, and a multivariate statistical analysis (Cluster Analysis) was applied to determine the Euclidean distance between the three species, using the Infostat program, v2009p.

Results
43 VOC was identified (over 95% of total), and only three were common in the three Tagetes species: Isothymol, β-caryophyllene and α-Humulene.
Majority VOC:
T. minuta: (E)-Tagetone (10.5 %), cis-Tagetenone (18.4 %) and trans-Tagetenone (46.2 %).
T. filifolia: Estragole (19.2 %) and trans-Anethole (55.20 %).
T. Argentina: (E)-Tagetone (9.1 %); Tagetone (10.2 %), trans-Tagetenone (30.2 %) and D-Verbenone (21.5 %).
There were significant differences in the composition of majority and minority components of three Tagetes species studied. From the results of cluster analysis, T. filifolia is separated from T. minuta and T. argentina, which form a group each.

Discussion and Conclusions:
A variability and diversity of the composition of VOC in the studied species was found. The results show that, from a chemical point of view, T. minuta and T. argentina have a greater similarity between them than with T. filifolia.
Also the main compounds found are equivalent to reported for those essential oil, but by HS-SPME/GC-MS most minority VOC was found, allowing better characterization of three specimens.

References:
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Introduction
The fraction V (FV), obtained from human plasma by alcohol fractionation (Method 6 of Cohn) is used as raw material in the formulation of intravenous injection of HUMAN SERUM ALBUMIN UNC. The analysis of the total proteins, pH, molecular distribution, and protein purity is part of the quality control to assess the suitability of such fraction for its subsequent processing.

Objectives
To analyze all quality parameters involved in obtaining the Cohn fraction V through statistical tools \(^1\) to determine whether the process is under statistical control.

Materials
39 batches of FV, sodium phosphate dehydrate p.a, monosodium phosphate monohydrate p.a, sodium chloride p.a, sodium azide p.a, water for injection, BIO-RAD gel filtration standard, cellulose acetate strips, sodium Veronal buffer for electrophoresis, glacial acetic acid p.a, sodium chloride p.a, sodium hydroxide p.a, copper sulfate pentahydrate p.a, sodium and potassium tartrate p.a, potassium yoduro p.a, NIST 7 albumin standard, HPLC equipment (Hewlett Packard Model1050), TSK gel 3000 SW column, TSK gel 3000 SW guard column, Ultrospec 3300 pro UV/ Visible Spectrophotometer, pH-meter (Consort P901) were employed.

Methods
Purity \(^2,6,7\): electrophoresis on cellulose acetate. Molecular Distribution \(^3,6,7\): High-performance Liquid Chromatography. Total Proteins \(^4,6,7\): Gornall method. pH \(^5,6,7\): potentiometry. A retrospective study was carried out, and statistics tools such as control graphics, histogram, etc. were employed \(^1\).

Results
1) A retrospective study about the values obtained from the Molecular Distribution, Total Proteins, pH, and Purity of 39 batches of FV was done.
2) Creation of Control Graphics: It was observed that the process variability is only due to a system of uncontrollable random causes (non assignable causes) since the distribution of observations (Molecular Distribution, Total Proteins, pH, and Purity) is normal \(^1\).
3) The values of the observations are within the 2SD limits (2 standard deviations), that is, within the warning lines, therefore the process is under statistical control \(^1\).
4) Creation of Histograms \(^1\): the higher frequency is within the following levels:
   • Total Proteins: from 21 to 23 g/% with the 15.4% (Specification: 15-35 g/%)
   • pH: 4.4-4.5 with the 35.9% (Specification: 4-5)
   • Molecular Distribution: 0-0.6 of polymers with the 43.6% (Specification: 0-3% of polymers)
   • Purity: two levels of 98-99% and 100%, each of them with the 29.73% are observed.

Conclusions
The behavior of Molecular Distribution, Total Proteins, pH, and Purity shows a normal random distribution pattern. The acquisition process of FV is under statistical control and the use of control graphics for the monitoring of the process is a very valuable tool.

References
(1) Course on Statistical Control of the Processes given by the engineer Silvina Suzuki. Instituto Argentino de Normalización y Certificación (IRAM).
(2) SOP LH-DT-CC-F-013 Electrophoresis on Cellulose Acetate.
(3) SOP LH-DT-CC-F-021 Determination of the Molecular Distribution by means of High-Performance Liquid Chromatography.
(4) SOP LH-DT-CC-F-010: Determination of Protein by the Gornall Method.
(6) United Stated Pharmacopeia, USP XXXI
DEVELOPMENT OF THE PRODUCTION OF INJECTABLE GENERIC DRUGS IN THE LABORATORIO DE HEMODERIVADOS UNC (BLOOD PRODUCT LABORATORY UNC)

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Introduction
Due to the Argentine economic crisis in 2002, the National University of Córdoba, through the Laboratory de Hemoderivados UNC and the Faculty of Chemical Sciences promoted the construction and the start-up of an injectable generic drug plant to meet the population’s needs.

Objectives
To assess the development of the production of both the own brand UNC-FARMACOS (A) and others produced for third parties (B) and development, implementation and validation of physicochemical techniques necessary to guarantee their quality.

Materials and methods
An evaluation of the batches produced from the year 2003 to December 2009 was carried out. The considered injectable drugs for (A) were: Dexamethasone, Diazepam, HCI Difenhidramine, Sterile Water for Injection, Potassium Chloride 15mEq/ml, Sodium Chloride 20%, Physiological Solution, HCI Ranitidine, HCI Metoclopramide, Furosemide, HCI Lidocaine. For third parties (B) HCI Metoclopramide, Diclofenac, Diazepam, Furosemide, Fenitoin, Ixosuprine, Dexamethasone, Adrenaline, Water for Injection, Dextropropoxifene + Dipirona, Sodium Chloride7%, Zinc-Copper-Magnesium-Manganese Sulfate, Chromium Chloride, Ammonium Molibdate, Selenious Acid.

Results
A) Physicochemical Department, Quality Control Area . 22 analytical techniques for identification and quantification of excipients and active principles, and 15 for finished product were implemented. 7 new techniques were developed for the process controls. 5 of these techniques has already been validated and contrasted with pharmacopeia methods.

B) Generic Production Area: From 2003 and December 2009, a total of 487 batches were produced. The 64% (311 batches) belongs to the production of UNC-FARMACOS. The remaining 36% (176 batches) belongs to batches B, that showed the following distribution: period 2003-2004: 14.3%, period 2005-2006: 28.6% and the 57.1% from 2007 to December 2009.

UNC-FARMACOS: The production percentages of the 311 batches were: 6.4% Dexamethasone, 3.5% Diazepam, 2.9% HCI Difenhidramine, 10% of Water for Injection, 13.0% of Potassium Chloride, 4.2% of Sodium Chloride, 20%, 7.4% of Physiological Solution, 28.1% of HCI Ranitidine, 11.3% of HCI Metoclopramide, 9.7% of Furosemide, and 3.5% of HCI Lidocaine. All batches were distributed in the province of Córdoba.

Conclusions
With the application of the requested Quality Standards, the plant was approved by the ANMAT (Administration National de Medicaments, Food y Technology Medical). The plant’s approval along with the gained experience and the authorization to produce such products in Argentina will allow their use in a much larger population, and will enable us to go on fulfilling our Laboratory’s mission.

References
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3) United Stated Pharmacopeia, USP XXXI:
DETERMINATION OF SILDENAFIL TRACES IN URINE AND HERBAL MEDICINES

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Introduction
Sildenafil, popularly known as Viagra, is an effective drug for treatment of erectile dysfunction (ED)\(^1\). Since sildenafil was launched, it has being one of the most widespread drugs of use and abuse\(^2\). The illicit addition of this drug in local herbal medicine for ED treatment represents other risk for the human health. Therefore, an accurate, sensitive and robust methodology for sildenafil determination is highly required. Complex matrices require a clean-up step before the analyte determination. A column of a polymeric resin XAD impregnated with a cationic surfactant retained effectively sildenafil. Drug was eluted with ethanol and determined by spectrofluorimetry. At optimal experimental conditions, several samples (urine, herbal medicine) containing the drug were analyzed with satisfactory results.

Experimental

Materials and methods
A column was filled with Amberlite XAD-1180 (Rhom-Haas) previously activated, and 10 mL (0.1 M) of hexadecyltrimethylammonium bromide (HTAB, Tokyo Kasei Industries, Japan) were added. The reagent excess was removed washing with bidistilled water. Samples of human urine and aqueous extracts of \(L.\) saururus, \(H.\) baylahuen, \(B.\) articulata, \(T.\) vulgaris and \(S.\) apiana spiked with sildenafil (Gador, Argentina) were fed. The column was then thoroughly washed with aqueous solution pH 11 and finally eluted with 5 mL of ethanol. A portion of 3 mL was collected and analyzed by spectrofluorimetry (\(\lambda_{ex}\) 310, \(\lambda_{em}\) 430 nm).

Results
HTAB micelles positively charged interact with negatively charged molecules of sildenafil\(^3\). Micelles adsorbed on XAD-1180 resin have the capability of retaining sildenafil with two important benefits: sildenafil was effectively separated of matrix sample, and the drug was adequately preconcentrated in column. Additionally, eluted ethanolic portion showed a great enhancement respect to drug native fluorescence (40-field). Lower detection limit was 0.02 ng mL\(^{-1}\), with a lower quantification limit of 0.06 ng mL\(^{-1}\).

Conclusions
Amberlite XAD-1180 impregnated with HTAB micelles has shown the capability of separating and concentrating sildenafil in alkaline aqueous medium. This methodology has been successfully applied for determination of sildenafil in biological and pharmaceutical samples.

Acknowledgments
The authors want to thanks INQUISAL-CONICET, FONCYT and UNSL (Project 22/Q828) for the financial support.

References

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DETERMINATION OF ESTROGENIC ENDOCRINE DISRUPTOR: ETHINYLESTRADIOL, USING MODIFIED MAGNETIC NANOPARTICLES

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Introduction
Ethinylestradiol (EE2) is a synthetic estrogen female sex hormone which is used as a contraceptive therapy. The determination of synthetic estrogen has attracted much attention since these compounds were included in the endocrine disrupting chemicals (EDCs) and its ability to cause the deleterious reproductive dysfunction of animals and humans (1). Due the problems mentioned previously, it is important the determination of EE2 in water samples. Therefore we present a method that combined the use of magnetic nanoparticles (MNPs) modified with anti-EE2 antibodies and square-wave voltammetry (OSWV). The advance in experimental electrochemical techniques in the field of analysis of drugs is due to their simplicity, low cost and relatively short time of analysis when they are compared with other techniques (2-7). In addition the use of MNPs also has attracted great interest for biological and clinical applications (8–11), and they are widely used as a new agent of preconcentration due to its features, between these: large surface area (12), strong magnetism, improve the adsorption capacity of analytes and avoid the time-consuming enrichment process of loading large volume samples through the rapid isolation of NPs with an adsorptive magnet.

Materials and method
Electrochemical experiments were performed in unstirred solutions using a BAS 100B/W electrochemical analyzer (Bioanalytical System, West Lafayette, IN). Cyclic and square-wave voltammograms were obtained using a three electrodes system consisted of a glassy carbon (GC) working electrode, an Ag|AgCl|3M NaCl reference electrode and a Pt wire counter electrode. All reagents were of analytical or biochemical grade.

Procedure
This method was applied to the determination of EE2 in water samples. 25 mL of sample solution were put into a beaker. Then 175 µg of magnetic nanobeads, previously modified with anti-EE2 antibodies were added to the sample solution. EE2 present in the sample was allowed to react immunological with the modified magnetic beads. Then, after stirring for 10 min, the magnetic nanobeads were recovered using an external magnet and washed three times with 0.01M PBS buffer (pH 7.2) to remove the excess of sample and they were resuspended in 250 µL of H₂SO₄ pH 2.00 and added in a voltammetric cell for the desorption of EE2; after 2 min, the signal was OSWV.

Results
The calibration curve was plotted using ∆I versus concentration of the standard solutions. Calibration curve was found to be linear over of the concentration range 0.03–25 µg L⁻¹. The calibration graph is described by the calibration equation ∆I(nA) = 76.08 + 7.67C_{EE2} with a correlation coefficient of 0.998, with a limit of detection DL=3Sᵦ/m was 0.003 µg L⁻¹.

Conclusions
The developed voltammetric technique is suitable for the determination of EE2 in environmental samples after a simple step of preconcentration using modified MNPs. It has the advantages of low cost, wide linear range, reproducibility, accuracy and, more important, low DLs. The developed procedure was successfully applied to water samples.

References

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Microfluidic Enzymatic Sensor for the Electrochemical Detection of Pipemidic Acid in Pharmaceutical Samples

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Introduction
Pipemidic acid (PA) is a synthetic quinolone that belongs to the first generation of this kind of compounds, which is used as antibacterial agent. The antibacterial property of the quinolones is associated with their potential to inhibit the bacterial topoisomerase II (1). PA is widely used for the treatment of urinary tract infections, showing high activity against gram-positive and negative bacteria. Several methods for the determination of PA have been developed (2-4) but the use of microfluidic enzymatic sensors with electrochemical detection represents an interesting option to be considered for the PA determination, because these devices offer many potential advantages (5). For these reasons, we have developed a very sensitive device based in the presence of tyrosinase immobilized on ACPG particles contained into the central channel (CC) of the microfluidic system, where occurs the enzymatic reaction for the indirect-PA determination.

Materials and methods
All reagents used were of analytical reagent grade. The main body of the sensor was made of Plexiglas. The gold electrode is at the end of the CC. The CC containing 0.3 mg of controlled-pore glass, and the end of the CC was blocked with glass fibers. The diameter of the CC was 150 µm and the diameter of the accessory channels were 100 µm. The potential applied to the gold electrode was 0 V vs the Ag/AgCl wire pseudo-reference electrode and a Pt wire was the auxiliary electrode. Amperometric detection was performed using a BAS LC-4C potentiostat and BAS 100 B/W (USA), which was used to voltammetric determinations. Syringe Pumps Systems were used for pumping, sample introduction, and stopping flow.

Results and discussion
The measuring principle of this biosensor for the determination of PA in pharmaceutical formulations is as follows. First, the tyrosinase immobilized on ACPG particles catalyzes the oxidation of catechol (Q) to o-benzoquinone (P), whose electrochemical reduction back to Q was obtained at potential of 0 V. Second, the detection of the PA was accomplished for the suppression of the substrate recycling process between tyrosinase and the electrode (denoted by the dotted arrow), decreasing the peak current obtained proportionally to the increase of the PA concentration. A linear relation, \( \Delta I (nA) = 311.37 - 4.443 [C_{PA}] \) was observed between the \( \Delta I \) and the PA concentration in the range of 0.02 to 70 µM. The linear regression coefficient and the detection limit (DL) were \( r = 0.998 \) and 18 nM, respectively. Reproducibility assays were made using repetitive standards solutions (n=5) containing 1.0 mM Q and 10 µM PA, and the coefficient of variation (CV) for this study was below 3%. The developed microfluidic-biosensor for the PA determination was applied to two commercial preparations. There is no need for any extraction procedure before of the analysis. No change of the peak height in the presence of the excipients was observed.

Conclusions
In this article we have showed the usefulness of the microfluidic-enzimatic sensor with electrochemical detection, applied to the determination of very low concentrations of pipemidic acid in pharmaceutical samples. This sensor provides a cost effective solution to obtain good quantitative information and wide applicability in the pharmaceutical industry as quality control method.

Acknowledgements

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The authors wish to thank the Universidad Nacional de San Luis, the Agencia Nacional de Promoción Científica y Tecnológica, and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) for their financial support.

References
MODIFIED GOLD ELECTRODE APPLIED IN THE METHIMAZOLE DETERMINATION

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Introduction
Methimazole (MT, 1-methyl-2-mercaptoimidazol) is a drug used to treat hyperthyroidism. Its administered orally and concentrated in the thyroid gland [1], where its action is decrease the iodide incorporation into tyrosine and thus inhibits the production of thyroid hormones [2]. The development of rapid and sensitive methods that allow its determination in pharmaceutical samples is of vital importance. For this reasons in this article we describe the development of a dynamic system for in batch detection of methimazole in drug samples.

Materials and methods
All reagents used were of analytical reagent grade. Horseradish peroxidase, catechol, thioctic acid, dimethylaminopropyl carbodiimide hydrochloride and N-hydroxy-succinimide were purchased from Sigma Aldrich Inc.

Cyclic voltammetry and timebase analysis were performed using the BAS 100 B (electrochemical analyzer Bioanalytical Systems).

Modificación of gold electrode
In the first stage of immobilization, gold electrode was immersed in a solution of thiotic acid (TA) 250 mM for 12 hours, then was rinsed with ethanol and dried with N2 gas. At this stage TA was covalently attached to the electrode surface through thiol groups, constituting a monolayer. After that, the TA modified electrode was placed in a succinimide-carbodiimide solution-EDC: NHS (EDC 1% (v / v), NHS 2.5% (v / v)). In this step occurs the activation of carboxyl groups of TA, which will be used for the covalent bond with the amino groups of the enzyme. Finally, on the modified surface of the electrode were added 20 µL of a solution 0.25 mg / µl of horseradish peroxidase in phosphate buffer, pH 7.40 for 18 h at 4 °C [3,4].

Determination of metimazol
The modified electrode was introduced in an electrochemical cell, where the enzyme peroxidase (HRP) catalyzed the reduction of hydrogen peroxide (H2O2) to water (H2O) with the consequent oxidation of the mediator catechol (Q) to o-benzoquinone (P) The electrochemical reduction of P to Q was detected on the surface of the electrode using timebase technique. The current response obtained was directly proportional to the activity of the enzyme and consequently to the concentration of H2O2 added to the system [5]. When MT was added to the solution, the thiol groups of this compound participate in a Michael type addition reaction with P to form the corresponding thioquinone derivatives; decreasing the reduction current proportionally to the increase of the concentration of added MT[6].

Results and Discussion
For determination of methimazole in pharmaceutical samples using a modified gold electrode for methimazole determination in citrate buffer pH 5.0, a calibration curve was obtained, the regression lineal equation was I (nA)=−338-6.38C_Met with a coefficient of lineal regression of r=0.998 and the detection limit obtained was 1.9 µM.

Conclusion
The proposed system, which incorporates a modified gold electrode for the determination of methimazole in pharmaceutical samples, showed selectivity and sensitivity, also has advantages such as; do not require

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highly trained personnel or expensive instrumentation. In summary proposed methodology has good applicability in the pharmaceutical industry as a routine method.

References
QUALITY CONTROL OF DOXYCYCLINE HYCLATE

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Introduction

A simple, sensitive and accurate spectrophotometric method was developed for the assay of doxycycline hyclate in raw material and tablets. The validation method yielded good results and included the range, linearity, precision and accuracy. The absorbance was measured at 273 nm for doxycycline hyclate solutions. This drug is a broad-spectrum antibiotic derived from oxytetracycline, used in Brazil and the rest of the world as both human and veterinary medicine, as well as in animal nutrition. Its method of analysis is not in the Brazilian Pharmacopoeia. On the other hand, it is described in the British Pharmacopoeia an UV spectrophotometry analysis, to which is recommended the preparation of solutions using a mixture of hydrochloric acid and methanol as solvent. The aim of this work was to investigate the use of water solutions in the UV spectrophotometric method of doxycycline hyclate, since it is soluble in this solvent.

Materials and methods

Spectrophotometer UV Shimadzu, quartz cells, aqueous solutions of doxycycline hyclate in concentrations 100 mg/ml (1); 50 mg/ml (2); 25 mg/ml (3); 12.5 mg/ml (4); 6.25 mg/ml (5) and 3.125 mg/ml (6). A standard calibration curve of doxycycline was constructed by plotting absorbance versus concentration. The dilutions were analyzed individually in the UV and using their respective absorbance values an absorption curve was constructed.

Results

In the spectrophotometer, peaks were obtained in two wavelengths: 273 and 345 nm. When we fixed the wavelength at 273 nm, we obtained the following absorbance values: 3.0277 (1); 1.5333 (2); 0.7834 (3); 0.4094 (4); 0.2272 (5) and 0.1272 (6). By setting the wavelength at 345 nm, we had the following absorbance values: 2.5630 (1); 1.2792 (2); 0.6464 (3); 0.3323 (4); 0.1790 (5) and 0.0984 (6). The construction of the concentration by absorbance graph showed correlations of 0.9999 and 0.9999, respectively. The precision and accuracy of the assay, as well as the linearity of the calibration curve were determined for intra- and inter-day on three different days. The precision was expressed as the percent coefficient of variation of each curve. The statistical data were calculated by ANOVA.

Conclusions

The doxycycline hyclate showed a linear relation between absorbance and concentration in both wavelengths. Doxycycline was shown to be stable during all the procedure. Thus, the results parameters demonstrated that the spectrophotometric method could be applied for the analysis of the pharmaceutical formulations assuring the quality and efficacy of the doxycycline hyclate under investigation. It was also found that the excipients in the commercial product did not interfere with the method.

References

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PRELIMINARY STRESS TESTING AND SPECTROSCOPIC IDENTIFICATION OF THE DEGRADATION PRODUCTS OF BENZNIDAZOLE

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Introduction
Chagas’ disease, a protozoan infection caused by the kitenoplastid *Tripanosoma cruzi*, constitutes a major public health problem for developing nations. Benznidazole (N-benzyl-2-nitroimidazolylacetamide, BEN) is a chemotherapeutic agent currently used for the treatment of *Tripanosoma cruzi* infections in their chronic and acute phases. The drug belongs to the nitroimidazole class of compounds, which have attracted much attention in chemotherapy (1).

Environmental conditions, including oxygen, light, heat, humidity, and the susceptibility of the substance towards hydrolysis, photolysis or oxidation can play an important role in the production of impurities. Stress testing provides evidence on how the quality of an Active Pharmaceutical Ingredient (API) may be affected under the influence of different stress conditions; therefore, it can help identifying degradation products and provide important information about the intrinsic stability of drug substances (2).

The test guideline Q1A issued by the International Conference on Harmonization (ICH) (3) requires stress testing studies to be carried out on drugs to establish their inherent stability characteristics, aid identification of degradation products and hence support the suitability of the proposed analytical procedures. The main aim of the present study was to establish the inherent stability of BEN through stress studies under the variety of ICH recommended test conditions (3). We also disclose the chemical synthesis of one of the observed degradation products.

Materials and methods
The experiments were performed with pharmaceutical-grade BEN, HPLC-grade solvents and analytical-grade reagents. Stress decomposition studies were performed in methanol-water media at an initial drug concentration of 5 mg ml\(^{-1}\). The stress conditions used are shown in Table 1. Photolytic stress studies were performed in a home-made photostability chamber. The impurities and their precursors were spectroscopically characterized. IR spectra were obtained with the sample as a KBr pellet and as film in NaCl according to impurity, with the aid of a Shimadzu Prestige 21 FT-IR spectrophotometer; \(^1\)H and \(^13\)C NMR spectra were acquired in a Bruker Avance 300 spectrometer, employing acetone-d\(_6\). Thin layer chromatographic analyses were carried out to monitor the degradation of the API. The plates were developed with CHCl\(_3\):MeOH (85/15, v/v) for all experiments and examined under short-wavelength UV light (254 nm) or by exposure to iodine vapours.

HPLC analyses were carried out at 30 ºC, employing a C\(_{18}\) column (Luna, Phenomenex, 250 × 4.6 mm, 5 µm particle size) and a 40:60 (v/v) mixture of MeOH:H\(_2\)O as mobile phase, pumped at a flow rate of 1.0 ml min\(^{-1}\) (4). Semi-preparative chromatographies were developed with the same phase mobile using a C\(_{18}\) column (Axia, Phenomenex, 50 × 21.2 mm, 5 µm particle size. Liquid samples were filtered through 0.45 µm nylon filters before use. The detection was accomplished at 224 nm. Solutions for analyses containing mixtures of the analytes were prepared immediately before use in volumetric flasks, by appropriate dilutions of the degraded samples with the mobile phase.

Results
Solution and solid-state stress testing of BEN

**Hydrolytic conditions:** Under neutral and acid conditions, the samples demonstrated to be stable. The drug was found to be labile to alkaline hydrolysis, where BEN furnished in two relevant degradation impurities, which increased with time. These impurities, identified as 1-phenylmethanamine and (5-nitro-1H-imidazol-1-yl) acetic acid, resulted from hydrolytic cleavage of the amide link.
Photolytic conditions: No major degradation products were observed after exposure of the drug solution to visible (fluorescent lamps) or long-wavelength UV-light (black-light) for at least 15 days. Minor degradation recorded was considered as irrelevant.

Oxidative conditions: The drug was stable when exposed to 0.7% methanolic H$_2$O$_2$ (2:1, v/v) for 8 hours.

Solid-state study: BEN proved to be stable when exposed to heat (70°C and 65% r.h.) for 60 days.

Isolation, synthesis and characterization of the impurities resulting from the alkaline hydrolysis of BEN

The impurities were isolated using a semi-preparative HPLC method. 1-Phenylmethanamine (benzylamine) was identified against a commercially available sample. On the other hand, (5-nitro-1H-imidazol-1-yl) acetic acid was also synthesized in order to obtain sufficient quantity and for final structural confirmation.

The acetic acid derivative was prepared in four steps from the inexpensive aminoacetal and O-methylisourea sulphate, which were first condensed to yield 2-amino-imidazole (5); this was subsequently oxidized to the related 2-nitroimidazole (6) and the latter was alkylated with ethyl bromoacetate in MeCN to furnish ethyl(5-nitro-1H-imidazol-1-yl)acetate (7) as a clear, yellowish oil. Basic hydrolysis of the ester afforded the corresponding acid. Structural elucidation of the impurities and reaction intermediates were based on their IR and NMR spectral data.

Conclusions

The results of stress testing of the API, undertaken according to the ICH guidelines, revealed that BEN is unstable under basic and photolytic conditions. Only in alkaline medium relevant degradation products were formed. The degradation impurities were identified, spectroscopically characterized and one of them was chemically synthesized.

Acknowledgments

The authors thank UNR, CONICET and ANPCyT for financial support. Thanks are due to Roche Argentina for the kind provision of a sample of BEN.

References


Table 1. Stress testing conditions and results for the degradation of BEN.

<table>
<thead>
<tr>
<th>Stress condition</th>
<th>Solvent (Sample conc.= 5 mg ml$^{-1}$)</th>
<th>Time (Days)</th>
<th>Temperature (°C)</th>
<th>Results</th>
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<td><strong>Hydrolytic</strong></td>
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<tr>
<td>Neutral</td>
<td>H$_2$O:MeOH (2:1, v/v)</td>
<td>0.33</td>
<td>reflux</td>
<td>Stable</td>
</tr>
<tr>
<td>Acid</td>
<td>1 N HCl:MeOH (2:1, v/v)</td>
<td>0.33</td>
<td>reflux</td>
<td>Stable</td>
</tr>
<tr>
<td>Basic</td>
<td>1 N NaOH:MeOH (2:1, v/v)</td>
<td>0.33</td>
<td>reflux</td>
<td>2 Products</td>
</tr>
<tr>
<td><strong>Oxidizing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>0.7% H$_2$O$_2$:MeOH (2:1, v/v)</td>
<td>0.33</td>
<td>reflux</td>
<td>Stable</td>
</tr>
<tr>
<td><strong>Photolytic</strong></td>
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<tr>
<td>Long-λ UV</td>
<td>H$_2$O:MeOH (1.5:1, v/v)</td>
<td>17</td>
<td>40</td>
<td>I.D.$^*$</td>
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<tr>
<td>Long-λ UV-visible</td>
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<td>36</td>
<td>50</td>
<td>I.D.$^*$</td>
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<tr>
<td>Visible</td>
<td>H$_2$O:MeOH (1.6:1, v/v)</td>
<td>21</td>
<td>50</td>
<td>I.D.$^*$</td>
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<td><strong>Solid state</strong></td>
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<tr>
<td>Heat, at 65% r.h.$^b$</td>
<td>-</td>
<td>60</td>
<td>70</td>
<td>Stable</td>
</tr>
</tbody>
</table>

$^*$I.D.: Irrelevant degradation, $^b$r.h.: relative humidity.
METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ANALYSIS OF MELOXICAM AND PRIDINOL MESYLATE IN TABLETS BY CAPILLARY ZONE ELECTROPHORESIS. INITIAL STUDIES

Vignaduzzo SE, Vera-Candioti L, Goicoechea HC, Castellano PM, Kaufman TS

Introduction
Tablets containing the pharmaceutical association between meloxicam and pridinol are employed as anti-inflammatory, analgesic and muscle relaxant agents. Meloxicam (MEL, 4-hydroxy-2-methyl-(5-methyl-2-thiazoly)-2H-1,2-benzo-thiazine-3-carboxamide-1,1-dioxide) is a non-steroidal anti-inflammatory drug (NSAID) (1), while Pridinol mesylate (PRI, 1,1-diphenyl-3-piperidinopropan-1-ol methanesulfonate) belongs to the group of central anticholinergics (2). Both drugs have very different acid-base characteristics.

In recent years capillary electrophoresis (CE) has expanded its scope and range in aspects of instrumentation and applications (3). The pharmaceutical use of capillary zone electrophoresis (CZE) is favored by the wide range of possible benefits that may be obtained, when compared to the well established and widely used high performance liquid chromatography (HPLC). Main advantages of CZE, which are likely to be obtained, include method simplicity, environmental friendliness and cost efficiency, due to the low amounts of solvents involved in the separations.

The aim of this work was to perform preliminary studies on the development and validation of a CZE method for the simultaneous determination of the MEL and PRI in tablets, using experimental design strategies.

Materials and methods
All experiments were performed with pharmaceutical-grade MEL and PRI, and analytical grade-reagents. Milli-Q quality water was used in all the CE experiments. Sodium borate was obtained from Merck. The pH of the solutions was measured employing a Hanna pH meter. These solutions, which were preserved at 4ºC in the darkness during the experiments, were filtered through a 0.45 µm nylon membrane (Sartorious-Germany) and degassed before use.

The CZE analyses were performed on an Agilent 3D CE apparatus consisting of an automatic injector, an autosampler, a variable wavelength diode array detector and a temperature controlling system (15-60°C). The CE Chemstation software was used for instrumental control, data acquisition and data analysis. Data handling was carried out on a Pentium 4 PC.

The experimental designs, data analysis and desirability function calculations were performed with Design Expert 7.0.

The electrophoretic separations were carried out using fused silica capillaries having 75 µm i.d. and 50 cm total length (41.5 cm effective length), in positive mode using constant voltage (20 kV). At the beginning of each working day, the capillary was rinsed with 0.1 N NaOH for 10 min. During the analyses and after each determination the capillary was sequentially washed with 0.1 N NaOH, water and running buffer. Injections were performed hydrodynamically at the anodic side employing pressure (50 mbar) for 10 s. The capillary temperature was thermostatted at 20°C. The analytes were detected at 200 nm.

Results

CZE method development
Considering the resolution of analytes and the analysis time, the best conditions were obtained using a chemometric experimental design. This procedure offers an efficient route for determining the best resolution from a selected number of conditions (4). For the determination of the most relevant variables a
screening experimental design using a factorial design with four factors was conducted: pH (7.0 and 9.0), temperature (20 and 25°C), concentration of the electrolyte (15 and 25 mmol L\(^{-1}\)) and running voltage (15 and 25 kV). These were evaluated by analysis of a sample of a mixed standard solution, analyzing in each case the peak resolutions between PRI and the electro-osmotic flow (EOF) and EOF and MEL. An ANOVA test was applied to the experimental data, concluding that concentration and pH of the running buffer as well as running voltage were significant (\(p<0.05\)) and should be considered for further method optimization. On the contrary, because the effect of capillary temperature proved not to be significant in the experimental domain, this was established at 20°C.

For method optimization, a central composite design was carried out for the statistically significant variables, in the following experimental domain: electrolyte concentration (range= 14–19 mmol L\(^{-1}\)), the pH of the electrolyte (range= 6.5–9.0) and the voltage (range= 18.6–25.4 kV). Three responses, peak resolutions (PRI-EOF and EOF-MEL) and overall analysis time were simultaneously optimized by using Derringer’s desirability function (5). The optimum conditions for the determination were found to be a 14 mmol L\(^{-1}\) sodium borate buffer solution of pH= 7.0 at a running voltage of 23 kV.

**Method validation**

Work towards validation of the optimized CZE method was carried out according to the ICH guidelines (6). The calibration curves (six points, in triplicate) were linear in the ranges from 5 to 65 ppm for PRI (\(r=0.996\)) and from 20 to 206 ppm for MEL (\(r=0.997\)). LOQ data obtained from the calibration curves demonstrated that these were below the working ranges of the analytes. Three standards at three different concentration levels were analyzed three times each within the same day in order to obtain repeatability information.

In order to evaluate the robustness of the developed method, a Plackett-Burman experimental design was built setting small changes in the studied parameters and evaluating the effect that these changes produced on the recovery of each of the analytes contained in the injected sample of mixed standard solution. An ANOVA test was applied to the experimental data, finding that the three variables, concentration and pH of the buffer and running voltage were found to be not significant at a \(p<0.05\) level, confirming that the developed method is robust.

**Conclusions**

A CZE method was developed and submitted to validation. Good results with respect to linearity, precision and robustness were obtained in the concentration ranges studied for MEL and PRI. Upon completion of the validation studies, the method will be applied to the simultaneous determination of MEL and PRI in their combined dosage form.

**Acknowledgments**

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**References**

ANALGESIC PROPERTIES OF NEW COPPER(II) COORDINATION COMPLEXES WITH FENOPROFEN

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Introduction
The analgesic effect of copper(II) coordination complexes with non-steroidal anti-inflammatory drugs (NSAIDs) is an issue of debate. Some authors sustain that the analgesic action becomes enhanced when the NSAID forms a coordination compound with copper(II) (1), meanwhile others have found that the complexation does not produce changes on the analgesic effect of the parent drug (2).

In this work, we investigate the analgesic effect of two new copper(II) coordination complexes with the non-steroidal anti-inflammatory drug Fenoprofen, Cu$_2$(Fen)$_4$(dmf)$_2$ (Fen: fenoprofenate anion; dmf: dimethylformamide) and Cu$_2$(Fen)$_4$(caf)$_2$ (caf: caffeine) using acetic acid-induced writhes and formalin test. These two different analgesic testing models were employed with the aim of identifying peripheral and central effects of the test substances. Acetic acid induces a painful reaction and acute inflammation in the peritoneal area and induces abdominal writhing which is a visceral pain model (3,4). The formalin test is considered a model for pain (5) and is an effective way to measure the peripheral and central pain.

Materials and methods
The synthesis of Cu$_2$(Fen)$_4$(dmf)$_2$ has been previously described (6). From this one dissolved in acetone, Cu$_2$(Fen)$_4$(caf)$_2$ was prepared by the addition of an ethanolic solution of caffeine. After the reaction, crystals were obtained by the diffusion of acetonitrile.

For the writhing test, four groups of mice were orally treated with Cu$_2$(Fen)$_4$(dmf)$_2$ (26 mg/kg), Cu$_2$(Fen)$_4$(caf)$_2$ (31 mg/kg), Fenoprofen calcium salt as uncomplexed parent drug (21 mg/kg), and the control one which received only vehicle (1% CMC, 0.1% Tween80). Then, 60 minutes later nociception was induced by an intraperitoneal injection of 0.5% acetic acid solution (10 mL/kg). After injection, each animal was isolated in an individual box to be observed during 20 minutes. The number of writhing and stretching was recorded.

For the formalin test, 20 µl of 2.5% formalin was injected into the dorsal surface of the left paw of mice, one hour after oral administration of complexes, Fenoprofen calcium salt or vehicle. The time that animals spent on licking the injected paw was recorded. Two distinct periods of intensive licking activity were identified and scored separately. The initial nociceptive scores normally peaked 5 minutes after formalin injection (early phase) and 15-30 minutes after injection (late phase), representing both the neurogenic and inflammatory pain responses, respectively.

Results
Acetic acid-induced writhing
Treatment with Cu$_2$(Fen)$_4$(dmf)$_2$, Cu$_2$(Fen)$_4$(caf)$_2$ and Fenoprofen salt groups significantly decreased the acetic acid-induced writhing response compared with the control: 89.7% (p<0.001), 89.8% (p<0.001) and 45.2% (p<0.05), respectively. When Fenoprofen calcium salt was compared to the complexes, both Cu$_2$(Fen)$_4$(dmf)$_2$ and Cu$_2$(Fen)$_4$(caf)$_2$ decreased more effectively the visceral pain (p<0.001).

Formalin test
The time spent on licking the injured paw was significantly attenuated in the early phase by complexes and Fenoprofen salt, but Cu$_2$(Fen)$_4$(dmf)$_2$ and Cu$_2$(Fen)$_4$(caf)$_2$ showed marked inhibition (p<0.001) of licking responses in the late phase when compared to the control and to the uncomplexed parent drug.

Conclusion
The results found in this work indicate that, although Cu$_2$(Fen)$_4$(dmf)$_2$ and Cu$_2$(Fen)$_4$(caf)$_2$ produce similar effect than Fenoprofen calcium salt in central pain, both complexes present a more potent analgesic effect for peripheral pain.

References


ANTI-INFLAMMATORY PROPERTIES OF TWO NEW COPPER(II) COMPLEXES WITH FENOPROFEN

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Introduction
The proposed curative properties of copper based non-steroidal anti-inflammatory drugs (NSAIDs) have led to the development of copper(II) complexes of NSAIDs with enhanced anti-inflammatory activity and reduced gastrointestinal toxicity compared with their uncomplexed parent drugs. No copper(II) anti-inflammatory drug is currently available for oral human use, although a gel base of copper-salicylate (Alcusal®) is available for topical temporal relief of pain and inflammation in humans in Australia(1).

Copper is an essential trace element, taking part in all aspects of metabolism(2). It is believed to possess anti-inflammatory activity and has been proposed an increased demand for copper during inflammatory conditions(3).

Fenoprofen, 2-(3-phenoxypyphenyl)propionic acid, is an antipyretic, analgesic and NSAID(4). Little is known about chemical structures of Fenoprofen complexes and copper drugs have yet to reach an extended human market, so in this work we present the enhanced anti-inflammatory activity of two new copper(II) complexes with Fenoprofen.

Materials and Methods
Copper complexes, of formula Cu₂(Fen)₄(caf)₂ and Cu(Fen)₂(im)₂ (Fen: Fenoprofenate; caf: caffeine; im: imidazole), were synthesized from Cu₂(Fen)₄(dmf)₂(5) dissolved in acetone and by the addition of a solution of caffeine in ethanol and imidazole, in acetone respectively. The diffusion of acetonitrile led to the formation of crystals which were studied by physicochemical techniques to confirm their molecular structures.

The studies of the anti-inflammatory properties were carried out employing the carrageenan induced paw edema in female mice described by Winter et al(6).

Test animals were administered orally an aqueous suspension of Cu₂(Fen)₄(caf)₂ (31 mg/kg), Cu(Fen)₂(im)₂ (28 mg/kg) and the calcium salt of Fenoprofen (21 mg/kg). The vehicle alone (carboxymethylcellulose and Tween80) was used as excipient for the control group. Drug and excipient were orally administered to each animal one-hour before inducing oedema in the left hind paw by subplantar injection of carrageenan.

The length of the paw was measured with a digital electronic caliber immediately before the injection of carrageenan and 3, 5, 7 and 9 hours after. The anti-inflammatory effect was expressed in terms of the percent inhibition of oedema produced by each drug-treated group.

Results
The study of acute anti-inflammatory test showed that the percentages of inhibition of inflammation for Cu₂(Fen)₄(caf)₂ were 84.3, 81.0, 81.5 and 73.4% at the third, fifth, seventh and ninth hour from the beginning of the experiment, meanwhile for Cu(Fen)₂(im)₂, the percentages were 30.0, 33.3, 58.5, 40.5% respectively. When Fenoprofen calcium salt was studied it presented 29.1, 42.3, 19.9, 10.5% of inhibition at the same hours.

Discussion
Cu₂(Fen)₄(caf)₂ presented the highest inflammation inhibition percent, sustaining it during all the time of the experiment. Cu(Fen)₂(im)₂ presented highest action than Fenoprofen salt from the fifth hour. Both complexes maintained enhanced action until the end of the experiment when compared to Fenoprofen calcium salt, demonstrating a more sustain activity in time.

Conclusion
Both copper(II) complexes with Fenoprofen presented enhanced anti-inflammatory action than the uncomplexed parent drug, being this characteristic improved for the complex containing caffeine in its structure. Complementary studies, such as analgesic and toxicological effects are being carried out to improve the knowledge of therapeutic properties of this kind of potential new anti-inflammatory drugs.

References


Schinus molle var. areira l.: CONSEQUENCES OF SUBACUTE EXPOSURE TO ETHANOLIC EXTRACT FROM ITS FRUITS IN MICE

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Introduction
It is known that Schinus molle var. areira (Anacardiaceae), whose vulgar names are “aguariibay” or “molle”, is a shrubby tree native of South America which exerts several biological effects such as: antibacterial, antiviral, antiseptic, diuretic, hypotensive, antitumoral, analgesic and anti-inflammatory (1). In our laboratory, abundant studies also indicated that this plant has repellent and insecticidal properties (2-3). This evidence suggests that this species could be very useful for the treatment of several pathologies and for some pest control, so we considered that it is necessary to investigate its safety. For this reason, the aim of the present work was to study the subacute oral exposure to ethanolic extract from fruits of Schinus molle var. areira in mice, by assessing its effects on the nervous system functionality and on hematological and biochemical parameters.

Materials and Methods
The experiment was conducted according to the protocols described by OECD (4). The plant extract was incorporated into the diet and fed daily to a group of 8 CF1 female mice, 8 weeks old, over a period of 28 days at a dose of 1000 mg/kg body weight/day. Control group was fed only with the standard diet. All the animals were observed for signs of toxicity. At the end of the exposure, behavioral and functional parameters of the mice were evaluated through a functional observational battery (FOB), which included a thorough description of the animals’ appearance, behavior, and functional integrity. Motor activity was assessed in an open field. Subsequently, blood samples were obtained for hematological analysis and for the determination of biochemical parameters like glucose, cholesterol, creatinine, urea and hepatic enzymes.

Results
Evaluations of the FOB showed that female mice exposed to extract had an incremented activity in their home cages and in the experimental arena compared to control group (p<0.01 and p<0.05 respectively). In the hematological analysis, mice exposed to the extract showed a significant decrease in the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin (MCH) (p<0.05 in both cases) compared to control mice, but all the values were within the reference range for mice. Finally, in the biochemical evaluations, the exposed group showed a significant increase in the urea and creatinine levels compared to control group (p<0.05 in both comparisons).

Conclusions
The subacute exposure of the ethanolic extract from fruits of Schinus molle var. areira increased the spontaneous activity of the exposed female mice in the evaluations of the FOB. This stimulant effect has already been observed after the acute and subchronic exposure of the extract in rats and mice (5). The incremented levels of urea and creatinine in plasma observed in the exposed group could be a sign of impairment in renal function. However, this alteration could be transitory because, after the subchronic exposure of the extract, it has been seen that the levels of both parameters were normal.

These results show that the ethanolic extract from fruits of Schinus molle var. areira did not produce serious effects on the nervous system functionality and on hematological and biochemical parameters after the oral subacute exposure in female mice.

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EVALUATION OF SUBACUTE TOXICITY OF AQUEOUS EXTRACT FROM CAPITULA OF Solidago chilensis IN MICE

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Introduction
Solidago species have been used in folk medicine for the treatment of several diseases. In South America, the most abundant species is Solidago chilensis Meyen (Asteraceae), widely used in traditional medicine of several countries, where is usually employed as a diuretic, analgesic, anti-inflammatory, and to treat gastrointestinal disorders (1). We have recently reported that aqueous extracts from inflorescences of S. chilensis protected gastric mucosa in mice subjected to an ethanol-induced gastric ulcer model (2). However, there are no toxicological studies available which would be necessary in order to confirm the safe usage of the plant. For this reason, the aim of the present work was to study the subacute oral toxicity of the aqueous extract from inflorescences of S. chilensis after a 28-day repeated exposure in mice by means of a Functional Observational Battery (FOB) and by assessing the motor activity in an open field.

Materials and Methods
The experiment was conducted according to the protocols described by OECD (3). A group of healthy CF1 mice (8 males and 8 females) were daily exposed to 1000 mg/kg of body weight of the extract mixed with the standard food for 28 days. A control group was fed only with the standard diet. All the animals were weekly observed for signs of toxicity. At the end of the exposure, behavioral and functional parameters were evaluated through a FOB, which included a thorough description of the animals’ appearance, behavior and functional integrity. This was assessed through observations in the home cage, while animals were moving freely in an open field, and through manipulative tests. The motor activity was assessed in an open field whose floor was divided into squares. The number of squares entered by mice with all four paws, rearings, groomings and fecal boluses were scored for 15 min.

Results
The subacute exposure to S. chilensis did not produce alterations in all parameters evaluated in the FOB or during the manipulative tests. No significant differences were observed between control and experimental groups in the different parameters analyzed during home cage, hand-held and open field observations (P>0.05). Motor activity evaluations in the square open field indicated that the subacute exposure did not modify neither the number of squares crossed nor rearings after the exposure. No significant differences were observed between control and experimental groups in emotionality parameters as the number of groomings and fecal boluses (P>0.05).

Conclusions
This study demonstrates that the aqueous extract from Solidago chilensis does not produce neurotoxicity due to it does not affect the functionality of the nervous system at neuromuscular, sensory and autonomic level. Considering the results it was demonstrated that the oral administration of the aqueous extract of the plant does not produce toxicity by subacute exposure in mice. This effect has already been observed in previous studies after the acute exposure in mice (4). Despite the lack of toxicity indicates that the possible therapeutic use of the plant may be safe, future research like potential chronic toxicity associated with this extract will need to be evaluated through long-term bioassays in order to ensure its safety.

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GLUTAMATE AND PSA-NCAM DEPENDENT HIPPOCAMPAL SYNAPTIC REMODELLING: CORRELATION WITH AN EXPERIMENTAL MODEL OF DEPRESSION AND ITS PHARMACOLOGICAL TREATMENT

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Introduction
Dysfunction of hippocampal plasticity and excessive glutamate (GLU) release has been proposed to play a critical role in the pathophysiology of depression. In experimental models of depression such as the learned helplessness paradigm (LH), hippocampal dendritic atrophy has been reported. We have previously shown in hippocampal CA3 region of LH animals decreased synaptic proteins PSD-95 and synaptophysin (SYN) and that chronic treatment with fluoxetine (FLX) reverses the behavioral deficit and recovers PSD-95 and SYN levels, pointing out the synapse as a target for antidepressant action. Moreover, it is known that adhesion molecule NCAM (Neural Cell Adhesion Molecule), one of the most abundant in glutamatergic synapses, contributes to hippocampal plasticity.

Materials and methods
We examined synapse morphology and cell adhesion molecule (CAM) expression in animals subjected to the LH paradigm: control animals that received no shock (C), shocked non depressed animals (SND) and shocked animals that showed the behavioural deficit (LH). We studied the effect of 21 day treatment with FLX employing four groups: control animals treated with saline (C-S) or fluoxetine (C-FLX) and LH animals that received saline (LH-S) or fluoxetine (LH-FLX). While CA3 synaptic morphology was analyzed by electronic microscopy, adhesion molecule expression was determined by Western blot and immunohistochemistry. Glutamatergic hyperstimulation effects on adhesion molecules and cytoskeletal, pre- and post-synaptic proteins were analyzed in primary neuronal cultures by immunohistochemistry.

Results
Concerning electronic microscopy, CA3 synapses of LH animals showed increased synaptic cleft width. While in control rats synaptic vesicles per synapse (SV/S) ratio was homogeneous, in LH group SV/S ratio presented extreme low or high values. Postsynaptic density (PSD) morphology was altered in LH rats: while PSD length decreased, PSD width increased rendering similar values in total area. These results are compatible with plastic and synaptic connectivity alterations. LH rats showed decreased immunostaining for CA3 NCAM and PSA-NCAM (NCAM polysialylated non-adhesive form). Glutamate hyperstimulation of hippocampal neurons in culture decreased immunostainings of the dendritic marker MAP2. NCAM and PSA-NCAM. It also diminished PSD-95(+) and SYN(+) synapse number and increased SYN(+) individual synapse area. Our results indicate that excessive neuronal exposure to GLU induces synaptic changes in vitro that resemble those observed in LH animals. FLX treatment of LH animals returned synaptic cleft width to control values and increased reserved synaptic vesicle number. Regarding cell adhesion molecules, FLX strongly reduced CA3 NCAM expression and most importantly, increased CA3 PSA-NCAM levels in LH rats.

Conclusions
Results support the hypothesis that GLU hyperactivity in the CA3 of LH rats could reduce CAM expression leading to alterations in synaptic connectivity. Since PSA-NCAM is considered a neuronal plasticity marker it can be suggested that fluoxetine action in LH animals may involve PSA-NCAM dependent synaptic remodelling that might lead to neuronal connectivity normalization.
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Efficacy of Ivermectin against *Triatoma infestans*

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**Introduction**

*Triatoma infestans*, the main domestic vector of Chagas disease in Latin America, is one of the most widely distributed triatomines in Argentina. Extensive populations of *Triatoma infestans* can be found in domestic and peridomestic habitats. The peridomestic habitats such as corrals and chicken coops can provide the source for reinfesting domestic habitats after the initial control interventions (pyrethroid spraying). Domestic chickens, dogs, cats and goats play an important role in the epidemiology of Chagas disease as frequent sources of blood meal for triatome bugs. Different techniques for eliminating peridomestic population of *T. infestans* have been developed, an alternative is the application of antiparasitic or insecticides agents to the domestic animals (1-2). In the context of these new trends this study tested the efficacy of Ivermectin 0.5% injected subcutaneously to hens at a dose rate of 200 µg/kg against fifth instars’ nymphs of *Triatoma infestans*.

**Materials and methods**

Insects used in this study were fifth instars’ nymphs of *T. infestans*. Nine hens were used during the experiment. Six hens were treated with Ivermectin 0.5% (subcutaneous route), and the other three animals were used as control group, the control group was manipulated similarly but did not receive Ivermectin. The effects of Ivermectin were measured in two different aspects: mortality and blood intake. Mortality was measured using six groups of 10 fifth instars’ nymphs each, fed over the individually identified hens. The groups were fed at two and seven days after the treatment with Ivermectin. Blood intake was measured as the difference between the weights of the groups, 10 fifth instars’ nymphs each, before and after each feeding.

**Results**

Ivermectin produced differential mortality rates in *T. infestans* fed on treated hens. Nymphs fed 2 days after treatment not show differences with the control group. Nymphs fed 7 days after treatment showed 10% mortality.

Groups fed 2 and 7 days after the treatment showed no difference in blood intake. On average each nymph consumed 210 ± 67 mg of blood.

**Conclusions**

This study shows that Ivermectin had only slight lethal effects after a single feeding on treated chickens. Further studies are needed to evaluate if changing certain parameters, such as route of administration and dose could increase the mortality rate of Ivermectin in *T. infestans*.

**Acknowledgments**

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FACILITATION OF THE LABILIZATION/RECONsolidATION PROCESS OF A RESISTANT FEAR MEMORY

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Introduction
The memory consolidation theory propose that recent learning can be transiently modified, however once this process is complete, this trace is consolidated and insensitive to further modifications, including pharmacological intervention (1). In the last years, numerous studies have proposed that recalling a previously consolidated memory can render this trace vulnerable to interference (2-5), for instance to benzodiazepine ligands such as Midazolam (MDZ) (6), this process is followed by a stable phase termed memory reconsolidation (4,7). There are, however boundary conditions that place constraints on the onset of the labile phase after retrieval (8). For instance, memory age, the duration of the reactivation period and the interaction between these factors have a crucial influence on retrieval-induced lability (9-11). In addition, activation of NMDA sites seems to be a prerequisite for the emergence of memory reconsolidation (12). It is known that exposure to a stressful event prior to fear learning induces resistance to the emergence of the unstable phase after recall (13).

The main goal of this study was to evaluate the vulnerability to MDZ after the retrieval of consolidated fear memory in animals that have experienced a single stressful situation and the influence of D-cycloserine (DCS), a partial NMDA agonist, on the disruptive effect of MDZ on memory reconsolidation.

Materials and Methods
Male Wistar rats (280-330g) were used in all the experiments. Animals were subjected to a contextual fear conditioning paradigm (three 0.3 mA footshocks with a 30 s interval among shocks) (7, 10, 13). Stressed animals were subjected to a 30 min restraint period one day prior to the fear conditioning procedure. Memory reactivation was conducted one day after learning by re-exposing the animal in the conditioning environment. Behavioral freezing was scored as an index of fear during the test session one day after memory reactivation. Half of the animals were injected with DCS (15 mg/kg, i.p.) or saline 20 min prior reactivation (13). Rats were systemically administered with MDZ (1 mg/Kg) or vehicle immediately after reactivation.

Results
The results showed that stressed animals were insensitive to the disruptive effect of MDZ. However, the vulnerability to MDZ was evident in stressed rats that were previously administered with DCS.

Conclusion
These results show that NMDA receptor activation promotes the onset of the labile phase following reactivation even in resistant memory traces, as those found in rats that have experienced a stressful event prior to fear acquisition. This study highlights the relevance of the combined treatment with DCS and MDZ for attenuating the undesired emotional disturbance associated with the emergence of traumatic memories.

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ROSUVASTATIN-CYTOPROTECTIVE PLEITROPIC EFFECTS DURING EXPERIMENTAL NEONATAL OBSTRUCTIVE NEPHROPATHY

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Introduction
Congenital obstructive nephropathy is characterized by oxidative stress, decreased proliferation and increased apoptosis. Oxidative stress represents the common denominator of multiple cellular alterations and contributes to tubulointerstitial mechanism damage. Both pro-apoptotic and anti-apoptotic effects of nitric oxide (NO) have been demonstrated. NO has been implicated in apoptosis for unilateral ureteral obstruction (UUO), being a controversial key. Furthermore, induction of the stress response includes synthesis of heat shock proteins (HSPs) been well characterized in injured cells. Hsp70 confers cellular protection by modulating the engagement and/or progression of apoptosis. In agreement, we have demonstrated an association between NO bioavailability and Hsp70 expression in UUO (1).

Strategies performed on slowing the progression of chronic kidney disease (CKD) have included increasing evidence for the beneficial, lipid-independent effects of 3-Hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase inhibitors (statins). Statins exert beneficial effects upon CKD, including restoration/normalization of endothelial function upregulation of NO, oxidative stress reduction and vascular inflammation. Interest during obstructive nephropathy, rosuvastatin has renoprotective effects in terms of morphology and inflammation, independent of the changes in blood pressure and plasma lipid levels (2).

Objective
To determine whether NO associated with Hsp70 expression is involved in rosuvastatin resistance to obstruction-induced oxidative stress and cell death in neonatal obstructive nephropathy experimental model.

Materials and methods
Neonatal rats (n=5) with and without UUO (OC and CC) and Ros treated rats (10mg/Kg/d) for 14 days, were nephrectomized to evaluate in cortexes oxidative stress and heat shock response.

Results
After 14 days of obstruction, oxidative stress markers as lipid peroxidation (MDA) (90±5 vs 70±4 nmol/mL) and NADPH oxidase activity (21682±234 vs. 8200±123 RUF/µg prot/min) increased, whereas hsf1 and Hsp70 expression (0.35±0.04 vs. 0.87±0.05) and lower endogenous nitric oxide levels (67±2 vs. 74±2 nM) decreased. Conversely, Rosuvastatin administration increased hsf1 and Hsp70 expression linked to increase in NO levels with absence of apoptotic response and decreased oxidative stress.

Conclusions
Rosuvastatin exerts cytoprotective effects against oxidative stress through NO restoration and upregulation of Hsp70 in UUO. These effects could be involved on delaying the development and progression of renal injury from obstruction.

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AMPA RECEPTORS IN THE NUCLEUS ACCUMBENS CORE ARE INVOLVED IN LONG TERM SENSITIZATION TO COCAINE AFTER A SINGLE RESTRAINT STRESS

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Introduction
Several evidences indicate that cocaine is able to induce behavioural and molecular sensitization in both, the dopaminergic (DA) and glutamatergic (Glu) mesocorticolimbic systems. It has been shown that a single restraint stress induces sensitization to behavioural and neurochemical effects of psychostimulants (Pacchioni et al., 2007). The aim of this work was to study whether or not AMPA receptors (AMPARs) participate in the behavioural manifestation of long-term cross-sensitization between stress and cocaine (21 days after a single stress session) and to investigate the participation of nucleus accumbens core (NAc Core) in this cross-sensitization.

Material and Methods
Male Wistar rats (250-350 g) were restrained for two hours (acute-stress group) while control animals were left undisturbed in their cages. Twenty-one days after this single stress episode, all animals were assigned to one of the following two experiments: I) Locomotor activity in response to saline or cocaine (15 mg/kg i.p.); and locomotor activity followed by intra-NAc Core microinfusions of increasing doses of AMPA (1 nM, 10 nM and 100 nM). II) AMPA receptor expression in NAc Core after i.p. injection of saline or cocaine (15 mg/kg i.p.). The animals were decapitated 45 minutes after the injection and the NAc Core were dissected. Brain slices were incubated for one hour in ice-cold sulfo-NHS-BLC-biotin. Samples were then centrifuged to pellet the insoluble fraction. Biotinylated surface proteins present in the remaining supernatant were immunoprecipitated with avidin-Bagarose beads for 2 h at 4°C. Beads were pelleted, and in the supernatant the surface fraction was subjected to quantitative immunoblotting for AMPARs using anti-GluR1 as a primary antibody.

Results
Ours results demonstrate that the expression of sensitization can be induced with AMPA in NAcc Core twenty-one days after an acute stress episode, as compared with the remaining control groups. AMPA produced a dose-related increase in horizontal photocell counts (1). After the highest dose of AMPA (0.1 ug/ul) in NAc Core, the number of horizontal photocell counts was greater in the sensitized than in the control animals. These results are discussed in the framework of an increase in the surface cellular expression of GluR1 (the AMPA receptors subunit), in NAc Core, in stressed animals following a long period after the stress episode (21 days), independently of saline or cocaine injection. There are significant differences between stress and non-stress groups.

Conclusions
In conclusion, the present behavioural and neurochemical findings reveal that AMPA receptors are critical to underlie the expression of behavioural sensitization to cocaine following a single restraint stress. These results extend and confirm our hypothesis of a common molecular mechanism underlying the drug- and stress-induced sensitization to cocaine’s behavioural effects (Pierce et al., 1996; Boudreau et al. 2005, 2007; Kourrich et al., 2007).

Acknowledgements
This work was supported by grants from FONCyT, CONICET, SECyT and Agencia Córdoba Ciencia (Argentina). The authors are grateful to Estela Salde for her laboratory technical assistance.

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RESIDUE DEPLETION OF ENROFLOXACIN AND CIPROFLOXACIN IN BROILER CHICKEN FEATHERS AFTER ORAL ADMINISTRATION

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Introduction
Antibiotics in animal feed are a public health concern. Drug residues could harm animals and eventually be detected in animal food products intended for human consumption (1). Pharmacokinetic studies in edible poultry tissues are required to establish withdrawal time. Although feathers after been processed are introduced in the food chain as a protein source in animal feed, withdrawal periods are not established.
The aim of this work was to study the residue depletion of enrofloxacin (ENR) and its metabolite ciprofloxacin (CIP) in broiler chicken feathers.

Materials and methods
- Broiler chickens (3 week-old) were treated with 10 mg ENR / kg body weight per day for 5 consecutive days. ENR was administrated into drinking water.
- Feather samples were taken from 10 random birds per day at 0, 1, 2, 3, 4, 5, 7, and 9 days.
- Extraction was performed following a technique described by San Martin et al. (2) and modified by us. Finely cut feathers were placed in centrifuge tubes and 5 ml of Acetone was added. Samples were shaken and centrifuged at 5000 rpm for 10 minutes. Supernatants were transferred into drying tubes. The extraction was repeated two times. Supernatants were combined and evaporated to dryness under nitrogen. Residues were dissolved in 75µl methanol 0.1% tetrahydrofuran. 475µl aqueous 0.1% tetrahydrofuran were added. Samples were centrifuged and supernatant was filtered and injected into the chromatographic system.
- Chromatographic conditions: mobile phase (water: acetonitrile: triethanolamine, pH 3), flow rate 1,2 ml/min, column Phenomenex Luna C18, fluorescence excitation 278 nm and emission 446 nm.

Results
High levels of ENR and CIP were found in feathers after the administration of the last of five daily intramuscular doses (10 mg/kg) for ten animals and every day. These compounds were detected in feathers for 9 days.

Conclusions
Feather meal is a potential source of drug residues that can pass through the food chain when contaminated meal is fed to food-producing animals. In the present study, feathers had high ENR and CIP concentrations. This finding cannot be explained by blood contribution to this tissue because feather vasculature reaches only the lower portion of the calamus. One possible source of feather contamination is secretion from the uropygial gland, which may reach the feathers via grooming behavior (3).
Further studies to establish a withdrawal time may be useful to avoid that drug residues could result in adverse health consequences like increases in antibiotic resistance.

References
3. Montalti D, Gutiérrez AM, Reboredo GR, Salibián A ;The difference between sexes in the uropygial gland secretion of rock dove V. Jornada Multidisciplinaria de la Sociedad Argentina de Biologia, Bs As, Argentina; Biocell 2004 28, 115
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Candida albicans INDUCES THE ACTIVATION OF THE ARGINASE PATHWAY IN THE HUMAN MONOCYTE CELL LINE (U937)

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Introduction
The phagocytes including Monocytes and Macrophages, play an important role in the defence mechanism against the infection with Candida albicans. In the metabolism of the L-arginine, the balance between the activation of the inducible Nitric Oxide Sintetase(iNOS) pathway and the arginase pathway, promotes in these cells two alternative states that have been associated with different immune response in the host. The “in vitro” host-pathogen models are a powerful tool to explore particular interaction and their modulation by therapeutic agents. In the present work we evaluated the activation of L-arginine pathway in a human monocyte cell line(U937), in order to validate previous results obtained with purified human cells(PHC), and to explore the effect of antifungal treatment in the balance of this metabolic pathway. Two antifungal agents used frequently in the treatment of this micosis, Fluconazole and Amphotericine B, were included in this study.

Material and Methods
We used the patogenic Candida albicans strain 387 as previously described(1); Fluconazole and Amphotericine B as antifungal agents; different stimulants for the macrophagic cell as LPS from Escherichia coli serotype 055:B5 and PMA from Sigma. The monocyte cell line U937 was cultured at a density of 3x10^5 cell/mL and incubated with C.albicans, in the absense or presense of the different stimulants or the different antifungal agents single or in combination with C.albicans. The supernatants were removed and used to determine nitric oxide and the monolayer was used to determine arginase activity(2).

Results
The contact of C.albicans with U937 induces the activation of L-arginine pathway in a dose dependent manner(p<0.05). Although the NO production was absent, the balance between both pathways favored the production of arginase. The level of stimulation was higher and the value obtained after fungus stimulation at 5:1(E:T) was similar to the classic activator PMA(p<0.05). It has been described that antimicrobial drugs may have broad immunomodulatory properties besides their antifungal activity. In the present study, neither Amphotericine B nor Fluconazole induce the NO production, but when the activation of arginase pathway was evaluted only in presence of Fluconazole, the U937 cells produce arginase. When the cells were cocultured with C.albicans and the antifungal agents, the same profile was observed.

Conclusions
In several host-pathogen interactions, mean the classic Mo/MØ activation is associated with the pathogen control, the alternative Mo/MØ way is considered to favored growth and establishment of infection. The relevance of balance of Mo activation pathway against Candida albicans is not completely understood and in vitro approach could contribute to explore this field. Here in we provide evidence of the ability of fungus to trigger the L-arginine pathway and favored the alternative activation of U937. These cells present the same profile of response that PHC, indicating that U937 could be used as a valid model to evaluate the molecular mechanism triggered by the pathogen during the host interaction. Interestingly the arginase pathway could be modulated in different manners depending on antifungal agents.

References
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Comparision of Antioxidant Properties of Apple Vinegar Market in Southern Brazil


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Introduction
Apples constitute an important part of the human diet, as they are a source of sugars, acids, and various biologically active compounds, such as phenolic compounds, which are responsible for most of the antioxidant activities of the fruit (1). Along with sugars and organic acids, phenolics determine the quality of the apples (2). The concentration of these phenolic compounds, particularly anthocyanins, is strongly dependent on the apple cultivar and the maturity of the apples and is closely associated with their nutritional and sensory qualities, such as taste and color (1). In special apple cider vinegar is a popular folk remedy for rheumatoid arthritis was found to be ineffective in suppressing the adjuvant-induced arthritis in rats as indicated by hind-paw measurements and body weights (3). Therefore, this research is focused on the analysis and comparison of the antioxidant activity of five apple vinegar marked in southern Brazil.

Materials and methods
Five samples of apple vinegars were purchased in local market and coded as A, B, C, D and E. Qualitative and quantitative evaluation of the antioxidant activity towards DPPH was performed by spectrophotometric measurements of consumption of the radical in the presence of antioxidants (4). Total monomeric anthocyanin (MA) content of the apple vinegar was measured using a spectrophotometric pH differential protocol (5).

Results
The results show the presence of antioxidant activity and phenolic compounds in five samples of vinegar, but with differences in the concentrations of these compounds. Samples B and C showed the lowest levels of anthocyanins, although, the sample with higher content of anthocyanin was sample A. The results showed differences between the samples sold in the market regarding the content of monomeric anthocyanins. Although current legislation does not provide for apple vinegar fuck quantity of anthocyanins, in recent years, anthocyanins have been associated with reduced risk of coronary heart disease and to prevent several chronic diseases (6). Samples A and E showed a higher antioxidant capacity from DPPH analisys. These findings go against the values found in an award for anthocyanins.

Conclusions
DPPH and MA methods can be recommended to evaluate antioxidant activities of apple vinegar. This kind of vinegar shows higher antioxidant activity than others sour wine. The samples marketed in southern Brazil have significant differences in the composition of phenolic compounds and brand A showed the best results.

References


POSTNATAL NITRIC OXIDE SYNTHESIS INHIBITION ALTERS Na⁺, K⁺-ATPase MODULATION BY PEPTIDE NEUROTENSIN

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Introduction
Neurotensin (NT) is a tridecapeptide widely distributed in brain and peripheral tissues of mammalian species (1). This peptide is closely related to dopaminergic system as well as to other neurotransmitter systems which are involved in the physiopathology of schizophrenia. Behavioural and biochemical effects of centrally administered NT resemble those of systemically administered antipsychotic drugs (2). Nitric oxide (NO) acts as an intercellular messenger (3). NO may influence the maturation of neurons and synaptogenesis during neuronal development. Therefore, a disturbance in NO release could interfere with the maturation of brain neurons as well as with their functional connections (4). Such alteration at early stages of development could lead to a dysfunctional CNS, which in turn, may exhibit schizophrenia symptoms (5).

We have previously studied cortical synaptosomal membrane Na⁺, K⁺-ATPase activity in the presence of NT, to observe that the peptide inhibits this enzyme activity, an effect entirely prevented by high affinity NT receptor antagonist SR 48692 (6). In order to study potential relationship between NO and Na⁺, K⁺-ATPase regulation, herein we tested NT effect in membranes isolated from rats early administered with an inhibitor of neuronal nitric oxide synthase (nNOS).

Materials and methods
- Male and female Sprague-Dawley rats maintained in a 12-h light:dark cycle and with access to food and water ad libitum were employed. On postnatal days 3-5 rat pups were injected (s.c.) with vehicle (saline solution), 10 or 100 mg/kg No-nitro-L-arginine (L-NoArg,) (5).
- Procedure for the open field behavioral task: a wooden box with lines dividing the floor into 12 equal squares was used. Rats were gently placed on the posterior left corner of the open field box and allowed to explore for 5 min.
- Synaptosomal membranes were isolated by differential and sucrose gradient centrifugation as previously described in this laboratory (7)
- ATPase activity was measured as described Albers et al.(8).
- [³H]ouabain binding assays were carried as described by Antonelli et al. (9)

Results
- Rats were subjected to the open field behavioral task; the number of crosses was counted. A transiently lower locomotor activity and an impediment in learning were observed in rats early administered with nNOS inhibitor.
- The presence of 3.5 x 10⁻⁵, 3.5 x 10⁻⁶ M NT produced 6%-34% inhibition of Na⁺, K⁺-ATPase activity in membranes isolated from 35 days old male and female untreated rats. This peptide concentration range failed to change Na⁺, K⁺-ATPase and Mg²⁺-ATPase activities in membranes isolated from treated rats. Basal Na⁺, K⁺-ATPase and Mg²⁺-ATPase activities in cortical synaptosomal membranes from male and female rats early administered with saline (vehicle) or nNOS inhibitor indicated no changes in the mentioned enzyme activities. [³H]ouabain specific binding decreased 37% and 53% in young (35 day old) and adult (56 day old) male rat membranes, respectively in the presence of 1.0 x 10⁻⁶ M NT. At variance, this peptide concentration decreased 12% [³H]ouabain in membranes isolated from control rats of either age.

Conclusions

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Results presented suggest that inhibition of NO synthesis at early stage of brain development produces a permanent alteration of the neurotensinergic system, which is involved in Na⁺, K⁺-ATPase activity inhibition by neurotensin.

Acknowledgments
Financial support was provided by CONICET and Universidad de Buenos Aires, Argentina.

References
INTRODUCTION

The effects of cytokines on cognitive processes have been extensively studied. Particularly, IL-1β significantly influences consolidation and persistence of memories that depend on hippocampus (1). However, the impact of IL-1β on memory reconsolidation, an important memory process, has not been yet established.

A variety of effects of central IL-1β administration are blocked by the melanocortin α-MSH. Five subtypes of melanocortin receptors (MC1R-MC5R) have been identified, being the MC3R and MC4R predominant in the central nervous system (2). Therefore, in the present work we studied the effect of IL-1β on the reconsolidation of a contextual fear memory. We also examined the influence of α-MSH and the role of MC4 receptors on the IL-1β effects on memory reconsolidation.

MATERIALS AND METHODS

Adult male Wistar rats, maintained on controlled conditions, were implanted bilaterally in hippocampus under ketamine -xylazine anesthesia. Each experiment consisted of three phases: conditioning, reactivation session and testing sessions. Training consisted in placing the rat in a chamber with a grid floor attached to a scrambled shocker to provide footshock. Rats were allowed a 3 min acclimation period. After this period, rats received three unsignaled footshocks (0.3mA; 2.5 s). Animals remained in the chamber for additional 2 min and immediately after they were placed in their home cages. Reactivation session: 24hs after training, rats were reexposed to the training context without shocks during 2 min. Test session: Contextual fear conditioning was assessed 24 h after training, by placing the rats in the training environment for a period of 5 min. Memory was assessed and expressed as the percentage of time that rats spent freezing. Different treatments (vehicle, rrIL-1β (R&D Systems), α-MSH (Peninsula Laboratories ) and HS024 (selective MC4-R antagonist (NeoMPS) were administered after reactivation.

RESULTS

We demonstrated that the injection of IL-1β (5ng/0.25ul) in dorsal hippocampus after reexposition to the context decreases freezing during the contextual fear test. The treatment with α-MSH (0.05ug/0.25ul) blocked this effect. Administration of the MC4 receptor antagonist HS014 (0.5ug/0.25ul) reverses the effect of α-MSH. The injection of α-MSH administered alone did not produce any significant effect on the reconsolidation of the fear memory (Table 1).

CONCLUSIONS

We determined that IL-1β has a detrimental effect on the reconsolidation of contextual memory, and also that α-MSH, through the activation of MC4-R, could reverse the effect of IL-1β on the reconsolidation of fear memory.

Our results are in agreement with the fact that melanocortins have potent neuroprotective effects. Besides, it is important to determine the specific melanocortin receptors that are involved in the effects of α-MSH. The importance of this is exposed by the fact that the pharmaceutical industry is investing great efforts in the search for agonists and antagonist of these receptors.

ACKNOWLEDGMENTS

This work was supported by grants from FONCYT, CONICET and SeCYT.
Table 1: Effect of intrahippocampal injection of IL-1β on the reconsolidation of a contextual fear memory and it modulation by α-MSH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>65 ± 15.7</td>
</tr>
<tr>
<td>IL-1β</td>
<td>34.5 ± 6.9*</td>
</tr>
<tr>
<td>IL-1β + α-MSH</td>
<td>58 ± 16.3</td>
</tr>
<tr>
<td>IL-1β + HSO14 + α-MSH</td>
<td>38 ± 9.8*</td>
</tr>
<tr>
<td>α-MSH</td>
<td>55.5 ± 11.5</td>
</tr>
</tbody>
</table>

*p<0.05, versus vehicle

References.

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ANXIOLYTIC EFFECT OF BLOCKING ANGIOTENSIN II AT₁ RECEPTORS ON THE CENTRAL AMYGDALA IN A FEAR POTENTIATED ANIMAL MODEL

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Introduction
The central amygdala (CAN) is one of the most important brain nuclei for emotional processes regulation (1). Recent evidence suggest a key role for the angiotensinergic system in the regulation of stress responses. Activation of brain angiotensin II AT₁ receptors is required for stress-induced hormone secretion, and stimulation of the central sympathetic activity (2). The aim of this work was to assess the role of losartan (Ang II AT₁ receptors antagonist) on the anxiety state in a fear potentiated animal model.

Materials and methods
Male adult wistar rats weighing 250-300g kept in a 12 h light-dark period under controlled temperature conditions, with food and water “ad libitum”. The animals were stereotaxically implanted under anesthesia with bilateral stainless-steel cannuli in CAN.

Fear potentiated model: the animals received three electric foot shocks, 24 hs later were reexposed to the conditioning box without electric foot shock stimulation. The control animals did not received electric shocks (3).

Both groups (stressed and non-stressed animals) were injected with 0.5 ml Losartan (4 μg / ml), or 0.5 ml AngII (1ng / ml). Controls were injected with 0.5 ml of saline solution.

The animals were tested on the elevated plus-maze 15 min after intraamigdalar injection of drugs (3). Percentage of open arm entries, time spent in the open arms, number of extreme arrivals of the open arm, and grooming, were determined as indexes of anxiety. Total number of arm entries, and total traveled distance, were recorded as a locomotor activity index.

Results
The injection of AngII, decreased time spent on open arms in non stressed and stressed groups compared to their respective controls (66,14 ± 13.0 sec vs 29,14 ± 6.2 sec) and (38,75 ± 6.2 sec vs 14,00 ± 4.6 sec). The stress-induced anxiogenic effect was equivalent to that of Ang II non-stress group. Losartan completely reversed the stress-induced anxiogenic effect.

The microinjection of Ang II decreased the preference for open arms under stress and non-stress conditions (0.62 ± 0.11 vs 0, 31± 0.06) and (0.62 ± 0.16 vs 0.36 ± 0.17) respectively. Losartan completely reversed the anxiogenic effect of Ang II under stress conditions.

The total distance average was 6, 7 m and no differences were found between groups. The stress-induced grooming increase was equivalent to Ang II non-stress conditions. Losartan decreased grooming to control levels.

Discussion
Since treatment with Losartan completely reversed the stress-induced anxiogenic effect, we conclude that Ang II AT₁ receptors of CAN are mainly involved in the generation of the anxiety state induced by the fear potentiated.

References


INHIBITION OF THE MUTAGENIC EFFECTS OF SULFATIAZOLE-NITRITE MIXTURE BY L-ASCORBIC ACID AND GREEN TEA

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Introduction
The concept of chemoprevention (use of natural or synthetic compounds to prevent cancers) has great appeal. N-nitroso compounds are mutagens which can be formed in vivo due to the reaction between amides and/or amines with nitrite (1). L-ascorbic acid (asc.) and green tea polyphenols can react with nitrite diminishing or removing the nitrosation risk. Sulfonamides, widely used by their properties, present potential risk of nitrosation in stomach because the presence of these functions.

In the present work we assay the chemopreventive action of asc. and green tea as antimutagens (AM) on the mutagenicity of a reaction mixture (RM) formed by sodium sulfathiazole (NaST) and NaN$\text{O}_2$ in acidic medium, whose mutagenicity was previously proved by us (2).

Materials and methods
Mutagenicity activity was evaluated in a bacterial reverse mutation assay by the standard Ames test (3). Variable doses of asc. 0.0244 M were tested. Aqueous extracts of green tea were prepared with commercial bags of 2 g in 100 mL of sterile distilled hot water, and were diluted 1/5 previous to test.

Results
Results of reversion coefficient, R.C. (R.C. = revertants with tested substance/spontaneous revertants) and % inhibition of mutagenicity of the RM by asc. are shown in Table 1. The % of inhibition (%inh.) was calculated with the following equation: % inh. = [{(CR without AM - CR with AM)} / (CR without AM - 1)] · 100.

According to the Ames test a compound would be a mutagen if the R.C. is ≥ 2. The R.C. diminished with the tested doses of asc., increasing the % of inhibition of the mutagenicity. In previous work (580-5800 and 5.8-58 nmol L-ascorbic acid/plate) we obtained an inhibition of 34% of mutagenicity in the minor range and 100% in the major one. In the present work good curves dose-response were obtained for the inhibition of mutagenicity of RM in the tested range.

With green tea the R.C. increased with the minor doses and diminished only for the major ones, in agreement with that only a strong green tea might inhibit the formation of nitrosamines (4).

Conclusions
Total inhibition of the mutagenicity of the reaction mixture NaST-NaNO$_2$ was achieved for equimolar quantity of L-ascorbic acid with nitrite. Only partial inhibition was achieved with diluted aqueous extract of green tea.

Acknowledgments
CONICET, Fundación Prats, UNR

References
Table 1. Effect of the L-ascorbic acid on the mutagenicity of sodium sulfathiazole-NaNO₂ reaction mixture with Salmonella typhimurium TA98 strain

<table>
<thead>
<tr>
<th>L-ascorbic acid /plate nmol (µg)</th>
<th>R.C.ᵃ exp 1</th>
<th>% inhᵇ exp 1</th>
<th>R.C.ᵃ exp 2</th>
<th>% inhᵇ exp 2</th>
<th>R.C.ᵃ exp 3</th>
<th>% inhᵇ exp 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°</td>
<td>3.17</td>
<td>0</td>
<td>3.27</td>
<td>0</td>
<td>4.41</td>
<td>0</td>
</tr>
<tr>
<td>119 (21)</td>
<td></td>
<td></td>
<td>2.56</td>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>363 (64)</td>
<td>2.62</td>
<td>25</td>
<td></td>
<td></td>
<td>3.16</td>
<td>37</td>
</tr>
<tr>
<td>727 (128)</td>
<td>1.70</td>
<td>68</td>
<td>1.63</td>
<td>72</td>
<td>2.05</td>
<td>69</td>
</tr>
<tr>
<td>1215 (214)</td>
<td>1.35</td>
<td>84</td>
<td>1.20</td>
<td>91</td>
<td>1.93</td>
<td>73</td>
</tr>
<tr>
<td>1703 (300)</td>
<td>0.89</td>
<td>100</td>
<td>1.17</td>
<td>92</td>
<td>1.23</td>
<td>93</td>
</tr>
</tbody>
</table>

ᵃ R.C.: reversion coefficient = revertants with tested substance/spontaneous revertants; b % inhibition of mutagenicity, calculated as follow: % inh. = \( [(CR_{without\ AM} - CR_{with\ AM})/ (CR_{without\ AM} - 1)] \cdot 100 \)

³Only reaction mixture: sodium sulfathiazole/plate: 539 nmol (164 µg); NaNO₂/plate: 1740 nmol (120 µg). Negative control: without tested compounds, spontaneous revertants/plate ± SD: exp1: 32.5 ± 2.5; exp2: 21.7 ± 2.4; exp3: 27.00 ± 5.6. Positive controls with 4-Nitro-α-phenylenediamine (diagnostic mutagen): R.C.: 3.19
MUTAGENIC AND PHYTOTOXIC STUDIES WITH COBALT COMPLEXES OF SULFA DRUGS

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Introduction
The synthesis of metal sulfanilamide compounds had received much attention because sulfanilamides were the first effective chemotherapeutic agents for the prevention and cure of bacterial infections in humans (1). In this paper we analyzed the direct mutagenicity of two cobalt complexes of sulfanilamide: [CoII(PST)(H2O)4]·2H2O (Co(II)-PST; PST: phthalylsulfathiazolate) and [CoIII(ST)2OH(H2O)3] (Co(III)-ST; ST: sulfathiazolate), which exhibited moderately antifungal activity and antibacterial activity similar to the parent sulfonamides, and the phytotoxicity of Co(II)-PST.

Materials and Methods
Mutagenicity activity was evaluated in a bacterial reverse mutation assay by the standard Ames test in the absence of S-9 mix, by using the Salmonella typhimurium histidineBrequiring test with TA98 and TA100 strains (2). As it has been proved that sulfanilamide drugs are not mutagenic, ligands were not tested.

Plant Genotoxicity Test (Allium cepa Test) was done following standard procedures (3). Onions bulbs were kept in mineral water for 48 h and then exposed to Co(II)-PST and phthalylsulfathiazole (H2PST) solutions for 24 h. Length of roots as index of toxicity and modifications in root consistency and shape were observed as macroscopic parameters. Microscopic parameter was mitotic index (MI, five slides, 1000 cells/slide) to evaluate cellular division rate. Chromosome observation: with light microscope (400X magnification), staining with Schiff and orceine reagents.

Results
The results of the Ames test for both complexes are shown in Table 1. According to the Ames test a compound would be a mutagen if the reversion coefficient, R.C. (R.C. = revertants with tested substance/spontaneous revertants) is ≥ 2 (2). Consistent with this, the tested complexes did not show direct mutagenicity in the assayed range.

Allium cepa has been frequently used to determine the cytotoxic, mutagenic and genotoxic effects of several substances, being considered the standard organism for quick tests, since it shows a high correlation with mammal test systems and have an oxidase enzyme system, which is essential for promutagen evaluations (4).

The results of phytotoxicity and MI of Co(II)-PST and H2PST with comparative purposes are shown in Table 2. Roots length showed similarity between the Co(II)-PST and the parent sulfa drug, phthalylsulfathiazole. MI was not affected at concentrations in which both drugs showed antibacterial effects. The inhibition in the MI was similar for both drugs at higher concentration.

Conclusions
Both complexes did not show direct mutagenicity with the Ames test in the tested range. With the Allium test the behavior of Co(II)-PST was similar to the phthalylsulfathiazole one, its parent sulfonamide.

Acknowledgments
UNR, Fundación Prats, CONICET

References

Table 1. Mutagenic activity of the Co(III)-ST and Co(II)-PST complexes with S. typhimurium TA98 and TA100 strains

<table>
<thead>
<tr>
<th>Tested complex</th>
<th>Dose µg/plate (nmol/plate)</th>
<th>TA98 Nº rev./plate± SD</th>
<th>R.C.</th>
<th>TA100 Nº rev./plate± SD</th>
<th>R.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co(III)-ST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td>24.00 ± 5.04</td>
<td>1.00</td>
<td>157.50 ± 16.87</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>33.2 (52.1)</td>
<td>23.14 ± 5.11</td>
<td>0.93</td>
<td>147.375 ± 19.29</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>99.6 (156.2)</td>
<td>21.43 ± 3.77</td>
<td>1.01</td>
<td>137.125 ± 8.33</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>166.0 (260.0)</td>
<td>23.62 ± 2.06</td>
<td>1.01</td>
<td>146.50 ± 19.92</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>232.4 (364.7)</td>
<td>20.75 ± 2.73</td>
<td>0.89</td>
<td>140.22 ± 25.45</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>332.0 (520.0)</td>
<td>20.43 ± 4.89</td>
<td>0.86</td>
<td>121.80 ± 25.65</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Co(II)-PST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td>24.83 ± 5.67</td>
<td>1.00</td>
<td>151.67 ± 8.71</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>102.2 (179.8)</td>
<td>26.17 ± 3.37</td>
<td>1.05</td>
<td>157.83 ± 9.64</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>306.6 (539.4)</td>
<td>25.33 ± 8.29</td>
<td>1.02</td>
<td>140.20 ± 10.23</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>511.0 (899.0)</td>
<td>30.50 ± 6.77</td>
<td>1.23</td>
<td>145.83 ± 7.73</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>715.4 (1258.6)</td>
<td>24.67 ± 6.98</td>
<td>0.99</td>
<td>132.40 ± 8.08</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>1022 (1798.0)</td>
<td>24.17 ± 6.85</td>
<td>0.97</td>
<td>151.80 ± 11.43</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>4-Nitro-o-phenylenediamine</td>
<td>1.25</td>
<td>76.50 ± 0.50</td>
<td>3.19</td>
<td>---</td>
<td>3.11</td>
</tr>
<tr>
<td>NaN&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.15</td>
<td>---</td>
<td>---</td>
<td>490.00 ± 2.00</td>
<td>3.11</td>
</tr>
</tbody>
</table>

<sup>a</sup>mean of duplicate experiments, with three replicates per experiment. <sup>b</sup>R.C.: reversion coefficient = revertants with tested substance/spontaneous revertants. <sup>c</sup>Negative control: without tested compounds, spontaneous revertants/plate. <sup>d</sup>Positive controls with the respective diagnostic mutagens

Table 2. Phytotoxicity (as root length) and mitotic index (MI) of Co(II)-PST and H<sub>2</sub>PST evaluated with the Allium cepa test

<table>
<thead>
<tr>
<th>M x 10&lt;sup&gt;-8&lt;/sup&gt;</th>
<th>Root length (% of control)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>MI&lt;sup&gt;a&lt;/sup&gt; (%) ± SD</th>
<th>Co(II)-PST</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;PST</th>
<th>Co(II)-PST</th>
<th>H&lt;sub&gt;2&lt;/sub&gt;PST</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.0 ± 0.9</td>
<td>6.7 ± 0.6</td>
<td>7.1 ± 0.2</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>4.4</td>
<td>72</td>
<td>83</td>
<td>5.2 ± 0.7</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>5.9</td>
<td>73</td>
<td>-</td>
<td>4.8 ± 0.9</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>8.8</td>
<td>68</td>
<td>62</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.1 ± 0.7</td>
</tr>
<tr>
<td>17.6</td>
<td>48</td>
<td>50</td>
<td>2.6 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>440</td>
<td>21</td>
<td>19</td>
<td>2.6 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>mean of duplicate experiments, with seven replicates per experiment. ∆ (cm): 1.45 ± 0.52 (36%); 1.20 ± 0.14 (12%). Positive control: K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 3.4 x 10<sup>-8</sup> M (1 mg/L): root length: 58 % of negative control; MI%: 5.9 ± 0.9.
INHIBITORY EFFECT OF NEUROTENSIN ON HIGH AFFINITY \(^{3}H\)-OUABAIN BINDING TO CNS MEMBRANES

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Inst Biol Cel y Neuroc “Prof. E. De Robertis”, Fac Med, y Cátedra de Farmacol, Fac Farm y Bioq, UBA. Paraguay 2155, 1121-Buenos Aires, Argentina.

Introduction
Neurotensin is a peptide widely distributed throughout the gastrointestinal tract and the central nervous system. Neurotensinergic system interacts with other neurotransmitter systems, including dopaminergic, cholinergic, serotonergic, opioid and aminoacidergic systems, among others (1). Neurotensin binds to a group of receptors (2, 3). Two of them, termed NTS1 and NTS2, are seven transmembrane domain receptors coupled to G proteins, which bind neurotensin with high and low affinity, respectively (3). This peptide acts as an agonist for all NTS1-mediated pathways, whereas it may exert either agonist or antagonist activities, according to the NTS2 mediated pathway involved (4). It has been shown that neurotensin inhibits the activity of synaptosomal membrane Na\(^+\), K\(^+\)-ATPase, this effect most likely involves NTS1 receptor, because it is blocked by antagonist SR 48692 (5). In order to further explore whether the K\(^+\) site was involved in neurotensin effect on Na\(^+\), K\(^+\)-ATPase activity, herein the effect of neurotensin on high affinity \(^{3}H\)-ouabain binding to cerebral cortex membranes was determined in the presence of neurotensin or NTS1 antagonist SR 48692 as well as after the administration of the later.

Materials and methods
\(\square\) Lots of six rats were administered i.p. with 100 µg/Kg, 250 µg/Kg SR 48692 or with vehicle (0.01% Tween 80 in saline). Thirty min later, animals were sacrificed, cerebral cortices harvested, homogenized (10% W/V) in ice cold 0.32 M sucrose (pH 7.4) and subjected to differential centrifugation to obtain the membrane fractions.
\(\square\) Stereotaxis: Wistar rats (250-300g) were anaesthetized with 300 mg/Kg chloral hydrate and injected into the lateral cerebral ventricle with neurotensin 3, 10 or 30 µg/ 10 µl, or saline (control) at a rate of 1 µl / min with a Hamilton syringe. Sixty min later animals were decapitated and cerebral cortex harvested to isolate membrane fractions (6).
\(\square\) \(^{3}H\)-Ouabain binding was carried out by a filtration assay using 45 nM \(^{3}H\)-ouabain, with or without neurotensin and/or SR 48692 to evaluate Na\(^+\), K\(^+\) - ATPase α3 isoform (7, 8).

Results
Results showed that neurotensin and NTS1 receptor antagonist SR 48692 diminished high affinity \(^{3}H\)-ouabain binding to cerebral cortex membranes in a dose-dependent manner. The simultaneous addition of both substances produced a synergic action. Neurotensin increased Kd without changes in Bmax, indicating a competitive type inhibition. The administration of either neurotensin or the antagonist, invariably decreased \(^{3}H\)-ouabain binding. Injection of SR 48692 failed to modify neurotensin inhibitory effect on ouabain binding.

Conclusions
Results suggested an inhibitory action of neurotensin and SR 48692 on \(^{3}H\)-ouabain binding and that SR 48692 did not prevent neurotensin effect on ouabain binding. It is concluded that neurotensin is able to modulate \(^{3}H\)-ouabain binding, an effect which hardly involves NTS1 receptor.

References

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EFFECT OF QUERCETIN ON ACUTE INFLAMMATION. ANALYSIS OF STRUCTURAL INTERACTION WITH 5HT₂ AND H₁ RECEPTORS

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Introduction
The symptoms of acute inflammation are the results of complex processes mediated by several molecules, characterized by an increase of vascular permeability with diffusion of fluid which forms exudates. Histamine and serotonin are important mediators in this early phase. The vasodilatation, pro edema, vascular permeability on skin rodent is mediated by activation of serotonin receptors: 5HT1 and 5HT2 (1, 2). Experimental results suggest primarily activation of 5HT2 (3). Histamine is involved on pruritogenic process; it produces vasodilatation on blood vessel by stimulation of H1 and H2 receptor. H1 receptor stimulation generate mediators such as nitric oxide which trigger vascular permeability and edema formation by endothelial cell contraction (4). H2 receptor activation also produces dilatation on vascular smooth muscle, it is mediated by AMPc and the response is slower than when H1 receptor is activated (4).

On the hand, the flavonoids showed anti-inflammatory properties (5, 6, 7). Present study investigates the inhibitory effect of quercetin, a flavonoid; on acute inflammation on rat paw edema induced by histamine and serotonin and its possible interaction with the 5HT2 and H1 receptors.

Materials and methods
Pharmacological assay
Wistar rats weighing (160-180g) were divided into control (CGs, CGh) and experimental (EGs, EGh) groups of six animals each. CG received intraperitoneally saline, EG received quercetin 80 mg/kg. After one hour, via intradermic in left hind paw, CGs-EGs received serotonin 0.1% (8) and CGh-EGh received histamine 1% (9). Post-treatment paw edema was measure plethysmographically at 15, 30, and 60 min for histamine and at 30, 60 and 120 min for serotonin. Percent inhibition of inflammation was calculated for each animal group respect control group.

Homology Modelling and Docking
The three-dimensional models of the serotonin and histamine receptors were constructed with MODELLER 9.4 (10) using as template the high resolution crystallographic structure of the human β₂ adrenergic G protein coupled receptor (HAGPCR) (PDB dataset 2RH1) which has 31.79 % sequence identity, 49.29 % similarity against the serotonin receptor and 31 % sequence identity, 54 % similarity against the histamine receptor. BLAST (11) was used to search for the template and to do the alignments. The quality of the model was assessed by WHAT_CHECK (12). Dockings of serotonin, histamine and quercetin into the receptors were done using AUTODOCK4.

Results
Quercetin inhibited inflammation induced for histamine only at 15 min, while showed significant anti-inflammatory activity from 30 to 120 min against serotonin (Table 1 and 2).

The docking of serotonin and quercetin into the 5HT₂ receptor showed that serotonin binds in two subsites (I, II) with a similar energy. Quercetin binds occupying subsites I and II with a similar binding energy. The docking of histamine and quercetin into the histamine receptor showed different results. Histamine binds very deeply in active site; defining subsite I. Quercetin binds at the top of histamine receptor defining subsite II. Binding energy for histamine and quercetin binding were similar (Table 3).

Conclusions
We observed a correlation between pharmacological results that show a higher effect of quercetin against serotonin than histamine possibly because of serotonin and quercetin binds into the 5HT₂ receptor in two subsites (I, II) with a similar energy, while histamine and quercetin bind into the receptor H₁ with similar binding energy but in two different sites.

References


7- Manthey JA. Biological properties of flavonoids pertaining to inflammation. Microcirculation 2000; 7:829-834.


<table>
<thead>
<tr>
<th>Table 1: Effect of quercetin on serotonin induced paw edema in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (Minute s)</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>120</td>
</tr>
</tbody>
</table>

Values are the mean ± SD for 6 rats. Dunnet’s test:* P < 0.05, ** P < 0.01

<table>
<thead>
<tr>
<th>Table 2: Effect of quercetin on histamine induced paw edema in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (Minute s)</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>30</td>
</tr>
<tr>
<td>60</td>
</tr>
</tbody>
</table>

Values are the mean ± SD for 6 rats. Dunnet’s test:* P < 0.05, ** P < 0.01

<table>
<thead>
<tr>
<th>Table 3: Results of the docking experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
</tr>
<tr>
<td>SHT₂</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
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<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

Quercetin
| Clustering | Binding Energy kcal/mol | Subsite |
| *3 1 | -7.76 | |

Histamine
| Clustering | Binding Energy kcal/mol | Subsite |
| *1 1 | -4.52 | I |
| *1 9 | -4.38 | |
| *2 1 | -4.40 | |

Quercetin
| Clustering | Binding Energy kcal/mol | Subsite |
| *1 1 | -7.64 | II |
| *1 2 | -7.10 | |
| *2 1 | -7.56 | |
| *2 2 | -7.47 | |
| *3 1 | -7.40 | |
| *3 2 | -7.30 | |

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MECHANISMS OF ACTION INVOLVED IN THE GASTROPROTECTIVE EFFECT OF PLANT EXTRACTS

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Introduction
The gastroprotective effect of five plants that previously shown reduction of gastric damage induced by ethanol were evaluated using three different experimental models.

Materials and methods
Hidroalcohols extracts (EHA) of Jodina rhombifolia (Jr), Lippia turbinata (Lt), Gnaphalium gaudichaudianum (Gg), Ruta chalepensis (Rch) and Plantago lanceolata (Pl) were used orally to develop the following essays in mice:

a) Determination of ulcerogenic effect by the model of induced gastric ulcer by cold restraint stress (1).
b) Determination of gastric pH in mice previously treated with EHA (2)
c) Comparison of pH for EHA, excipient compose by carboximetil celulosa and Tween 80 (Ex) and solution of Ex with referent drugs using a digital pHmeter.
d) Evaluation of EHA effect on time for gastrointestinal emptiness using X Rays (3).

Results
a) The EHA of Gg reduced the develop of gastrics ulcers in 83% (P<0,01), Lt in 99% (P<0,01), Jr, Rch, and Pl in 100% compared to the Control Group.
b) The medium pH for the control group was 2,1 ± 0,08, ranitidine 1,6 ± 0,02 (ns), Jr 4 ± 1,1 (P< 0,05), Lt 2,3 ± 0,08 (ns), Gg 3 ± 1,3 (ns), Rch 3 ± 0,07 (ns) and Pl 2,6 ± 0,06 (ns).
c) The pH were: Jr 5,66; Lt 6,5; Gg 5,43; Rch 5,10 y Pl 4,43; Ex 7,33 and solution of ranitidine 7,30.
d) Gastrointestinal emptiness for the Control Group was 30’ and for the referent drugs, hyoscine N-butylbromide and neostigmine was 2hs and 30’. The Groups treated with Gg did not show differences with the Control Group ( Lt 4h, Rch 6 h, Pl 4h and Jr 30’).

Discussion and conclusion
The EHA significantly prevented the stress ulcers in all cases. Only the EHA of Jr shown differences compared to the control group regarding the pH over the surface of the gastric mucosa. Under the conditions of the present experiment, there was not relationship between gastric pH measure post administration of the EHA and the gastroprotector effect. In addition, no correlation was observed between the pH of Ex, ranitidine and the extracts with respect to the gastrics pH, excluding the expected antacid effects. For EHA from Pl, Lt and Rch the decrease of gastric motility could also reduce the erosive mechanical effect, while the gastroprotector of Gg could be related to cytoprotectors mecanisms.

References
DNA AND LIPID DAMAGE IN DIABETIC RATS SUBMITTED TO FORCED SWIMMING TEST: THE INSULIN AND CLONAZEPAM EFFECT

Wayhs CAY*, Manfredini V, Sitta A, Deon M, Ribas GS, Vanzin CS, Biancini GB, Nin MS, Barros HMT, Vargas CR

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Introduction
Diabetes Mellitus is a hyperglycemic chronic state that may modify central nervous system functions (1) and is associated with moderate cognitive deficits and neurophysiological and structural changes in the brain, a condition that may be referred to as diabetic encephalopathy (2). Psychiatric manifestations seem to accompany this encephalopathy, since the prevalence of depression in diabetic patients is much higher than in the general population (3) and clonazepam (CNZ) is being used to treat this complication (4). There is growing evidences that excessive generation of highly reactive free radicals causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications, since free radicals induce a variety of lesions in biomolecules like DNA and lipids. So, the aim of this study was to evaluate the effect of insulin and/or clonazepam on oxidative damage to lipids and DNA from streptozotocin (STZ)-induced diabetic rats submitted to forced swimming test (FST).

Materials and methods
Diabetes was induced by a single i.p. dose of STZ 60 mg/kg in male wistar rats. Insulin acute i.p. treatment (4 IU/kg) and/or CNZ acute i.p. treatment (0,25 mg/kg) were administered 24, 5 and 1 hour before the FST. Nondiabetic control rats received i.p. injections of saline (1 ml/kg). The damage to DNA was determined using the alkaline (pH > 13) comet assay in peripheral whole blood leukocytes from streptozotocin-induced diabetic rats submitted to FST (5). The oxidative lipid damage was determined by measuring malondialdehyde (MDA) levels in plasma from diabetic rats submitted to FST (6).

Results
It was verified a significantly increased damage index in diabetic group submitted to FST when compared to the control group. Additionally, diabetic animals submitted to FST treated with insulin and clonazepam presented a significant decrease in damage index. Furthermore, in diabetic rats submitted to FST treated with insulin plus clonazepam there was a reversion in damage index to equal statistical levels presented by control group (p<0.05). The oxidative lipid damage was significantly increased in blood from diabetic rats submitted to FST when compared to controls and significantly reduced by insulin acute treatment. In addition, insulin plus clonazepam acute treatment reverted the oxidative lipid damage to equal statistical levels presented by control group (p<0.05). Also, a significant positive correlation was observed between the DNA damage index and MDA in streptozotocin-induced diabetic rats submitted to FST.

Conclusions
These results may suggest that the treatment with insulin plus clonazepam could protect against DNA and lipid damage in diabetic rats submitted to forced swimming test and that a significant positive correlation was observed between the DNA and the lipid damage index in streptozotocin-induced diabetic rats submitted to FST.

Acknowledgments
CNPq, CAPES, FIPE/HCPA, PROPESQ/UFRGS.

References


LATRUNCULIN IN NUCLEUS ACCUMBENS BLOKS STRESS-INDUCED SENSITIZATION TO COCAINE.

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Introduction
Drug addiction is associated with long-term changes in the synaptic function, including the actin cytoskeleton. There is evidence about the proactive influence of stress on drug addiction, a process that is exerted on excitatory synapses by the activation of common mechanisms between drugs and stress. The present study sought to investigate whether the neurobiological mechanisms that modulate repeated cocaine administration also occur in a stress-induced cocaine sensitization model. These experiments were designed to evaluate whether repeated stress induces alterations in actin rearrangement in the Nucleus Accumbens.

Material and Methods
Male Wistar rats (250–350 g) were restrained daily for 2 hours for 7 days. Control rats were left undisturbed in their home cages. Three weeks after the last restraint stress, the animals were decapitated 45 minutes after an injection of saline or cocaine (30 mg/kg i.p.), and the nucleus accumbens was dissected. After subcellular fractionation by differential centrifugation to separate the F-actin from G-actin (Toda et al., 2006), the immunoreactivity of the actin (1:500, Santa Cruz), homer 1 b/c (1:100, Santa Cruz), Arp2 (1:100, Santa Cruz), p-cofilin (1:100, a gift from Dr. J. Bamburg), PSD 95 (1:250; Santa Cruz Biotechnology, Inc.) and p-cortactin (1:200; Santa Cruz Biotechnology, Inc.) was detected by Western blot, using tubulin (1:2,000, Sigma) as a loading control. Locomotor activity was monitored in a photocell apparatus (actographs). Motor activity was quantified as total photocell counts. For these sessions, animals were allowed to habituate to the activity chambers for 60 min before latrunculin A or DMSO (1%) microinjections, which were followed by cocaine (15 mg/kg, i.p.) or saline, and behavior recorded was for 120 min over 10 min interval-bins.

Results
Our experiments revealed that repeated stress induces changes in protein levels involved in synaptic plasticity, such as actin and proteins that regulate actin cytoskeleton. Thus, stress lowered F-actin levels and acute cocaine administration restored F-actin values to the levels observed in non-stress animals. The stress-induced decrease in F-actin levels may arise from a pronounced decrease in p-cofilin. Binding of the ADF/cofilin family of proteins to F-actin promotes filament disassembly, and actin binding by cofilin is terminated by phosphorylation (Ono, 2003). Thus, a decrease in p-cofilin may increase F-actin depolymerization, thereby reducing F-actin in the stressed animals. Along these lines, cortactin is known to activate the Arp2/3 complex which promotes the nucleation of the new actin filaments from F-actin, and this action of cortactin is inhibited by phosphorylation (Lua and Low, 2005. Thus, the cocaine-induced reduction of p-cortactin in stress-pretreated subjects may contribute to the restoration of F-actin. Latrunculin A binds to G-actin thereby preventing its polymerization into F-actin (Morton et al., 2000), and interestingly the cross-sensitization between repeated stress pretreatment and cocaine was prevented by intra-accumbens lantrunculin A (0.5 µg/µl) administered 5 min before saline or cocaine.

Conclusions
This is the first evidence that increased actin cycling in the NAc is one of the shared underpinning molecular mechanisms of stress and drug-induced sensitization to cocaine.

Acknowledgements
This work was supported by grants from FONCyT, CONICET, SECyT and Agencia Córdoba Ciencia (Argentina). The authors are grateful to Estela Salde for her laboratory technical assistance.
References


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#Corresponding author. Tel +54 351 4334437, fax +54 351 4334420; e-mail: lcancela@fcq.unc.edu.ar
PHARMACOLOGICAL ACTIVITY IN AN ACUTE ANIMAL MODEL OF SEIZURES OF NOVEL ANTICONVULSANTS IDENTIFIED THROUGH COMPUTATIONAL CHEMISTRY

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Introduction

Epilepsy is the most prevalent chronic brain disorder, affecting about 50 million people worldwide, 90% of which come from developing countries. (1) Current available chemotherapies fail to control epilepsy seizures in around 30-40% of the patients, (2) and even the new generation of anticonvulsant drugs present significant and frequent side-effects, e.g. drowsiness, sedation, ataxia, nausea and other gastrointestinal symptoms and liver toxicity. (3,4)

Recently we have reported the discovery of anticonvulsant activity in the Maximal Electroshock (MES) test of abietic acid, propylparaben (PPB) and methylparaben (MPB), through the application of a discriminant function based in topological molecular descriptors in the virtual screening of Merck Index 13th database (5,6). The three drugs have shown activity at 30 mg/kg (mice, ip), which is the lowest dose tested in phase I of the NIH Anticonvulsant Drug Development Program. MES test is an acute animal model of epilepsy that identifies phenytoin-like anticonvulsants, whose mechanism of action involves blockade of sodium and/or calcium channels.

Here we report the effects of abietic acid, PPB and MPB (AT4, AT2 and AT5, respectively) on another species and another acute animal model of epilepsy, the Pentylenetetrazol (PTZ)-induced seizures, which identifies anticonvulsant drugs that enhance the GABAergic pathway.

Materials and methods

Male Wistar rats of 250-300g received a single administration of PTZ (100 mg/kg, i.p.) 30 min after the injection of saline solution (1 ml/kg, i.p.) or AT2, AT4 or AT5 (30 mg/kg, i.p.). Latencies to the first myoclonus, clonic and tonic seizures as well as the mortality rate were evaluated during 1 h after PTZ administration.

Results

The three drugs tested increased the latency to the myoclonic, clonic and tonic components of PTZ induced seizures in Wistar rats (See table 1). All the experimental groups as well as controls showed 100% of mortality. The AT2 drug presented the higher efficacy to enhance the latency to the different components of PTZ-induced seizures.

Conclusions

Based on the results in the PTZ-induced seizures, we may conclude that the three drugs tested induce inhibitory effects that result in an enhanced latency to present the PTZ-induced seizures. Further experiments using experimental models such as kindling, are necessary to determine if these drugs are able to modify the epileptogenesis process.

Acknowledgments

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References.

Tables
Table 1. Increase of the latency time to different components of PTZ-induced seizures by the three tested drugs (related to the control group).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Myoclonus</th>
<th>Clonus</th>
<th>Tonic Seizure</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT2</td>
<td>216%</td>
<td>238%</td>
<td>228%</td>
</tr>
<tr>
<td>AT4</td>
<td>65%</td>
<td>103%</td>
<td>56%</td>
</tr>
<tr>
<td>AT5</td>
<td>68%</td>
<td>78%</td>
<td>206%</td>
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MECHANISM OF PROTECTIVE EFFECTS OF DEHYDROLEUCODINE ON GASTROINTESTINAL TRACT. ROLE OF CAPSAICIN-SENSITIVE SENSORY NERVES

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Introduction

Infusions of fresh leaves of Artemisia douglasiana Besser (Asteraceae), popularly known as “matico”, are used in folk Argentinean medicine as a cytoprotective agent, to treat peptic ulcers, and as antispasmodic (1). Dehydroleucodine (DhL), a sesquiterpene lactone of the guaianolide type isolated from Artemisia douglasiana, shows a pharmacological cytoprotective effect and prevents the formation of gastric lesions induced by various necrotizing agents (2). DhL prevented the damage and inflammation in acetic acid-induced colitis in rats and TNBS-induced colitis in mice (3). Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active ingredient accounting for the pungency of hot peppers and has important pharmacological actions. Capsaicin is uniquely selective for stimulation and then blockade of the subset of mammalian afferent neurons of dorsal root ganglia with C and Aδ fibers. Sensory nerves have been shown to participate in the maintenance of the gastric mucosal homeostasis and in the protection against mucosal damage in the gastrointestinal tract (4).

In the present study, the role of capsaicin-sensitive neurons in the cytoprotection of DhL on gastric damage and experimental colitis was evaluated.

Materials and methods

Extraction and purification of DhL: Artemisia douglasiana was collected in the mountains of the province of San Luis, Argentina, and a voucher specimen was deposited in the Herbarium of the Universidad Nacional de San Luis (UNSL No. 55). DhL was extracted as previously described (2). Briefly, the air-dried material was soaked in chloroform at room temperature. The extracts were evaporated in vacuo and dissolved in 95% ethanol. After addition of 4% aqueous lead tetraacetate solution, the aqueous cloudy solution was filtered through a celite pad, and the filtrate was concentrated under vacuum. The mixture was extracted 3 times with chloroform and the solution was concentrated under vacuum. The final residue was chromatographed in a medium-pressure chromatography system using 1:9 EtAcO/hexane as eluent. DhL (100% purity) was identified by 1H or 13C-nuclear magnetic resonance, mass spectrometry, or melting point analysis, and its structure was identical to that cited by others authors (2).

Animals: Male Wistar rats, weighing 200-250 g were used. They were maintained in a restricted access room, which was temperature controlled at 26ºC. The light-dark cycle of the room was 12 h/12 h. They were provided with food and water ad libitum. All experiments were in compliance with the ANMAT No. 6344/96 for animal care guidelines.

Functional ablation of afferent neurons: Rats were treated subcutaneously with capsaicin in increasing doses (20, 30, and 50 mg/kg) on three consecutive days in a regimen shown to deplete neuropeptides in primary afferent neurons. The animals were pretreated with orciprenaline 0.2 mg/kg, atropine 0.2 mg/kg and theophylline 20 mg/kg; i.m., just before capsaicin injection to prevent the respiratory impairment associated with capsaicin injection (5). Experiments were performed two weeks after completion of the capsaicin treatment in animals fasted 24 h. One day before the start of treatment, functional ablation of the capsaicin-sensitive nerves was confirmed. One drop of 0.1 mg/ml capsaicin was instilled into one eye. Vehicle-pretreated rats responded with immediate wiping of the front paw against the eye instilled with capsaicin. The test eye was rinsed with water. The wiping response was absent in the capsaicin-pretreated rats.

Production of acute gastric lesions: Gastric lesions were produced according to the methods of Robert et al. (6). Absolute ethanol administered orally was employed as the necrotizing agent, and 1 h later the animals were sacrificed by CO₂ asphyxiation. DhL (100 mg/kg) was administered 1 h before the absolute
ethanol. The stomachs were removed, opened along the greater curvature, and washed gently with ice-cold saline solution. The degree of erosion in the glandular part of stomach was assessed from a scoring system designed by Marazzi, Uberti and Turba (7).

Production of experimental colitis: Colitis was induced by 2 ml 10% acetic acid (i.r.). Colon rats received saline, acetic acid or capsaicin alone. Another group of rats received DhL 1 h prior to damage induction. Rats were sacrificed 24 h after damage induction, the colon isolated and damage was quantified by the scoring system of Wallace et. al. (8). Diarrhea was graded according to the criteria: 0 (normal) to 3 (severe) (9).

Results
Administration of absolute ethanol produced severe band-like lesions with congestions in the corpus mucosa; the mean lesion index in the ethanol control group was 4.60 ± 0.24. DhL inhibited the formation of gastric lesions (0.10 ± 0.10, p<0.001 vs. ethanol control). In the control group of chemical ablation of capsaicin-sensitive sensory neurons and absolute ethanol, the lesion score was 4.50 ± 0.28. Sensory desafferentation abolished the protective effects of DhL on ethanol-induced gastric ulceration (0.85 ± 0.14, p<0.01 vs. DhL + ethanol).

All acetic acid-treated rats experienced diarrhea manifested as watery, loose stools. Acetic acid induced extensive colonic damage (8.2± 0.58). No damage was observed in the colon of rats treated only with saline or capsaicin. DhL pretreatment significantly decreased the macroscopic damage (1.21 ± 0.34, p<0.001 vs. acetic acid) and the diarrhea (p<0.001 vs. acetic acid). Capsaicin pretreatment resulted in significant reduction of the cytoprotective action of DhL (3.25 ± 0.45, p >0.05 vs. DhL + acetic acid).

Conclusions
In order to study the role of sensory nerves in the protection on gastrointestinal tract, we used capsaicin, an excito-toxin known to acutely stimulate unmyelinated (C) or thinly myelinated (Aδ) afferent neurons. With high doses or prolonged exposure to capsaicin, afferent neurons are functionally desensitized, exhibiting long-lasting loss of responsiveness to capsaicin itself or other stimuli of sensory neurons. The gastric mucosa is densely innervated by capsaicin-sensitive afferent neurons and these are known to increase the resistance of the gastric tissue to injury, facilitating the repair of damaged tissue by releasing peptide transmitters from their nerve endings. As a consequence, functional ablation of afferent nerves by a neurotoxic dose of capsaicin fails to induce acute injury by itself, but aggravates the mucosal lesions caused by topical irritants.

Our results suggest that the protective activity of DhL on gastrointestinal tract is mediated, at least in part, through the afferent sensory neurons.

Acknowledgments
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References.
BIOEQUIVALENCE ASSESSMENT OF THREE IVERMECTIN ORAL FORMULATIONS IN LAMBS

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Introduction

Ivermectin (IVM), a member of the macrocyclic lactones antiparasitic drugs, exhibits a broad-spectrum of activity against gastrointestinal (GI) and lung nematodes, as well as against ectoparasites of domestic animals. Two pharmaceutical formulations containing the same active ingredient are considered to be bioequivalent when they reach a similar systemic availability after administration at the same dose rate under standardised experimental conditions (1). The goal of the current work was to investigate the relative bioavailability/bioequivalence of three different oral IVM commercial formulations in lambs.

Materials and methods

Twenty four Corriedale lambs were allocated into three groups (n= 8 each). Animals were orally treated (parallel design) with different IVM formulations (0.2 mg/kg): formulation A (reference), B (test 1) or C (test 2). Plasma samples were collected over 15 days post-treatment and drug concentrations were measured by HPLC. Non-compartmental analysis was used to calculate the plasma pharmacokinetic parameters.

Results

Peak plasma concentrations (Cmax) of 5.14±2.46 (formulation A), 5.82±2.53 (B) and 4.96±1.21 (C) ng/mL were measured. (P=0.776). The time to peak plasma concentration (Tmax) were observed at 0.81 (formulation A), 0.71 (formulation B) and 0.60 (formulation C) days post-treatment (P=0.340). Areas under concentration vs. time curves (AUC0-t) were 6.70±3.00 (formulation A), 8.65±4.47(B) and 7.55±2.78 (C) ng.day/mL (P=0.577). No significant differences (P>0.05) were observed on the MRT, elimination half-lives and Cmax/AUC0-t among formulations. The 90% CIs for the log-transformed ratio (formulations B or C vs. the reference formulation) were 0.81-1.58 (Cmax) and 0.93-1.73 (AUC0-t) (formulation B) and 0.78-1.39 (Cmax) and 0.87-1.54 (AUC0-t) (formulation C). The variation coefficients obtained for Cmax and AUC0-t were upper 30% in all test/reference comparisons.

Conclusions

IVM showed similar disposition kinetics after its administration as different formulations in lambs. However, based on the internationally accepted criteria, the bioequivalence among the reference preparation and the test formulations B and C, could not be demonstrated under the current experimental conditions.

Acknowledgments

This work was supported by ANII (Project FCE 703), Uruguay

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URINARY EXCRETION EVALUATION OF Aloe saponaria METABOLITES

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Introduction
The 1,4-linked β-D-mannopyranosan and 1,4-linked α-D-mannopyranosan are two partially acetylated mannan, isolated from the pulp of Aloe saponaria (iii). They have been associated to many biological activities, including antiviral, anti-bacterial, anti-inflammatory and immunostimulatory (iv). It is known that the α and β polisaccharides can be catabolyzed to a lower molecular compounds (mannose or mannose oligosaccharides) by the human intestinal microflora (v).

Many Gram (-) bacteria have mannose-specific adherence due to the presence of lectins onto the bacterial surface, which bind to mannose residues (vi).

Studies of polymicrobial sepsis in mice showed that the i.v. administration of Aloe vera fresh gel markedly enhanced the urinary bacterial clearance (vii).

The objective was to evaluate the urinary excretion of saccharides metabolites after Aloe saponaria fresh gel oral intake in volunteers to estimate its potential utility as a coadjuvant in urine tract infections. For this purpose a systematic study of quantification and identification of total mannose present in urine was performed.

Materials and methods
The fresh gel contained in 250 g of Aloe saponaria leaves obtained from Calamuchita Valley, Cordoba, Argentina was orally administered after dinner to five volunteer during seven days. This study was evaluated by the Clinical Trial Commission of the Clinicals Hospital according to the Declaration of Helsinki. All volunteers gave their written informed consent prior to study inclusion. Urine samples were collected fasted at t=0 days (Mt₀) and t=7 days (Mt₇) and subjected to the determination of:

Mannose through total carbohydrates by Dubois method (phenol/ sulfuric acid)(viii) with mannose reference. Glucose presence was discarded by the selective Enzymatic Method.

Additionally TLC Chromatography, ¹H NMR and HPLC were performed using urine Mt₀ and distilled water spiked with mannose as references.

Results and Discussion
The carbohydrate total concentration determined in the urine samples was 622, 289, 480, 356 and 287 mg/l. No glucose was detected. Then these concentrations were inferred as mannose. The differences among volunteers were related to the variation in absorption and metabolism of the natural product in each individual.

In agreement TLC showed a unique spot at the same Rf (0.81) than mannose reference. Also HPLC chromatogram of Mt₇ samples showed a peak with retention time (14,91 min) matching mannose reference (14,84min). In addition, peaks overlapped at slightly higher retention times were observed. They may be due to mannose oligosaccharides present in the biological sample.

¹H NMR of Mt₇ spectra presented signals at δ: 3, 42 (s)-3.55(s) 3.76, 3.77 (d)(CH OH) δ: 4.51 4.53(d) (C₁ αH), δ: 5.10 5.11(d) (C₁ β H) which are characteristic of carbohydrate. The signals are similar to that previously described for polysaccharides from Aloe saponaria (2).

Conclusions
The results showed a significant metabolism of the acetylated mannan and excretion of mono and/or oligosaccharides from Aloe saponaria fresh gel after oral administration. The TLC and HPLC chromatograms detected mannose in the analyzed biological material. In agreement ¹H NMR signals from carbohydrates are present. Further research is necessary to establish if concomitant intake of Aloe saponaria fresh gel would be useful as a coadjuvant for the treatment of patients with urinary tract infections.

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THERAPEUTIC EQUIVALENCE FOR CITALOPRAM TABLETS THROUGH IN VIVO AND BIOWAIVER STUDIES

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Introduction
Assessing therapeutic equivalence (TE) through dissolution studies (Biowaiver), is nowadays one of the experimental designs to prove interchangeability of pharmaceutical products. Some regulatory agencies accept these studies for Class 1 drugs (high solubility-high permeability) according to the Biopharmaceutical Classification System (BCS), when the drug is formulated in a rapidly dissolving solid oral dosage form, excluding drugs with a narrow therapeutic window. To demonstrate interchangeability, similarity in dissolution profiles between reference and test products has to be assessed (1,2,3). Experimental conditions of dissolution profiles for biowaiver are different from those of compendial methods like USP. The latter considers only one dissolution condition; in the case of citalopram tablets, dissolution medium is a pH 1.5 buffer (4). In contrary, for a biowaiver application, at least 3 different pH have to be tested, in the range of the gastrointestinal pH. Several guidances suggest the following pH values: 1.2 – 4.5 and 6.8, trying to represent pH ranging from the stomach to the middle part of the jejunum.

Citalopram can be classified as a Class 1 drug according to BCS. Is used as solid dosage forms, 20 mg of dose. Its therapeutic window is 20-200 ug/L. According to these characteristics, citalopram tablets would applied for a Biowaiver study. (4,5).

The aim of this study was to evaluate the feasibility of a biowaiver protocol to assess TE for citalopram tablets, comparing the results obtained in an in vivo bioequivalence study with those of an in vitro dissolution study, in order to evaluate the concordance of the TE conclusions obtained from both protocols.

Materials and methods
Citalopram tablets, 20 mg dose, Reference (R) and Test (T) products.
In vivo study: crossover 2x2, n= 24 healthy volunteers. The protocol of the study was approved by the Ethical Review Board of the Clinical Hospital of the University of Chile, where the clinical part of the study was performed. A validated LC-MS-MS method was used for plasma citalopram assay. AUC, Cpeak, tpeak and K were obtained from plasma profiles. Comparison between T and R was assessed using the confidence bioequivalence interval of 80-125%. Absorption profiles were obtained assuming a one compartment model.
In vitro study: dissolution profiles (n=12) were obtained with USP Apparatus 2 (paddle) , 75 rpm, 37°C, 900 mL of: HCl 0,1 N – acetate buffer pH 4,5 and phosphate buffer pH 6.8. Profiles obtained for R and T products in the 3 media were compared through the similarity factor f2.

Results
T and R formulations were pharmaceutical equivalents. Formulations were bioequivalent when AUC and Cmax , both log transformed , were compared. 90% Confidence intervals were [99.8% - 101.2%] and [97.8% - 101.7%] respectively. Dissolution profiles showed no differences between R and T when pH 1.2 medium was used. At pH 4.5 and 6.8 differences were found at the first sampling times, specifically during the first 10 minutes. However, these differences don’t have a physiological meaning considering that at 15 minutes practically all the drug was released in the 3 media. Considering 20 minutes as the average gastric emptying times, the results of the study showed that R and T are completely dissolved when they are still at the stomach. From this point of view, T and R could be classified as a very rapid dissolving products (over 85% dissolved in 15 minutes at the pH range of the GI tract) and application of f2 for dissolution profiles comparison was not necessary (1,2). Besides, due to this rapid dissolution behavior, no mathematical correlation could be found between dissolution and absorption profiles. According to the results of the dissolution study, both formulations can be considered as therapeutically equivalent and, therefore, interchangeable

Conclusions
Through the *in vivo* and *in vitro* studies the same conclusion regarding therapeutic equivalence was obtained. Biowaiver is a feasible model to evaluate therapeutic equivalence for citalopram tablets.

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EXPLANATORY VARIABLES OF THE LYMPHOCYTE PgP VARIABILITY IN HEALTHY INDIVIDUALS

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Introduction
Great population variability has been detected in drug pharmacokinetic and toxicity. Partly, these have been explained by the physiological function of the P glycoprotein (P-gp), which reduces plasmatic membrane drug permeability (1). P-gp is expressed and active in human peripheral blood lymphocytes (2).

The aim of the work is to provide a data base describing lymphocyte P-glycoprotein activity distribution in healthy individuals and to explore biochemical, anthropomorphic and clinical measures associated with PgP.

The data were described by a linear multiple regression model (3) linking fluorescence activity with the measured covariables. We have explored the factors that explain pgp activity variability and have found the only significant factor was cholesterol.

Materials and methods
Population evaluated: This protocol was approved by the Clinical Investigation Ethics Committee and sixty-one healthy volunteers from Buenos Aires city were included. They were evaluated by a questionnaire and routine laboratory tests, samples were collected after informed consent was signed by the donors.

Variables recorded: information requested was age, sex, race, height, weight, nationality, smoking and nutritional habits, consume of alcohol and drugs. Biochemical determinations performed were hemogram, erythrosedimentation, glycemia, creatininemia, total cholesterol, triglycerides, total bilirrubin and transaminases

Rhodamine 123 efflux assay: P-gp activity was determined by Rhodamine 123 (Rh123) efflux assay (4) in healthy volunteers lymphocyte. P-gp activity was calculated as the ratio of mean fluorescence intensity for Rh123 in Rh123 + Verapamil/Rh123 as recommended by Marie (5).

Statistic analysis: the first step was to examine the simple relation between each potential explanatory variable and FR ignoring the others. Then a multiple linear regression model was fitted, with each subgroup of variables. At last we fitted a stepwise multiple regression model considering the best explanatory variable of each subgroup.

Results
The age range was from 19-90 years. Between the simple linear regressions, the only statistically significant was \( FR=4.07 -0.008 \text{Cholesterol} \) (The regression of FR on age wasn’t significant)

The stepwise multiple regression analysis, at its starting point enter the single variable with the strongest association with FR. Next, the variable, not in the model, that explains the largest amount of the remaining variability, repeating this steep until no extra variable is significant for the remaining variability. This procedure arrived to the predictive equation: \( FR=3.92 -0.38(\text{Cholesterol}) – 0.18(\text{Age}) \), but the coefficient of age was not significant, so it can be said that age was over fitted and could be omitted.

Conclusions

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We have found that plasma cholesterol concentrations have a linear inverse relationship with PgP activity. The study of the relationship between the other biochemical, anthropomorphic and clinical variables and P-gp activity showed no statistical significance.

Acknowledgments
This research was supported by the grants B067 from the University of Buenos Aires.

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FENPROPOREX AND AMPHETAMINE LEVELS IN ORAL FLUID FOLLOWING ADMINISTRATION OF DESOBESI-M®

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Introduction
Fenproporex (FEN) is an anorectic drug used in the treatment of moderate to severe obesity and its biotransformation originates mainly amphetamine (AMP), another potent CNS stimulant. (1) FEN is one of the three most widely used anorectic in world (2), and in Brazil it’s highly misused, especially as stimulant for professional drivers. On the other hand, AMP is not marketed as a medicine in the country. The detection and time/concentration profile of FEN together with his major metabolite AMP has been described in classical biological matrices,(1-3-4) however, there are no reports of such data in oral fluid. Oral fluid is a mixture of saliva (secretion of three main salivary glands, parotis, submandibularis and sublingualis) and other constituents present in the mouth, like water, enzymes, glycoproteins and electrolytes. (5) The aim of this work is to estimate the pharmacokinetic profile of FEN and AMP in oral fluid, in order to assist in the development, application and interpretation of positive saliva tests applied to monitor the consumption of stimulants on roads.

Materials and Methods
Were administered orally 25 mg of FEN (one capsule of Desobesi®) to three volunteers and oral fluid samples collected with Quantisal® device during 24 hours, one before drug administration and the others in periods of 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours after the drug ingestion. FEN and AMP extraction was performed by solid phase microextraction (SPME) and the analysis carried out on a gas chromatography-mass spectrometry detector (GC-MS), using selected ion monitoring (SIM) and methamphetamine as internal standard.

Results
With the obtained data was established a preliminary pharmacokinetic profile of FEN and AMP in oral fluid. After FEN administration, both analytes could be detected in oral fluid of all volunteers with an initial detection time varying from 0.5 to 1 hour. FEN peak concentration occurred in samples collected between 0.5-2 hours after administration, with maximum between 72.58 and 192.27 ng/mL. For AMP, peak concentration occurred between 1-6 hours, reaching 35.22 to 156.32 ng/mL.

Conclusion
It was observed that oral administration of FEN results in significant amounts of FEN and AMP in oral fluid, showing that saliva can be a biological matrix suitable for pharmacokinetic studies of these substances, and be able to infer the pharmacokinetic models for both analytes. According to the data obtained, the FEN and its metabolite AMP follow different pharmacokinetic models. FEN follows one-compartment model, while its major metabolite AMP obeys two-compartment model.

References


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IN VITRO AND IN VIVO ASSESSMENT OF THE INTERACTION BETWEEN DANOFLOXACIN AND IVERMECTIN IN SHEEP

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Introduction
A potential drug interaction refers to the possibility that one drug may alter the intensity of the pharmacological effects of another drug given concurrently (Nies and Spielberg, 1996). Although less frequent compared to drug interactions involving the cytochrome P450 system, clinically significant ATP binding cassette (ABC) transporter-mediated drug interactions have been reported (Lin, 2007). ABC transporters are physiologically located in tissues involved in the pharmacokinetic processes such as the brain-blood barrier, luminal surface of hepatocytes and ducts cells, kidney tubules and enterocytes (Lin, 2003). Concomitant administration of multiple drugs is often used in current veterinary practice, which may drastically induce changes on the disposition kinetics and pharmacological activity of different therapeutically used drugs. The aim of the current trial was to evaluate the pharmacokinetic disposition of an antimicrobial drug (danofloxacin) and an antiparasitic compound (ivermectin) given either separately or co-administered to sheep. To further understand the basis of their kinetic interaction, complementary in vitro assays with the Ussing Chamber system were carried out.

Material and methods
Corriedale sheep (in vivo experiment) received ivermectin (IVM) (0.2 mg/kg) by subcutaneous (s.c.) route (Group A). Group B received danofloxacin (DFX) by s.c. route (6 mg/Kg, twice every 48 h) and Group C received the co-administration of IVM+DFX, both administered at the same dose rate. Jugular blood samples were collected and the IVM and DFX concentrations were analyzed by HPLC using fluorescence detection. Additionally, the effect of IVM and DFX on Rhodamine 123 (Rho 123) intestinal transport was studied in diffusion chambers. The ileum segment of sheep intestine was opened along the mesenteric border and then partially stripped from underlying muscle layers. Resulting flat sheets of gut mucosa were mounted into Ussing chambers. Rho 123 (5 µM) was added to both mucosal (M) and serosal (S) sides either alone or with IVM (25 µM) and DFX (25 µM), used as P-glycoprotein (P-gp) modulators. Buffer samples were taken between 30 and 240 min for determination of drug flux across the mucosal and serosal membranes. The Rho 123 concentrations were measured by spectrofluorometric detection and the apparent permeability coefficients, per unit of membrane surface area (Peff) (cm/s), were estimated.

Results
No significant changes were observed in the IVM disposition after its co-administration with DFX. However, the IVM presence affected the DFX plasma disposition in sheep. IVM enhanced the DFX plasma availability (between 32 and 35 %) (P<0.05) and prolonged its elimination half-life (between 40 and 52 %) (P< 0.05) in co-administered group. The efflux transport of Rho 123 in sheep intestine mounted in the Ussing chamber system was significant decreased in the presence of IVM. The efflux coefficient (PeffM/S/PeffS/M) decreased from 6.49 (Rho 123 alone) to 1.12 (Rho 123 + IVM) (P<0.05). However, no significant differences were observed in the efflux coefficient in the presence of DFX (PeffM/S/PeffS/M = 5.52).

Discussion
IVM affects the disposition kinetics of DFX in sheep. IVM is a recognised P-gp inhibitor (Schinkel et al., 1994). The Ussing Chamber assay corroborated the inhibition of the P-gp-mediated intestinal secretion of Rho 123. However, DFX did not modified the Rho 123 intestinal secretion. The fluoroquinolone antibacterials have been reported as substrates of different ABC transporters. IVM is also a weak inhibitor of the breast cancer resistance protein (BCRP) (Muenster et al., 2008). Therefore, a drug to drug interaction with BCRP should not be ruled out in the in vivo changes to the kinetics of DFX co-administered with IVM.
References
ENHANCEMENT OF SULPHAMERAZINE SOLUBILITY BY ITS INTERACTION WITH MEGLUMINE

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Introduction
Sulphamerazine (SMR) is a weak acid drug (pKa1 2.6; pKa2 6.9) (1) that presents antibacterial activity (2). In its crystalline form, is sparingly soluble in water (16 mg/ml at 20 °C, in neutral pH) (3). This fact gives rise to difficulties in the pharmaceutical formulation. To overcome this drawback, the generation of its amorphous state may be the solution (4).

In this way, meglumine (N-methyl-D-glucamine) (MEG), a polyhydroxy base (5) able to interact with weak acidic drugs, was used in this study to obtain greater water solubility products. Consequently, the bioavailability of the drug could be improved and so its therapeutic efficacy.

Materials and methods
The binding constants (Ks) and the stoichiometry for the SMR:MEG systems were determined in water and in phosphate buffer solutions of pH 2, 7 and 8, by means of phase-solubility studies. The different pH values were used to study the influence of the ionization state of the drug on the interaction with MEG.

In solid state, the binary systems, prepared by means of simple physical mixture (PM) or by lyophilization (LYO), were studied by Infrared Espectoscopy (IR), Differential Scanning Calorimetry (DSC) and Thermogravimetry (TG).

Results
Soluble systems of A1 type diagrams were obtained (according to Higuchi and Connors) (6) indicating the presence of 1:1 systems, except at pH 2. In this case the phase solubility plot lacks of a tendency, not allowing to determine the Ks and stoichiometry, and showing a 2 folds decrease in the solubility of SMR. At water (Ks=3,4 x1011), pH 7 (Ks=2,4 x109) and pH 8 (Ks= 9,15 x 108) solubility enhancements of about 2400, 54 and 41 have been respectively observed.

The solid state studies showed that an amorphous SMR:MEG system is obtained when it is prepared by means of lyophilization. This fact is important for the bioavailability of the drug when it is administrated in solid pharmaceutical formulations.

Conclusions
An important increment of SMR solubility and high binding constants were obtained for SMR:MEG system, mainly in water. Also it presented amorphous form in solid state. These facts gives rise to include this product in pharmaceutical formulations which contains SMR as active ingredient and so will improve its therapeutic performance.

References

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OBTENTION OF PARACETAMOL TASTE MASKED MICROCAPSULES BY A SINGLE LOW-TECHNOLOGY TECHNIQUE

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Introduction
Microencapsulation of APIs is currently widely used in the pharmaceutical industry. High tech fluid bed systems allow to obtain film coated particles with taste masked, enteric or sustained release properties from polymethacrylate substances (1). Many drugs applied for pediatric medicines require to be taste masked in order to obtain a better acceptance by the patient (2); paracetamol and ibuprofen are used as drug models for this application. The aim of this study is to obtain taste masked paracetamol microcapsules, starting from drug crystals and using a single and low cost novel technology.

Materials and Methods
Paracetamol crystals were granulated in a planetary mixer, with monohydrated lactose and PVP 10% w/w water solution. Powder was eliminated from dry granules using a 149 µm mesh.

The spheronization of the granules was carried out in a conventional pan by addition of a “growing agent” and wetted with a PVP 10% water solution (3). Starch and lactose monohydrate (Granulac® 200) were evaluated as growing agents for this procedure according to table 1.

Obtained spheronized granules were coated using an alcoholic solution of EUDRAGIT® E PO (aminomethacrylate copolymer) (4), mixed with talc and magnesium stearate as anti-attacking agents. Coating process were performed using 0.2 bar atomization pressure and product temperature between 26 ºC - 28 ºC. Final weight gain of polymer was of about 13%.

Dosage of paracetamol on coated granules was carried out by liquid chromatography. Microcapsules were first solved in HCl 0,1 N and diluted in the mobile phase (water:methanol, 70:30). The chromatographic procedure was carried out with a stainless steel column packed with stationary phase C18 and using a detection wavelength of 243 nm.

Results
Two types of growing agents were used for the spheronization of paracetamol granules; both starch and lactose gave similar size dispersion of granules. Additionally, rounded shape granules were observed by optical microscopy in both cases. Obtained microcapsules had a final concentration of about 42% of paracetamol (table 2).

Taste masking effect was tested with 7 volunteers; coated granules could remain in their mouths more than 1 min longer than uncoated granules without any bitter taste (table 2).

Conclusion
A single procedure was evaluated for the spheronization of paracetamol granules. Both starch and lactose monohydrate gave rounded granules of similar size. About 13% weight gain of EUDRAGIT® E PO was applied to reduce the bitter taste of the drug. Based on the fact that the polymer is soluble only in acid solutions (pH 1-5), microcapsules could be included in an extemporaneous suspension by buffering it to a final pH 6 – 7. This buffered media would keep intact the granules time enough to permit the administration of this API in pediatric patients (table 2). Same low technology technique could be of help for a single masking of bitter drugs, such as ibuprofen or clarithromycin, commonly used for pediatrics.

References
(4) EUDAGIT® Specifications, [www.eudragit.com/products](http://www.eudragit.com/products)

Table 1. Spheronization Procedure - Experimental conditions.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Paracetamol Granules</th>
<th>Lactose Monohydrate</th>
<th>Starch</th>
<th>PVP K-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>I - Starch as “growing-up” agent</td>
<td>336 g</td>
<td>-</td>
<td>224 g</td>
<td>21 g</td>
</tr>
<tr>
<td>II - Lactose as “growing-up” agent</td>
<td>360 g</td>
<td>240 g</td>
<td>-</td>
<td>22 g</td>
</tr>
</tbody>
</table>

Table 2: Evaluation of Paracetamol Coated Granules.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Starch as “growing agent”</th>
<th>Lactose Monohydrate as “growing agent”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle size (average)</td>
<td>438 µm</td>
<td>457 µm</td>
</tr>
<tr>
<td>Particle size (≥ 90%)</td>
<td>300 / 545 µm</td>
<td>300 / 545 µm</td>
</tr>
<tr>
<td>EUDRAGIT® E PO (content in coated granules)</td>
<td>10,8 %</td>
<td>11,2 %</td>
</tr>
<tr>
<td>Paracetamol dosage</td>
<td>42,6 %</td>
<td>40,8 %</td>
</tr>
<tr>
<td>Disintegration time HCl 0,1 N</td>
<td>7 secs</td>
<td>11 secs</td>
</tr>
<tr>
<td>Disintegration time Buffer Phosphates pH 6,8</td>
<td>&gt; 30 min</td>
<td>&gt; 30 min</td>
</tr>
<tr>
<td>Taste masking (7 volunteers)</td>
<td>&gt; 1 min</td>
<td>&gt; 1 min</td>
</tr>
</tbody>
</table>

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DRUG-POLYELECTROLYTE COMPLEXES OF EUDRAGIT E WITH DICLOFENAC. CHARACTERIZATION AND DELIVERY PROPERTIES.

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Introduction
The interaction between a polyelectrolyte (PE) and an ionizable drug of opposite charge leads to the formation of complexes, nanostructures with different physical and chemical properties to the original molecules, in which the polymer acts as a carrier's drug (1-3). In this study, the PE used is Eudragit E (Eu) (4) and the drug diclofenac (D). By acid-base interaction in an organic medium, it is possible to obtain different degrees of neutralization of Eu with the drug and with different percentages of a second counterion (chloride (Cl)). This strategy lets to produce solid complexes with different types of behavior. The purpose of this study is evaluating some properties related to these complexes.

Materials and methods
The complexes EuD50Clx were obtained by neutralization of Eu with fifty percent in moles of D and different molar proportions (x) of hydrochloric acid (HCl), using the solvent evaporation method (3). They were characterized by infrared spectrophotometry (IR) and X-ray diffraction (XRD). Delivery rates of free acid D were measured in a Franz-type two-compartment device using water and NaCl 0.9% solution as receptor media (4). Matrices prepared by compaction of EuD50Clx were subjected to measurements of solvent up-take and drug release, in water and NaCl solution (6-8).

Results
The complex formation was assessed by IR and XRD analysis. The complexes in dispersion behave as a reservoir of diclofenac that releases the drug at a slow rate, being the NaCl solution the receptor medium which presents the higher releases. Solvent up-take studies shown that the sorption rate in water is higher than that in NaCl solution. This trend is similar to the drug release from matrices but opposite to the release from the complexes in dispersion as evaluated in Franz cells. Drug release from matrices and from the dispersions of EuD50Clx show a constant delivery rate (zero order kinetics) in both, water and NaCl solution. Regarding the properties, sorption and release rates of the complex EuD50, are very low while EuD50Cl25 exhibited the highest release rates.

Conclusions
The present research shows that delivery rate of diclofenac, can be modulated by varying the composition of EuD50Clx and follows zero order kinetics. Polyelectrolyte–drug complexes like EuD50Clx exhibit interesting delivery properties and could be useful in the design of monolithic as well as multiparticulate delivery systems.

Acknowledgments
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NANO AND MICRO TECHNOLOGY APPLIED FOR THE TREATMENT OF CHAGAS’ DISEASE.

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Introduction
Chagas’ disease is a highly prevalent infection in the American Continent. The disease affects nearly 20 million people. Currently, there is no effective alternative for chronic cases, no vaccine, and no preventive treatment. In the acute, recent or congenital disease, the most important drugs available to control the disease are: nifurtimox and benznidazole (BZL) a nitroimidazole derivative. The only trypanocidal chemotherapies available for Chagas’ disease are solid dosage forms, but they have the disadvantages associated with oral absorption of poorly soluble drugs. (1).

The loading of BZL into biodegradable polymeric microparticles provides an attractive alternative to improve the drug solubility and bioavailability. Microparticles were prepared with chitosan (CH) by both ionotropic gelation, and a liquid-liquid phase separation with sodium lauryl sulphate and Na(OH), using two different methodologies: dripping and spraying. Then, physical chemical parameters such as yield, encapsulation efficacy (EE), size and morphology of the microparticles were evaluated. Also it was prepared pharmaceutical dosage forms with the solid systems (tablets) and they were characterized. (2-4)

Materials and methods
CH and BZL were solubilized in acetic acid (50% v/v). Na(OH) or sodium lauryl sulphate (SLS) were solubilized in water. Finally the acid solutions were sprayed or dripped on the basic ones and stirred for 24 h generating the microparticles. The polymeric particles were centrifuged and washed twice and collected in a drying chamber at 40 ºC. The pharmaceutical solid forms (tablets) were prepared by wet granulation method. The pharmacokinetic of the BZL-microparticles were evaluated in vivo employing Wistar rats, of 100 days old.

Results and discussion
The microparticles obtained by dripping technique exhibited a quasi-prismatic shape with a regular and flat surface. The use of a spray device led to a significant decrease in particle size range with showed an acceptable spherical shape whist a porous surface. The yield of the microparticles obtained employing both methodologies were high (70-80 %). The dissolution profiles obtained from different formulations was contrasted against isolated BZL without any treatment. The microparticles formulated showed an enhanced dissolution rate for BZL in comparison with the drug alone, confirming that the novel formulations conferred improved dissolution properties to the drug.

Conclusions
BZL microparticles were successfully prepared using CH as carrier and NaOH or SLS as a counterion in high yield. The EE employing spraying method was better than the obtained using dripping method and size of microparticles was smaller in the first case indicating that spraying is a good methodology to obtain it. The pharmaceutical dosage forms were successfully prepared and evaluated to improve the current therapeutic alternatives to control the Chagas’ disease.

References:

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PHASE BEHAVIOR OF ASCORBYL PALMITATE IN PEG400 SOLUTION

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Introduction

Liquid crystals (LC) have a high degree of internal order and symmetry, combined with a large interfacial area and balanced hydrophobic and hydrophilic domains content. These properties make the LC excellent universal drug carriers, with numerous advantages over most other systems currently used20. That is the reason why phase behavior of Ascorbyl Palmitate (Asc16) in solution of polietileneglycol 400 (PEG 400) have been developed. The knowledge of phase behaviors is important to describe possible zones where mesophases are present. Stabilization of these phases may carry out to new drug carrier.

Materials and methods

Ascorbyl Palmitate (AP) was purchased from (Flukka-Italia). Redistilled water by Allchemistry (Buenos Aires, Argentina) was used in all experiments. PEG 400 was purchased from (Parafarm-Argentina). Solvent was prepared with 25% Peg 400 and 75% water. Nine samples were prepared; 10, 20, 30, 40, 50, 60, 70, 80 and 90 % weight/weight (w/w).

Calorimetric measurements were performed with a Q20 Differential Scanning Calorimeter (TA Instruments). Samples were prepared using sealed aluminium pans which have been weighed with a fifth cipher balance Sartorius (Germany). All runs were performed at the rate 5 ºC.min⁻¹. The samples were treated cooling to -20ºC during 5 minutes. Then, they were heated to 150ºC at a rate of 5 ºC.min⁻¹. Eventually, they were kept at this temperature for a minute.

Optical microscopy was performed with a Nikon Eclipse E-200 POL polarizing (Tokyo, Japan) microscope. The scatters were heated until the temperature that DSC thermograms have been shown a phase change.

Results

The phase diagram showed the existence of three different lamellar liquid crystals (LLC) depending on the concentration, which show different textures. One of them exists below 60% (w/w) of surfactant, with typical thin, smooth and a few birefringent oily streaks. Other kind of LLC is in between 60 and 90 % showing thick oily streaks with transversal groove and very birefringent. Finally, at very high proportions of surfactants, the system shows thin low birefringent oily streaks, a texture quite similar to that appeared below 60 % Asc16-solution. Below 60% (w/w) the mesophase has free solvent. Between 60 and 90 % (w/w) of surfactant, all solvent is associated to polar surface. Above 90 % (w/w) there is only solvation (strongly attached) solvent and pure Asc16.

Below 60º C isotropic solution, crystals and gel phase has appeared below 60% (w/w). Above this concentration there is no free water; different types of crystals of surfactant appear.

Conclusions

The phase diagram of the system Asc16-solution (25% (w/w) PEG400 in 75% (w/w) has been done. The PEG 400 added to solution reduces the transition temperatures because it decreases the compactness of the lamellar structure21. The degree of diminution of the transition temperatures produced by this proportion of PEG400 with regard to Asc16-water system was not singinificant. However, to work with LC at room temperature, we have to achieve stabilization of this mesophase. Stable liquid crystals phase is the future of the development of Asc16 applications.

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References.

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INJECTABLE DRUG RELEASE SYSTEMS USING THERMOSENSITIVE POLOXAMER GELS

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Introduction
The utilization of polymer solutions capable of gelling in situ at temperatures closer to the physiological represent an attractive alternative for the development of drug delivery systems. The aim of the present work was to explore the potential of the combination of poloxamer and carrageenan for their application as an injectable drug release system. Progesterone is used as model drug.

Materials and methods
Materials used are: poloxamer 407 (PO407), poloxamer 188 (PO188), κ-carrageenan (CA), progesterone and sodium chloride.

Preparation of thermosensitive gel: Poloxamer gels were prepared by "cold method" described by Schmolka (1).

Dissolution of the gel and drug release: The release experiments were performed using the membraneless model.

Measurement of gelation temperature (Tgel): Tgel was determined as the temperature displayed on the thermometer when the bar magnet stops moving due to gelation.

Measurement of gel strength: The gel strength was determined by the time that the apparatus took to sink 5 cm down through the poloxamer gel.

Results
Gel dissolution: The formulations PO407/PO188/CA were prepared in the following proportions: 28/10/0 % w/w (abbreviated 28/10/0); 28/15/0 % w/w (abbreviated 28/15/0); 28/10 / 0.1 % w/w (abbreviated 28/10/0.1) and 28/15/0.1% w/w (abbreviated 28/15/0.1). Based on the results it was observed that erosion is significantly influenced by the concentration of PO188, increasing 5% its concentration, erosion decreases 12% in the gels without CA. The gels constituted by poloxamers as sole component, dissolve at a higher proportion (2). Apparently, carrageenan would strengthen gel structure, exhibiting erosion between 35-40% at the 48 hours in poloxamers gels containing the macromolecule.

Drug release: Carrageenan decreases the release of progesterone from the gels containing 10% of PO188, but this effect is not observed in the gels with 15% of PO188. Kinetic data were processed using the power model. The values of n obtained are between 0.5 and 1, suggest that, besides the diffusion mechanism, the erosion of the gels is involved in the kinetic control of drug release.

Gelation temperature: The temperature-dependent gelation of poloxamer solutions could be explained by configuration change. By increasing temperature, hydrophobic interactions between the segments of polyoxypropylene in the molecule of poloxamer increase, resulting in a most compact and more viscous gel. Tgel decreases with increasing the concentration of PO 188. Carrageenan generated a small variation, but different, in the poloxamer gels. In the case of 28/15/0 gels, the gelation temperature increase, but to 28/15/0 gels decreases. This particular behavior was also observed in dissolution studies of these gels.

Gel strength: At 5% increase in the concentration of PO188, gel strength increases about 50%. By incorporating carrageenan, the gel strength values increase considerably, about 400% for 28/15/0.1 gels and 500% for 28/10/0.1 gels. These results correlate with the obtained dissolution data.

Conclusions
Poloxamer-carrageenan gels appear to be versatile release platforms that provide a range of release rates and erosion in function of their composition. The possibility of incorporating carrageenan would reinforce the integrity of the gel, which would allow getting injectable matrices with drug release properties optimal and adequate gelation temperature.

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CHAGAS DISEASE. HYDROPHILIC POLYMERS APPLIED TO ENHANCE THE SOLUBILITY OF BENZNIDAZOLE.

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Introduction
Chagas disease is a major cause of morbidity and mortality in Latin America. Actually, it is also an emerging opportunistic infection among immunocompromised patients. One of the chosen drugs to treat this infection is benznidazole (BZL). The unique formulation available is a tablet of 100 mg and, however, despite of the growing incidence in pediatric populations, there is not any BZL liquid formulation. Therefore, in this work, different polymers were evaluated as carrier for improving the solubility of BZL, in order to preformulate liquid dosage forms of that compound.

Materials and Methods
BZL was a gift from Roche. Polyvinylpirrolidone K-30 (PVP) was kindly donated by ISP (Argentina). Pluronic was purchased from Merck. Polyethylene glycols (PEG_{6000} and PEG_{400}) were purchased from Parafarm.

Solid dispersions. The systems were prepared by the solvent method. 50 mg of BZL and the corresponding polymer were dissolved in 3 ml of ethanol and water respectively. Both solutions were mixed and the solvents were removed under reduce pressure at 50 ºC until complete evaporation.

Physical mixtures. Powders were mixed in a mortar with a pestle until a homogeneous mixture was obtained.

Solubility determinations. Drug solubility was determined by adding excess amounts of BZL solid dispersions to water at 25 ± 0.5 ºC for 48 hs and then filtered. BZL concentrations were determined by UV spectroscopy at 324nm.

Cosolvent systems. PEG_{400} and water mixed at different ratios were selected as cosolvents to study the solubility of BZL-PVP dispersions.

Physicochemical characterization. Differential Scanning Calorimeter (DSC), Scanning Electron Microscope (SEM), and Nuclear magnetic resonance (NMR) were applied to analyze potential drug-polymer interactions.

Results
The solubility of BZL was greatly increased by means of PVP and Pluronic-based solid dispersions at 1:5 drug:polymer ratio, while PEG 6000 did not influence the drug solubility. In addition, water-PEG_{400} cosolvent mixtures were more efficient than water alone for improving BZL solubility, particularly at 9:1 PEG_{400}:water ratio. By DSC, it was observed the complete absence of BZL crystalline peaks when dispersed into the polymers. SEM analysis showed that the solid dispersions appeared in the form of irregular particles in which the original morphology of both components disappeared and amorphous pieces of irregular size were present.

Conclusions
The increased solubility of BZL was achieved by solid dispersions as well as using cosolvent mixtures. The solid characterization of BZL-polymer mixtures showed that a decreased crystallinity, increased wettability, and reduction of drug particle size modified the drug solubility. In addition, NMR experiments provided potential to study the interactions of BZL with PVP.
ASSESSMENT OF SAFETY AND EFFICACY OF A NOVEL INTRAUTERINE CIPROFLOXACIN SUSTAINED RELEASE HYDROGEL FORMULATION IN MARES WITH ENDOMETRITIS

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Introduction

Several therapeutic strategies with antimicrobials were developed to reduce the bacterial infection in mares susceptible to endometritis. Fluoroquinolone antibiotics offer some alternatives to current veterinary therapy. Enrofloxacin given intravenously and formulated in solution at 5 mg/kg q 24h by 7-10 days is the often therapeutic used in clinical practice with controversial efficacy. Enrofloxacin undergoes biotransformation given a more potent metabolite named ciprofloxacin (CIP). Both moieties exert the antibacterial mode of action in a dependent concentration way by which the intrauterine administration of a sustained release formulation of those compounds may be a potential tool to improve the efficacy against this bacterial disease.

Objectives

The main goals of this research were a) To assess the safety of the intrauterine administration of a novel hydrogel formulation CIP- based, b) To assess the efficacy of the novel formulation when given at single dose in mares with endometritis.

Materials and Methods

A new carbomer hydrogel CIP-based was developed for intrauterine administration. NaOH was used to adequate the viscosity and also the pH=7 of the formulation. The pharmaceutical formulation was prepared in a concentration of 0.4 mg of CIP/100 mL, sterilized and upon given intrauterine at single dose in a final volume of 50 mL). Previous studies demonstrated that the hydrogel system when confronted with biological fluids (in vitro study), release supra-inhibitory concentrations of CIP over 24 post administration.

Study 1: Seven mares (10-23 year old) were involved in this trial. Three of them without endometritis, (confirmed by histopathological and microbiological studies) and, four mares recruited with clinical endometritis. Endometrial biopsies to evaluate the inflammatory process were taken in estrus before and after the intrauterine administration of the novel formulation at 0, 24, 72 y 120 h post-treatment. Samples were stored and analyzed for histopathology by conventional methods.

Study 2: Nine mares (12-23 year old) with confirmed clinical endometritis (ultrasonography, endometrial biopsy and pathogens positives), were involved in this efficacy trial. The assessment of the efficacy of the novel formulation was made by microbiological culture and endometrial biopsies at pre-treatment (0 h) and post-treatment (168 h). Microbiological samples were taken with protected swabs, stored in Amies medium and cultured and typified.

Results

Study 1: After the intrauterine administration of the CIP hydrogel endometrial free fluid was not detected by ultrasonography at 24, 72 and 120 h post-treatment. In health mares was observed on the luminar epithelium an increase of Polymorph Nuclear Cells (PMN) (P<0.001) at 24 h post-treatment when compared with samples taken pre-treatment (0h). PMNs back on to normal at 72 and 120 h post-treatment indicating that the formulation induces a slight inflammatory process. The same trend was observed in mares with endometritis.

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Study 2: *Escherichia coli* and *Streptococcus zooepidemicus* were the main bacteria isolated in mares with endometritis. After the intrauterine administration at single dose of the hydrogel system CIP-based, 6/9 mares resulted in clinical and microbiological cure, showing a 66% of efficacy. **Conclusions**

The application of the novel sustained release hydrogel CIP-based system showed safety on the uterine tissue and high efficacy at single dose being a potential alternative for using in equine veterinary therapeutics.
EVALUATION OF ANTIBIOTIC AND ANTIBIOTIC/ANALGESIC HYDROGELS WITH POTENTIAL UTILITY AS TOPICAL THERAPY

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Topical therapy is a convenient way to administer drugs, optimizing concentration in the target site and/or minimizing systemic exposition. Hydrogels are an important class of biomaterials. Since their ability to uptake exudates, sustained release of drugs and convenient biocompatibility, rheological and bioadhesive properties, these systems are potentially suitable for therapeutic uses. A fundamental understanding of mechanical properties, drug release and underlying molecular mechanisms is crucial for determining whether these biomaterials.

Goal.
Develop a ciprofloxacin (or enrofloxacin)-loaded hydrogel and a ciprofloxacin/lidocaine-loaded hydrogel, with antibiotic and antibiotic/analgesic sustained release properties with potential utility in human and/or veterinary topical therapy.

Materials and Methods
Series of carbomer-ciprofloxacin (or enrofloxacin) and carbomer-ciprofloxacin-lidocaine hydrogels were prepared by neutralizing a 0.5-1% dispersions of carbomer with variable amounts of NaOH solution, followed by addition of constant concentration of aqueous dispersions of the drugs (ciprofloxacin and enrofloxacin 0.3%, lidocain 0.67%). The hydrogels were sterilized (autoclave) and subjected to visual observation, weight changes, pH determination, rheology measurements and kinetic release studies in simulated biological fluids. Experiments were carried out at temperatures simulating conditions of ambient (25ºC), eye (32ºC), skin (35ºC) and mucosa (37ºC). Stability was assessed at 23ºC y 40% HR during 6 months by HPLC methodology. Irritation and refreshing potential (using silver sulfadiazine cream as a reference) were assessed by Draize1 and modified Tail Flick tests.

Results
The pH values ranged 6.80-7.01. Differences in NaOH produced small modifications of pH, suggesting high buffer capacity, related to the overlapping of acid-base equilíbrium of drugs and carbomer. Although pH increases with temperature (p<0.05), owing to ionic pairs dissociation2,3, the systems maintain within the physiologically recommended pH range for topical administration. The hydrogels obtained at 0.5% of carbomer showed the best subjective and rheologic characteristics (plastic flow, yield stress=90 Pa/seg, independent of temperature) to be applied to skin and mucous tissues.

No physical or chemical modifications neither of the drugs nor the formulations were observed after sterilization and storage period, suggesting they are stable and can be obtained as sterile products. In addition, no irritation signs emerged after application to skin (primary irritation index=0). Longer reaction times to the heat were observed after application of hydrogels to healthy skin compared to reference, suggesting an immediate refreshing effect due to water evaporation.

Release profiles fitted to Higuchi release kinetics, with sustained release up to 24 h in presence of biological fluids. Bactericidal and minimal analgesic concentrations were achieved in the first 30 and 10 min, respectively. Enrofloxacin released 1.96 folds faster than ciprofloxacin probably due to the steric hindering produced by ethyl portion of enrofloxacin. An increase in release rate with temperature (p<0.05) was also observed, matching pH observations. When lidocaine was incorporated, a reduction in ciprofloxacin release rate was observed. Although statistically significant, differences observed are too small to have a clinical impact.

Conclusions
The obtained systems have a smart release behavior. They are stable and can be obtained as sterile products. Their antibiotic and antibiotic/analgesic properties suggest they could be potential candidates for the topical prophylaxis and/or treatment of susceptible infections such as wounds burns and endometritis equine. Uterous biocompatibility and efficacy studies are in progress.
Acknowledgment

This work was supported by SECyT-UNC, FONCyT and Ministerio de Salud de la Nación Argentina, Breda S thanks for the CONICET fellowship.

References


RHEOLOGICAL AND TENSILE PROPERTIES OF SODIUM HYALURONATE HYDROGELS AND FILMS

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2Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina.
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Introduction

The hyaluronic acid sodium salt, also called hyaluronan (HA), is a naturally occurring high molecular mass linear polysaccharide, consisting of alternating units of N-acetyl-β-D-glucosamine (GlmNAc) and β-D-glucuronic acid (GlcAc). HA is distributed in the human body forming part of synovial fluid, skin, umbilical cord and vitreous humor; and widely used in medical practice in many pathological conditions such as osteoarthritis, wound repair and eye surgery (1-5). Several studies related to biocompatibility and biodegradability (6) have suggested the use of HA as a promising biomaterial to design modified drug delivery systems with ophthalmic applications. In this work we present preliminary studies in order to evaluate rheological and mechanical properties of hydrogels and films based on HA prior to further structural modification needed to improve the biomechanical properties of HA.

Materials and methods

HA solutions were prepared at 1, 2 and 4% w/w concentrations using distilled water as solvent. Additional solutions were also prepared containing 2% w/w HA and different concentrations 0.25, 0.5 and 1% w/w of polyethyleneglycol 400 (PEG) (Merk). The solutions have pH near 6, avoiding polymer degradation (7,8).

Hydrogel films from the 2% w/w HA and 2% w/w HA/PEG solutions were prepared by casting at room temperature. The rheological characterization of the different solutions was carried out in small-amplitude oscillatory shear flow using a rotational rheometer from TA Instruments (AR-G2) 25°C. Stress-strain plots were obtained in an Instron 3369 tester in traction mode at 2 mm/min at room conditions (23°C and 40% relative humidity).

Results

Both storage (G’) and loss (G’) modules values increase with HA concentration. In all cases, the module G’ at low frequencies is higher than G’ to certain point where the crossover of the G’ and G’” curves occurs. Increasing HA concentration moves the crossover point toward lower frequencies indicating an earlier transition from viscous-like towards elastic behavior. This shift is less significant in HA/PEG solutions. The incorporation of PEG slightly increases the magnitude of G’ and G’” on the whole frequency range, although no significant difference was observed with different PEG concentrations used in this work.

Table 1 shows elongational properties for the pure HA films and those containing 11, 20 and 33% w/w of PEG. Multiple dog-bone shaped probes for each sample were tested in order to obtain mean values for each property. Increasing PEG content diminish the elastic modulus inducing a change from fragile to ductile behavior characterized by a yield zone at low deformations followed by drawing with no significant changes in the nominal stress up to the rupture point.

Future adhesion assays will be performed to complete preliminary studies.

Acknowledgments

The authors thank financial support from Universidad Nacional de Sur (UNS), the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC).

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References


Table 1. Elongational properties

<table>
<thead>
<tr>
<th>Sample</th>
<th>Thickness (µm)</th>
<th>Elastic modulus (MPa)</th>
<th>Maximum Tensile Strain (%)</th>
<th>Yield Stress (MPa)</th>
</tr>
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<tr>
<td>2% HA</td>
<td>38 ± 3</td>
<td>2564 ± 165</td>
<td>10.9 ± 2.3</td>
<td>77.9 ± 2.7</td>
</tr>
<tr>
<td>2% HA – 0.25% PEG</td>
<td>42 ± 3</td>
<td>2061 ± 282</td>
<td>17.1 ± 0.7</td>
<td>65.3 ± 4.5</td>
</tr>
<tr>
<td>2% HA – 0.5% PEG</td>
<td>47 ± 3</td>
<td>1593 ± 158</td>
<td>29.7 ± 2.6</td>
<td>48.1 ± 4.9</td>
</tr>
<tr>
<td>2% HA – 1.0% PEG</td>
<td>59 ± 5</td>
<td>853 ± 91</td>
<td>50.9 ± 4.9</td>
<td>26.4 ± 2.4</td>
</tr>
</tbody>
</table>
EVALUATION OF RAPIDLY DISINTEGRATING TABLETS BASED ON SOLID DISPERSIONS.

Castro SG*, Allemandi DA and Palma SD.

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Ciudad Universitaria, Córdoba, Argentina CP: 5000

Introduction:
Solid dispersions (SDs) have been proposed as an alternative to improve the dissolution rate of low solubility drugs.
In previous studies, SDs containing albendazole (ABZ) and poloxamer188 (P188) were formulated. The dissolution profiles indicated that ABZ incorporated in SDs (as multiparticles) was rapidly released compared with free ABZ.
The aim of the present study was to develop and evaluate rapidly disintegrating tablets (RDT) containing ABZ – P188 DSs.

Materials and methods:
DSs were prepared by the melting method. Directly compressible rapidly disintegrating tablets using 200 mg of ABZ, 732 mg of Ludiflash™, 60mg of sodium crosscaramelllose and 8mg of magnesium stearate were prepared at different compaction forces (250, 500 and 1000 mPa). Tablets containing only ABZ were used as controls.
Powder flowability and tablet mechanical properties were measured.
The dissolutions tests were performed using 0,1N HCl and Gastric Simulated Solution pH:1,2 (GSS) + Sodium Lauryl Sulfate (SLS) 0,25 % p/v as medium.

Results:
Table 1 shows the effect of compaction force on the hardness and disintegration time of the tablets. ABZ incorporated in SDs tablets was released faster than multiparticle systems. So, this formulation would be advantageous from a pharmaceutical point of view, compared to powdered solid dispersions. As might be expected, tablets containing ABZ without P 188 showed lower dissolution rate than those containing DSs.
The presence of SLS improved drug dissolution. The surface tension of the medium and the wettability of the drug would be playing a central role in the dissolution of ABZ. (Table 2 and 3).

Conclusions:
ABZ dissolution was improved when formulated in SDs. However, this property has to remain unaltered when these SDs have to be vehiculized in tablets. In this work, we developed a RDT by using a coproccesed granular excipient (Ludiflash™). The main characteristic of these tablets was its very fast disintegration which facilitated the immediate exposure of powdered SDs to the dissolving medium. So, drug release was even faster. Besides, the deleterious effect of compaction force on disintegration time was practically negligible.

References.
2- Vasconcelos T., Sarmento B and P. Costa. Solid Dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discovery Today 12: 1068-1075

* Corresponding author. Tel +54 351 4334163, fax +54 351 4334127; e-mail: castrosilvina@fcq.unc.edu.ar
<table>
<thead>
<tr>
<th>Force (mPa)</th>
<th>ABZ Hardness (Kg/cm²)</th>
<th>Disintegration time (min)</th>
<th>ABZ (SDs) Hardness (Kg/cm²)</th>
<th>Disintegration time (min)</th>
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<tr>
<td>250</td>
<td>1,08 ± 0,29</td>
<td>0,75</td>
<td>2,66 ± 0,46</td>
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<tr>
<td>500</td>
<td>1,48 ± 0,28</td>
<td>0,75</td>
<td>4,34 ± 0,53</td>
<td>2,66</td>
</tr>
<tr>
<td>1000</td>
<td>2,34 ± 0,59</td>
<td>1</td>
<td>8,12 ± 1,53</td>
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Table 1

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<th>Time</th>
<th>% of ABZ dissolved in 0,1N HCl</th>
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<tr>
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<td>ABZ F250 F500 F1000 DS F250 F500 F1000</td>
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<td>0</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>1</td>
<td>- 2,93 3,45 4,14 40,13 18,13 20,76 9,19</td>
</tr>
<tr>
<td>3</td>
<td>- 9,81 11,94 12,63 42,59 36,21 39,33 19,86</td>
</tr>
<tr>
<td>5</td>
<td>3,01 15,55 19,76 20,28 46,33 45,42 46,68 28,58</td>
</tr>
<tr>
<td>10</td>
<td>- 26,06 28,99 27,87 50,58 55,08 56,35 41,07</td>
</tr>
<tr>
<td>15</td>
<td>3,44 31,55 34,72 34,79 52,37 59,48 59,56 49,29</td>
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Table 2

<table>
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<th>Time</th>
<th>% of ABZ dissolved in GSS pH:1,2 + SLS 0,25 % w/v</th>
</tr>
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<tr>
<td></td>
<td>ABZ F250 F500 F1000 DS F250 F500 F1000</td>
</tr>
<tr>
<td>0</td>
<td>0 0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>1</td>
<td>4,09 28,31 11,87 13,23 47,70 32,65 32,49 11,54</td>
</tr>
<tr>
<td>3</td>
<td>7,63 31,24 32,50 30,88 62,44 57,18 57,15 34,49</td>
</tr>
<tr>
<td>5</td>
<td>33,92 42,68 51,99 44,07 69,22 70,65 70,54 48,71</td>
</tr>
<tr>
<td>10</td>
<td>- 59,93 62,88 63,30 72,36 78,28 82,15 70,76</td>
</tr>
<tr>
<td>15</td>
<td>49,88 66,56 72,78 70,18 78,76 84,79 81,49 82,54</td>
</tr>
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Table 3
NEW THERMO/pH -RESPONSIVE MATERIALS CONTAINING OFLOXACIN. PREPARATION AND PHYSICOCHEMICAL AND IN VITRO RELEASE BEHAVIOUR

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Introduction
A hydrogel is a kind of polymeric three-dimensional network that exhibits ability to absorb and to swell in water, maintaining its form until attaining a certain equilibrium balance. This ability makes them interesting materials as drug controlled release systems (1-4). In this work, the development of new hydrogels based in N-isopropylacrylamide/acrylic acid copolymer with potential utility as drug delivery systems by via oral was performed. Ofloxacin (Ofx), a fluoroquinolone antimicrobial agent, was selected as model drug.

Materials and methods
All hydrogels were prepared by free-radical crosslinking polymerization using N-isopropylacrylamide (NIPA) and acrylic acid (AAc) as monomers and diallyltartradiamide (DAT) as crosslinking agent. NIPA(100) and NIPA(70)/AAc(30) were yielded and analyzed by IR, equilibrium weight swelling ratio ($q_w$), swelling kinetic, determination of rate of diffusion of water within the matrix, structural parameters of the network [molecular weight between crosslinking points (Mc), volume fraction of polymer in swollen state ($v_{2s}$) and pore size ($\xi$)] and DSC and rheological studies. Release studies of materials as matrices were performed using USP-dissolution apparatus 2, at 50 rpm with 900 mL of simulated gastric (SGF, pH= 1.2) or intestinal (SIF, pH=6.8) fluids at 37 °C. Ofloxacin dissolved was measured by UV-spectroscopy at 287 nm. Kinetics data was calculated from release profiles.

Results
The products [NIPA(100) and NIPA(70)/AAc(30)] were obtained in rod shape with high yields. They presented different $q_w$ values (Table 1) and kinetic of swelling according the monomers composition and pH (6.8 and 1.2) or temperature (25 and 37°C) of the swelling medium.

In general, NIPA(100) presented very high swelling ratios at both pH values at 25°C, while a drastic collapse was observed when the temperature increased at 37°C. At 25°C, the load of the matrix with Ofx produced a diminution of the swelling (at both pH values) due at a possible interaction drug-matrix, although a major rate of swelling was observed respect to the uncharged gels.

The product NIPA(70)/AAc(30) presented high swelling behavior at 25°C at pH 6.8 while a diminution in swelling was observed at pH 1.2. A less pronounced collapse was observed, respect to NIPA(100), when the temperature increased at 37°C. Additionally, the drug loading in this material (31.2 % p/p) results 12.5 times higher than NIPA(100).

A low release rate of Ofx (0.4 mg/min) and zero order kinetic was observed in SGF. Whereas, an increase close to 7.4 times in the release rate was observed with the pH change of dissolution medium. Additionally, a change in kinetic control (anomalous kinetic) was observed. These results could be associated with the physicochemical properties of hydrogels.

Conclusions
- New hydrogels were prepared from NIPA, AAc and DAT. The effect of the incorporation of AAc into polyNIPA structures was analyzed.

It was concluded that:
- The composition of monomers in the products and the temperature and pH of swelling influenced the network parameters of the hydrogels.
- High drug loading was reached
- The release rate of Ofx and the kinetic control was sensible to change of pH dissolution medium.

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The new material showed good properties to develop oral drug delivery systems with application on site-time controlled drug release.

Acknowledgments
The authors thank SECYT, FONCyT and CONICET for financial assistance. J. Cuggino also acknowledges receipt of a fellowship from CONICET.

References

<table>
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<td>Sample</td>
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<tr>
<td>NIPA(100)</td>
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<td></td>
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<tr>
<td>NIPA(70)/AAc(30)</td>
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COMPARATIVE STUDY IN VITRO OF THE RELEASE OF METRONIDAZOLE FROM DERMATOLOGICAL PRODUCTS

Costa Edda1*, Correa O1, Muñoz JP2, Plasencia MJ2, Escalona A1

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Introduction
Rosacea is a chronic skin disorder characterized by transient or permanent redness of the skin, usually limited to the center area of the face, which may be accompanied by pustules, papules and telangiectasia as well as itching and burning sensation on the skin (1-7). Topical metronidazole is one of the first-line treatments used in this disease. In our country, topical metronidazole is marketed by different pharmaceutical companies as a gel and a cream at 0.75%. It is also prescribed as a compound custom medication in gel form at similar concentration. In this study we evaluated the in vitro release of metronidazole from a compound medication prepared in gel form and two commercial products, a gel and a cream.

Materials and Methods
The analytical methodology proposed by the USP 30 for metronidazole was a UV spectrophotometry technique. This was validated in terms of linearity, range, precision, accuracy and specificity (8,9). We standardized the method to evaluate the release of metronidazole using the modified apparatus 5 of the USP by means of its precision (Category III). For this, the values obtained in each vessel were utilized to obtain the coefficient of variation.

The method used to evaluate the release from metronidazole formulations was a modification of the Apparatus 5 of the USP 30 (paddle over disk), originally used for the evaluation of the release from transdermal patches. The experimental conditions for testing the in vitro release were 50 rpm, 900 mL, 32°C and deaerated distilled water as the dissolution medium.

The kinetic order was calculated using the equation of Peppas. Also other kinetic models (zero order, first order and Higuchi) were studied in order to deepen about the release mechanism of metronidazole from the tested products considering the factor of determination (r²) as a basis for comparison. The statistical treatment of the values of the constants of average release rate of metronidazole from gels studied (P1 and P2) was conducted using analysis of variance and multiple range test (Statgraphics Plus 5.1 software).

Results
The analytical methodology to quantify metronidazole by UV was validated and met the acceptance criteria outlined in the ICH and USP 30.

The release method was validated with regard only to its precision, as pointed out by the validation recommendations of the USP 30. The results show a similarity between the products P1 and P2 and a noticeable difference with the product P3 (cream).

The results obtained by Peppas equation indicate that the release kinetics of metronidazole given for the three products is approaching a Higuchi model. The release kinetics of metronidazole was adjusted to an apparent first order for the three products studied.

The results showed significant differences between gels and cream. The gel products (reference and compound medicine) turned out to be similar, according to the calculation of the difference factor f1 and f2 similarity factor.

Conclusions
In this study was compared the in vitro release of metronidazole from a compound medicine prepared in gel form and two commercial products, gel and cream obtaining significant differences between gel and cream products, while products in gel form were similar.

These results indicate that the commercial and the compounding gel should have similar efficacy in vivo.
References
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SWELLABLE DRUG POLYELECTROLYTE MATRICES: PRELIMINARY RHEOLOGICAL STUDIES ON CARBOMER-CIPROFLOXACIN-SODIUM SYSTEMS


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2Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina.
3Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

Introduction
Many controlled drug delivery systems have been introduced in pharmacotherapy along time exhibiting a number of advantages over conventional dosage forms. In the early works, novel swellable drug polyelectrolyte matrices (SDPM) obtained by compaction of powdered complexes of a polyelectrolyte (PE) fully or partially neutralized with a ionizable drug (D) have been developed using current tabletting technology. Unlike traditional swellable hydrophilic matrices, SDPM’s contain a molecular dispersion of D in the mass of the matrix where D is ionically bonded to the functional groups of PE as a (PE-D) complex. This is relevant to obtain: drug controlled release, drug chemical stability and aqueous compatibility increase, and/or multiparticulate delivery systems. Extended release SDPM, containing Carbomer-Ciprofloxacin (500mg) complex (CB-Cip), were prepared and evaluated by Bermúdez et al. They showed that water sorption and release properties were highly sensitive to material composition. It is known that rheological studies can offer detailed structural information about dispersed polymeric system.

For this reason, a preliminary rheological study was conducted to associate the viscoelastic characteristics (G’, G’’, tan δ) of the hydrated matrices as a function of Na+ and water contents with the water sorption and Ciprofloxacin release behavior from SDPM.

Materials and methods
(CB-Cip)50 complexes were prepared (subindex 50 meaning the Cip molar percentage with respect to the CB ionizable carboxylic groups). Then, NaOH 1M was added in order to obtain a molar concentration of 5, 10 and 20% of sodium in the complexes. The resulting samples, labeled as (CB-Cip)50, (CB-Cip)50Na5, (CB-Cip)50Na10 y (CB-Cip)50Na20, were characterized by Differential Scanning Calorimetry (DSC) in a PerkinElmer Pyris I calorimeter.

The sodium containing complexes were hydrated using physiological solution (PS) at different concentrations, ranging from 19 to 29% w/w, depending on the ability of the powdered samples to absorb PS to acquire adequate consistency for the rheological analysis. Rheological characterization was performed in a TA Instruments AR-G2 rheometer, in small amplitude frequency sweep tests using 25 mm diameter parallel plates, at room temperature. In order to avoid dehydratation during the test the samples located between the plates were protected with thin layer of a low viscosity silicon oil.

Results and conclusions
DSC analysis showed that the Cip characteristic fusion peak of neat Cip (265-268°C) was not observed in any of the (CB-Cip)50Na complexes confirming the complete interaction between CB and Cip. The complexes showed a narrow window on their capacity to absorb PS in order to obtain the desired consistency for the rheological analysis. A further addition of PS caused the formation of a second phase.

From the preparation of the samples for rheological tests, it was inferred that increasing the sodium concentration on the (CB-Cip)50Na complexes, increases the capacity of the complexes to absorb PS up to Na contents of about 10%. Higher contents of Na do not further augment the amount of PS absorbed by the complexes.

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The samples with different Na content showed similar rheological behaviour, $G'$ being always higher than $G''$ in frequency sweep shear tests. Log $G'$ increased almost linearly with respect to log $\omega$ in the frequency range studied, while $G''$ evolved from a plateau until approximately $1 \text{ rad/s}$ and then followed a growth pattern similar to that of the $G'$ curve.

Acknowledgments
The authors thank financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional del Sur (UNS) and Universidad Nacional de Córdoba.

References
INFLUENCE OF BIOPOLYMERS ON SURVIVAL OF MICROENCAPSULATED
*LACTOCoccus LACTIS* IN THE PRESENCE OF ACID, BILE SALTS AND
PHOSPHATE BUFFER.

**Dusso, D.; Wendeler, L.; Ramanzín, F.; Salomón, C. J.**


**Introduction**
Probiotics are live microorganisms that administered in adequate amounts, $10^6-10^7$ CFU/mg, confer a beneficial effect on the health of the host, being able to be used for therapeutic purposes. However, these bacteria are sensitive to gastric secretions and bile salts. Therefore, is necessary to develop systems capable of protecting them. Cell microencapsulation in alginate matrix has been widely used to improve their viability in gastric medium. However, the protection of probiotics using alginate mixed with other biopolymers has been not investigated deeply.

**Materials and Methods**
Microencapsulation methodology: sodium alginate and each of the polymers (Carbopol®, carboxymethylcellulose, pregelatinized starch, chitosan), were dissolved in distilled water. The encapsulated *Lactococcus lactis* NZ9000 were grown in liquid medium (M17). The cells were centrifuged and resuspended with sodium alginate solution and the polymer. The suspension formed drip was added to a solution of calcium chloride 3% with stirring yielding alginate microparticles with encapsulated bacteria. The solid was filtered and washed with sterile distilled water and dried at 30 °C. Encapsulation test: The microparticles were dissolved in buffer pH 7.40. It took an aliquot of the solution and serial dilutions were made, sowed on plates with half –Agar M17, incubated 24 hours at 30 °C and then came the plate count.

Tolerance acid and bile salts test: The microparticles were incubated 2 hours at pH 1.5. Another batch of microparticles was incubated for 2 hours in a solution of 0.6% bile salts pH 7.4. An aliquot of each batch and dilutions were planted in half M17-Agar (24 hs at 30 °C) and then counting the colonies, expressing the results as CFU / g of microparticles.

Release rate of bacteria: The microparticles were dissolved in buffer pH 7.4 and aliquots were taken at different times, appropriate dilutions were made, sowed on plates with half –Agar M17, and then counting the number of colonies after 24 hours incubation at 30 °C.

**Results**
High cell loading (> 70%) was achieved by formulating alginate with carboxymethylcellulose. Survival of the microorganisms was found to increase on alginate-carboxymethylcellulose in comparison with free cells, and alginate pure encapsulation at different conditions of pH and bile salts. Carbopol®, chitosan, and pregelatinized starch did not show any advantanges over pure alginate microcapsules on the viability of the cells. In size and shape the microparticles, characterized by light and electron microscopy, we observed that ranged in size from 550-650 microns.

**Conclusion**
Microencapsulated cells of *Lactococcus lactis* NZ9000 in alginate beads formulated with cellulose derivatives resulted in better survived than for free cells after sequential incubation in simulated gastric, intestinal and bile salts fluids.

**References**
POLYURETHANE/POLY(2-(DIETHYL AMINO) ETHYL METHACRYLATE): A POLYMERIC BLEND FOR DRUG DELIVERY APPLICATIONS

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Introduction
A previous study of 2-(diethyl amino) ethylmethacrylate (DEA)-based microgels (1) shows that they are film forming. A simple way to improve the physical characteristics of films formed from pure polyDEA (PDEA) is by blending PDEA with polymers like polyurethanes (PU) that also reduces the cost of the material. In this work, blends 50:50 of PU and PDEA were prepared and characterized for water swelling capacity, water vapor transmission and in vitro drug release using theophylline as delivery marker.

Materials and methods
The materials and methods used for the synthesis of polymers dispersions are already described (2,3). For release details see reference 1. Data were adjusted to a power-law type relationship (4):

\[ C_t = K t^n \]  

where \( C_t \) is the theophylline cumulative concentration and \( n \) is the exponent depending on the release process.

Water vapor transmission (WVT, g m\(^{-2}\) s\(^{-1}\)) through the films was determined using an ASTM method (5).

For dynamic swelling studies, the material was immersed in water at 25\(^\circ\)C. Data were adjusted to a first order equation (6).

Results
Drug delivery. \( C_t \) as a function of release time is well fitted by Eq. [1], with \( n = 0.67 \) and \( k = 1.2 \times 10^{-6} \) M. s\(^{-n}\). The \( n \) value points to an anomalous drug transport (4).

Water Vapor Transmission (WVT). The weight gain of the permeation cells as a function of time showed linear behavior. A WVT of \((2.20 \pm 0.18) \times 10^{-3} \) g s\(^{-1}\) m\(^{-2}\) was obtained, at 29 °C and relative humidity of 86 %.

Swelling Dynamics. The water swelling behavior is well fitted by \( Q_t = Q_{\text{max}} (1 - e^{-kt}) \). Data up to 80 % of \( Q_{\text{max}} \) have been analyzed by \( Q_t = k' t^m \) (7). The \( m \) value of 0.51 indicates that the water uptake process is mainly diffusion controlled.

Conclusions
PDEA and PU can be blended to prepare films, suitable for drug delivery applications. This material shows a maximum water uptake of 54 % with a diffusion controlled process. This film has a moderate water vapor transmission and shows non-Fickian behavior in drug release.

References

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STUDY OF THE INFLUENCE OF TERNARY SYSTEMS ON THE AQUEOUS SOLUBILITY OF SULFADIZINE. PREPARATION AND CHARACTERIZATION OF COMPLEXES IN SOLID STATE.

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Introduction
Sulfadiazine (Sdz) is used for the cure of infections. The solubility of SDZ in water is very low, approximately 0.074 mg/ml at 25°C (1), which affects negatively its bioavailability. Cyclodextrins (CDs) interact with poorly-water soluble compounds to increase their apparent solubility. However, the amount of CD that can be used in most pharmaceutical formulations is limited. Therefore, in order to reduce the amount of CDs necessary to obtain the desired drug solubilizing effect, it is important to find effective methods to adequately improve their performance (2).

The aim of the present work was to study the influence of βCD and Leucine (Leu) on the aqueous solubility of SDZ and also, the preparation and characterization of Sdz-βCD-Leu complexes.

Materials and Methods
Sdz was obtained from Parafarm, βCD was a gift from Ferromet S.A. and Leu was obtained from Sigma-Aldrich. All experiments were performed with analytical grade chemicals and solvents.

Solubility diagrams were obtained according to Higuchi and Connors (3). Excess amounts of Sdz were added to water solutions containing varying concentrations of βCD and a constant concentration of Leu. Ternary solid systems were prepared with an equimolar ratio of Sdz, βCD and Leu, according to the previous phase solubility studies, using two distinct methods: physical mixtures (PM) and freeze-drying (Free). The IR spectrum and the DSC-TG curves of the Sdz-βCD-Leu freeze-dried product were compared with those of the physical mixture and the pure β-CD, Sdz and Leu.

Result and Discussion
The phase-solubility diagram of the Sdz-βCD-Leu system, showed a linearly increase in the solubility of the drug with increasing βCD concentration at a constant concentration of Leu. This diagram could be classified as A_L, according to the model proposed by Higuchi and Connors, and related with the formation of a soluble inclusion complex. The stability constant (Kc) values for the corresponding complexes, were calculated from the slope of the phase-solubility diagram. The Kc for the Sdz-BCD-Leu system was greater than that of Sdz-βCD one, while the solubility values were very similar. The aqueous solubility of Sdz exhibited no change in the presence of Leu only.

The results obtained by IR, DSC and TG showed the presence of a true inclusion complex, Sdz-βCD-Leu, when it was prepared by freeze-dried, and noting a slight interaction when it is formed by PM.

Conclusion
An increment in water solubility was obtained for Sdz and the combination of the different analytical methods used (DSC, TG and IR) provided evidence of complexation, which will allow the development of pharmaceutical products using these complexes containing Sdz as active ingredient.

Acknowledgments
We thank Ferromet S.A (agent of Roquette in Argentina) for its donation of β-cyclodextrin.

References

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SUPERAGREGATES FROM AMPHOTERICIN B MICELLES: A NEW WAY TO DECREASE ITS TOXICITY

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Introduction
The Amphotericin B in a micellar system (AmB) is a drug largely used for the treatment of systemic fungal infections, especially in immune-compromised patients. However, its use is limited due to its high toxicity, which is the main cause of side effects such as nephrotoxicity (1). There are several intravenous amphotericin B lipidic formulations that are less toxic, but presenting high cost. According to the literature, in aqueous solution there are the monomeric and aggregated forms of AmB, these latter being responsible for its side effects (2). Lately, it has been observed that the heating of AmB solutions generates “superaggregates” forms, which are produced by the condensation of monomeric and aggregate forms. This new state has been demonstrated to be less cytotoxic while keeping its activity (1). The aim of this work was not only to investigate the changes in the spectrum of the unheated and heated AmB solutions, following the bands of “superaggregates”, monomer and aggregates forms, but also to evaluate its toxicity.

Materials and methods
A spectroscopic study was performed in the wavelengths from 300nm to 450nm at four different concentrations (from 50 to 0.05mg.L⁻¹) of unheated and heated AmB. It is important to attempt that those values to the blood concentration following intravenous administration. For the in vitro toxicity assay, red blood cells from healthy human donors were used representing a mammalian (cholesterol containing) cell model. Then, potassium and hemoglobin leakage from red blood cells were monitored, respectively, as a measure of acute and chronic toxicity. Such protocol was previously approved by the UFRN’s Ethical Committee.

Results
The spectrum of heated AmB revealed a decrease in the monomers concentration (409nm) at high concentrations, and an increase on the “superaggregates” form (323nm) when compared to the unheated ones. Concerning the toxicity, unheated and heated AmB showed different behavior: the hemoglobin leakage from unheated AmB, especially at high concentrations, was higher than the one found to the heated AmB, whose values tended to zero. Similar profile was found to the K⁺ leakage.

Conclusions
The heated AmB showed to be much less toxic than the unheated ones, highlighting this new procedure as a simple, inexpensive and safe alternative for the future treatment of systemic fungal infections.

Acknowledgments
CNPQ, CAPES and UNICAT

References

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TECHNOLOGICAL DEVELOPMENT AND EVALUATION OF MICROBIOLOGICAL EMULSIFIED SYSTEM FOR THE TREATMENT OF DIAPER DERMATITIS

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Introduction
Diaper dermatitis, also known as diaper rash, refers to inflammation of the skin covered by nappy, affecting 50% of infants (1-4). Treatment usually involves increasing the frequency of diaper changes, using superabsorbent disposable diapers, and applying topical agents such as ointments or creams using zinc oxide (ZnO). When secondary \textit{Candida albicans} infection is present, a topical antifungal agent is beneficial (1, 5-8). The aim of this work was to develop and characterize emulsion with sesame oil, a scientifically proven medicinal oil (9, 10), as lipophilic phase, added of zinc oxide.

Materials and methods
Phase diagrams were built by visual inspection of the surfactant (S) and co-surfactant (CS) admixtures [Tween\textsuperscript{®} 20 - Vetec Química fina Ltda, Brazil (S) and Span\textsuperscript{®} 80 Vetec Química fina Ltda, Brazil (CS)] at the percentage rate of 10:0 to 0:10. To this S/CS admixture, sesame oil (Vital Átman Ltda, Brazil) was added in the proportions from 1:9 to 9:1, respectively and the final mixture was titrated with distilled water. The dispersed systems obtained during the performance of the phase diagrams were classified according to their physicochemical aspects. After the construction of the phase diagram, seven cream emulsion formulations were obtained from a certain region of these pseudo-ternary diagrams. The ZnO (Mapric) was incorporated into the CEMs at the concentration of 10\% (w/w), obtaining thus, a total of fourteen formulations. The stability of all formulations was analyzed by the evaluation of their particle size, stability under storage, stability under centrifugation, pH value and conductivity measurements. To evaluate the antifungal activity a strain of \textit{C. albicans} ATCC 64548 was used. The microdilution method was used to assess the antifungal activity according to the document M27-A2 Clinical and Laboratory Standards Institute (CLSI), with modifications.

Results
As a result of the centrifugation process, only three formulations remained stable (F1, F8 and F10). Among these, two contain ZnO, indicating that the presence of this powder increases the stability of the formulation. The first signs of instability, evaluate by visual analysis, were observed after 4 weeks at both 4 and 45°C. The formulations showed small droplet size. However, those with ZnO had lower droplet size, which may be due to the powder measurement detected by the equipment. The presence of ZnO also increased the pH, changing the formulations from acidic to neutral. Concerning the conductivity assay, except for F10 formulation, the presence of ZnO increased its value. The microdilution method reveals a minimum inhibitory concentration of 0.6\% of the formulation.

Conclusions
After performing the stability studies, F10 formulation present the better result in all tests. Therefore, this formulation was further subjected to the test of antifungal activity and has presented good results. As a conclusion, this formulation could be used not only as a prevention product, but also as an aid treatment of diaper dermatitis associated with secondary infection by \textit{C. albicans}.

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References
SIZE DISTRIBUTION OF MAGNETIC POLYMERIC MICROPARTICLES BY OPTICAL MICROSCOPY AND LASER LIGHT SCATTERING

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Introduction
Many techniques have been developed for particle size analysis for pharmaceutical applications (1). The determination of the particle size and the size distribution is frequently performed in many laboratories by using laser light scattering (2). The biggest advantage of this method is its especially outstanding broad measuring range. Moreover, this technique presents two major drawbacks, the expensive price of the equipment and the inaccessibility of the morphology of the product. Therefore, a direct observation is needed and particle size and morphology must be measured together (3). A simple and inexpensive alternative technique to measure the size distribution is the microscopy analysis. The aim of this work was produced magnetic particles and magnetic Eudragit-coated particles with amoxicillin for drug delivery and evaluates two methods for particle size characterization (laser scattering and optical microscopy) in order to compare the efficiency of both methods.

Materials and methods
First, magnetite particles (MP) were produced by co-precipitation of iron salts in alkaline medium. The second step consisted of coating the particles with Eudragit®S100 and the antibiotic amoxicillin using the spray-drying technique (4). The microscopy analyses (MA) of the particles were performed according to the Ferret’s diameter principle using an optical microscope calibrated with a stage micrometer scale (1). Additionally, the diameter of MP and polymeric magnetic particles (PMP) was examined using a laser scattering particle size analyzer (LSA).

Results
According LSA, the mean diameter of the MP was found to be 6.1 µm. It was also determined that 90%, 50% and 10% of the sample was smaller than 15.1, 5.6 and 0.9 µm, respectively. For the PMP, the mean diameter was found to be 78.7 µm and 90%, 50% and 10% of the sample was smaller than 163.2, 76.0 and 1.7 µm, respectively. On the other hand, for the MA a mean diameter of 8.95 µm was found for MP. It was also determined that 90%, 50% and 10% of the sample was smaller than 18.4, 9.6 and 5.9 µm, respectively. The data concerning PMP was found to be 11.1 µm and the 90%, 50% and 10% percentiles were smaller than 16.8, 10.0 and 8.0 µm, respectively. The span index was used to analyze the polydispersity in the particle size distribution. The span index of MP and PMP using the LSA was 2.54 and 2.13, indicating high polydispersity. For the MA these values were markedly decreased to 1.3 and 0.88 for MP and PMP, respectively.

Discussion/Conclusions
According the obtained results, the size of the particles was not uniformly distributed around the median value. Instead, the size distribution of the MP, as well as the size distribution of the PMP samples, showed a tendency towards a bimodal distribution with a left-sided tail, and larger weight for small particle diameters. Studying the differences between particle-sizing instruments for analyzing the size of polymeric magnetic particles demonstrated that the LSA were shown to be either insensitive to the presence of large particles or overestimated it.

Acknowledgements
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References

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pH-RESPONSIVE POLY(HYDROXYETHYL METHACRYLATE)/POLY(2-(DIISOPROPYL AMINO) ETHYLMETHACRYLATE) COPOLYMERS FOR THERAPEUTIC OPHTHALMIC APPLICATIONS

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Introduction
Useful polymers for soft contact lens are silicone-based elastomers or hydrogels based on poly(hydroxyethyl methacrylate) (polyHEMA) (1). However polyHEMA lenses could be difficult to handle and a convenient way to modify their physical characteristics is by copolymerization. Several hydrophils monomers were used but, to our knowledge, no work was reported using poly(2-(diisopropyl amino) ethylmethacrylate (DPA). The copolymerization of HEMA and DPA produce low Tg, good film-forming and high water content materials. In this work, copolymers containing HEMA polymerized with different amounts of DPA monomer (10 and 30 wt.%) and different amounts of crosslinking agent (1, 2 and 3 wt.%) were prepared and characterized for FTIR, DSC, water content, drug uptake and in vitro drug release at different pHs using Rhodamine (Rh6G) as drug model and dexamethasone as typical anti-inflammatory drug.

Materials and methods
The materials and methods used for the synthesis and characterization of copolymers are described elsewhere (2). The Rh6G and dexamethasone were incorporated by soaking the film in aqueous solutions of the drugs. The drug uptake was performed at pH 7 following the kinetics by UV-visible spectroscopy. Release studies from loaded films were performed at pH in the range of human eyes (6.5 – 8.5, average pH ~ 7.4) in buffer solutions at 34°C by using UV-visible spectroscopy and data were adjusted to a power-law type relationship (3):

\[
m_t/m_\infty = kt^n \quad [1]
\]

Results
The water content changes depending on the composition, the medium pH and the degree of crosslinking, ranging from 30 to 80 wt. % for pH 6.5 and from 25 to 48 wt. % for pH 8.5. For pure polyHEMA with 3 wt. % of crosslinking agent and for low DPA content (10 wt. %) the amount of Rh6G released at pH 7 is very low (~ 30 %). For poly(HEMA/DPA):70/30 and 1 wt. % of crosslinking agent the release of drug is almost total. For copolymers with 1 wt. % of crosslinking agent, the release change from 10 % to 100 % depending on the composition and pH.

Conclusions
DPA and HEMA monomers can be copolymerized to prepare soft contact lens for therapeutic ophthalmic applications with variable water content and variable release kinetics depending on the DPA/HEMA ratio and the degree of crosslinking.

Acknowledgments
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References

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PRODUCTION AND CHARACTERIZATION OF CORE-SHELL BIOPOLYMER VEHICLES FOR MULTIPLE DELIVERY STRATEGIES

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Introduction
In the pharmaceutical sector, there are many potential applications for nano and microparticles developed with engineered biomaterials (proteins and polysaccharides). These particles could be used to encapsulate, protect, and deliver functional agents such as bioactive peptides, proteins, enzymes or lipophilic drugs (1,2). In this contribution, experimental information about the preparation and molecular characterization of core-shell biopolymer particles produced by electrostatic deposition of an anionic polysaccharide (sodium alginate, SA) on the surface of soluble protein aggregates (heat-denatured whey protein isolate, hd-WPI) will be presented.

Materials and methods
Whey protein isolate (WPI) was provided from Davisco (Minnesota, USA), and low viscosity sodium alginate (SA) was supplied by Cargill (Buenos Aires, Argentina). WPI aqueous solutions (6.0% wt) were heat treated over the temperature range 55-85ºC. The incidence of thermal treatment on the hd-WPI aggregates formation was monitored using a set of complementary spectroscopic techniques: UV/Vis absorption (3), intrinsic and extrinsic fluorescence (4). Polysaccharide electrostatic deposition on the surface of the hd-WPI aggregates (core-shell particles formation) was performed at pH 4.0. The influence of protein-polysaccharide ratio, Pr:Ps (1:1-6:1), on molecular structure of the core-shell biopolymer particles was studied spectroscopically at 0.3% wt total biopolymer concentration. On the other hand, in order to determine the macroscopic phase behavior (cosolubility, complexation and coacervation) of aqueous mixed systems as a function of pH (6.0-3.0), transmittance studies were performed (5). In all cases, the results were compared with their respective controls (thermally untreated WPI). Analysis of variance (ANOVA) was carried out, and the statistical differences ($p<0.05$) were determined using the LSD test.

Results
The hd-WPI aggregates formation was observed at the highest heating temperature (85ºC) as it can be deduced from: (i) an increment in the aqueous system turbidity, (ii) changes in protein conformation (increased flexibility and molecular rearrangement), and (iii) a greater exposure of protein hydrophobic patches. SA electrostatic deposition on the surface of the hd-WPI aggregates had a great impact on the core-shell particles formation depending on the magnitude of Pr:Ps. The increase in Pr:Ps value caused: (i) an increased turbidity of mixed systems, and (ii) occlusion of protein surface hydrophobic patches. Transmittance studies revealed that SA-hd-WPI system mixed at 4:1 ratio showed a typical behaviour (soluble complex formation followed by an associative phase separation, i.e. coacervation) at pH values of 4.3 and 3.3, respectively. However, for the other evaluated systems, the onset of complex coacervation could not be determined experimentally possibly due to cold-set gelation of SA-hd-WPI mixed systems.

Conclusions
Results derived from this research could be useful to determine structural and environmental (pH, Pr:Ps) conditions in order to: (i) control polysaccharide electrostatic deposition on the surface of soluble protein aggregates and/or develop core-shell biopolymer particles with specific characteristics and multiple applications. The core-shell biopolymer vehicles prepared in this work could be used as drug carriers and/or as delivery systems of heat insensitive lipophilic agents.

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Acknowledgments
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References
COPROCESSED EXCIPIENTS PRODUCTION FOR DIRECT COMPRESSION OF ORALLY DISINTEGRATING TABLETS

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Introduction
The tablets are currently the most widely used solid form for administering oral medications because they can be accurately dosed, easily manufactured and give good patient compliance (1-2). In particular, the preparation of orally disintegrating tablets (ODTs) requires adequate excipients to ensure a high disintegration rate in the mouth without need of water (3-4). The use of ODTs has been growing, however the excipients to be used for these formulations are limited since they have to accomplish desired properties such as: water solubility, pleasant taste and mouth feel, sweetness and rapid dissolution (5-6). Many of the excipients used nowadays have been developed in the last decades, therefore there is still room to explore new formulations with better flowability, compressibility, hygroscopicity, palatability, dissolution and disintegration properties.

Materials and methods
In this study, different co-processed excipients with flow characteristics suitable for the production of ODTs, using a single processing step (spray drying) prior to direct compression, were obtained. For this purpose, different low-cost and easy accessibility excipients available in the market were first selected: lactose monohydrate (LACT), mannitol (MAN), microcrystalline cellulose (MCC) and silicon dioxide (SiO₂). A Buchi Spray Drier B-191 was used to process different aqueous solutions containing different excipients and concentrations. Powders were obtained using one, two (binary mixtures) or three (ternary mixtures) excipients. For all the cases, the solutions had 10wt% as total solid concentration and they were processed using the same spray drying operating conditions. The powders were subjected to different characterization tests: repose angle, Carr and Hausner indexes and tablets wettability and strength obtained at different compression pressures.

Results
The ternary mixture of MAN:MCC:SiO₂ 13:6:1 showed the better flowability properties, tablet strength (higher than 3 Kg) and adequate wettability times (of about 30 seconds) when tablets of 200 mg were obtained using a pressure of 0.3 tons. The SiO₂ was added in order to improved the yield of the process, however higher quantities are not recommended because the wettability times increase considerably.

Conclusions
The use of lactose was not found suitable, even this excipient allows to improve the powders flowability, the yield of the process drops to unfeasible levels.

Acknowledgments
I would like to thank the scholarship given by the Universidad Nacional del Sur, which allows me continuing my PhD.

References.
(2) Rasenack, N., and Muller, B. W., Crystal Habit and Tableting Behaviour, Int. J. Pharm., 244: 45-57, 2002.


Table 1: Selected Operating Spray dryer conditions

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<td>Asp (%)</td>
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<td>Atom (l/h)</td>
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<td>Pump (%)</td>
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Table 2. Selected experiments. Content and properties of excipients.

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<th>Hum., %</th>
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<td>9.25</td>
<td>2.18</td>
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<td>1</td>
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<td>62.05</td>
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<td>-</td>
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<td>25.57</td>
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<td>55</td>
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<tr>
<td>#14</td>
<td>14</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>49.00</td>
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<td>1.78</td>
<td>44</td>
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<td>#15</td>
<td>13</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>54.12</td>
<td>--</td>
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<td>1.78</td>
<td>44</td>
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Table 3. Wetting time and hardness of selected tablets

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<td>Wetting time, seg</td>
<td>Hardness, force Kg</td>
<td>Wetting time, seg</td>
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<td>30.7</td>
<td>1.1</td>
<td>56.1</td>
</tr>
<tr>
<td>#13</td>
<td>41.5</td>
<td>1.4</td>
<td>60.1</td>
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<td>#14</td>
<td>41.5</td>
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<td>57.6</td>
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<td>#15</td>
<td>30.4</td>
<td>3.1</td>
<td>60.6</td>
</tr>
<tr>
<td>Ludiflash</td>
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<td>9.7</td>
</tr>
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<td>--</td>
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<td>9.5</td>
<td>--</td>
<td>9.6</td>
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</table>
PREFORMULATION STUDIES TO DEVELOP AN INTRARUMINAL CONTROLLED RELEASE FORMULATIONS

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Introduction
Anatomy and physiology of ruminants are quite different from humans. The need of controlled release formulations in order to treat different illnesses and plagues make necessary the development of very long controlled release formulations (1-2 months) (1)

Dosage forms dense and heavy enough to stop in the ruminal-reticular sac, are retained there rather than pass to the alimentary tract. High density provides the mechanism to retain the dosage form over a long period of time while the therapeutic ingredient is released through solubilization or erosion by the ruminal fluid (2).

The utility of lipid-based oral formulations has been recognized for many years (3). The most important release mechanism from matrix formulated with lipid excipients is erosion. Different class of lipid excipients could be used to modulate the release pattern. Among them, Gelucires® are a family of lipid-based excipients comprising glycerides and esters of polyethylene glycol (PEG), these two components conferring hydrophobic and hydrophilic properties to the vehicle. Gelucires® come in a variety of grades with different melting points (from 33 °C to 65 °C) and HLB values (from 1 to 14). Compritol ATO 888® is glyceryl behenate, its melting point is 74°C and has a HLB of 2.

The objective of this study was the development of a controlled release delivery system, using lipid excipients, designed for the incorporation of active ingredients for the oral administration to ruminants (cattle and sheep)

Materials and methods
Gelucire 50/02, Gelucire 44/14, Compritol ATO 888, stearyl alcohol, cetyl alcohol, iron powder, PEG 6000 were the excipients used

Formulations were obtained by melting the excipients in a water bath (60°C), with the exception of powder iron. The mixture was stirred at room temperature and when reached 35°C powder iron was added. Molten mixtures were poured over cilindric plastic forms, containing 100 g of excipients for cattle and 30 g for sheeps. Erosion of the samples was assessed in vivo in fistulated cattle or sheeps. Samples were weighed and placed into the rumen. Once a week they were removed, dried gently in an oven and then weighed.

Results
The table shows the formulations lasting for longer times into the rumen.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Melting Point</th>
<th>HLB</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F16</th>
<th>F18</th>
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</thead>
<tbody>
<tr>
<td>Gelucire 50/02®</td>
<td>50</td>
<td>2</td>
<td>39.8</td>
<td>30</td>
<td>30.8</td>
<td>15</td>
<td>13.5</td>
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<tr>
<td>Compritol ATO 888®</td>
<td>74</td>
<td>2</td>
<td>13.4</td>
<td>---</td>
<td>---</td>
<td>35</td>
<td>31.3</td>
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<tr>
<td>Cetyl alcohol</td>
<td>45-50</td>
<td>15</td>
<td>---</td>
<td>18.2</td>
<td>---</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Stearyl alcohol</td>
<td>55-60</td>
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<td>---</td>
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<td>PEG 6000</td>
<td>42-44</td>
<td>---</td>
<td>1.8</td>
<td>1.8</td>
<td>1</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Iron (powder)</td>
<td></td>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td>Time lasting in the rumen (weeks)</td>
<td></td>
<td></td>
<td>6</td>
<td>&gt;12</td>
<td>&gt;12</td>
<td>9</td>
<td>4</td>
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Melting point played a fundamental role in the erosion process. High melting points produces a low erosion and would produce an excessive delay in the release of the active principle. No relationship was obtained with the HLB values. Small proportions of PEG 6000 as porous former also had an influence in the erosion process, making the process faster. We develop formulations lasting between 1 and 12 weeks.
into the rumen. Therefore, these formulations can incorporate different active principles for different purposes, according to the releasing times needed.

**Conclusions**
Combination of lipid excipients allowed the production of lipid matrix with different times for its complete erosion. They can incorporate different drugs.

**Acknowledgments**
Proyecto FIA PI-C-2004-1-P-025

**References.**
1.- Rathbone M J. and Martinez M N. Modified release drug delivery in veterinary medicine. DDT 2002. 7(15) 823-29
IN VITRO DISSOLUTION OF CEPHALEXIN EXTEMPORANEOUS SUSPENSIONS DURING SIX MONTHS OF STORAGE

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Introduction
Most problems linked with extemporaneous suspensions are associated with physical stability. In general, suspension stability studies consider only changes in chemical stability, pH, caking, and re-dispersability (1, 2), with no focus on dissolution stability (3).
Previous studies carried out on suspension dissolution do not consider changes during the administration period of the constituted suspension (stored at room temperature as well as under refrigeration) throughout the shelf life of the powdered product (4-10). During aging, absence of dissolution changes suggests that bioavailability could remain intact (3).
Our research attempted to evaluate dissolution stability of three cephalexin extemporaneous suspensions from Argentinian market, throughout the recommended administration period of constituted forms, during six months of powders storage under natural and accelerated aging conditions (11).

Materials and methods
Two samples (B and C) of cephalexin extemporaneous suspensions (250mg/5mL) were purchased from pharmacies in Bahía Blanca city, a third sample (A) was produced by a local state laboratory.
Cephalexin content, dissolution profiles, pH, specific gravity and organoleptic characteristics were determined (12-14) at constitution time, after 7 days of storage at room temperature and 14 days under refrigeration, repeating this scheme at time zero, and throughout the storage of the powders for oral suspension (3 and 6 months). The formulations were stored under ICH accelerated (40°C/75% R.H.), and natural conditions (25°C/60% R.H.).
Dissolution profiles were compared in terms of Dissolution Efficiency (DE). Analysis of variance (ANOVA) was used to compare both assay average results (chemical stability) and DE values (dissolution stability).

Results
Color changes and unpleasant odor were observed during aging of all constituted suspensions, and pH values remained in the range of 3.0–6.0, satisfying pharmacopoeia specifications.
Due to major changes in appearance after three months of storage under accelerated aging conditions, studies could not be continued on sample A. Microbiological assays and chemical interaction tests are being performed.
A cephalexin content decrease trend was observed during the administration period, throughout the storage of powders, but in almost all cases the values were between 90.0-120.0%. For sample A, there were two assay values lower than 90.0%, in accordance with the results of maximum percentage dissolved and DE, possibly due to reconstitution volume.
In all cases, a 100.0% was dissolved in 5 minutes. Almost all DE values were above 100.0%, which indicates an excellent dissolution performance. In some cases, statistical comparison showed differences between DE values during the administration period of the suspension.

Discussion / Conclusions
Cephalexin concentration remained within 90% of the initial value throughout the stability study, in all brands. All samples showed a high dissolution rate with large dissolved percentages at early time points of the dissolution profile. Although statistical differences were found between DE values throughout administration period, they do not have an important clinical significance.

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The constituted forms were chemically stable and had acceptable dissolution stability during the administration period, throughout the powders aging study.

Acknowledgments
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References
NOVEL EUDRAGIT E100 SYSTEM FOR ZERO-ORDER DEXAMETHASONE PHOSPHATE RELEASE

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Introduction
New controlled release materials were obtained by loading the cationic polyelectrolyte EudragitE100 with oppositely charged drugs. In a previous work we showed that the dimethylamino moieties of EudragitE100(EuE) can form ionic complexes with phosphate and phosphonate drugs. In this work we present the in vitro release characterization of this systems using dexamethasone phosphate(DP) as a model drug.

Objectives
Characterize the affinity and release behavior of DP from dispersions of EuE-DP systems.

Materials and Methods
A series of solid complexes of EuE loading variable amounts of DP were obtained. The complexes were subjected to titration of ionic pairs with NaCl solution. The pH changes produced by ionic exchange were regarded. Rate and kinetic release in water and physiologic solution was assessed in Franz cells. The impact of the direct addition of increasing NaCl amounts (50%-300%) in the release behavior was also assessed. EuE-Benzonic Acid and EuE-Diclofenac were also obtained as references of carboxylic model drugs. Additional characterization of the interaction was achieved by 1H and 31P RMN spectroscopy.

Results and discussion
As NaCl solution was added to the system, an increase in the pH was observed. The equilibrium was achieved after addition of 800% of NaCl, 4 times the amount needed to reach the equilibrium in the systems containing carboxylic acid moieties. The systems showed a smart behavior, with DP release in water below 2% in eight hours and an increase (10%) as media was replaced by NaCl. Interestingly, the kinetic release fitted zero-order. Direct addition of NaCl to the dispersions showed no modifications nor in the rate neither in the release, proving that no burst effect is expected. In contrast, significant modifications were observed in the systems containing carboxylic acid moieties. The 1H NMR evaluation showed a noticeable widening and reduction of some proton signals of DP after complexation. This fact could be related to an increase in relaxation time in 1H nuclei of DP as a consequence of EuE complexation. 31P NMR spectra showed a unique signal at frequencies higher than free DP. When DP is in excess, the phosphorous signal approaches to free DP, proportional to the DP: EuE ratio. However, when EuE is in excess, the chemical displacement showed a landslide higher than expected, probably related to the participation of the second acidic group of phosphate. This fact can be related with the affinity and kinetic observed.

Conclusion
DP has significantly higher affinity for the dimethylamine groups of EuE than carboxylic acid groups. The complexes EuE-DP present zero-order release, which is sustained for at least 26 hs. The participation in the interaction of the 2 acidic groups of the molecule may play a role. These novel systems can find utility as controled release systems for administration of DP. EuE is a promising carrier of other phosphate drugs such as biphosphonates, nucleotides, plasmid or proteins.
LIQUID CRYSTALLINE EMULSIONS AS POSSIBLE CONTROLLED RELEASE SYSTEMS FOR TOPICAL APPLICATION

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Introduction
In emulsions with liquid-crystal characteristics emulsifier molecules (including long-chain alcohols and fatty acids, among other amphiphilic substances) are adsorbed in the oil-water interface forming a laminar structure 1, 2. This multi-layer surrounding the emulsion droplets reduces van der Waals interactions between oil droplets and acts as a barrier against coalescence 3. Susuki, Takei y Yamazaki 4 attributed the stability of emulsions with liquid crystals to the increase of the mechanical strength of the oil-water interface and the setting of the emulsion droplets to the liquid-crystalline structure. Lyotropic liquid crystals proved adequate systems for the controlled release of active ingredients 5, thus a similar behaviour is expected of the liquid-crystalline emulsions.

The objective of this study was to develop a methodology that allows to obtain a liquid-crystalline emulsion by dilution with water, or an aqueous solution, of a liquid crystalline concentrate formed by the emulsifier, the lipophilic ingredients and part of the aqueous phase.

Materials and methods
The oil phase of emulsions was formed by stearic acid (15.00%), mineral oil (20.00%) and propylparaben (0.03%), while the aqueous phase was formulated with triethanolamine (4.14%), water (60.76%) and methylparaben (0.07%). Liquid crystalline concentrate was prepared by adding stearic acid, mineral oil and propylparaben, in warm and with shaking, to a mixture consisting of triethanolamine and part of the water. After homogenising, the previous mixture was diluted with the remaining water that contained the methylparaben. The stability of the emulsions was evaluated by centrifugation during 30 minutes to 3000 rpm and by storage during 6 months at 40 ºC. The formation of liquid-crystalline structures was verified using a polarizing microscope Carl Zeiss model Axiolab with a digital camera Olympus SP 35.

Results
An emulsion with liquid-crystalline characteristics was obtained that turned out to be stable against centrifugation and to storage during 6 months at 40 ºC.

Conclusions
The propose methodology allows to obtain potentially suitable liquid-crystalline emulsions like topical systems of controlled release of drugs.

References
SWELLABLE ANTI-TUBERCULAR DRUG-POLYELECTROLITE MATRICES: CHARACTERIZATION AND DELIVERY PROPERTIES

Lucciani Giacobe C, Ramírez Rigo MV, Catani Y, Romañuk C, Manzo RH, Olivera ME.

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Introduction
Rifampicin (RIF) and Isoniazid (INH) are administered in Fixed Dose Combination (FDC) solid formulations to improve patient compliance in the treatment of tuberculosis. In FDC, RIF bioavailability problems are frequent because of its acid decomposition, catalyzed by INH. RIF degradation is proportional to INH concentration in acidic media. These problems could be overcome by developing an FDC in which the delivery of the drugs is site-specific and segregated, with RIF released in stomach and INH in small intestine (1). The aim of this work is to obtain and characterize swollen drug-polyelectrolyte matrixial systems of RIF and ISO for a further development of a bi-layer tablet that sequentially release RIF and INH.

Materials and methods
Alginic acid (AA), carboxymethylcellulose (CM), RIF and INH were used to obtain solid acid-base complexes. The solid powders were characterized by FT-BIR, DSC-TG and powder X-Ray diffraction. The flow properties were also evaluated. Matrices prepared by compression of the complexes were subjected to measurements of solvent up-take and release kinetics in simulated gastric and intestinal fluid. RIF stability in the dissolution media was determined.

Results
The AA-ISO and CM-RIF material were easily prepared as particulate materials. Characterization through FT-infrared spectroscopy, powder X-ray diffraction and DSC indicates the ionic nature of the interaction between the carboxylic groups of the polyelectrolytes and the basic group of the drugs. The complexes were granulated using ethanol to obtain powders with improved flow properties. Fluid uptake from CM-RIF matrix was fast reaching quickly a plateau; water diffuses promptly through the matrix pores to completely wet it in a few minutes. On the other hand, sorption rate of AA-ISO matrix was slow and decreased with time as a consequence of the development of a continuous hydrogel layer on matrix surface. Experimental results indicate that delivery rate from matrices is a function of its composition. When the CM-RIF matrix was immersed in the dissolution media to determine release rates, it takes solution quickly, swell, and finally disintegrate in a very short period of time. RIF release in acidic media approached 100% as a consequence of the fast exchange between the H⁺ or Na⁺ of the dissolution medium and RIFH⁺. This behavior was previously observed for CM-model basic drugs matrices (2). In contrast, ISO release from matrices of AA–ISO exhibited a slow delivery rate, by a diffusion mechanism, in simulated gastric fluid followed by a complete release in simulated intestinal fluid. The mechanism is different of previously developed AA- complexes (3). RIF stability in simulated gastric medium was improved in the matricial system in relation to RIF/INH acid solutions as a result of the slower INH concentration in the dissolution medium.

Conclusions
The release rate of RIF and INH could be modulated by acid-base complexation with different polyelectrolytes. The in vitro release behavior is reasonably satisfactory to continue with the formulation step of a bi-layer tablet that sequentially release RIF and INH.

Acknowledgments
The authors thank Carrillo-Oñativia Grant of the Ministry of Health of Argentine.
References
CHARACTERIZATION OF BIODEGRADABLE IN SITU FORMED AND PREFORMED MICROSPHERES

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Introduction
Controlled drug delivery technology is concerned with the systematic release of a pharmaceutical agent to sustain a therapeutic level of the drug in the body for a period of time. This may be achieved by incorporating the therapeutic agent into a biodegradable polymer vehicle, releasing the agent continuously as the matrix erodes. Poly(lactic-co-glycolic) acid (PLGA) microspheres are widely used for this purpose (1). There are two types of pharmaceutical dosage forms of PLGA-based release microspheres: a) conventional preformed microspheres (PFM), and b) novel injectable in situ forming microspheres (ISM). This contribution is concerned with a comparison of preparation methods and microstructure characterization of PFM and ISM, as fundamental work to develop a better understanding of essential features to choose the most appropriate drug-eluting platform for a given application.

Materials and methods
As first preparation step, polymer was dissolved in a solvent-system (phase1) and then emulsified into a second solvent-system (phase2). For PFM, volatile no-biocompatible phase1 is removed from emulsion to precipitate the polymer in phase2 under controlled laboratory conditions. For ISM, precipitation takes place when preformed emulsion comes in contact with physiological fluids diluting the biocompatible organic phase1 (2, 3). For ISM, a solution of 20%PLGA50:50 comprising Glycerol Formal (GF) was tested as phase1 and emulsified with Miglyol812. For PFM, equal quantity of PLGA was dissolved in Ethyl Acetate (EA) and emulsified with 1%Poloxamer188 as phase2. The resulting emulsions were cast into an aqueous surfactant solution and magnetic stirred to form the microspheres by exchange of solvents (for ISM) or evaporation of EA (for PFM). After analysing size distribution (mean±SD), shape and sphere surface by optic microscopy and scanning electron microscopy (SEM), following conclusions arisen up to the present.

Results
For both solvent systems, shape of the microparticles was spherical. PFM provides the lowest microsphere size (14,5±7,5\,\mu m; 3,5-40,9\,\mu m) compared to ISM method (61,5±27,4\,\mu m; 14,6-156,6\,\mu m). SEM characterization shows that sphere surface was more porous for system using GF than EA. Both size and surface characteristic may be related to the slow PLGA precipitation rate during EA evaporation that allows shrink the emulsion droplets, with the consequent polymer concentration and formation of a dense/non-porous surface. In contrast, high diffusion rate of a water soluble solvent like GF forms a dense polymer shell around emulsion droplets that are unable to shrink, creating particles with a hollow core that collapsed forming porous when the remaining solvent is extracted from the core (2, 4).

Conclusions
Results show that in vitro ISM and PFM are spherical and non-aggregated particles characterized by a Gaussian size distribution. PFM are small enough for injection of the suspension using standard needles and for provide a fast degradation and deliver of drug. In contrast, ISM exhibits higher particles sizes but its higher porosity additionally favours the diffusion and erosion phenomena, which increase the PLGA biodegradation rate. Therefore, from the viewpoint of the effects of structural differences between the both systems we do not argue in favor of a particular one. However, from a practical and economical
viewpoint, there is clear advantage in favour of ISM systems due to its cheaper preparation methods and easier injectability.

References
FILMS BASED ON CHITOSAN/CARBOPOL POLYELECTROLYTE COMPLEX CONTAINING ECONAZOLE NITRATE FOR THE TREATMENT OF CUTANEOUS CANDIDIASIS

Marinelich, D.M.; Frattini, A.; Luque, A.G.; Biasoli, M.S.; Olivieri, A.C.; Leonardi, D.

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Introduction:
One of the aims of dermal delivery is to localize a drug within the skin in order to enhance a local effect. In the case of dermato-pharmacotherapy for the treatment of skin inflammation, acne and skin fungal infections, the dermal delivery of active ingredients is desirable. (1) The conventional dosage forms for the treatment of skin micosis are creams, ointments, solutions and powders (1 %). The affected zone must be covered with the dosage form three times daily, generating an erratic drug concentration in the target site. Bioadhesive films based on polyelectrolyte complexes are a good alternative to conventional dosage forms, because they allow to apply an exact drug concentration on the target site and to control the release of the drug. This could be reflected in a reduction of the number of applications. The polyelectrolyte complexes are obtained by electrostatic interactions between two polymers with opposite charge, without the use of organic solvents. The aim of this work was to develop bioadhesive films employing chitosan and carbopol 940®, for the control of release of econazole nitrate at different concentrations.

Materials and Methods:
Chitosan (3 % w/v) was dissolved in lactic acid (2 % v/v) and carbopol 940® (1 % w/v) in water. 20 ml of each solution were mixed for 5 minutes and the pH was fixed at either 2 or 5. Finally, 1 ml of glycerol (as plasticizing) and econazole nitrate at different concentrations (0; 0.01; 0.05; 0.10; 0.25; 0.5 y 1% w/v) were added. Film forming solutions were mechanically stirred at 800 rpm for 2 hrs, cast on Petri-dishes and dried at 35 ºC for 48 hrs. Dried films were conditioned in a desiccator containing a saturated solution of NaCl at 25 ºC (75% RH) (2). The interactions between the polymers were investigated by infrared spectroscopy (IR) and differential thermal analysis and thermogravimetry (DTA-TG). The film surfaces were studied by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The *in vitro* activity of the complex was evaluated by the diffusion method in an agar Müeller Hinton medium, using *Candida krusei* ATCC 6258 as control. Plates were incubated at 37 ºC and the activity of the the films was evaluated by transfers until 24 hs (measuring the inhibition hales).

Results:
The IR spectra showed interactions between the polymers, but the absorption peaks corresponding to the drug were unchanged. Similar results were obtained by DTA-TG. The interactions were stronger in films prepared at pH 5 than those prepared at pH 2. The AFM analysis showed more irregular surfaces in films prepared at pH 5, probably due to the strong interactions between the polymers. Films formulated at pH 5, with a concentration of econazole nitrate lower than 1% w/v, showed *in vitro* activity during 24 hs, and their matrices remained unaltered after 24 hs assay.

Conclusions:
Polyelectrolyte complexes formulated at pH 5 showed a adequate *in vitro* activity during 24 hs. This formulation could generate a new pharmaceutical form that can be applied only once a day.

References:

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FLEXIBLE POLYMERS AS CARRIERS OF NON-SOLUBLE ANTIFUNGAL DRUGS: FLUORESCENCE STUDIES

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Introduction
Over the last ten years superficial mycosis have become increasily prevalent. Some antifungal drugs commonly used are water non-soluble, which induces a low biodisponibility and biological activity. The need for a topical drug delivery system of griseofulvin and ketoconazole arise due its poor oral bioavability and numerous side effects. Several investigations regarding the effectiveness of these antifungicals in topical systems may be attributed to inappropriate selection of the vehicle. The use of flexible chain polymers (FCP) in semisolids of topical application has a enormous potential for creating satisfactory drug dosage forms. In this work we show the application of fluorescence techniques to study molecular interactions of the drugs in the microdomains of micellar aggregates. The goal of this work was to increase the solubilization of non soluble antifungal drugs in aqueous medium by using FCP.

Materials and Methods
The polymers tested were: Cellulose derivatives, Guar derivatives, poloxamers (Pluronic ® 68, 87, 108) and acrylates polymers (Hyspagel 200®, Polygel W30®, Stabylen 30®, Cosmedia SP®).
The solubilization of Ketoconazole (KTO) and Griseofulvin (GRI) in the systems was studied by fluorescence spectroscopy. The mesurements where carried out in a Jasco FP770 spectrofluorometer.

Results
The modification in the fluorescence emission band is the result of an interaction fluorophore-FCP. The extinction of the fluorescence emission band was proportional to the concentration of polymer in the medium. This property was used to quantify the maximum solubilization capacity of the polymers assayed. The results obtained are showed in the following table:

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Solubility mg antifungical / mg polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cosmedia G®</td>
<td>2.5</td>
</tr>
<tr>
<td>CMC</td>
<td>1.0</td>
</tr>
<tr>
<td>Cosmedia SP®</td>
<td>0.92</td>
</tr>
<tr>
<td>Stabylen®</td>
<td>0.47</td>
</tr>
<tr>
<td>KTO</td>
<td>GRI</td>
</tr>
<tr>
<td>0.040</td>
<td>0.025</td>
</tr>
<tr>
<td>0.015</td>
<td>0.010</td>
</tr>
</tbody>
</table>

The other polymers solubilized 0.1 – 0.05 mg of KTO and 10⁻⁴ mg of GRI which can be considered of poor solubilization capacity.

Conclusions
KTO has a solubility of 4.7 µg/ml in water; its molecule presents two basic groups, a piperazine and an imidazole with pKa values of 2.94 and 6.51 respectively; its solubility and antimicotic activity are dependent on the percentage protonation of the piperazine group. The efficient solubility capacity showed by the anionic polymers (sodium carboximethilcellulose, Cosmedia SP® and Stabylen®) may be explained in basis to a coulombic interaction with the piperazine group of KTO. The high solubilization capacity of Cosmedia G®, a cationic polymer, mat be explain in basis to the numerous polar groups of guam present in this polymer. The same polymers showed a poor performance to solubilize GRI.

Acknowledgements
Organización Stengel (Rosario) and Cognis Argentina for the supply of the polymers used in this work.
References
LAYERED DOUBLE HYDROXIDES SUCH AS AN EFFECTIVELY RESERVOIR FOR INDOMETHACIN AND SODIUM INDOMETHACIN

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Introduction
Layer double hydroxides (LDH) have been used as catalysts, ion exchangers, adsorbents(1-3). LDH of Mg-Al is biocompatible and has pharmaceutical applications, such as antacids, drug stabilizer, for the therapy of digestive disorders, and others(4). These materials consist of nano-layers of hydroxides, positively charged, and interlayer exchangeable anions. In the present work, Indomethacin (Indo) and Sodium Indomethacin (IndoNa) was incorporated into LDH Mg-Al-CI by the anionic exchange method.

Materials and methods
The host solid LDH-CI was obtained by co-precipitation from a solution of metals chlorides and NaOH (molar ratio (Mg²⁺:Al³⁺)=3)(3).
Drugs intercalated LDH-CI were prepared by ion-exchange method. An aqueous solution containing the drug selected in distilled and deionized water (H₂Odd) was added to a suspension of LDH-CI in H₂Odd. The pH=8 was reached with NaOH 0.1M. This mixture was magnetically stirred at 70°C with N₂ flow for 3 days. The product was washed and filtered under vacuum and finally dried at room temperature.
X-ray Powder diffraction (XRD) patterns were recorded with a X’Pert Pro-PANalytical. Infrared spectra (FT-BIR) were obtained on a Jasco FT/IR 5300 spectrophotometer by the KBr disk method. Drug content was determined by UV absorption spectrophotometer Jasco 7800 at λ=320 nm: a known amount of intercalated LDH was dissolved in 10 mL of HCl 1M and then diluted with phosphate buffer at pH=7.4.

Results
The table shows the exchangeable LDH, drug content and the interlayer spacing. The exchange was realized with an excess of drug with respect to Al content.
By XRD the reflection plane 003 was studied, this value corresponds to the interlayer size.
The intercalated samples showed the first band at approximately 3° (in 2θ), indicating a higher basal spacing. The different values found with both drugs, suggests that the molecules are not oriented same(5).
The bands recorded in the FT-IR spectra of these samples should be due to the drug-intercalated. The spectrum revealed the presence of carboxylate group, indicating that the drug was incorporated. After the exchange in the samples intercalated with Indo, the bands related to carboxylic group, was not observed.

Conclusions
The Indo and IndoNa by anion-exchange method were inserted in the LDH-CI. By XRD and FTIR was not observed free drug, after the incorporation. The value for the drug content was around 40%.

References

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<table>
<thead>
<tr>
<th>Sample</th>
<th>Drug Incorporated</th>
<th>d(Å)</th>
<th>Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH-Cl</td>
<td>--------</td>
<td>8.02</td>
<td></td>
</tr>
<tr>
<td>LDH-Indo</td>
<td>Indomethacin</td>
<td>24.85</td>
<td>46.06 %</td>
</tr>
<tr>
<td>LDH-IndoNa</td>
<td>Sodium Indomethacin</td>
<td>23.79</td>
<td>38.99%</td>
</tr>
</tbody>
</table>
DEVELOPMENT AND IN VITRO EVALUATION OF CHITOSAN FILMS FOR ESTRADIOL DELIVERY

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Introduction
Estradiol (E2) transdermal systems are indicated for the relief of menopausal and postmenopausal symptoms. Polymeric membranes with skin-imitating permeability properties appear as attractive models in the evaluation of transdermal drug delivery systems. Some examples are carbosil membranes and chitosan (CHT) membranes (1, 2). The aim of this work was to prepare a CHT membrane as a mimic of human epidermis and evaluate its applicability for the development of a CHT film for E2 delivery.

Materials and methods
Human epidermis was obtained by enzymic digestion with trypsin. Epidermal sheets were used in release studies to evaluate the cumulative amount of E2 released per unit time. E2 transdermal therapeutic system commercially available was used as donor compartment. CHT membrane as a mimic of human epidermis was prepared by dipping a CHT membrane in a sodium tripolyphosphate (TPP) solution during 15 minutes. E2 transdermal therapeutic system commercially available was used as donor compartment. CHT films for E2 delivery were prepared by pouring on a polycarbonate petri dish a solution containing CHT, glycerol, E2 and poloxamer 188; and drying at 30 ºC (3). Release studies were performed according to the USP Apparatus 5 paddle over disk method (4).

Results
CHT membrane as a mimic of human epidermis: an optimization procedure based on response surface methodology was applied in order to find out the combination of CHT solution concentration, TPP solution concentration and cross-linking time that allows the preparation of an optimized membrane. This membrane reproduced the cumulative amount of E2 released per unit time of human epidermis. Optimized membrane was prepared with the following experimental condition: 3.76 % w/v for concentration of CHT solution, 5.0 % w/v for concentration of TPP and 15 minutes of cross-linking time. The dissolution profile of the optimized membrane was compared to the dissolution profile of human skin epidermis according to the model independent approach utilizing difference factor (f1) and similarity factor (f2) (5). f1 and f2 values were 5.18 and 74.5, respectively and indicated equivalence between both profiles. CHT film for E2 delivery: the optimized membrane was applied to the development of a release system. For this purpose a prototype of E2 transdermal patch was prepared. The release profile of the prototype alone and prototype plus the skin-mimetic membrane were similar to that obtained for commercial path and commercial patch plus the skin-mimetic membrane.

Conclusions
Synthetic membranes with skin-mimetic permeability properties are economical and result an alternative to in vivo studies or in vitro experiments with excised skin. The results presented show a successful application of the in vitro model proposed with CHT mimetic membranes for transdermal patch development. In addition, this system also provides a potential tool for modelling transport phenomena.

Acknowledgments
The authors thank Consejo Nacional de Investigaciones Científicas y Técnicas and Laboratorio Industrial Farmacéutico SE-Provincia de Santa Fe for the financial support.

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References.


DEVELOPMENT AND CHARACTERIZATION OF AN INNOVATIVE TAMOXIFEN MICROEMULSION

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Introduction
Breast cancer is the most common cancer among women and the second leading cause of disease deaths. Tamoxifen (TMX) is the drug of first election in premenopausal patients with estrogen receptor (+) \(1\). It shows poor water solubility and vulnerability to enzymatic degradation in intestine and liver \(2\). Its oral bioavailability is affected by the first pass effect \(3\). The objective of the work was to optimize and characterize a microemulsion containing TMX that could present a high solubilization capacity and a low \textit{in vitro} toxicity profile.

Materials and methods
Screening of the microemulsion region was performed using the titration method at 37°C \(4\). Polisorbate 80 and other pharmaceutically acceptable excipients were chosen; compositions were then represented in Pseudo Ternary Diagrams. To determine the equilibrium solubility of the drug in formulations, excess of drug was added to the formulations, they were left to equilibrate for 72 hs, filtered and finally analyzed by HPLC (Shimadzu, Japan). Droplet size was evaluated using a Nanozetasizer, Malvern Instruments, UK (37°C). Citotoxicity evaluation of the microemulsions and excipients were carried out using MCF-7 breast cancer cell line. The selected compositions diluted with culture media were incubated 48 hs and finally viable cells were determined using CellTiter 96® Non-Radioactive Cell Proliferation Assay (MTS).

Results.
Considering solubility and citotoxicity assays, phosphatidylcholine was selected as oil phase and ethanol and propyenglycol as cosurfactants. During the screening, only formulations containing ethanol were able to form microemulsions at the desired excipients levels. Finally, 5 different compositions were selected for physicochemical characterization. Solubility studies showed an improvement of drug solubilization of 10000 fold compared with water. Polisorbate 80 was the excipient with the highest rate of toxicity but the dilutions used were the usual ones.

Discussions/conclusions.
Microemulsions are thermodynamically stable dosage forms, widely accepted that can improve oral bioavailability or can achieve the desired dose at the tumor site for a longer period. The formulations prepared and characterized in this work showed a high solubilization capacity and a low toxicity profile, allowing different anti cancer strategies to be challenged \textit{in vitro}.

Acknowledgments
Financial support was obtained from PICT 2007-00595.

References.

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Introduction
Hydrochlorothiazide (HCT), a benzothiazide diuretic, is an ionizable acid (pKa of 8.75 and 9.88), sparingly soluble in water and a chemically stable substance. Considering the poor water solubility of HCT, this compound has been complexed with β-cyclodextrin (β-CD). CDs are cyclic oligosaccharides with hydrophilic outer surface and a somewhat lipophilic central cavity. In aqueous solutions CDs are able to solubilize hydrophobic drugs by taking up some lipophilic moiety of the drug molecule into the central cavity, i.e. through formation of hydrophilic inclusion complexes (1). CDs are known to form nanosized aggregates in aqueous solutions and thus have the potential to develop into sophisticated drug delivery systems. The largest aggregates are observed for β-CD, which can be up to several micrometers in diameter. The anomalously low solubility of β-CD is explained by the intensity of aggregate formation, which becomes notable at β-CD concentrations above 3mM and it interaction with the surrounding water structure (2). In the present work, the complexation mechanism between β-CD and HCT has been investigated by phase-solubility diagrams under various experimental conditions, with the aim to obtain a wide range of information about the molecular interactions that drive the complexation process.

Materials and Methods
Phase-solubility studies of HCT in aqueous solutions of β-CD were carried out according to the Higuchi–Connors procedure (3). The phase solubility profiles of complexes were prepared in water and in phosphate buffers at pH values of 5.5, 6.8 and 7.4, using two temperature values (25 and 37ºC).

Results
Phase solubility profiles of HCT in the presence of β-CD showed deviations in the slope at different sections of the solubility isotherms. Based on the shape of the generated phase-solubility relationships, several types of behaviors could be identified. Aβ-type phase-solubility profiles of HCT with β-CD were found at the two temperature values (25 and 37ºC) in phosphate buffers at pH values of 6.8 and 7.4 and in water at 25ºC. These results might be ascribed to that the HCT:β-CD complex aggregates by ionic interaction. Meanwhile, Aα-type phase-solubility profiles were found in water at 37ºC and in phosphate buffer at pH 5.5 at 25ºC. This might be attributed to hydrogen bond which are affected by the temperature increase, so aggregates dissociate upon heating increasing the relative concentration of complex monomers. An increase of the HCT solubility with the temperature was also observed.

Conclusions
Results of this study provide some insight into the aggregation equilibrium of the HCT:β-CD complex so the aggregate transformations greatly influence macroproperties of the solutions.

Acknowledgements
The authors thank FONCyT Préstamo BID 1728/OC-AR, PICT 1376, the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT), and the Consejo Nacional de Investigaciones Científicas y Tecnológicas de la Nación (CONICET) for financial support. We also thank the Ferromet S.A. (agent of Roquette in Argentina) for their donation of β-cyclodextrin.

References
PARDINI FM\textsuperscript{1,2}, ECHEVERRÍA MG\textsuperscript{1}, PARDINI OR\textsuperscript{1,2}, AMALVY JI\textsuperscript{1,2,3,}\#

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Introduction
A previous study of 2-(diethyl amino) ethylmethacrylate (DEAEMA)-based microgels (1) shows that they are film forming and pH-sensitive materials useful for drug delivery. However depending on the conditions they are difficult to handle. A convenient way to improve the physical characteristics of films is to copolymerize with vinyl-terminated polyurethanes (VTPU) to produce good film-forming hybrid materials. In this work, hybrid polymers containing VTPU polymerized with different amounts of DEAEMA monomer (10 and 30 wt.\%) were prepared and characterized for FTIR, DSC and \emph{in vitro} drug release at different pHs using Rhodamine 6G (Rh6G) as delivery marker (2). VTPU film was used as reference material.

Materials and methods
The materials and methods used for the synthesis of hybrid polymers are already described (3, 4). FTIR and DSC were performed according to reference 3 and water absorption experiments were performed by gravimetric analysis. Release studies of Rh6G from films were performed at pH 4 and 9 by using UV-visible spectroscopy and data were adjusted to a power-law type relationship (5):

\[ \frac{m_t}{m_\infty} = k t^n \]  

[1]

Results
The amount of Rh6G released at pH 9 is very low for VTPU and for both VTPU/DEAEMA hybrid films. The release curves at pH 4 for hybrid samples are well fitted by Eq. [1]. The \( n \) values indicate a Fickian transport (diffusion-controlled mechanism) for VTPU/DEAEMA:90/10 and a non-Fickian process for VTPU/DEAEMA:70/30 (5). Data are discussed taking into account the water absorption capacity. FTIR and DSC results are also discussed.

Conclusions
DEAEMA monomer and VTPU can be copolymerized to prepare films suitable for drug delivery applications with variable release kinetics depending on the VTPU/DEAEMA ratio. The \( n \) exponent varies depending on the composition and the medium pH, indicating that the mechanism for the drug transport change accordingly. These cationic polymers have potential applications in protective formulations to mask unpleasant tastes and odor, to protect the active ingredient and also to improve drug storage stability.

Acknowledgments
CICPBA and ANPCyT are thanked for financial assistance. ORP and JIA are members of CICPBA.

References

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DESIGN OF NANOSTRUCTURED BIOINTERFACES FOR ENCAPSULATION AND CONTROLLED DELIVERY APPLICATIONS

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Introduction
Nanotechnology applications to the development of nanostructured biomaterials involve the manipulation of biopolymers (proteins and polysaccharides) at the nanoscale (1). In this contribution, we report the impact of the enzymatic hydrolysis of a model protein on the formation of self-assembled biopolymer nanoparticles, based on protein/polysaccharide electrostatic complexation, and on the structuration of biopolymer films adsorbed at fluid interfaces or “nanoshells”. The design of nanoshells with specific properties is a new technology based on nano-encapsulation that could solve industrial problems related to controlled release of drugs (2).

Materials and methods
Biopolymers used were: whey protein isolate (WPI) obtained from Danisco Ingredients (Denmark), sodium alginate (SA) and high-methoxyl pectin (HMP) both supplied by Cargill (Buenos Aires, Argentina). WPI hydrolysates (WPI-h) at different hydrolysis degree (HD: 1.0-5.0%) were obtained by enzymatic reaction using immobilized bovine α-chymotrypsine on agarose microparticles. Preparation of self-assembled biopolymer nanoparticles was carried out at pH 4. For this, WPI and WPI-h concentrations were remained constant at 1.0% wt, whereas polysaccharides (PS) were analyzed in the 0.1-0.5% wt concentration range. The size distribution and particle average size ($Z_{av}$), were determined by dynamic laser light scattering (2). Air/water interface was chosen as interfacial model due to its relative simplicity. Dynamic surface pressure and surface rheological characteristics of adsorbed films or nanoshells were measured by tensiometry and dilatational rheology, respectively (3).

Results
At pH 4, it was observed that the magnitude of the interfacial dynamic properties (surface pressure and surface rheological characteristics) of WPI/PS systems were affected by protein limited hydrolysis, depending on the HD, biopolymer relative concentration and/or combination of these variables. In general, this phenomenon could be explained in terms of modification in WPI size distribution and $Z_{av}$ as a result of limited enzymatic hydrolysis, electrostatic complexation with the PS and/or combined action of both processes. In absence of PS, the incidence of limited hydrolysis on WPI adsorption at the air/water interface could be associated to an alteration in the molecular size, flexibility and protein exposed hydrophobicity. On the other hand, the influence of limited hydrolysis on the interfacial adsorption behaviour of WPI/PS systems could be attributed to the protein surface charge modification. Consequently, WPI-h ability to interact electrostatically with PS depended on polysaccharide chemical structure and biopolymer relative concentration in solution.

Conclusions
Hydrolysis of WPI affected the structuration of biopolymer nanoshells depending on the extent of enzymatic treatment, presence of PS and biopolymer relative concentration. Self-assembled biopolymer nanoparticles and its interfacial behaviour could be explained in terms of the modification of WPI molecular size, and WPI electrostatic complexation with the PS and/or a combination of both effects. The results derived from this research could find applications in the design of engineered biomaterials with mechanical and barrier properties adequate for strategies of both nano-encapsulation and controlled release of drugs.

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Acknowledgments
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References
ACRYLIC POLYELECTROLYTES AS TASTE MASKING AGENTS IN ORAL SOLUTION DOSAGE FORMS

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Introduction
Masking of undesirable taste in oral pharmaceuticals is one of the key aspects for patient compliance, especially for the pediatric population. The most widely used approaches for taste masking are the addition of flavors or sweeteners (1), sometimes ineffective for very bitter compounds. Other approaches such as the use of lipophilic vehicles or coating with hydrophilic vehicles, could impair drug dissolution and bioavailability. One interesting technique is the use of polyelectrolytes to form ionic complexes. In this work we tested the feasibility of achieving taste masking of the model drug ranitidine by the use of acrylic anionic polymers belonging to the Eudragit family.

Materials and methods
Solutions of ranitidine and the acrylic polymers were prepared. Three factors were evaluated:
- The molar ratio polymer:ranitidine were varied between 1:1 and 30:1, at a fixed pH of 6.5 adjusted by means of NaCO\textsubscript{3}.
- The pH was varied between 6-9 at a fixed polymer:ranitidine ratio of 1:10.
- The use of Eudragit L100, L100-55 or S100.
A single blind study was designed for the taste masking test. Six volunteers participated in the test. They rated the bitter taste of the different formulations using a scale of 0–5. When the score was 1 or less, the taste was considered as acceptable. Scores above 1 were considered as unacceptable (2).

Results
The higher the concentration of polymer employed, the lower the rate of bitterness determined by the taste panel. At ratios of 10:1 or higher the score was 0-1 for all the volunteers. Higher ratios did not improve taste masking and presented difficulty in the dissolution of the polymer. The order of effectiveness for the Eudragit polymers was L100-55\textgreater S100\textgreater L100. For the formation of ionic complexes the polymer as well as the drug must present opposite charges; as a result, the optimum pH range was limited by the neutralization of the carboxylic groups generating an insoluble polymer at low pHs, and the pKa of the drug that turns ranitidine neutral at high pHs.

Conclusions
Acrylic polymers were effective in masking the unpleasant bitter taste of ranitidine. Unlike resins of drugs, ionic complexes with Eudragit are soluble in water allowing the formulation of solutions such as syrups, instead of suspensions. Besides the formation of the complexes, the rheological modification of the solutions caused by the presence of the polymer, could improve the masking effect. Studies by infrared spectroscopy and RMN will be presented at the RICiFa meeting.

References.

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ENHANCE OF TRASCORNEAL PERMEATION USING NOVEL EUDRAGIT-FLURBIPROFEN COMPLEXES

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Introduction
This work was carried out in the framework of a project concerning the development of new ophthalmic drug delivery systems. It is based on the properties of Polymethacrylates (Eudragit®, Eu)-drug complexes, which are able to increase the aqueous compatibility of low solubility drug. The aim of the work was the assessment of physico-chemical, pharmaceutical and biopharmaceutical properties of these complexes. Flurbiprofeno (Fl) was selected as active and Eudragit®-Fl complexes were formulated in aqueous solution, which was subjected to transcorneal permeation assays as well as the evaluation of its potential ocular irritation in rabbits.

Materials
Eu-Fl complexes, containing Fl 0.1%, were formulated using 5% dextrose and saline solutions as vehicles. As comparison a commercial product (Tolerane®) and Fl 0.01% aqueous solution (control) were studied. (Table1)

Methods
Properties such as osmolality, partition coefficient, pH, electrokinetic potential, solubility were measured and analysed. Drug release and transcorneal permeation assays were performed in bicompartamental diffusion cells. The potential ocular irritancy and/or damaging effects of the formulations were evaluated using a slightly modified version of the Draize test and inspection of histological sections of cornea.

Results
The Eu-Fl complex solutions were transparent, stable, with high and positive electrokinetic potential (+35 - +55mV). A high proportion of Fl associated to the Eu by ionic condensation was corroborated. As consequence, an increase of the solubility of Fl was observed in pHs where it exhibits low aqueous solubility. The kinetics of permeation through rabbit cornea of 4 formulations showed similar tendency than those obtained in the release assays. The comparative analysis of Fl release and permeation rates showed that solutions containing Eu-Fl50 NaCl 0.9% promoted Fl permeation faster than expected. These systems produced slight irritation characterized by a discrete to moderate vessel congestion of the conjunctiva, without corneal or intraocular injury.

Conclusions
Eu-Fl complexes, vehiculized in aqueous solutions, showed an increment in drug permeation through rabbit cornea, especially in saline solutions. Two factors would the responsible for this effect. By one side, Fl apparent solubility was augmented as consequence of complexation. On the other side, the presence of anionic ions (i.e. Cl-) promoted Fl release from the complexes as consequence of the activation of an ionic interchange process. Finally, the use of these formulations seem to be promissory since have shown to be practically no aggressive for ocular mucosa.

Acknowledgements
CONICET, FONCYT and Universidad Nacional de Córdoba

References.

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Table 1: Formulations used for topical ocular application.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Characteristics</th>
<th>pH</th>
<th>Osmolaridad (osmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen *</td>
<td>Solución fisiológica ajustado con HCl 1N</td>
<td>4.87</td>
<td>0.256</td>
</tr>
<tr>
<td>Tolerane®**</td>
<td>Tetraborato sodico decahidratado, Ácido bórico Cloruro de Sodio, EDTA sal disódico; Beta ciclodextrina agua purificada</td>
<td>7.66</td>
<td>0.292</td>
</tr>
<tr>
<td>Eu-Fl50**</td>
<td>Solución fisiológica</td>
<td>4.87</td>
<td>0.289</td>
</tr>
<tr>
<td>EukFl50**</td>
<td>Dextrosa 5%</td>
<td>5.53</td>
<td>0.287</td>
</tr>
</tbody>
</table>

*[F]:0,01%; **[F]:0,1%
POLYELECTROLYTE-LISINOPRIL COMPLEXES: PHARMACEUTICAL AND BIOPHARMACEUTICAL PROPERTIES

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Introduction
Lisinopril (LS) is a peptide-mimetic drug, angiotensin-converting enzyme inhibitor, used for the treatment of hypertension and congestive heart failure and to alleviate strain on heart damage as a result of a heart attack. LS is absorbed in the first segment of the intestine, by PEPT 1 and 2 transporters. It is slowly and incompletely absorbed after oral administration, with a bioavailability of 25-30%.

In a R&D project on drug delivery systems, LS was selected to be associated to polyelectrolyte carriers (PE). Besides, its amphoteric nature allows the interaction of LS with acid and basic PEs.

The aim of this work was to study some pharmaceutical and biopharmaceutical properties of PE-LS complexes.

Materials and Methods
Alginic acid (polyanion model), Eudragit E100 (polycation model) and LS were used to obtain new materials as solid at various drug-to-polymer weight ratios by a wet granulation method, and drying at 40°C in an oven. Similarly, LS-loaded hydrogels with the same composition were prepared by dispersing the obtained solids in water.

The complexes were studied in solid state (DSC, PXRD and FTIR) as well as in dispersion (release in Franz cells) to establish affinity and type of interactions. Drug permeation was evaluated by the rat everted gut sac technique from LS-Eudragit E100 dispersions, at a concentration of LS of 20 and 40 mg/250 ml, to simulate concentrations of LS after oral administration at the lowest and highest recommended dose.

Results
The PE-LS materials were easily prepared as solid particulates by mixing the powders with small proportions of hydroalcoholic solutions. No residues of crystalline or unreacted LS were present in the solids, regardless of the PE. In solid state, the amine group of LS is ionically linked to the carboxylic groups of alginic acid. Likewise, one of the carboxylic groups of LS is ionically linked to the dimethyl amine groups of Eudragit E100. The aqueous dispersions are physically stable and clear when the functional groups of the PEs are 50% neutralized with LS and the remaining 50% with NaOH (in alginic acid) or HCl (in Eudragit E100). Final pH of the formulations were in the range of 4-6.

The PE-LS dispersions show in Franz cells slow and prolonged release when the receptor medium is water. Release rate is increased upon contact with physiologic simulated fluid, indicating the reversibility of the interaction. Data fits the fickian kinetic model. Interestingly, the release in water from both PEs was faster than previously studied monofunctional drug-PE dispersions (1, 2). This fact can be explained by the zwitterionic nature of LS, which in dispersion appears as a bipolar ion able to migrate from the electrical gradient of polyelectrolyte.

LS intestinal permeation, at the both concentrations tested, was not affected by the presence of neither of the PE.

Conclusions
The ionic interaction between alginic acid or Eudragit E100 and LS modulates the release rate. This attribute could be beneficial to develop long acting formulations to circumvent problems related to LS current treatment, not only for oral but also for transdermal administration.

Acknowledgments
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SOLUBILITY OF AMPHOTERICIN B IN WATER LECITHIN DISPERSIONS AND O/W LECITHIN-BASED MICROEMULSIONS

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Introduction
Cutaneous leishmaniasis is the most prevalent clinical form of the disease caused by parasites of the genus Leishmania spp.; there is a need of topical dosage forms for the treatment of this pathology as monotherapy or associated with other therapies (1). Since amphotericin B (AmB) is an effective polyene-type antibiotic with antileishmanial activity, it would be useful to count with a low-cost topical formulation. In the present work, O/W lecithin-based microemulsions (MEs) and water-lecithin-dispersions (WLD) were prepared. ME are clear, low-viscosity, isotropic and thermodynamically stable colloidal dispersions (2) while WLD are systems obtained by dispersing lecithin in water using extensive mixing at 60°C to obtain good hydration (3).

The aim of the work was to compare the solubility of AmB in MEs and in WLD in order to determine the effect of lecithin on the solubilization of the active compound so as to understand the influence of composition and microstructure of each one of the dosage forms on their drug loading capacity.

Materials and methods
WLD and MEs were prepared by dispersing lecithin (Phospholipon 90G, Lipoid, Germany) in water at 60°C with a magnetic stirrer; for MEs, isopropyl miristate and Brij 97 were mixed and then added while stirring with a high shear mixer. AmB was added in excess to MEs and WLD and shaken for 24 h at room temperature. Drug concentration was analyzed using UV detection at 406 nm. Stability studies were carried out for a month. Statistical analyses were performed using unpaired t-Student Test.

Results
WLD and different microemulsions with either 1.2 or 2.4 % of lecithin were prepared. WLD with 2.4% lecithin show a 10-fold increase in solubilization of AmB compared with 1.2% lecithin WLD. Microemulsions with 1.2% lecithin show an increase of over 400 times in solubilization compared with WLD containing the same concentration of lecithin, whereas MEs with 2.4% lecithin show an increase of over 40 times. Drug solubilization in MEs with 2.4% lecithin is not significantly greater than in those containing 1.2% lecithin. The content of surfactant has a significant influence on drug solubilization (P<0.05).

Conclusions
Results indicate that a synergic, not just additive effect exists on AmB solubilization given by the components of the microemulsion. It can be assumed that solubilization is then due to the formulation microstructure and not to the separate components themselves.

Acknowledgments
Financial support was obtained from UBACyT B003 and PICT 2007-00595.

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INMUNOADYUVANT EFFECT OF OLIGODEOXYNUCLEOTIDS WITH CPG MOTIFS (CPG-ODN) LOADED IN NANOSTRUCTURETED SYSTEMS (COAGELES)

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Introduction
CpG-ODN has shown very promising immunoadyuvant activity. Despite successes demonstrated in early clinical trials, the clinical use of free CpG-ODN remains still uncertain because some troubles (enzymatic degradation, unfavorable pharmacokinetics, a lack of specificity for target cells and poor cellular uptake) (1, 2). In order to improve the biodisponibility of CpG-ODN, we used CpG-ODN formulated in nanostructures (coagels) of 6-O- ascorbyl palmitate (CoaBASC16), which has the ability to form supramolecular aggregate that are produced by phase cooling below a critical micellar temperature(3).

Materials and methods
Mice: 3- months old- female BALB/c, C57BL/6 or TLR4B/B mice. The mice were housed in our animal facility until use under specific pathogen-free conditions. Our animal facility meet the terms of the Guide to the Care and Use of Experimental Animals, published by the Canadian Council on Animal Care and has the assurance number A 5802-01 delivered by the Office of Laboratory Animal Welfare (NIH).
Antigen: OVA was purchased from (grade V) SigmaAldrich (St. Louis, MO).
Oligodeoxynucleotides: CpG-ODN, the sequence 1826: TCCATGACGTTCCTGACGTT. CpG-ODN was synthesized with a nuclease-resistant, phosphorothioate backbone, (Operon Technologies-Alameda, CA).
CoaBASC16, was prepared by heating ASC16 in isotonic dextrose (with or without CpG-ODN) to above the phase transition temperature and allowing the temperature to fall to room temperature[3].
Immunization: Mice were immunized on day 0, 7 and 15 with OVA mixed with CpG-ODN (OVA/CpG-ODN) or OVA mixed with CpG-ODN formulated with CoaBASC16 (OVA/CpG-ODN/CoaBASC16). Each mouse was injected subcutaneously in the tail, in the neck region, and in both hind limbs. The dose of OVA was 60µg/animal/dose. CpG-ODN was administered at a dose of 75µg per animal.
Antibody assays: specific antibody against OVA were determined by enzyme-linked immunosorbent assay (ELISA).
Cytokine-specific ELISA: level of IL-6 was measured by capture ELISA.
Flow cytometry analysis: cells were pre-incubated for 20 min at 4˚C with anti CD32/CD16 monoclonal antibody and then stained with primary monoclonal antibodies conjugated with an appropriate fluorochrome for 30 min at 4˚C. Cells were acquired on a FACS Canto II cytometer and data were analyzed using FlowJo software.
Statistical analysis: Data were analyzed by Graph Pad Prism4 software (Graph Pad Software. San Diego, CA). All data were considered statistically significant if p values were <0.05.

Results
In previous studies we have observed that this pharmaceutical strategy improved the CpG-ODN immunoadyuvant activity. In such studies, the immunization of mice with OVA/CpG-ODN/CoaBASC16 resulted in a significant increase in specific antibody titer (IgG, IgG1 e IgG2a) and IFN-γ in comparison with mice immunized with OVA/CpG-ODN (4). This effect was maintained for a long time (117 days post immunization). In this work we evaluated if CoaBASC16 induces “per se” inflammation. Mice were intraperitoneal injected with CoaBASC16 or dextrose solution (5%) (control group) and 6 h later the peritoneal lavages were taken. CoaBASC16 induced a strong infiltration of neutrophils (Ly6G<sup>high</sup>, CD11b<sup>+</sup>, Ly6c<sup>+</sup>), F4/80<sup>neg.</sup> (47.3±3.6 vs 0.81±0.03, p<0.001), monocytes (CD11b<sup>+</sup>, Ly6G<sup>neg.</sup>, Ly6C<sup>neg.</sup>, F4/80<sup>neg.</sup>) (26.2±1.2 vs 1.03±0.06, p<0.001) and dendritic cells (CD11c<sup>-</sup>) (1.65±0.06 vs 0.65±0.05, p<0.001) into peritoneal cavity. In addition, CoaBASC16 induced an increase of the innate cytokine IL-6
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(1438±349pg/ml vs 48,4±2,2 pg/ml, p< 0,01). These experiments were also carried out in TLR4-/- animals obtaining the same results.

Conclusions
We observed that Coa-ASC16 is able to induce an inflammatory condition in the peritoneal cavity. The inflammation induced by Coa-ASC16 could be one of the mechanisms to explain how Coa-ASC16 increases the effect of CpG-ODN. The use of Coa-ASC16 as strategy for delivering the CpG-ODN may represent an optimal approach to modulate the immune system.

Key words: adjuvant, CpG-ODN, vaccine

References
DEVELOPMENT OF A TOPICAL FUNCTIONALIZED FORMULATION FOR THE PROPER SKIN CARE: PREFORMULATION, FORMULATION, AND QUALITY CONTROL STUDIES

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Introduction
The skin is one of the most delicate organs of the body. May be affected by many external factors such as sun exposure, wind or cold temperatures and it is therefore necessary the use of creams formulated to reduce or prevent damage. There are many treatments aimed at keeping it in good condition and prevent or reduce wrinkles, such as daily cleaning, adequate hydration and use of sunscreen formulations. One reason of the lack lubrication in the skin is the incorrect functionality of sebaceous glands. Some cares that could help solve the problem are: not to use too aggressive facial cleansers, to avoid excessive sun exposure, a balance diet, not to abuse of scrubs products and to use creams for each type of skin.
In this work we developed the study of preformulation, formulation and quality control of a pharmaceutical dosage form suitable for the proper skin care in dry climates or extreme temperatures, providing protection from daily irritants such as wind and salt or chlorinated water.
The pharmaceutical dosage forms was formulated whit the following active principles: Karité butter, avenal milk, Aloe vera glycolic extract, omega 6. This formulation serves to promote cell desquamation resulting in an increase of epidermal water content which restores elasticity, brightness, tone and skin functionality.

Materials and methods
Studies were performed in triplicate, following the protocols required by Argentina Pharmacopoeia VI th edition, Food and Drug Administration and Spanish Pharmacopoeia I st edition.
The semi-solid pharmaceutical form was subjected to stability studies at different times (0, 2, 7, 15,21 and 60 days).
- Pre formulation studies: design and elaboration of three pharmaceutical topical dosage forms: emulsion and creams with different exipients were development.
- Stability tests: pH , water loss, reversibility, thermal effects, mechanical stress (extensibility and centrifugation assays), stability , emulsion type determination, spontaneous separation of phases, organoleptic test, sensory tests, microscopy study, gross appearance study, short-term physical stability, long term physical and functional stability.
-Microbiological studies: antimicrobial activity against pathogenic bacteria and fungi, environmental fungal contamination test and health monitoring.

Results and conclusions
The microbiological studies showed that the formulations contain the optimal dose of methylparaben and propylparaben ensuring the conservation of the pharmaceutical dosage forms.
Stability studies allow characterized these formulations in relation to stability and represent a valid indication of the suitability of the manufacturing processes used and the correct choice of components in the formulation. Organoleptic and sensory tests showed emulsion as a very good acceptance in terms of appearance, smell and color, uniformity and good absorption, making it the product of choice in the double-blind study. Accelerated stability studies showed preservation for a period of one year.
A NOVEL ANTI-AGING SKIN CARE CREAM. DEVELOPMENT AND PHYSICAL STABILITY EVALUATION.

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Introduction
Skin aging is a complex and dynamic process in which are involved genetic, hormonal and environmental factors. In a biochemical level, the aging process (AP) is just an inevitable process of oxidation and cellular death. Lifestyle is an important factor in the AP. A balanced diet, exercise and basic care such as using antiaging creams will be reflected in the delay of aging.

The interest in the process of photo aging study emerged from the sixties, due to the great importance that society attributes to maintain a longer youthful appearance.

The aim of this work was the study of preformulation, formulation, stability control and microbiological assays to a pharmaceutical dosage form that can be trade in something, used to treat dryness, damaged and old, promoting cellular metabolism of skin. The pharmaceutical dosage forms was formulated with the following active principles: Rosehip oil, wheat germ oil, glycolic extract of Centella asiatica, glycolic extract of red grape seeds, vitamin A and retinol.

Materials and methods
Semi-solid pharmaceutical forms (creams and emulsion) was subjected to stability studies at different times (0, 2, 7, 15.21 and 60 days).

Pre formulation studies: design and elaboration of three pharmaceutical topic dosage forms: emulsion and creams with different exipients were development.

The tests conducted were:
Stability assays: pH, water loss, reversibility, thermal effects, mechanical stress (extensibility and centrifugation assays), stability, emulsion type determination, spontaneous separation of phases, organoleptic test, sensorial tests, microscopy study, gross appearance study, short-term physical stability, long term physical and functional stability.
-Microbiological assays: antimicrobial activity against pathogenic bacteria and fungi, environmental fungal contamination test and health monitoring.
Antioxidant activity assay.

Results
The developed formulation emulsion was found to be stable and homogeneous in the time period analyzed. There was no change in pH influenced by temperature or time. No microbiological contamination was detected. Organoleptic and sensorial tests showed a very good acceptance in terms of appearance, smell and color, uniformity and good absorption, making it the product of choice in the double-blind study. Accelerated stability studies showed preservation for a period of one year.

Discussion and Conclusion
Stability and microbiological tests showed that this preparation complies with the conditions of Argentina Pharmacopoeia VI th edition, Food and Drug Administration and Spanish Pharmacopoeia I st edition. This formulation may be applied topically to prevent or treat skin aging by oxidative stress.
INTERACTION BETWEEN ASCORBYL PALMITATE AND WATER

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Introduction
The knowledge of the interaction between water and drugs having pharmaceutical applications is of fundamental importance for the design of applications. The interplay of experimental, structural and molecular dynamics simulation gives a complete picture of this interaction. We have performed differential scanning calorimetry (DSC) experiments and have interpreted the results on the basis of a geometric model of hydrated crystals and lamellar liquid crystals of ascorbyl palmitate (Asc16) and molecular dynamics simulation (MDS).

Materials and methods
Ascorbyl Palmitate (AP) was purchased from (Flukka-Italia). Redistilled water by Allchemistry (Buenos Aires, Argentina) was used in all experiments. Samples were prepared having 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 95 % weight/weight (w/w).
Calorimetric measurements were performed with a Q20 Differential Scanning Calorimeter (TA Instruments). Samples were prepared using closed hermetic aluminium pans which have been weighed with a fifth cipher balance Sartorius (Germany). All runs were performed at the rate 5 ºC.min⁻¹. The samples were treated cooling to -20ºC during 5 minutes. Then, they were heated to 150ºC at a rate of 5 ºC.min⁻¹. Eventually, they were kept at this temperature for a minute.
Optical microscopy was performed with a Nikon Eclipse E-200 POL polarizing (Tokyo, Japan) microscope. The scatters were heated until the temperature that DSC thermograms have been shown a phase change.
The simulation of a solvated ascorbyl-6-O-dodecanoate monomer was performed by molecular dynamic (MDS) using the AMBER10 molecular simulation suite. After a minimization to adjust the angles and bond lengths, the monomer was solvated with 7524 TIP3 model waters. First the system was equilibrated at 300 K (Langevin thermostat) and 1 bar. Afterwards, a dynamic trajectory (canonical ensemble NPT) of 10 ps was performed, saving the configurations every 0.1 ps.

Results
DSC thermograms show that there are tree kinds of water. We have identified one of them as hydration (i.e., water strongly attached to the polar headgroups), corresponding to a hydration number of 11.5 ± 1.3 water molecules per Asc16 molecule. Other kind of water was identified as water associated to the polar surface of crystals, which amounts 54 ± 4 water molecules per surfactant one in very dilute samples, but diminishes with increasing concentration and disappears at C ≈ 0.48 wt %. All water exceeding the above amounts is free (bulk) water. The fact that the N_{water}/N_{surf} of water associated to the surface diminishes with increasing C indicates that this water is loosely related to the surface and an increasing crowding of the polar groups will free some of these water molecules. These findings agree with literature ones.
MDS shows that the Asc16 headgroup has a hydrophobic side and there is a folding of the chain producing a hydrophobic bonding between the hydrophobic face of the polar head group and the chain. There is a first hydration layer, extended up to 3 Å which are directly attached via hydrogen bonds to the oxygen and hydroxyl groups of the polar headgroups, having 11.47 ± 0.95 water molecules per surfactant molecule and coincides with the number of water molecules which are undetectable by DSC, i.e., those appertaining to the first hydration layer (11.5 ± 1.3). A second hydration layer is extended up to 9 Å from the polar headgroup, formed by water molecules associated with that of the first hydration layer trough

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hydrogen-bonded strings of molecules. This second hydration layer has $59 \pm 17$ water molecules per surfactant one (excluding those in the first hydration layer).

Finally, a physical steric model of a bilayer of surfactant molecules with the polar heads pointing to the central plane and having water between the two surfactant layers was made using molecular dimensions. This model showed that when the distance between the two polar surfaces is 6 Å (i.e., two first hydration layers) the content of water per surfactant molecule corresponds to about 11, and when the distance is augmented to 18 Å, the increase in water is about 50-60 water molecules. Then, the findings obtained by DSC, MDS and the model agree perfectly.

Conclusions
DSC, MDS and the physical model results agree in that the polar headgroup of Asc!6 has a first hydration layer which is not detectable by DSC, having about 11 water molecules per surfactant one, strongly attached to the oxygen and hydroxyl groups of the polar head of Asc16 and extended up to 3 Å. A second hydration layer is formed by strings of hydrogen-bonded water molecules associated to those of the first hydration layer. That layer is extended up to 9 Å from the polar headgroup surface, and contains about 60 water molecules in diluted samples, but the number is reduced when the concentration augments. All other water present in the system is “free” or bulk. Moreover, the polar headgroup has a hydrophobic side and in dilute (molecular) solution the molecule folds forming an hydrophobic bond between that face and the chain.

Acknowledgments
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References.
DEVELOPMENT OF A SELF-EMULSIFYING DRUG DELIVERY SYSTEM CONTAINING COQ10

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Introduction
Ubiquinone (CoQ10) deficiency is associated with five clinical presentations which showed clinical improvements after oral drug administration (1). Co-Q10 exhibits extreme lipophilicity and high molecular weight, therefore it is necessary to develop special formulations for its bioavailability. It is well-known that bioavailability can significantly improve using dosage forms which contain CoQ10 dissolved (2). SEDDS (Self-emulsifying drug delivery system) are isotropic mixtures of oil, surfactant, co-surfactant and drug with ability to form oil in water emulsion upon mild agitation following dilution with aqueous phase (3). The objective of the present work was to optimize a liquid auto-emulsifying formulation so as to load a clinical relevant CoQ10 dose to be administered in pediatric patients.

Materials and methods
Materials: Cetiol OE (Dicaprylyl Ether), Imwitor 408 (Propylene Glycol Caprylate), Isopropyl myristate, Miglyol 812 (Caprylic/Capric Triglyceride), Laureth-4 and Propylene glycol (PG).
Methods: these excipients were chosen from previous CoQ10 solubility experiments, so a wide range of binary mixtures (oil phase: surfactant) could be obtained. PG was added and Ternary phase diagrams were used to evaluate the isotropic domain. To determine the equilibrium solubility, drug in excess was added. The samples were left to equilibrate using a Rotating Bottle apparatus and then filtered. Samples were analyzed by HPLC (Waters, MI, USA). Compositions with highest capacity of drug solubilization of each one of the oil phase used were selected for physicochemical (viscosity, rate and capacity of emulsification) characterization. A stability test that included microscopic observation was carried out during a month.

Results
Imwitor 408 was the oil phase which showed the biggest region of isotropic ternary mixtures and Cetiol OE the one with the smallest one. The dissolved concentrations obtained were equal or even higher than the ones previously presented in literature (6% w/v). No significant changes in physicochemical parameters or in physical stability were observed in the selected composition.

Conclusion
CoQ10 belongs to Class II in BCS Classification, nowadays SEDDS are considered to be a promising way to administer this kind of drugs. Their optimization can be carried out properly if the main physicochemical characteristics of the involved components are considered. In this way, an appropriate liquid dosage form for oral administration of CoQ10 for pediatric patients in a hospital environment could be proposed.

Acknowledgments
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References

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STUDIES OF THE INTERMOLECULAR INTERACTIONS BETWEEN PILOCARPINE AND CARBOMER IN SOLID STATE

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Introduction
Pilocarpine (PIL) is a drug used in the treatment of glaucoma. To obtain an optimal stability of the drug, the eye drops are formulated at pH 4-5, although in this range of pH the drug is fully ionized, which can reduce the ocular absorption of PIL (1). It has been reported that the anionic polyelectrolyte Carbomer (CBR), was effective for retarding the degradation rate of drugs, through the formation of ionic complexes (2, 3). In previous work we reported the positive effect of complexation with CBR on the chemical stability of PIL in aqueous solution (4). The aim of the present study was the preparation and characterization of an ionic complex between PIL and CBR in solid state.

Materials and Methods
The PIL:CBR complexes were prepared by lyophilization or physical mixture of the components. Their interactions were studied in the solid state by fourier-transform infrared spectroscopy (FT-IR), solid state nuclear magnetic resonance (ssNMR), X-ray powder diffraction, scanning electron microscopy (SEM) and thermal analysis [differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)].

Results and Discussion
When analyzing the FT-IR and ssNMR spectra corresponding to the binary systems prepared by physical mixture or freeze-drying, marked differences are evident with respect to the spectra of the raw materials. These changes are attributed to the complex formation between the drug and the polyelectrolyte thought electrostatic interactions. The powder x-ray diffraction pattern and SEM microphotographs demonstrated that the complexes obtained are amorphous solids. The DSC and TG curves indicated that the binary systems PIL:CBR obtained by physical mixture of the components or liophilization present the same thermal behaviour.

Conclusion
The anionic polymer CBR showed good capability to interact with the drug PIL giving rise to ionic drug/polymer complex in solid state. The complex can be prepared by physical mixture of the components or liophilization, although the latter method is the best suitable for obtaining this system at industrial scale.

Acknowledgments:
The authors thank the Laboratorios Beta for their donation pilocarpine.

References:

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DEVELOPMENT OF A DIRECT COMPRESSION ROBUST HYDROPHILIC MATRIX OF AN INSOLUBLE MICRONIZED ACTIVE USING NIFEDIPINE AS A MODEL DRUG

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Introduction
Nifedipine is a practically water insoluble, calcium channel blocker used in the treatment of hypertension. Due to the low solubility of the drug, an extended release formulation of a hydrophilic matrix may be challenging. This is mainly due to the requirement of using a low viscosity polymer couple with low concentration of the polymer to ensure timely and complete drug release from the matrix system. Low concentration of polymer may negatively impact the robustness of the formulation. In addition, application of a low viscosity polymer will result to low gel strength of the matrix. These two formulation practices will lead to variable in-vitro drug release as well as, potential in-vivo burst release.

Particle size reduction of poorly soluble drugs through micronization is commonly used to increase the surface area of the particles, enhancing their wettability and dissolution rate. In addition, it is vital to micronize low dose drugs for content uniformity within the batch as well as within a single extended release tablet.

The main objective of this work was to develop and characterize a directly compressible formulation of a robust extended release Nifedipine 30 mg matrix using hypromellose (HPMC).

Materials and Methods
The formulation and list of materials used in the study are shown in Table 1. Nifedipine, METHOCEL™ K15M CR and E15 LV, StarCap 1500®, anhydrous lactose and colloidal silicon dioxide were weighed, screened through a 30 mesh screen and mixed in a cubic blender for 10 minutes at 150 rpm. The magnesium stearate was screened through a 60 mesh screen and added to the initial mixture, and blended for a further 3 minutes. The blended formulation was characterized for flow.

Tablets with a target weight of 300 mg were manufactured using an instrumented 10 station rotary press (Junior Express, Talleres Sanchez, Argentina), fitted with 9.0 mm standard concave tooling. Tablet mechanical strength was determined using hardness (Erweka TBH 220) and friability (Ashitsu FAB145B07) testers. Drug release was measured in a USP 31-B Test 4 compliant dissolution bath (AT7smart – Sotax - Switzerland) using apparatus II (paddle at 100 and 150 rpm), 900 mL of 0.1N HCl with 0.5% SLS.

Results and Discussions
Powder flow of the formulation was good (Table 2) and physicotechnical properties of the tablets were excellent (Table 3). Tablet weight variation was low, indicating good flow of the formulation during compression. Nifedipine release profiles of the matrix tablets were analyzed at 100 and 150 rpm and the results met the USP 31 specifications for nifedipine 30mg ER formulation. In addition, the calculated F2 similarity values when comparing samples at 100 and 150 mg drug release profiles was 87.5, indicating similarity between samples in spite of their different agitation rate.

Conclusions
Robust tablets with good physico-mechanical characteristics were produced by direct compression process. StarCap 1500® provided good flow and compressibility, in addition to enhanced content uniformity to this formulation containing a micronized API. METHOCEL™ K15M and E15LV blend, showed a balanced gel strength and desired release profile. The formulation was robust and produced similar profiles at 100 and 150 rpm agitation rate

References
1. The United States Pharmacopeia USP 31, NF 26
4. www.rxlist.com
Table 1 Nifedipine ER formulation

<table>
<thead>
<tr>
<th>Material</th>
<th>% w/w</th>
<th>mg/tablet</th>
</tr>
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<tbody>
<tr>
<td>Nifedipine micronized</td>
<td>10.00</td>
<td>30.00</td>
</tr>
<tr>
<td>HPMC (METHOCEL™ K15M PR CR)</td>
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<td>30.00</td>
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</tr>
<tr>
<td>Magnesium stearate</td>
<td>1.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Total:</td>
<td>100.00</td>
<td>300.0</td>
</tr>
</tbody>
</table>

Table 2 Powder properties of the Nifedipine ER formulation

<table>
<thead>
<tr>
<th>Test</th>
<th>ADS Formula (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g/ml)</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>Tapped density (g/ml)</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Angle of repose (°)</td>
<td>24.00 ± 3.0</td>
</tr>
<tr>
<td>Carr’s index (%)</td>
<td>21.7 ± 0.00</td>
</tr>
<tr>
<td>LOD (%)</td>
<td>4.50 ± 1.50</td>
</tr>
<tr>
<td>Psd (% retained) (150 mesh)</td>
<td></td>
</tr>
<tr>
<td>Psd (% retained) (75 mesh)</td>
<td></td>
</tr>
<tr>
<td>Psd (% retained) (45 mesh)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Tablet physicotechnical properties for nifedipine ER formulation

<table>
<thead>
<tr>
<th>Specification</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tooling diameter (mm)</td>
<td>9.0 ± 0.2</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>Hardness (kp)</td>
<td>9.0 ± 2.0</td>
</tr>
<tr>
<td>Average weight (mg)</td>
<td>300.0 ± 3%w/w</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>&lt; 0.8 (4 min)</td>
</tr>
<tr>
<td>Weight uniformity (mg, n=30)</td>
<td>Min = 265.3, Max = 275.8, Av. = 271.73, %RSD = 1.13</td>
</tr>
<tr>
<td>Hardness (kp, n=10)</td>
<td>Min = 7.1, Max = 8.4, Av. = 7.8, %RSD = 5.64, 0.34</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Figure 1. Drug Release at different rpm dissolution configurations.
A NEW PATHWAY TO IMPROVE AMPHOTERICIN B ENTRAPMENT EFFICIENCY ON LIPIDIC CARRIERS AS MICROEMULSIONS

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Introduction
Systemic fungal infections induced by opportunistic microorganisms remain the major problem during the hospitalization of neutropenic patients (1). Amphotericin B (AmB) is the drug of choice for the majority of systemic fungal infections in immunocompromised patients because of its broad spectrum of activity (2). However, its conventional formulation, Fungizone®, a sodium deoxycholate micelle system containing 50 mg of AmB and 41 mg of sodium deoxycholate, is frequently limited by its high incidence of adverse reactions, mainly fever chills and nephrotoxicity (3). Developing new effective formulations of AmB is a goal in the pharmaceutical technology field. However this becomes a great challenge due to the fact that this molecule is, also, practically insoluble in the majority of solvents (1). The aim of this work was to evaluate a new pathway to improve the AmB entrapment efficiency into microemulsions (ME). This new method consists of to play with the pH range in which the AmB presents the maximum solubilization behavior, emphasizing the pKa values of the amphoteric groups (5.7 for –COOH and 10.0 for NH₂) (4).

Materials and methods
The ideal amounts of AmB were weight and added, at the concentration of 5 mg/mL, in a sodium hydroxide solution (NaOH 1 M). The solubilization process was observed not only by the clarity of the solution, but also spectrophotometrically by the presence of the AmB typical monomeric band at 408 nm. Following, the AmB alkali solution was titrated with hydrochloric acid solution (HCl 1 M) to induce changes on the pH values and to verify its influence on the AmB aggregation state. The full process was pH monitored and the spectrophotometric analysis was performed to determine the AmB content and aggregation state. The reverse procedure, in which the first solvent was HCl, was, also, verified. Indeed, the same process was performed to load the AmB into a ME system. The ME system was produced from a pseudo-ternary phase diagram (PTPD) technique. The final formulation was the following composition: Miglyol® 812N as oil phase (11 %), phosphate buffer solution pH 7.4 as aqueous phase (68 %) and Lipoid® S100 and Tween® 80 as surfactants (21 %).

Results
Initially, the AmB was solubilized in alkaline pH (13.00). The drug becomes insoluble when the pH of the media decreases to values above 10.00. At first, 100 % of the AmB nominal content was found on the monomeric form. The spectrophotometric readings at pH values of 10.00, 7.0 and 5.7 showed a decrease on its form for 99.10%, 45.75% and 34.40%, respectively. This proofs that AmB insoluble product starts to appear. In fact, the presence of some coacervates (crystals) was observed on the solution. Below pH 5.7 the relationship between insoluble and soluble AmB reach the equilibrium and at pH 1.8 the AmB monomeric band presents a value of 36.74 %. However, such behavior in alkali media was not observed on the reverse procedure. In acidic media the AmB solubility were not changed at all pH ranges and the drug remained insoluble. Similar behavior was found during the loading process of AmB into ME. The AmB was totally incorporated (100 %) into the system when the pH values reached alkaline ranges. However, at pH 7.0, only 70.20% was incorporated. This demonstrates that the drug was partitioned into the oil and aqueous phase. Therefore, this method favored the incorporation of the AmB into the ME.

Conclusions
The AmB molecule has an amphoteric behavior due to the presence of ionizable carboxyl and amine groups. This zwitterionic nature brings as a consequence its poor solubility in all aqueous neutral solvents and in many organic solvents at the physiological pH (6-7) (5). According to the literature, at extreme pH values (below 2 or above 11), AmB is water soluble. However, this molecule is not stable under these extreme conditions because of its probability to form salts (6). Our results showed, therefore, that AmB
was nicely soluble only at pH above 10. The acidic solubilization was not observed. Moreover, these results were of great help on the entrapment process of this drug into lipidic structures, such as ME. The alkaline environment promotes the better performance in terms of content in these structures.

Acknowledgments
The authors wish to thanks BNB and CNPq (Brasília, Brazil) for financial support in the development in this work.

References

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**IN VITRO EVALUATION OF PREABSORPTIVE EVENTS OF ORAL MULTIVITAMIN FORMULATIONS BY DYNAMIC LIGHT SCATTERING.**

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**Introduction**

The development of methodologies to evaluate *in vitro* the events that take place in the upper gastrointestinal tract upon administration of an oral dosage form have received considerable attention along time. In fact, well defined official tests are available to appraise disintegration and drug dissolution on tablets, hard capsules and some oral suspensions. Less attention has been paid to evaluate preabsorptive events of aqueous formulations consisting of supramolecular aggregates loaded with active pharmaceutical ingredients. Within this kind of dosage forms, mixtures of lipophilic and hydrophilic vitamins (A, D and C) dispersed in a water/glycerin/polysorbate-80 vehicle are formulated as oral drops, widely used in the pediatric population (newborns and infants, usual dose 0.3 mL).

In this report, a methodology to follow the contact of the drops with simulated gastrointestinal fluids was designed based on dynamic light scattering (DLS).

**Materials and methods**

Three multivitamin supplements with marketing authorization in Argentine[1], Trivisol® (P1), Ostelin® (P2) and Tanvimil® (P3), were selected and their composition is reported in table 1. A reference solution (RS) having the same proportion of polysorbate-80 glycerin and water was prepared. To mimick the preabsorptive events, the formulations were appropriately diluted (1/2, 1/5 and 1/10 v/v) with water, simulated gastric fluid (SGF) and simmulated intestinal fluid (SIF). DLS and electrokinetic ζ-potential of all samples were performed, at 25 and 37°C, using Zetasizer (Beckman-Coulter). Diffusion coefficients and apparent hydrodynamic diameters (dH) were reported (table 2).

**Results and Discussion**

As table 1 reports, there are only minor differences among formulations and therefore they may be considered as essentially similar products, in which the main components are polysorbate-80 (19.5%), glycerin (42.0%) and water to complete 100%.

**Undiluted formulations.** The DSL analysis of the products showed monodisperse supramolecular aggregates, with a polydispersity index ≤ 0.1 and diffusion coefficients ranging from 2.7 to 16.3E10 cm²/s. The complexity of the systems prevents the assignment of a physical meaning to dH values (table 2). However, they were useful to follow a comparative analysis since the formulations exhibited differences in dH (P3 > P2 > P1).

**Diluted formulations.** 1/2 to 1/10 dilutions with water exhibited at 25°C quite similar dH in P1 and P2 and a higher one in P3. At 37°C, dH became higher in P2 and P3 while those of P1 and the RS dispersion remain unchanged. Samples 1/10 diluted with SGF and SIF showed similar dH among P1, P2, P3 and the RS at 25°C. At 37°C, P2, P3 and RS rise their dH while that of P1 remained unchanged, as observed in water. It’s known that a polysorbate-80 aqueous dispersion at this concentration consist of spherical micelles[2].

All diluted samples exhibited negative close to zero ζ-potentials, (-0.6 to -2.0 mV), which is consistent with the non-ionic character of polysorbate-80.

**Conclusions.**

Diluted samples exhibit dH close to that of the polysorbate-80 reference dispersion. So, it appears that they also consist of sferical micelles loaded with the lipophilic vitamins. Therefore, it’s expected that the micellar supramolecular aggregates will be in contact with the gastrointestinal membranes upon administration. It is worth mentioning that the amount of polysorbate-80 contained in a newborne dose is higher than the WHO recomended daily intake of polysorbate-80 (25 mg/Kg).

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8 Corresponding author. Tel.: +54-351-4334163 (Int. 111), fax: +54-351-4334127; E-mail: rubmanzo@fcq.unc.edu.ar
References.


Table 1: Quali and quantitative composition of multivitamin products and reference solution, expressed in g. (Data extracted from product patient information leaflets)

<table>
<thead>
<tr>
<th>Components</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A palmitate</td>
<td>833.3 IU</td>
<td>833.3 IU</td>
<td>833.3 IU</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>8.33</td>
<td>8.33</td>
<td>8.33</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin D&lt;sub&gt;2&lt;/sub&gt;</td>
<td>166.6 IU</td>
<td>166.6 IU</td>
<td>166.6 IU</td>
<td>-</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>0.028</td>
<td>NA</td>
<td>0.030</td>
<td>-</td>
</tr>
<tr>
<td>Polysorbate-80</td>
<td>19.14</td>
<td>NA</td>
<td>19.50</td>
<td>19.50</td>
</tr>
<tr>
<td>Glycerin</td>
<td>41.72</td>
<td>NA</td>
<td>42.00</td>
<td>42.00</td>
</tr>
<tr>
<td>Methylparaben</td>
<td>211.64</td>
<td>NA</td>
<td>251.80</td>
<td>-</td>
</tr>
<tr>
<td>Propylparaben</td>
<td>0.023</td>
<td>NA</td>
<td>0.023</td>
<td>-</td>
</tr>
<tr>
<td>Sodium saccharin</td>
<td>0.088</td>
<td>NA</td>
<td>0.500</td>
<td>-</td>
</tr>
<tr>
<td>Aroma</td>
<td>0.313</td>
<td>NA</td>
<td>0.320</td>
<td>-</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>2.07</td>
<td>c.s.pH=6</td>
<td>c.s.pH=6</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol 96°</td>
<td>-</td>
<td>1.6</td>
<td>0.500</td>
<td>-</td>
</tr>
<tr>
<td>Butylhydroxytoluene</td>
<td>-</td>
<td>-</td>
<td>0.100</td>
<td>-</td>
</tr>
<tr>
<td>Purified water to</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
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</table>

Table 2: Hydrodynamic diameter values (nm) of different undiluted and diluted products calculated by DLS, at 25 and 37 °C.

<table>
<thead>
<tr>
<th>Product</th>
<th>T (°C)</th>
<th>Water</th>
<th>SGF 1/10 (v/v)</th>
<th>SIF 1/10 (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>undiluted</td>
<td>1/2 (v/v)</td>
<td>1/5 (v/v)</td>
</tr>
<tr>
<td>P1</td>
<td>25</td>
<td>44.6 ± 5.1</td>
<td>17.5 ± 0.7</td>
<td>14.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>25</td>
<td>73.6 ± 3.2</td>
<td>35.7 ± 0.9</td>
<td>13.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>25</td>
<td>153.0 ± 6.4</td>
<td>29.1 ± 0.7</td>
<td>17.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RS</td>
<td>25</td>
<td>12.7 ± 0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>
EVALUATION OF COQ10 SOLUBILITY IN DIFFERENT PHARMACEUTICALLY ACCEPTABLE EXCIPIENTS

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Faculty of Pharmacy and Biochemistry. University of Buenos Aires, Argentina.
Junín 954, (1113) Buenos Aires.

Introduction
The mitochondrial respiratory chain disorders are the most common kind of hereditary neurometabolic diseases. Ubiquinone (CoQ10), is a very important component in the oxidative phosphorylation. It is well known that its bioavailability depends mostly on the drug release system used, formulations containing dissolved drug are the most bioavailable ones (1) (2). The objective of the work was to evaluate its solubility in different pharmaceutically acceptable excipients (oil phases and surfactants). Afterward, binary mixtures were prepared with different excipients ratios and their capacity of CoQ10 solubilization was evaluated.

Materials and methods
Materials: Ubicaderone (2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone); liquid petroleum jelly, Dicaprylic ether, Propylene Glycol Caprylate, Isopropyl myristate, Glyceryl Caprylate/Caprate, Caprylic/Capric Triglyceride, Lecithin, castor oil; Polyoxyethylene (20) sorbitan monooleate, Caprylocaproyl macrogol-8-glycerides, Polyoxy (40) hydrogenated castor oil, Polyoxyethylene (6) C9-C11 alcohol, Polyoxyethylene (6.5) C13 alcohol and Laureth-4.
Methods: To determine the equilibrium solubility, drug in excess was added to excipients. The samples were left to equilibrate using a Rotating Bottle apparatus and then filtered. Binary mixtures were prepared with the four oil phases and the two surfactants which had shown the best values of solubility and in 1:2; 1:1; 2:1 weight ratios. Samples were analyzed by HPLC (Waters, USA).

Results
The excipients which showed the most efficiency in drug solubility were: Caprylic/Capric Triglyceride, Dicaprylic Ether, Propylene Glycol Caprylate and isopropyl myristate (82.98 to 173.06 mg/g); Polyoxyethylene (6.5) C13 alcohol (HLB 11) and Laureth-4 (HLB 9.7) were able to solubilize 15.94 and 63.55 mg/g, respectively. As polar and dispersion forces of selected excipients were similar every evaluated mixture ratios generated homogeneous system. The binary mixtures exhibited the best capacity of solubilization.

Conclusions
Lipid-based drug delivery systems are of increasing interest because of their potential to solubilize drug that may be otherwise difficult to develop (3). Isopropyl myristate showed the highest value of CoQ10 solubilization which is promising data for biopharmaceutical evaluation, because it has a parameter solubility similar to cell membranes. A number of binary mixtures able to solubilize a clinical amount of drug were obtained without any instabilization sign during a month at room temperature of storage. These results encourage further studies for CoQ10 oral administration in soft capsules.

Acknowledgments
Financial support was obtained from PICT 2007 00595.

References

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ABAMECTIN AND ALBENDAZOLE PLASMA PROFILES AFTER THEIR COMBINED ADMINISTRATION AS A CONTROLLED-RELEASE CAPSULE IN LAMBS

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Introduction
In an attempt to overcome the inconvenient of the development of resistance to the available anthelmintic chemical groups, pharmaceutical formulations combining either two or three chemical entities have been developed. Several preparations combining benzimidazole and macrocyclic lactone compounds are available in the veterinary pharmaceutical market, including an intra-ruminal controlled release capsule for use in sheep. The goal of the current work was to investigate the disposition kinetics of both abamectin (ABA) and albendazole (ABZ) after their oral administration as a combined controlled-release capsule in lambs.

Materials and methods
Eight (8) Corriedale lambs were treated with an ABA/ABZ controlled-release capsule (Bionic®, Ancare, New Zealand) by the oral route. Plasma samples were collected over 100 days post-treatment and drug concentrations measured by HPLC. Non-compartmental analysis was used to calculate the plasma pharmacokinetic parameters.

Results
ABA and ABZ were detected in plasma up to 92 days post treatment. The area under the concentration vs. time curve (AUCₜₜ) for ABA was 839.6 ± 377.5 ng.day/ml, with a peak plasma concentration (Cₘₚₜ) of 16.5 ± 7.7 ng/ml. ABZ parent drug was not detected in plasma at any time post-treatment. Its active metabolite, ABZ-sulphoxide (ABZSO), reached a Cₘₚₜ value of 0.2 ± 0.1 µg/ml, with an AUCₜₜ of 8.6 ± 3.4 µg.day/ml. The ABA and ABZSO plasma concentrations increased gradually to achieve the steady-state peak plasma concentration (10.3 ng/ml and 0.1 µg/ml, respectively) at approximately 8 days post-treatment, which was maintained over 77 days.

Conclusions
Sustained concentrations of both ABA and ABZSO were measured in the bloodstream. The steady-state plasma concentrations of both compounds were maintained up to 77 days post-treatment, which may be relevant to control helminth parasites susceptible to the assayed anthelmintic molecules.

Acknowledgments
This work was supported by INIA (Project FPTA 273), Uruguay
TABLETS FORMULATION OF Solidago chilensis BY DIRECT COMPRESSION USING A NOVEL DRY PLANT EXTRACT

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Introduction
Folk medicine has employed plant extracts since ancient times to treat gastrointestinal diseases. Solidago chilensis Meyen (Asteraceae) is a native species from South America commonly known as “vara dorada”, widely used in the popular medicine of different countries (1). Recently we reported the antiulcerogenic activity of the aqueous extract of the plant in mice subjected to an experimental model of ethanol-induced gastric lesions (2). The active component for the formulation of a phytotherapy in the form of tablets is usually a dry plant extract. However many dry plant extracts, including S. chilensis, have poor flow properties for direct compression (DC) (3). In the present work the development of solid pharmaceutical dosage formulations using a novel dry plant extract (NDPE) of Solidago chilensis is proposed for the first time.

Materials and methods
The fluid plant extract (FPE) was prepared by decoction of the inflorescences of the plant in water. The solid residue (SR) content of the FPE was determined by evaporation of the solvent under reduced pressure and oven drying the SR to constant weight at 80°C. The NDPE was prepared by drying the FPE and colloidal silicon dioxide in a ratio of 1:1 (colloidal silicon dioxide:SR) (4). The solid pharmaceutical formulations were formulated using a 2² factorial experimental design. The physical-mechanical properties (repose angle and Carr’s Index), hardness, friability and disintegration time were evaluated. The statistical evaluation of the results was carried out by analysis of variance (ANOVA).

Results
The NDPE and the four formulations showed good and acceptable flow properties for direct compression (Table 1). The hardness, friability and disintegration time were acceptable. The presence of Lactose DC improved the repose angle and Carr’s Index in formulations (P < 0.05). On the other hand, the presence of Acdisol had a significant impact on disintegration time (P < 0.05).

Conclusions
Solidago chilensis NDPE possesses suitable rheological properties which makes possible its use as an active ingredient in anti-ulcer tablet formulation through the use of direct compression technology. The use of this factorial experimental design is a useful tool to properly select excipient combinations to design solid formulations with adequate pharmaceutical properties.

References
2) Bucciarelli A, Skliar MI. Medicinal plants from Argentina with gastroprotective activity. Ars Pharm. 2007;48,361-369.
Table 1. Experimental matrix and studied responses according to the $2^2$ factorial design.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Fillers</th>
<th>Disintegrants</th>
<th>Repose angle (º)</th>
<th>Carr’s Index (%)</th>
<th>Disintegration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Emcompress</td>
<td>Avicel PH101</td>
<td>30.75</td>
<td>22.15</td>
<td>6.32</td>
</tr>
<tr>
<td>2</td>
<td>Emcompress</td>
<td>Acdisol</td>
<td>24.06</td>
<td>18.68</td>
<td>1.36</td>
</tr>
<tr>
<td>3</td>
<td>Lactose DC</td>
<td>Avicel PH101</td>
<td>22.75</td>
<td>15.40</td>
<td>16.43</td>
</tr>
<tr>
<td>4</td>
<td>Lactose DC</td>
<td>Acdisol</td>
<td>23.16</td>
<td>15.41</td>
<td>1.34</td>
</tr>
</tbody>
</table>
BIOCOMPOSITE FILMS AS SUPPORT OF METHYLENE BLUE

**Cavallo JA¹, Angel Villegas N², Miranda J², Paraje G², Strumia MC¹, Gomez CG¹**#

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²Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba
Medina Allende esq. Hayas de la Torre, Ciudad Universitaria, CP:5000

**Introduction**

The synthesis of biocomposite materials obtained from the combination of a natural and a synthetic polymer is being broadly studied, since this type of material displays interesting properties. Biocomposite material has proved useful in such varied fields as biomedical materials, controlled delivery systems, biological tissues engineering and food packaging (1). The biopolymer chitosan is a polysaccharide chemically composed of linkage \(-\text{\(1,4\)}}\text{-}(1,4)\)-2-amino-2-deoxi-D-glucose, it shows attractive properties such as biocompatibility, biodegradability and antimicrobial activity, particularly useful in biomedical material and vegetable conservation (2). On the other hand, methylene blue is a thiazidic dye and it was considered leading compound in clinical areas, including therapeutics for malaria, schizophrenia and cancer. Therefore, this work aimed at attaining biocomposite films based on polypropylene and modified with chitosan as support of methylene blue in order to study the dye activity against the development of *Staphylococcus aureus* and *Escherichia coli*.

**Materials and methods**

Chitosan (CS) low molecular weight, Aldrich-USA; commercial polypropylene film (PP) was supplied by Converflex S.A-Argentina; acrylic acid (AAc), Merck-Germany; benzophenone (BP), p.a. Mallinckrodt-USA; methylene blue (MB), p.a. Anedra-Argentina.

First, the PP film was functionalized with carboxyl groups from AAc photo-graft polymerization at room temperature. Then, CS was immobilized through electrostatic bond to the grafted film with PAAc (PP-g-PAAc), obtaining the biocomposite film (PP-g-PAAc-CS). The MB dye was fixed to modified films using two techniques, where either MB was attached to PP-g-PAAc-CS film by diffusion (A) or the dye was mixed with CS and then anchored to PP-g-PAAc (B). Finally, the antimicrobial and gas permeation property of the film was study.

**Results**

Biocomposite films (PP-g-PAAc-CS) based on PP were generated and utilized as support of MB. Using a photograft polymerization of AAc, the PP films was functionalized with carboxylic groups (PP-g-AAc), which attached CS by electrostatic bond. Immobilized MB confirmed to possess redox activity from its reaction with ascorbic acid (UV-Vis. Spectrometry), yielding the technique B a dye content (0.14 mmol/g) higher than in A (0.12 mmol/g). The antimicrobial property of modified film with MB was studied by the disc plate method, using the bacteria *S. aureus* and *E. coli*, where it was found that MB retains its antimicrobial activity after being immobilized (**Table 1**). In addition, the oxygen permeation study (**Table 2**) confirmed the film permeability to oxygen.

**Conclusions**

Biomedical materials obtained from the combination of natural and synthetic polymers are being broadly used. Taking advantage of its good mechanical properties, PP is modified by AAc photograft polymerization. MB is immobilized to AAc by electrostatic bond, attaching then a CS layer, which provides biocompatibility properties to the film surface.

**Acknowledgments**

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References

Table 1: Study of antimicrobial property of films

<table>
<thead>
<tr>
<th>Square sample (diameter: 1 cm)</th>
<th>S. aureaus</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATCC$^3$</td>
<td>20$^4$</td>
</tr>
<tr>
<td>PP</td>
<td>I (1 cm)$^a$</td>
<td>I</td>
</tr>
<tr>
<td>PP-g-AAc</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>PP-g-AAc-CS-MB</td>
<td>I (2,5 cm)$^b$</td>
<td>I (1,4 cm)</td>
</tr>
<tr>
<td>PP-g-AAc-(CS/MB)</td>
<td>I</td>
<td>I (0,7 cm)</td>
</tr>
</tbody>
</table>

I) The sample inhibits bacterial grown, NI) bacterial growth is not inhibited, a) inhibited region under the sample, b) circular diameter of inhibited zone.

Table 2: Oxygen permeation study of modified films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>P [barrer]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>0,8105</td>
</tr>
<tr>
<td>PP-g-AAc</td>
<td>0,0121</td>
</tr>
<tr>
<td>PP-g-AAc-CS</td>
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</tr>
</tbody>
</table>
CROSS-LINKED XYLAN MICROPARTICLES: A PHYSICOCHEMICAL APPROACH

Marcelino HR, Silva AE, Oliveira EE, Nagashima Junior T, Egito EST #

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Introduction
Xylan is a hemicellulose present in hardwoods, softwoods and cereals. The main structure of xylan found in corn cobs is composed of a backbone of D-xylose with glucuronic acid and L-arabinose on side chains (1-2). Microcapsules are drug delivery systems generally produced by emulsification techniques as interfacial cross-linking reaction. Xylan-based microcapsules are suggested for colon delivery because this polysaccharide is exclusively degraded by enzymes produced by the colonic microflora (3). The goal of this work was to evaluate the presence of cross-linked/polymer bonds by X-Ray Diffraction analysis (XRD), FT-IR spectroscopy and TGA/DTA analysis.

Materials and methods
Four samples were evaluated: xylan-based microcapsules produced by interfacial cross-linking polymerization (XBM); xylan-based cross-linked microcapsules dried by freeze-drying (lyophilized XBM); raw xylan and terephthaloyl chloride.

Results
XRD showed that raw xylan is a completely amorphous polymer with no peaks. The XBM showed a unique large peak between 20°-30°. Lyophilized XBM presented two single peaks between 20°-30° suggesting the cross-linking reaction that occurs to connect the polymer chains by terephthaloyl chloride, which shows the same characteristic peak between 25°-30°. The FT-IR spectrum of raw xylan showed different peaks that correspond to O-H and C=O vibrations, which are typical bonds in polysaccharides, while the peaks detected near 1280cm⁻¹ in XBM analysis are typical of ester bonds as formed between terephthaloyl chloride and xylan during cross-linking polymerization to form the microparticles (4). All these results corroborate with TGA/DTA analysis that showed weight loss events at different temperatures for raw xylan with the first weight loss between 61.7-111.0°C, which may be related to the residual moisture (8.87%). The second weight loss between 244.1-316.2°C is probably due to the degradation of saccharides. No endothermic peaks were detected at DTA analysis. TGA analysis of lyophilized XBM detected one weight loss between 327.3-363.3°C and DTA showed an endothermic peak in 346.4°C.

Conclusions
These results suggest that bonds between xylan and terephthaloyl chloride occurred during the process of formation xylan-based microparticles. In fact the FT-IR showed ester bonds characteristic of this cross-linking agent. XRD showed the presence of terephthaloyl chloride crystalline peaks and the TGA/DTA evidenced the likely formation of a new compound with different characteristics when compared to the thermal profile of xylan, such as the weight loss at a different temperature. Probably, terephthaloyl chloride is also mixed and linked with xylan chains.

Acknowledgments
The authors are grateful to NEPGN (CT-INFRA/LIEM) for XDR analysis and to Professor Maria de Fátima Vitória de Moura for FT-IR analysis.

References.

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PHYSICOCHEMICAL CHARACTERIZATION OF EMULSIONS FROM *Carapa Guianensis* (ANDIROBA) OIL

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**Introduction**

*Carapa guianensis*, a popular medicinal plant known in Brazil as “Andiroba” (1), has been used in traditional medicine as an insect repellent and anti-inflammatory product. Additionally, this seed oil has been reported in the literature as a repellent against *Aedes aegypti* (2,3). The aim of this work was to report on the emulsification of vegetable oils such as “Andiroba” oil by using a blend of nonionic surfactants (Span 80® and Tween 20®), using the critical HLB and pseudo-ternary diagram as tools to evaluate the system’s stability.

**Materials and methods**

**Materials**
The surfactants (Tween 20® and Span 80®) were purchased from Sigma; *Carapa guianensis* oil was obtained from Laboratório Santa Maria, Manaus-AM, Brazil.

**Methods**
The emulsions were prepared by the inverse phase method. Several formulations were made according to a HLB spreadsheet design (from 4.3 to 16.7) and the products were stored at two different temperatures (25°C and 4°C). Their physicochemical behavior was evaluated by the micro-emulotocrit technique and the long-term stability. The more stable preparation was used for the pseudo-ternary diagram study (4).

**Results**
The experimental data showed that the range of the lowest creaming index (CI) for the micro-emulotocrit test was correlated to the most stable formulations. Likewise, higher CI values for the micro-emulotocrit test indicated unstable systems. The emulsions stabilized by a couple of surfactants, presenting lower values of HLB, showed high CI, coalescence, and/or phase separation. In the same way, high values of HLB implied in more stability, and the final required HLB for “Andiroba” oil (*C. guianensis*) was found to be 16.7 by using only Tween 20®. For this study, the combination Tween 20®/Span 80® was unable to stabilize the system. The phase diagram performed for the oil allowed the visual identification of several dispersed systems such as microemulsions, emulsions, and creams; as well as the maximum and minimum limits of component proportions for achieving the different phase behavior.

**Conclusions**
The stable emulsions and creams from “Andiroba” oil can be eligible products for topical use either by incorporation of active ingredients or as insect repellents.

**Acknowledgments**
The authors are grateful for the financial support received from CNPq (477131/2007-7), FAPERN/MCT/CT-INFRA/CNPq (PPP 2007), and UFRN (Propesq/Pibic).

**References**


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QUANTITATIVE ANALYSIS OF Schinopsis brasiliensis Engl. EXTRACT WHEN INCORPORATED INTO OIL-IN-WATER MICROEMULSION

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Egito EST¹⁹

Introduction
The Schinopsis brasiliensis Engl. from the Anacardiaceae family, is a plant-sized tree that can reach 12 m in height and 60 cm in diameter. Also known as barauna or brauna (1, 2) this tree occurs in the Caatinga region at the Northeast of Brazil and is considered an endangered species, as mentioned in the IBAMA ordinances No. 83 (26/09/91) and No. 37BN (3/04/1992) (1, 3). The extract derived from this plant has low water solubility and antimicrobial and antioxidant properties as shown in recent studies (4), which qualifies to be entrapped in a biocompatible oil-in-water microemulsion (ME) (5). This work aimed to develop an analytical technique to evaluate the extract content into ME systems.

Methods
The leaves of Schinopsis brasiliensis Engl. were collected at the city of Mirandiba (Pernambuco, Brazil). After the drying period, the leaves were crushed and weighed and, then, exhaustively extracted in n-hexane and methanol, respectively. The hexane and methanol extracts were filtered, dried at 45ºC and weighed. Its yield was calculated according to the literature. This final extract was incorporated into an oil-in-water ME formulation selected from a pseudo-ternary diagram previously developed. The extract nominal concentration of incorporated was 10 mg/mL. From the incorporated ME, 1:1000 dilutions were made in ethanol, followed by readings on a UV-VIS spectrophotometer, in triplicate, using a wavelength of 279 nm, which represents the maxima wavelength for these extracts. The absorbances were, therefore, plotted at an analytical curve previously prepared by the stationary cuvette method (6).

Results
The main principle of analytical chemistry in pharmaceutical technology is to develop methods able to detect substances inside pharmaceutical dosage forms. To measure the content of the Schinopsis brasiliensis Engl. extract into a new lipidic carrier as MEs, one analytical curve has been developed. The mentioned methodology allows us to calculate the concentration of extract incorporation, which was 99.78 % (9.97 ± 1.6 mg/mL) of the nominal concentration. Although using a no chromatographic method, the spectrofotometry was quite reliable and no interference of the ME system was found during the analysis. Therefore, this methodology met the expectations of a fast method to evaluate the entrapment efficiency of brauna extracts into ME systems.

Conclusion
The UV-VIS spectrophotometry approach may be considered an excellent tool to derive analytical curves for analyzing drug contents in raw materials and medicines. To quantify the Schinopsis brasiliensis Engl. extract into ME, this method proved to be simple, fast and it meets the needs intended, becoming a tool of prime importance for some analytical studies.

Acknowledgments
The authors wish to thanks BNB and CNPq (Brasília, Brazil) for financial support in the development in this work.

References

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DESIGN OF CÁSCARA SAGRADA TABLETS BY DIRECT COMPRESSION USING A SPRAY DRYED EXTRACT

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Introduction

Pharmaceutical tablets are the principal dosage form for drug delivery, occupying two-thirds of the global market (1). Generally, direct compression (DC) is one of the most advantageous processes for tablet manufacture; it offers several advantages such as simplicity, low processing time and power consumption. On the other hand, the DC primary limitations are good flow properties and compressibility requirements (2).

In the case of phytomedicine tablets, the active component is a dry plant extract (DPE). Usually, DPEs have poor flow properties and compactability and consequently they cannot be utilized for DC (3). Sprays dryers are the most common technique used in the herbal processing industries. One of the main reasons is the ability to generate a product with precise quality specifications in continuous operations. The most normally required product specifications are moisture content, flow properties and hygroscopicity (4).

The goal of this study was to optimize the attainment of a vegetal solid extract (SDE) with suitable flow and compression characteristics in order to develop tablets containing Cáscara sagrada solid extract as active component. The spray drying process was selected as method for the SDE attainment and the composition of tablet formulae was proposed. The pharmaceutical performance of the formulation was evaluated.

Materials and methods

The extract was spray dried in a Mini Spray Dryer Büchi B-290 utilizing colloidal silicon dioxide as drying aid. The most efficient experimental conditions for spray drying were previously determined from a $2^5-1$ fractional factorial design. Such conditions were: drying air inlet temperature 130ºC, atomization air volumetric flowrate 400 l/h, feed volumetric flow rate 15% (expressed as % of the maximum pump rate), drying air volumetric flowrate 100% (given as % of the maximum aspiration rate) and dispersion concentration 7.32 % (w/w) with 1:1 colloidal silicon dioxide:solid residue ratio. The quantitative determination of the actives components (hydroxyanthracene derivatives and cascarrasides) was assayed by spectrophotometric measurements (5) and High Performance Liquid Chromatography.

Four solid pharmaceutical formulations, containing the SDE and common excipients for DC, were formulated by means of a $2^2$ factorial experimental design. The fluidity and compressibility, hardness, friability, disintegration time and dissolution rate were evaluated.

Results

The four formulations presented good flow properties and acceptable hardness, friability and disintegration time. The dissolution test indicated, for some formulations, a rapid release of the active components. Two of the four formulations, one constituted by SDE (146 mg), Lactosa CD (70 mg), Avicel PH101 (70 mg), Acdisol (70 mg) and magnesium stearate (4 mg) and another containing SDE (146 mg), Lactosa CD (70 mg), Emcompress (70 mg), Acdisol (70 mg) and magnesium stearate (4 mg), showed the best pharmaceutical performance. The presence of Acdisol lead to the lowest disintegration time and highest extract release.

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Conclusions

The spray drying technology allowed the production of a SDE with good flowability for the formulation of phytomedicine tablets by DC. The manufacture of tablets by a $2^2$ factorial design was a good strategy to evaluate the influence of different excipients and their proportions on the analyzed responses.

References

PROTEIN/POLYSACCHARIDE MIXED GELS AS MATRICES FOR BIOACTIVE DRUG DELIVERY SYSTEMS

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Introduction
Biomolecules for medicinal use are susceptible to hydrolysis, chemical changes and denaturation during storage and administration in the human body (1). One alternative to preserve its biological activity is to incorporate the bioactive molecules in an appropriate matrix in order to ensure the controlled release at the specific action sites (2). In this work, we evaluate the mechanical and fracture properties, pore size and structure of gels based in whey protein and a regional polysaccharide (Espina Corona gum) mixtures for their possible application as controlled release system of bioactive drugs.

Materials and methods
Whey protein isolated (WPI) was provided by Davisco Foods International, Inc. Espina Corona gum (EGC) was obtained from Idea Supply Argentina S.A. Firstly, we evaluated the effect of protein concentration (in the range of 12-16% wt) on the WPI gel characteristics. Gels were obtained by heat treatment of biopolymer aqueous solutions (80°C, 30 min). Secondly, we analyzed the impact of GEC and its relative concentration (WPI:GEC = 1:0.01-0.05) on the gel characteristics at 12% wt protein. Measurements of mechanical and fracture properties of the gels were obtained by uniaxial compression assays using an INSTRON universal testing machine. For this, gels were compressed until fracture at a compression rate of 1 mm/s. Pore size was estimated applying the following equation: ξ = (3K_B.T/E).r_0^2/r_f^2 1/3 (3), where ξ is the pore size, K_B is the Boltzmann constant, T is the absolute temperature, E is the Young's modulus, derived from the uniaxial compression parameters (4) and (r_0^2/r_f^2) is the front factor can be regarded as the average deviation of the network chains from the dimensions they would assume if they were isolated and free from all constraints (3). Finally, in order to estimate the gels structure arrangement, opacity index was measured with a Minolta 508d colorimeter. Analysis of variance (ANOVA) was carried out, and the statistical differences (p<0.05) were determined using the LSD test.

Results
It can be observed that the increment in protein concentration caused: (i) a lower pore size of gel network (from 157.0 to 63.2 nm) and (ii) a higher stress at fracture (from 22.5 to 101.4 kPa) which could be associated with a closer proximity among protein chains allowing a better distribution of applied stress against rupture and collapse of gel network. In mixed gels, the presence of GEC caused: (i) an increment in the stress at fracture (27.4 to 41.9 kPa) producing harder and less deformable gels, (ii) a reduced pore size (from 157 to 87 nm), and (iii) an increased opacity index. These observations suggest that the addition of GEC could produce much softer gels but with a pore size comparable with those WPI gels at higher protein concentration.

Conclusions
Information derived from this study could be useful to determine: (i) WPI gelation conditions in order to obtain matrices with nanometric pore size, and (ii) WPI:ECG ratio conditions that produce gels with pore size comparable to those WPI gels at higher protein concentration, but with different mechanical properties. We conclude that the modification of biopolymer concentration in WPI/EGC mixtures could be an interesting formulation strategy for to design gels with a desired pore size and specific mechanical properties for multiple delivery systems of bioactive drugs.

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Acknowledgments
Authors would like to thank the financial support of CAI+D-2009 tipo II PI 57-283 project.

References
DESIGN OF A NEW UROGENITAL PROBIOTIC FORMULA CONTAINING SALIVARICIN, LACTOBACILLI AND OTHER BIOACTIVE SUBSTANCES

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Introduction
The urogenital infections (UGI) affect over 1 billion women worldwide each year (1). The conventional treatments are based in prescribed antibiotic therapies. However, they produce an imbalance of indigenous microbiota, adverse effects and antibiotic resistance, being not recommended for pregnant women. A promising alternative for the UGI is the administration of probiotics, defined as “live microorganisms which, when administered in adequate amounts, exert a beneficial physiological effect in the host health” (2). The main objective of our research group is the design of novel probiotic pharmaceutical products containing lactobacilli strains combined with antagonistic substances, as for example bacteriocins, which are antimicrobial peptides ribosomally synthesized by some bacteria. Salivaricin CRL1328 is a bacteriocin produced by Lactobacillus salivarius CRL 1328, a strain isolated from healthy human vagina, with potential applications for preventing urogenital infections (3,4). Salivaricin is active against pathogens including Gardnerella vaginalis, Enterococcus faecalis, E. faecium, Listeria monocytogenes, Streptococcus agalactiae, Staphylococcus saprophyticus and Neisseria gonorrhoeae. The long term storage of microorganisms is a challenge in the pharmaceutical area. Lyophilization is one of the process usually employed and the carefully selection of lyoprotectors plays a major role in the stability of bioactive compounds. The aim of this work was to evaluate the resistance of beneficial lactobacilli combined with salivaricin and other bioactive substances to the freeze-drying, and their stability during their shelf-life.

Materials and methods
Lactobacillus salivarius CRL 1328 and Lactobacillus gasseri CRL 1263 were grown in LAPTg medium at 37°C. Sixteen different combinations of bacteria, salivaricin, lactose, inulin and ascorbic acid were designed and evaluated. Later, based on these results, a new set of eight combinations was assayed. The samples were lyophilized and stored in gelatin capsules at 4°C during 180 days taking samples periodically. The viability of the strains (CFU/g), as well as salivaricin activity (AU/g) were evaluated to determine the resistance to the lyophilization process and during the storage. The data were statistically analyzed by applying ANOVA test and a model proposed to evaluate the experimental results obtained.

Results
The selected microorganisms were compatible between them and with salivaricin. The ANOVA analysis showed that the protective agents and the storage time were the two factors that affect both, the activity of salivaricin and the viability of Lactobacillus strains. The viability of both strains was higher when lactose was present in the mixture. L.g.CRL 1263 was highly conserved with ascorbic acid or lactose, but not with inulin assayed individually. The negative effect of inulin disappeared when the three substances were present in the mixture. Ascorbic acid was detrimental for L.s.CRL 1328 when lyophilized simultaneously. However, when added after freeze-drying process, the survival rate increased significantly. The antimicrobial activity of salivaricin was lost in the samples combined with L.g.CRL 1263, probably by its adsorption to the bacterial cell. A progressive reduction of salivacin activity during storage was detected in the samples with inulin. However, when combined with lactose, ascorbic acid or L.s.CRL 1328, the activity was maintained for 180 days.

Conclusions
This is the first study that provides new information for the potential application of salivaricin as bioactive principle, alone or combined with probiotic microorganisms, for their inclusion in probiotic pharmaceutical formulations.

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References
PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON TOTAL ALKALOID FRACTION OF SOLANUM PSEUDOCAPSICUM SPECIES GROUP (SOLANACEAE)

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Introduction
The Solanum pseudocapsicum species group includes 7 Americans species (1). It is strongly promising as new potential natural resource. One of its worldwide species, S. pseudocapsicum, has antimicrobial (2), antifungal (3), antiviral (4), antispasmodic (5), cytotoxic (5), antihypertensive (5), hepatoprotective (6), anti-tumor and antioxidant properties (7,8) that have been proved with crude extracts or extracts enriched in alkaloids. From the phytochemical point of view, this species is peculiar by having an alkaloid of unusual structure, the solanocapsine (9,10), along with other minor alkaloids (11).

The objectives in this work are: 1) to verify the presence of solanocapsine in the remaining native South American species in order to determine its chemotaxonomical value; 2) to test anticholinesterase and antiviral activities (against non endemic and Argentinean endemic viruses); 3) to check if solanocapsine is the principal responsible of the pharmacological activities proved.

Material and methods
The alkaloid extraction of four species was made from dry leaves. The identification of solanocapsine was performed by Nuclear Magnetic Resonance Spectroscopy of Carbon (¹³C NMR), through the presence of the unique and characteristics signal of the 18 carbon of solanocapsine (spectrum of ¹³C RMN, δ= 96,77 ppm). ¹³C NMR spectra of extracts enriched in alkaloids were made for S. pseudocapsicum L., S. delicatulum L.B. Smith & Downs, S. argentinum Bitter & Lillo, and S. tucumanense Griseb.

The anticholinesterase activity was proved by inhibiting the enzyme acetylcholinesterase, using the Ellman et al. (12) methodology. For the antiviral activity, the Herpes simplex virus type I (VSH-I, strain Kos p31), the virus of the Encephalitis Equina Venezolana (EEV, strain TC83), the virus of the Encephalitis of San Luis (VESL, strain 78V6507) and the virus Junín (VJ, strain XJ Cl3) were used. The activity was measured by the method of neutral red uptake (13).

Results
The presence of solanocapsine was detected in S. pseudocapsicum, S. argentinum and S. tucumanense. A high inhibition on the acetylcholinesterase was observed in extracts of S. pseudocapsicum while the inhibition was moderate for S. argentinum and S. tucumanense. Solanum delicatulum’s extracts did not show inhibition. Solanum pseudocapsicum showed high antiviral activity against VJ and EEV while S. delicatulum have low activity against EEV and VSH-I. The extracts of S. tucumanense were negative against these viruses.

Conclusions
Apparently, solanocapsine is a chemotaxonomic marker since it is present in most species of the S. pseudocapsicum species group. The solanocapsine is not responsible for the pharmacological activities tested due to anticholinesterase and antiviral activities were not evidenced in the species with solanocapsine. Solanum pseudocapsicum is the species pharmacologically more active. Finally, further studies are necessary to determine the compounds responsible of the pharmacological activity demonstrated.

Reference

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SCREENING OF ANTIHELMINTIC PLANT COLLECTED IN LA PAMPA PROVINCE

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Introduction
The systematic use of anthelmintic has produced, basically in the bovine and ovine livestock, phenomena of resistance (Mc. Kenna, 1996). In the present study was investigated the vegetable extract anthelmintic activity of naturalized and native plants of La Pampa province, Argentina with the objective to find new therapeutic alternative.

Material and Methods
Experimental Model: A screening was done to determine the anthelmintic effect of different vegetable extract. The experimental models consisted in expose larva III of Haemonchus spp. to different concentrations of vegetable extract and observe the motility activity.

Extract Preparation: 100 mg of hydro alcoholic extract was reconstituted in 5 ml of distil water, and were placed in micro plates the following volumes 25 µl, 50 µl, 100 µl and 200 µl of the suspension.

Control drug: Phosfamisol® of Biogenesis – Bagó Laboratory.

Larva of parasite: ovine faeces were cultured and the larvas were recovered by the Henriksen and Korsholm (1983) technique.

Anthelmintic screening: 1 ml of larva were cultured and placed in wells, at 4 different concentrations of the hydro alcoholic extract. Two plates were utilized, one as control and other as positive control with phosfamisol.

Results
The results with 4 different concentrations of the extracts were obtained at 24 hours, and were classified as total motility inhibition, moderate motility inhibition and no effect.


No effect: Urtica urens, Plantago lanceolada, Phyla canescens, Verben a bonariensis, Atriplex undulata, Baccharis spartioides, Tamarix gallica, Trichocline sinuate, Berberis ruscifolia, Heliotropium curassavic um, Maytenus vitis idara, Acaena myriophylla, Prosopis flexuosa var flexuosa, Sarcocornia perennis, Verbascum thapsus.

The parasite exposed to the phosfamisol solution showed total inhibition of motility.

Conclusions
Out of 53 vegetable extract tested, 20 showed total inhibition of motility, 18 showed moderate inhibition of motility and 15 showed any effect at all. The extracts with activity warrants further research to determine the ovicidal and larvicide effect.

References

VEGETAL RESOURCES RELATED TO VETERINARY MEDICINE IN THE SIERRAS OF CÓRDOBA (ARGENTINA)

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Introduction
There has been a growing interest in traditional veterinary research during the last decades (1). Salient features of ethnobotanic veterinary practiced by peasants in the sierras of Córdoba have been described, as well as the role of medicinal plants in healing domestic and breed animals.

Materials and Methods
This study was developed with the criollo peasants devoted to agricultural and cattle-raising activities in sites to the South East (Paravachasca and Calamuchita) and West (near La Calera) of the sierras of Córdoba. Several species and vegetal applications for animal healing were documented by means of participatory observation, open and semi-structured interviews (2).

Results
A total of 127 medicinal uses corresponding to 70 species of botanic families were documented. Vegetal resources related to veterinary medicine in the sierras of Córdoba (Argentina). The species used in veterinary medicine are mainly represented by shrub and herbaceous biological forms, being also relevant the employment of wild native species. The most common means of preparation are decoctions and infusions in water (62 % of uses), macerations (6%), direct applications (5%) and smoke bath (4%) and the plants aerial parts are the most frequently used (71%). Washes, compresses and frictions are all common external applications (60%). According to the consensus of the interviewed participants, the most widely used applications are the following: The use of “ligas” (Ligaria cuneifolia and Tripodanthus flagellaris) for treating retained placenta; the use of “polvillo del diablo” (Calvatia cyathiformis), “espinillo” (Acacia caven) y “moradillo” (Schinus longifolia var. longifolia) for the scarring of wounds; lastly, the smoke bath of “maíz” (Zea mays) for animal distemper. Taking into account the number of different species used for distinct medicinal applications, (Table 1) the most important are those related to the scarring of wounds, followed by the treatment of digestive problems, osteomuscular pains, parasitism and respiratory problems.

Table 1: Frequency of medicinal applications of vegetal species used in traditional veterinary medicine in the sierras of Córdoba.

<table>
<thead>
<tr>
<th>Medicinal applications of plants</th>
<th>Absolute frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>cicatrizant, disinfectant, antiulcer</td>
<td>41</td>
</tr>
<tr>
<td>digestive, stomachic, antiidiarrheic, intestinal, hepatic, purging</td>
<td>16</td>
</tr>
<tr>
<td>osteomuscular anti-inflammatory</td>
<td>13</td>
</tr>
<tr>
<td>antiparasite (vermifuge and anti-mange)</td>
<td>11</td>
</tr>
<tr>
<td>pectoral decongestive</td>
<td>9</td>
</tr>
<tr>
<td>Oxytocic</td>
<td>8</td>
</tr>
<tr>
<td>anti-ophthalmic</td>
<td>10</td>
</tr>
<tr>
<td>diuretic, nephritic</td>
<td>6</td>
</tr>
<tr>
<td>anti-poison, treatment of intoxications</td>
<td>3</td>
</tr>
<tr>
<td>Febrifuge</td>
<td>1</td>
</tr>
</tbody>
</table>

Conclusions
The great amount of medicinal applications demonstrates the ethnobotanical relevance of the traditional veterinary. As with other studies of this type, more than half of the medicinal applications and almost all
of the plants used in traditional veterinary of the sierras of Córdoba have been registered in popular medicinal usage for humans (3), which suggests a strong alignment between human and animal health.

References.


MICROGRAPHIC PARAMETERS FOR THE RECOGNITION of “SUELDA CONSUELDA”: Catasetum macroglossum Rchb. f. (Orchidaceae).

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Introduction

Catasetum macroglossum is a plant originary from Ecuador, which grows in the litoral provinces of Guayas, Los Ríos, Manabi and El Oro. The pseudo bulbs of C. macroglossum are used topically in folk medicine as anti-inflammatory and antirheumatic. As best of our knowledge, there are no reports about anatomy of Catasetum macroglossum (1,2). In order to validate the popular use and provide elements that contribute to the identification and quality control of this species we studied the anatomy of stem (pseudo bulbs), root and leaves of C. macroglossum.

Materials and methods

A voucher specimen was deposited in LPE “Carlos Spegazzini” (Facultad de Ciencias Exactas, UNLP). Plant material was preserved in FAA. We made the analysis by microscopic observation of diaphanized leaves and transverse section of leaves, stem (pseudo-bulb) and root. Transverse section was made with Ranvier microtome and stained with FastGreen-bsafranine technique.

We used a stereoscopic microscope Olympus and an optic microscope Olympus CH with drawing tube. Photomicrographs were taken with digital camera Olympus

Results

Leaf: Cuticle smooth. Hypostomatic. Stomata superficial, tetracytic (58%) and anomocytic (42%). Epidermal cells polygonal, angular, and equally large in both surfaces. Mesophyll homogeneous. Fiber bundles and phloem sclerenchyma associated with stegmata arranged in rows with silicon conical bodies with a rough surface. There are numerous papillae, especially in the upper epidermis. Scarce hairs eglandular of three cells. Basal cell deeply buried in the epidermal cells (cells in crypt). Often, these hairs are lost during handling of the leaves. Observed under a stereoscopic microscope, the leaf has a characteristic brightness due to the presence of silicon bodies. It is also possible to observe the presence of eglandular trichomes.

Stem (pseudo bulbs): atactostela, with xylem/phloem sclerenchyma. Presence of smooth cuticle. Outer walls of epidermal cells thickened. There are a few starch grains. Idioblasts with raphides in bundles. Steg mata associated with phloem sclerenchyma. Absence of hairs and stomata

Root: Tilosomas absent. Cortical cells walls with bands, vascular tissue with embedment parenchymatous. Velamen: multiseriate epidermis, showing thickening and tracheoidal idioblasts characteristic.

Discussion/conclusions

Characteristics of the starch grains, the presence of raphides, layout and percentage of both type of stomata, the presence and characteristics of the velamen and pseudovelamen, trichomes, stegmata and papillae, are useful micrographic diagnostic characters for identify the species C. macroglossum and can eventually contribute to their quality control.

Acknowledgments

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References
(1) Moraes, Cristiano Pedroso de. Fenologia e anatomia dos órgãos reprodutivos de *Catasetum fimbriatum* Lindley cultivados sob diferentes intensidades luminosas. Tesis de Maestría (2002) Piracicaba, Brasil
THERAPEUTIC POTENTIAL OF ANTIOXIDANTS IN BLOOD CELLS STRESS INDUCES BY BACTERIAL TOXINS

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Introduction
Natural antioxidants from vegetal species, have been investigated to develop new therapeutic and preventive strategies against different pathologies, since they are considered to be important sources of radical scavenging (1,2). Shiga toxin (Stx)-producing Escherichia coli (STEC) is able to provoke Hemolytic Uremic Syndrome (HUS) with microvascular thrombosis and acute renal failure. The link between HUS and oxidative damage has been demonstrated by different authors, associated with a direct effect of Stx on polymorphonuclears that can contribute to tissue injury, since these cells are activated and release reactive oxidant species (ROS). In addition, in the acute phase, the erythrocytes also are exposed to an oxidative imbalance, which is associated with a decrease of membrane fluidity and hemolysis. This study was undertaken to elucidate the antioxidant effect of Zizyphus mistol and Prosopis alba, with the hypothesis that indigenous fruits could be antioxidants able to reduce oxidative stress, and could be a potential nutritional or pharmaceutical treatment in SUH.

Materials and methods
Z.mistol or P.alba fruits were extracted with different solvents, acetone, hexane, ethylic alcohol and water to test the best conditions. The extracts were dried until reaching constant weight in a rotary extractor; then they were analyzed by the assay of phenolic compounds, using the Folin-Ciocalteu reactive and Na₂CO₃. Results were expressed in µg gallic acid/mg of dry extract. The flavonoid content was determined with AlCl₃ and CH₃COOK, these compounds were expressed in µg of quercetin/mg of dry extract. Ferric Reducing Antioxidant Power (FRAP) was tested by using a reaction in acetate buffer with FeCl₃.6H₂O and 2,4,6-tripyridyl-s-triazine; the OD at 593nm was determined. Standard FeSO₄ diluted into different concentrations was employed to express the results in µM of Fe SO₄.

Results
A protective role of both plants was detected by chemiluminescence, due to the natural antioxidants that significantly decreased the levels of ROS induced by E.coli STEC and its toxins in blood. The FRAP was found to be higher in Z.mistol than in P.alba. The chemical analyses of phenolic compounds and flavonoids present in the fruit extracts indicated that the FRAP correlated with the amount of phenolic compounds, but not with the flavonoids analyzed.

Conclusions
Both fruits studied reduced the induction of ROS, and in this way could help to prevent the development of complications related to oxidative stress generated in the blood of patients with HUS. Our results are reinforced by authors that found in extract of other medicinal plants, phenolic compounds but not flavonoids, able to protect against oxidant injure in other pathologies (3).

Acknowledgements
This work was supported by grants from BID 1728 PICTO 36163 and SECyT-UNC. Albrecht C is a PhD fellow of FONCyT, and Pellarin MG and Rojas MJ are PhD fellows of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF ESSENTIAL OIL OF Salvia microphylla GROWING IN TUCUMAN, ARGENTINA

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Introduction
Salvia species is constituted by approximately 900 species widely distributed in the world. Some essential oils of others species of Salvia have demonstrated anti-bacterial, carminative, diuretic, hemostatic and antispasmodic activity. Previous work reported on Salvia species presence of compound as well as acids and glycosides phenolic, flavonoids, anthocyanins, coumarins, polysaccharides, sterols, terpenoids and essential oils (1)

Salvia microphylla Kunth Fam. Lamiaceae known as “oregano de jardín” or “oregano español” has been used in folk medicine in the treatment of respiratory affections (2). In Mexico this specie is known as “mirto” and used for some stomach ailments (3)

Materials and methods
Aerial parts of Salvia microphylla were collected at the full flowering stage in March from El Mollar, Tafi del Valle, Tucumán (at approximately 1800 m a.s.l). A voucher specimen has been deposited at the Herbarium of Miguel Lillo Foundation (voucher Nº LIL 608048), Tucumán, Argentina.

Essential oil of aerial parts was obtained by hydrodistillation using a Clevenger type apparatus. The GC-MS analysis of essential oils was carried out on a GC-HP 6890 with mass selective detector (quadrupole) HP 5973, source 70eV, fitted with a HP-5MS column (5% phenyl methyl siloxane, 30 m x 0.25 mm i.d., film thickness 0.25 µm) with helium as carrier gas at 1.0 ml/min. Quantitative data were obtained from automatic of area percent data (FID) without the use of an internal standard or response factors. Identification of components of the oil was based on comparison of their mass spectra with those found in the literature (4) and mass spectrometry data bank (NBS75K, NIST, WILEY) and computer search Wiley library.

Antimicrobial activity was evaluated by the agar diffusion method on Staphylococcus aureus ATCC 25923 and clinical isolated samples. Microorganisms were grown in BHI (Brain Heart Infusion) at 37°C for 18 hours and re-suspended in sterile physiological saline solution with reference to the value 0.5 of the McFarland scale (1.5 x 10⁸ CFU/ml). Petri dishes were prepared with a base layer of Mueller Hinton agar (10 ml) and wells (6mm of diameter) were made on the surface of the medium. 25 µl of essential oils with different concentrations were placed in the wells. Microorganisms were incubated at 35°C aerobically and after 24 hrs of incubation the extension of the inhibition zones was measured. Minimal inhibitory concentration (MIC) values were determined by conventional agar plate dilution methods (5,6).

Results
The yield of the essential oil from fresh aerial parts was 1%. 75 components were identified representing 95.3% of essential oil. The major chemical constituents were alpha-eudesmol (21.8%), transcaryophyllene (10.8%), bornyl acetate (9.7%), germacrene B (4.8%), y delta-cadinene (4.0%). On the other hand S. microphylla showed antimicrobial activity on all assayed strains.

Conclusions
This oil showed elevates proportions in sesquiterpenes. Chialva and Monguzzi (7) reported similar composition of essential oil of S. microphylla from Italy but is quantitatively different. Antimicrobial data presented here may stimulate the therapeutic use of S. microphylla essential oil.

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Acknowledgments
We thank Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT) for financial support.

References
ANTIOXIDANT AND SUNSCREEN ACTIVITY OF Leiothrix spiralis RUHLAND EXTRACTS (Eriocaulaceae)

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Introduction

Leiothrix spiralis Ruhland is popularly known as “sempre-viva” because it appears to be alive even after years of being harvested. Chemical investigations of this specie resulted in the isolation of important phenolic compounds(1). Plants containing phenolic compounds, in particular flavonoids have been reported to possess strong antioxidant and sunscreen properties. Ionizing radiations, included UV ray, cause a massive generation of cytotoxic reactive oxygen species and induce cellular DNA damage. The plant kingdom is a large source of natural active ingredients among which it is possible to find some plant extract with UV absorption properties that can be used in photo-protective products as enhancers of physical or chemical sunscreens(2). The aim of this study was to evaluate the in vitro antioxidant and sunscreen activity of L. spiralis extracts.

Materials and methods

The specimen were collected at Serra do Cipó–MG–Brazil. Voucher specimen was deposited at the Herbarium of Departamento de Botânica IB–USP (Sano4789). For this work we used methanol extracts from capitula, scapes and leaves. Antioxidant activity was determined using the ABTS radical scavenging method(3). The sun protection factor (SPF) was determined by spectrophotometric method(4).

Results

The results obtained from the antioxidant activity showed significant antioxidant capacity when compared to luteolin standard, and the methanol extract of L. spiralis leaves showed better result (IC50=1.53mg/mL) (Table 1). Throug the evaluation of sunscreen activity by spectrophotometric analysis, we obtained the results presented in Table 2.

Conclusions

It was found that the methanolic extract of L. spiralis possesses strong antioxidant activity and the phenolic compounds presents in this specie may explain the mechanisms involved in the activity. According to Mansur et al. (1986) the SPF is internationally rounded to the integer number. In accordance with Brazilian law, RDC237/2002(5) a product suitable for use in cosmetics to sunbathing or photoprotection must lodge an SPF equal or greater than 2. However, under the conditions used, the results for FPS were lower, and cannot be consider sunscreen specie. However the proposed UV spectrophotometric method is simple, rapid, employs low-cost reagents and can be used in the in vitro determination of SPF values in many plant extracts.

Acknowledgments

CAPES.

References.


Table 1. Inhibitory concentration (IC₅₀) from the *L. spiralis* extracts and respective standard

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luteolin</td>
<td>1.208</td>
</tr>
<tr>
<td><em>L. spiralis</em> (capitula)</td>
<td>3.005</td>
</tr>
<tr>
<td><em>L. spiralis</em> (scapes)</td>
<td>6.032</td>
</tr>
<tr>
<td><em>L. spiralis</em> (leaves)</td>
<td>1.530</td>
</tr>
</tbody>
</table>

Table 2. Spectrophotometrically calculated sun protection factor values of *L. spiralis* extracts

<table>
<thead>
<tr>
<th>λ/nm</th>
<th>EE x I*</th>
<th>Capitula</th>
<th>scapes</th>
<th>leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0,0150</td>
<td>0,002</td>
<td>0,002</td>
<td>0,002</td>
</tr>
<tr>
<td>295</td>
<td>0,0817</td>
<td>0,011</td>
<td>0,010</td>
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<tr>
<td>300</td>
<td>0,2874</td>
<td>0,037</td>
<td>0,033</td>
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<tr>
<td>305</td>
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<tr>
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<tr>
<td>320</td>
<td>0,0180</td>
<td>0,003</td>
<td>0,002</td>
<td>0,003</td>
</tr>
</tbody>
</table>

Σ₂₉₀⁻₃₂₀nm 0,13 0,12 0,12

SPF value calculated 1 1 1

*Value determined by Sayre et al. (1979) (6).
ANXIOGENIC EFFECT OF Aloysia polystachya – DERIVED ESSENTIAL OIL IN MOUSE.

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Introduction
Aloysia polystachya (Griseb.) Mold., is a commonly used plant in the folk medicine to treat gastrointestinal disorders. The major components found in the essential oil of the leaves are the terpenes carvona and/or α-thujone (1, 2). Since there are not enough studies about the effect of Aloysia polystachya-derived essential oil on the central nervous system, the aim of this work was to evaluate the chemical composition and the effects of Aloysia polystachya essential oil on the anxiety behavior in mice.

Materials and methods
The plants were collected in Córdoba city and the essential oil was obtained under hydrodistillation extracted by hexane. The analysis and quantification was done using a gas-liquid chromatography method. It was used male adult albino Swiss mice weighing 30-35g kept in a 12 h light-dark period under controlled temperature conditions, with food and water “ad libitum”. The experimental groups were: **Group 1:** Control: injected with 0.3 ml of 2 % of Tween 80 solution i.p. (vehicle). **Group 2:** injected with 100, 10 and 5 mg/kg i.p. of essential oil. **Group 3:** injected with 5 mg/kg of essential oil plus diazepam 1 mg/kg. **Group 4:** injected with 4,13 mg/kg of α-thujone. **Group 5:** injected with 4,13 mg/kg of α-thujone plus diazepam 1 mg/kg.

The animals were tested on the elevated plus-maze 30 min after injection of drugs. Open/closed arm quotient and time spent in the open arms were determined as indexes of anxiety. Total number of arm entries and closed arm entries were recorded as a locomotor activity index.

Results
The percentage of essential oil obtained was 3% and the principal component was α-thujone 82.6%. The injection of 100 mg/kg i.p. of A. polystachya essential oil induced convulsions and dead in 100 % of the cases but the dose of 10 mg/kg only induced convulsions. 5 mg/kg of essential oil did not induce convulsions but markedly reduced the time spent in the open arms and the open/closed arm quotient without changes in locomotor activity index. The same response was obtained with the administration of α-thujone alone. The anxiety index was fully reversed by diazepam in all cases.

Discussion
Since the decrease on the anxiety index induced by 5 mg/kg of A. polystachya essential oil were similar to that induced by α-thujone administrated in the same concentration found in the essential oil, we conclude that the behavioral effects are due to their principal component α-thujone. A possible explanation for the behavioral effects induced by A. polystachya essential oil involved the inhibition of GABA_A receptors because they could be fully reversed by low doses of diazepam administration.

References
TOXICITY ASSAY OF Gunnera manicata L. EXTRACTS USING Artemia salina LEACH.

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Introduction
Gunnera (Gunneraceae) comprises more than forty species occurring mainly in the Southern Hemisphere (1). Some cyanobacteria are involved in symbioses with Gunnera. The most common cyanobacteria found in symbioses with plants belong to genus Nostoc (2). Because of this symbiosis may occur the production of beta-N-methylamino-L-alanina (BMAA), a non protein amino acid (3). BMAA is a neurotoxin that has been found in high concentrations in brain tissues of patients with tauopathies such as Amyotrophic Lateral Sclerosis-Parkinsonism-Dementia Complex (4). In Southern Brazil is found the species G. manicata L. on which there is few scientific data about phytochemical, pharmacological and toxicological studies. Considering that G. manicata plants were able to be colonized by N. punctiforme (5) and as result may occur the formation of the neurotoxin BMAA, the present study determined the Medium Lethal Concentrations (LC$_{50}$) of aqueous leaves and roots extracts of G. manicata in Artemia salina L. (Artemiidae), used as an alternative test to determine toxicity of chemical and natural products (7).

Materials and methods
Dried roots and leaves was extracted in water bath at 50°C for 3h. The aqueous extracts were filtered, dryness under reduced pressure and the residue dissolved in water to analysis. The concentrations tested were 100, 200, 300, 400, 500, 750, 1000, 1250 and 1500 mcg/mL (w/v). To performed the bioassay, brine shrimp larvae were placed in seawater (30 g/L sea salt), under constant flow of oxygen and artificial light for 48 hours until larvae hatching. Eppendorfs tubes were used with 10 ~ 13 brine shrimp larvae in witch. Final volume was adjusted to 1.5 mL (water + salt solutions tested). Each test was run in triplicate, and seawater was used as the control. After 24h the number of living larvae was verified. The LC$_{50}$ determined by probit analysis in MINITAB 14.0 software. (6)

Results
The LC$_{50}$ values for aqueous leaves extracts was 133.83 ± 1.24 mcg/mL, and for aqueous roots extract was 230.92 ± 1.06 mcg/mL.

Discussion and conclusion
The present work was the first one to investigate the G. manicata toxicity. Despite of it is an appreciated ornamental plant in Brazil, there was no data in scientific literature about toxicity proprieties. The A. salina bioassay was choose because it is versatile, viable, fast and low cost screening assay (7). Furthermore, there is good correlation between the in vivo and the in vitro tests, and this method is a useful tool for predicting oral acute toxicity in plant extracts (8). Considering the results, the aqueous leaves extracts was more toxic than roots extracts. The data of this test encourage to complementary and more in-depth studies about chemical composition and toxicity proprieties of the G. manicata extracts.

References


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EFFECTS OF MATÉ (Ilex paraguariensis) ON ADIPOGENESIS IN VITRO

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2. Unidade Integrada de Farmacologia e Gastroenterologia, Universidade São Francisco, Bragança Paulista, SP.

Introduction
Ilex paraguariensis A. St.-Hil. is a South American tree from which leaves and twigs are used to prepare a tea known as maté (“yerba mate”). It is one of the most commonly consumed beverages in several South American countries, including Brazil (especially the Southern states), Uruguay, Paraguay and Argentina. Since a long time, leaves from Ilex species are being studied(1). Efforts are being made to study also the saponins present in the fruits of maté in order to use this raw material as a source of industrial saponins(2). More recently, our group initiated medicinal chemistry studies in order to find relationships between the chemical structure of saponins and their biological activity, as for example antimalarial one(3). In addition to polyphenols such as flavonoids (quercetin and rutin) and phenolic acids (chlorogenic and caffeic acids), maté is also rich in caffeine and saponins(4). Recently, evidences have shown some beneficial effects of maté which include antioxidant activity(5), a protective effect against induced DNA damage(5), vasodilatation effects(6), and antiobesity effects(7). This study presents the action of fractions from the hydroethanolic extract of I. paraguariensis leaves in reducing adipogenesis in cell culture.

Materials and methods
Maté tea extract
Leaves from Ilex paraguariensis A. St. Hil. were harvested in a cultivated area. Fresh leaves were grounded and submitted to maceration in EtOH 70% (1:10, plant:solvent, 2 x 7 days). After ethanol elimination, one half part of this residual aqueous phase was fractionated with ethyl acetate to obtain the ethyl acetate fraction and the aqueous residue. The other half part was subjected to column chromatography using molecular permeation and a gradient of H2O: EtOH as eluent. Collected fractions were grouped together according similar profile at thin layer chromatography (TLC). Substances were visualized at TLC using Si gel, BAW (4:1:5) as eluent. and spraying with anisaldehyde sulfuric acid/100 ºC. It was obtained two fractions: the saponin and the flavonoid fractions. All fractions were chemically characterized by TLC with reference substances found in maté as rutin and saponins.

Determination of triglyceride accumulation
Cells pre adipocytes 3T3-L1 were acquired from American Type Culture Collection (Manassas, VA) and maintained in culture until the period of maturation according to the manufacturer. Fractions of Ilex paraguariensis (50 to 100 µg/mL) were added to cultured cells until mature adipocytes when the amount of their fat were measured through the test of Oil Red O. Data in triplicate were analyzed by Student’s t-test (p ≤ 0.05).

Results
Maté extract was fractionated in order to find which groups of compounds are responsible for the biological activity. Fractions presented rutin and saponins by TLC. The flavonoid fraction and the aqueous residue presented a better result in the reduction of fat accumulation in adipocytes in comparison to the control.

Conclusions
The flavonoid fraction and the aqueous residue from maté may be potentially useful in obesity treatment by demonstrating a decrease in fat accumulation in 3T3-L1 adipocytes.

Acknowledgements
We are grateful to Capes, CNPq and Programa de Pós-Graduação em Ciências Farmacêuticas/UFRGS for grants and fellowships (Brazil).
References


**Limonium brasiliense** (Boiss) Kuntze, AN ALTERNATIVE TO ITS MEDICINAL PROPERTIES

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³CONICET

**Introduction**

Continuing our research of bioactive natural products on medicinal plants from southern Argentina and its potential applications, in particular its insecticidal activity against *Rhizopertha dominica*, we present the results obtained with the extract, sub-extracts and metabolites isolated from the polar extract from roots of *Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae).

*Limonium brasiliense* is a perennial herb, known as “guaycurú”, distributed in Argentina, Uruguay and South of Brasil. Infusions from the roots are used in the treatment of hemorrhage, menstrual disorders, rheumatism and it is believed to have cardioprotective properties [1].

*Rhizopertha dominica* is one of the most widespread and destructive primary insect pests of stored products. Control of these insects is primarily dependent upon continued applications of synthetics insecticides. Although effective, their repeated use has resulted in the development of resistance; it has had undesirable effects on non-target organisms, environment and human beings. Because of this, interest has been put in plant products for fumigant action.

**Materials and Methods**

Dried roots from *L. brasiliense* were milled and extracted with refluxing methanol. This extract was partitioned with different solvents of increasing polarity to obtain sub-extracts that were fractionated by silica gel column chromatography, for isolation and purification of the active compounds. Gallic acid and catechin derivates were identified. The elucidation of the isolated compounds were determined by ¹H and ¹³C NMR spectra and confirmed by comparison with literature data. [2-3]

*Rhizopertha dominica* had been reared on wheat in the laboratory at 30°C, 65- 75% r.h., under a photoperiod of 12 h light/ 12 h dark. A bioassay to evaluate the effect on the survival of beetles was conducted with the ethyl acetate and chloroform sub-extracts and with gallic acid, the major component isolated from the ethyl acetate sub-extract. To determine the fumigant toxicity the protocol was followed as previously described by Pascual Villalobos. [4]. Mortality was evaluated daily during 5 days. Probit analysis was used to estimate LT₅₀ (lethal time) at 30% (W/W) by Micro Probit 3.0 software.

**Results**

Table 1 shows the results of LT₅₀ of the two sub- extracts and gallic acid.

**Discussion and conclusion**

Both ethyl acetate and chloroform sub-extracts demonstrated to be toxic against *R. dominica* and there were no significant differences between them based on LT₅₀. In particular, gallic acid was more effective than the ethyl acetate sub-extract from which it was isolated, which may be attributed to the fact that this is one of its major constituents. Similar results were founded by Regnault Roger et al [5] with gallic acid isolated from five Lamiaceae against *Acanthoscelides obtectus*.

Further research is in progress to isolate other active compounds from *L. brasiliense* to explain the results obtained with these sub- extracts.

These results suggest that *Limonium brasiliense* is an important resource of biological relevance.

**Acknowledgments**

CONICET, ANPCYT y UNS.
References

Table 1

<table>
<thead>
<tr>
<th>Sub- extract/ compound</th>
<th>LT$_{50}$</th>
<th>95% CI</th>
<th>Slope± SE</th>
<th>$X^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>96.90</td>
<td>(79.20-143.96)</td>
<td>2.67±0.83</td>
<td>0.51</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>96.38</td>
<td>(79.66-137.02)</td>
<td>2.83±0.85</td>
<td>0.84</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>75.49</td>
<td>(50.37-171.53)</td>
<td>1.58±0.55</td>
<td>1.59</td>
</tr>
</tbody>
</table>

LT$_{50}$: Lethal Time 50 (hours); CI 95%: Confidence Interval of 95%; SE: Standard error.
ON PHYTOTHERAPEUTICAL MEDICINES AND DIETARY SUPPLEMENTS

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Introduction
There was an increase in the last decade in the consume of products considered “natural”, this is, those that contain medicinal plants (MP) as constituents. Thus, it is possible to find products with MP of different origin and quality. Taking into account that pharmacies are nowadays the most accessible sanitary establishment for population, it is very important that Pharmacy students became familiar with the different marketed products containing them or their products.
Among them, it is possible to identify Medicinal Specialities (MS), Phytotherapeutic Medicines (PM) and Dietary Supplements (DS), each one with a particular legal regulatory framework (1B3).
The aim of this work was to do a survey on PM and DS in order to determine if they fulfil with their respective legal regulations. At the same time, to identify and to differentiate (between both), the available products.

Materials and methods
The activity was planned together among the subjects Public Health, Medicinal Plant Substances Quality Control Bases and Pharmacy Professional Practice (PPP).
The strategy was that the students of PPP working in their centre of practices developed the above mentioned objectives.
They had to complete a table with the following data: R.N.E.; R.N.P.A.; certificate nº; laboratory; composition; suggested use; cost; and sale frequency.
The study was developed in 40 drugstores of the city of Córdoba (Practice centre). Finally, each student processed the obtained results and all the conclusions were discussed in a seminar.

Results
In total, 1141 products were listed, among them 39.6 % were PM and 60.4 % were DS. There were 58 suggested uses reported and a big variety of MP for PM as well as for DS.
In relation to the form, the DS are principally as sold as pills (75,4%), the PM as bags for infusions (44%), drops (23%) and pills (16%). 22 laboratories produced PM and 30, produced DS.
In relation to the regulations the 0.14% of the DS did not fulfil with the R.N.E. publication in the primary package, and an 8.46% of the PM lack of the certificate number, as is established by the regulation.

Conclusions
The students were very motivated by the activity, they wanted to learn more about the legal framework and to discuss about the implications in relation to the impact on the public health.
The proposed methodology allow the students to fulfil the all the planed objectives.

Acknowledgments
To the students and instructors.

References.

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PHARMACOBOTANY EQUIVALENCE OF COMMERCIAL DRY CARQUEJA: TRIMERA, ARTICULATA OR CRISPA?

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Introduction
Several species of Baccharis are known under the name of “carqueja” (Baccharis articulata (Lam.) Persoon, Baccharis crispa Sprengel and Baccharis trimera (Less) DC). The latter was selected according to its hepatoprotective activity(1), as raw material for the development of a drug formulation for oral solid herbal medicines (tablets)(3), which maintains the original drug components ensuring their safety, efficacy and quality as well as synthetic drugs.

Drugs whose active principles are plants or their extracts have characteristics that distinguish them from drugs whose active ingredients are pure synthetic chemicals or natural. These features make that the demonstration of efficacy, safety and quality is different than for medicinal preparations.

The pure products are accurately described by their chemical structure, are identified and valued by chemical analysis. Herbal products have variably chemical composition and are defined by the extraction process. The comparison between the characteristics of Baccharis trimera and chromatographic profiles its qualitative analysis, will provide the needed data to control the quality of commercial dried extracts of the species commonly known as “carqueja”, establishing identity parameters for the plant material under study. There are important factors in the industrialization of herbal products: the quality of the used raw material, the extraction solvent; find the excipients to form a matrix that keeps the components.

As the objective of this work is to find a simple and rapid method to detect the typical components Thin Layer Chromatography, commercial dry extract of Baccharis trimera, since the market dried extracts are also other species of Baccharis.

Materials and methods
To characterize the dried extracts we used a chromatographic profile of Baccharis trimera.


Reference substance: three dry extracts(4): dichloromethane, methanol and ethanol, made in the laboratory from plant Baccharis trimera(5) of known provenance.

Samples: Dry extracts from the market. Designated as Commercial A and Commercial B.

Chromatographic profiles (Thin Layer Chromatography) Dry extracts of dichloromethane, methanol and ethanol of known provenance Baccharis trimera, which are used as a basis for comparison with the behavior of commercial samples A and B.

Results
One of the commercial samples analyzed presented a chromatographic profile clearly different from Baccharis trimera. Sample A and reference substance was of identical profile and sample B was characteristic profile of Baccharis articulata(6).

Conclusions
Is important to have found an easy and quick method to distinguish the dry extracts of Carqueja. Nevertheless testing protocol has not a specified extraction method or the species used to do the extract data, that are extremely important because of them, depend upon the pharmacological activity. This method will be useful for the checking process during the tablets preparation.

Referencias.


TYROSYNASE INHIBITORY ACTIVITY OF NATIVE PLANTS FROM CENTRAL ARGENTINA: ISOLATION OF AN ACTIVE PRINCIPLE FROM Lithrea molleoides AND ITS SYNERGISTIC ACTIVITY WITH COMMERCIAL COMPOUNDS

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Introduction
Tyrosinase (EC 1.14.18.1) is an enzyme capable of catalyzing melanin synthesis (1), a pigment produced by organisms in all kingdoms (2). In mammalian, melanin is responsible for protecting the skin against ultraviolet (UV)-induced damage and it is involved in dermatological disorders such as hyperpigmentation (3) and Parkinson’s disease (3,4). Tyrosinase is responsible for enzymatic browning of botanical or fishery products (5,6). In insects, tyrosinase is involved in sclerotization of cuticle, encapsulation and melanization of foreign organisms and wound healing (7). On the other hand, melanin reduces the susceptibility of melanized microbes to host defence (8).

In this sense, tyrosinase inhibitors result in the best strategy for providing therapeutic or cosmetic agents (9), insecticides, antimicrobials or food additives. Numerous tyrosinase inhibitors have been described, however many of them show side effects (1,6,10-14). Plants are a vast source of compounds with different activities including tyrosinase inhibitors (15).

According to previously described we have screened 91 extracts prepared from plants native to Argentina. From one of the most effective, Lithrea molleoides, an alkylresorcinol with high anti-tyrosinase effect was isolated.

Materials and methods

Plant material
Extract from Lithrea molleoides was obtained by maceration with ethanol of crushed aerial plant material.

Isolation of the tyrosinase inhibitor compound
The extract from Lithrea molleoides was dissolved in ethanol and subjected to successive column and radial chromatographies. Finally one compound was isolated and identified as (Z,Z)-5-(trideca-4,7-dienyl)resorcinol (16).

Tyrosinase inhibitory assay
Tyrosinase inhibitory activity was determined spectrophotometrically as described in Chiari et al. (16) Mushroom tyrosinase dissolved in phosphate buffer was mixed with different concentrations of (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol dissolved in ethanol or with ethanol as control. After incubation, L-tyrosine or L-DOPA was added and dopachrome formation was monitored in the reaction mixture.

Synergistic activity assay
Synergism among (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol and kojic acid or hydroquinone was measured as explained above by adding to each well different combinations of mentioned compounds till reaching concentrations of each compound corresponding to 4, 8, 16 and 32 times below its IC₅₀.

Results

Tyrosinase inhibitory activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µg ml⁻¹)</th>
<th>Monophenolase activity</th>
<th>Diphenolase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithrea molleoides</td>
<td>3.77 (2.40, 5.92)</td>
<td>79.44 (27.48, 229.67)</td>
<td></td>
</tr>
<tr>
<td>(Z,Z)-5-(trideca-4,7-dienyl)-resorcinol</td>
<td>0.49 (0.22, 1.09)</td>
<td>14.94 (5.85, 38.09)</td>
<td></td>
</tr>
<tr>
<td>Kojic acid</td>
<td>18.25 (9.37, 35.53)</td>
<td>2.64 (1.06, 6.57)</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>1.55 (1.24, 1.92)</td>
<td>120.07 (30.63, 455.76)</td>
<td></td>
</tr>
</tbody>
</table>

*values IC₅₀ with 95% confidence limits (lower, upper)

Synergistic activity
**Conclusions**

(Z,Z)-5-(trideca-4,7-dienyl)-resorcinol showed a remarkable anti-tyrosinase activity being 8, 37 and 3 times more effective than *L. molleoides* extract, kojic acid or hydroquinone, respectively for monophenolase activity (L-tyrosine as substrate). In regard to diphenolase activity (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol was 5 and 9 times more effective than the extract of *L. molleoides* and hydroquinone respectively, but less active than kojic acid. (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol also showed a strong synergistic activity when combined with kojic acid. This combination exhibited about 90% inhibition when (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol and kojic acid were added at concentrations corresponding to 4 and 32 times below their IC$_{90}$ respectively. No synergistic effect was observed with hydroquinone. According to these results (Z,Z)-5-(trideca-4,7-dienyl)-resorcinol could arise as an effective anti-tyrosinase compound or being used in combinations with the commercial lightening agent kojic acid, with the aim of decreasing the effective doses of this last and in this way minimizing its side effects.

**References**


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PHYTOCHEMICAL ANALYSIS AND TOXICITY STUDY OF Passiflora SPECIES FORWARD TO Artemia salina LEACH.

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2Laboratório de Toxicologia. Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Av. Ipiranga, 2752, Porto Alegre, RS, 90610-000, Brazil.

Introduction

Passiflora species (Passifloraceae) are native plants from South America that provide appreciated fruits known in Brazil as “maracujá” (passion fruit). Some species like P. incarnata, P. alata and P. edulis have been traditionally used to treat anxiety and nervousness(1). Antiinflamatory and antioxidant activities are also reported for P. alata and P. edulis(2,3).

Since ancient times, people have used plants as medicines and this use has great importance, because plants can provide drugs to widen the therapeutic arsenal. However, many plants are known to be toxic. The United States FDA has reported cases of toxicity occurring with Passiflora species(1).

Artemia salina L. (Artemiidae), the brine shrimp larva, is an invertebrated component of the saline aquatic and marine ecosystems fauna. It has good correlation with “in vivo” test and it is used as an alternative method to determine toxicity of chemical and natural products. This method, which determines the LC$_{50}$ value of the active compounds and extracts in saline medium in µg/mL(4), has been used in research on medicinal plants carried out in different countries in order to evaluate toxicity as a screening method mainly for products of plant origin. In toxicity evaluation of plant extracts by brine shrimp bioassay, an LC$_{50}$ value lower than 1000 µg/ml is considered bioactive(5).

The present study describes the phytochemical study and toxicity assay of P. alata, P. morifolia, P. tricuspis, P. galbana and P. amethystina when submitted to Artemia salina LEACH.

Materials and methods

The hydroethanolic extract from five Passiflora species were prepared using EtOH 40º GL under reflux for one hour. Phytochemical profiles of these species were established by thin-layer chromatography according to Birk(6).

For every plant extract, seven concentrations from 1330 to 33 µg/ml (in triplicate) were tested in order to determine dose-response relationship, and a control group was set with artificial salt water and tween solution. Each test tube with sample contained 10 brine shrimp larvae, including the control group(7). After 24 hours, live larvae were counted and the LC$_{50}$ value was estimated using the statistical method of Probits(8).

Results

Flavonoids and saponins are good chemical markers to provide differentiation between closely related species. The chemical profile of these extracts was similar to that shown by Birk(6) where the P. alata extract presents saponins as main metabolites, whereas flavonoids are the major metabolites to the other studied species. The quadranguloside is a saponin previously isolated from the leaves from P. alata and can be used as chemical market(9). Saponins are metabolites with known biological activity otherwise many saponin are toxic compounds(10).

The hydroethanolic extract from P. alata showed LC$_{50}$ = 172.38 µg/mL ± 1.12 and the hydroethanolic extracts of P. tricuspis, P. morifolia, P. galbana and P. amethystina displayed LC$_{50}$ > 1330 µg/mL.

Conclusions

Five hydroethanolic extracts from Passiflora genus were tested in the brine shrimp bioassay and only P. alata extract displayed toxicity (LC$_{50}$ < 1000 µg/mL). Considering that P. alata has saponins as major metabolite it is possible that its toxicity is due to this class of compounds. Further studies are needed to evaluate which compound present in P.alata extract is responsible for its toxicity.
Acknowledgements
The authors thank to CNPq, CAPES, FAPERGS and Programa de Pós-Graduação em Ciências Farmacêuticas/UFRGS for scholarships and financial support. We are grateful to Prof. Gilson R. P. Moreira (Departamento de Zoologia, UFRGS, Brazil) for locating, collecting and identifying the plant material.

References


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ANTIFUNGAL EFFECT OF CHLOROFORM EXTRACT OF *Larrea cuneifolia* ON PLANT PATHOGENS

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**Introduction**

The control of plant diseases depends primarily upon the application of chemical fungicides. However, increasing interest in environmentally friendly sustainable agriculture and horticulture as organic farming has resulted in increasing demand for pesticides produced by plants (1). In the search for novel antifungal agents with high activity, the aerial part of *Larrea cuneifolia* has been evaluated against plant pathogens *in vitro*.

**Materials and methods**

The plant was collected in Córdoba province, Argentina in May 2008. The vegetable was powdered and n-Hexane (HE), Chloroform (CE), Methanol (ME), Cold Aqueous (CAE) and Warm Aqueous (WAE) extract were obtained. The effect of the extracts on fungal growth was evaluated by microbroth dilution technique (2) against *Fusarium graminearum*, *Fusarium solani*, *Fusarium verticillioides* and *Macrophomina phaseolina*. Preliminary phytochemical screening of the extracts was carried out using silica gel TLC sheets and revealed with different spray-reagents, according to reported procedures (3).

**Results**

The activity of *L. cuneifolia* extracts against different plant pathogenic fungi is summarized in Table 1. The chloroform extract significantly inhibited *F. graminearum* and *M. phaseolina* growth, with Minimum Inhibitory Concentration (MIC) values of 250 µg ml\(^{-1}\). *Fusarium verticillioides* and *F. solani* MIC were of 500 and 2000 µg ml\(^{-1}\). The other extracts tested had lower activity. Phytochemical screening revealed that chloroform extract contains flavonoids, lignans and flavones. The presence of detectable quantities of alkaloids, coumarins, quinones, terpenoids, steroids and sapogenins in the extract were discarded.

**Conclusions**

Results revealed that *L. cuneifolia* chloroform extract had significant activity against pathogenic *F. graminearum*, *M. phaseolina* and *F. verticillioides*.

**Acknowledgments**

The authors would like to thank CONICET, Universidad Nacional de Río Cuarto and PICTOR program, BID 1728 /OC-AR, for financial support.

**References**

Table 1
Antifungal Activity of *L. cuneifolia* extracts microbroth dilution assay.

<table>
<thead>
<tr>
<th>Extract</th>
<th><em>F. graminearum</em></th>
<th><em>F. solani</em></th>
<th><em>F. verticillioides</em></th>
<th><em>M. phaseolina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanic</td>
<td>500</td>
<td>2000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Chloroformic</td>
<td>250</td>
<td>2000</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>Methanolic</td>
<td>1000</td>
<td>2000</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>Cold Aqueous</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>1000</td>
</tr>
<tr>
<td>Warm Aqueous</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

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PHARMACOGNOSTIC PARAMETERS OF Cucurbita SEEDS IN CHACO: PRELIMINARY STUDY

Cravzov AL, Tauguinas AL, Baez M, Gimenez MC

Introduction
In a lot of undeveloped countries, most of the population is served as traditional medicines that modern science hasn't always know how to study, recognize or appreciate. Today the big pharmaceutical industries worldwide depend in numerous ways of medicinal plants. A lot of modern medicines come from plants that have been being used for centuries. The search of vegetal origin compounds, biologically actives is a task in which a lot of researchers are involved (1).

A tradition exists about the use of Cucurbita as an antiparasitic and other therapeutic aims nevertheless there isn't available in our region any studies about their phytochemistry composition. This work has the objective of comparing three varieties of pumpkin aiming to obtain some criteria that allows the selection of the most adequate for its pharmaceutical use.

Materials and methods
Three varieties of calabash seeds were compared: Tetsokabuto (hybrid among C. moschata y C. maxima), Cucurbita mixta (calabaza rayada) y Cucurbita moschata (coreanito), for seed mass, desiccation loss at 105 ºC, total ashes by muffle incineration at 800 ºC and insoluble ashes determination in hydrochloric acid incinerated at 650 ºC (2).

Results
Obtained results in the determination of the studied pharmacognostic parameters of each seed (mass, residual humidity, total ashes and soluble ashes - are shown in Table 1. They match the ones reported by Abreu Payrol, J. et al (3).

Best performance on seed mass and total ashes percentage are presented by C. mixta, while the worst results are shown by Tetsokabuto seeds,

Conclusions
This study allows us to propose in a preliminary way that C. mixta is the most advantageous variety for its use with pharmaceutical goals.

References.

Table 1: Pharmacognostic parameters from the pumpkin seed

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tetsokabuto</th>
<th>C. mixta</th>
<th>C. moschata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (mg)</td>
<td>163,21 ± 1,11</td>
<td>288,97 ± 1,28</td>
<td>252,77 ± 0,76</td>
</tr>
<tr>
<td>Residual humidity %</td>
<td>5,86 ± 0,74</td>
<td>5,34 ± 0,70</td>
<td>4,69 ± 0,25</td>
</tr>
<tr>
<td>Total Ashes %</td>
<td>3,23 ± 0,2</td>
<td>5,02 ± 0,68</td>
<td>4,55 ± 0,30</td>
</tr>
<tr>
<td>Soluble sustances %</td>
<td>0,19 ± 0,04</td>
<td>0,20 ± 0,13</td>
<td>0,30 ± 0,04</td>
</tr>
</tbody>
</table>

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PRELIMINARY STUDIES ON *Mulinum spinosum* (Cav.) AQUEOUS EXTRACT: BIOCHEMICAL PARAMETERS

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**Introduction**

*Mulinum spinosum* (Cav.) Persoon (Apiaceae), popularly known as “neneo”, has a broad distribution in the Patagonian steppe. Traditionally, an aromatic bitter decoction made from the root of this plant is used in the control and management of Type 2 diabetes. Previous experiments in our lab with alloxane-induced diabetic mice shown that aqueous extracts of neneo exhibit a potential anti-hyperglycaemic activity, but further studies are needed to know more about the mechanisms of this action.

Due to the widespread utilization of the plant as a traditional remedy, it is essential to investigate the potential effects of the crude extract on liver and kidney for the evaluation of potential health risks to humans. In view of the lack of information on the pharmacological and toxicological properties of neneo, the present work investigated the effects of its decoction in mice, providing preliminary biochemical and hepatic tissue parameters.

**Materials and methods**

*M. spinosum* were collected in February 2008 from Jacobacci (Río Negro) steppe, and were identified by Dr. Gandullo (Universidad del Comahue, Facultad de Agronomía). Voucher specimens were deposited at CInIByC herbarium. Root were dried and crushed. Plant material was prepared according to the traditional method (decoction): 1g or 10g of root with 100 ml distilled water were boiled for 10min. Thereafter, the aqueous extract was filtered and administered to the mice.

Adult male mice, weighing 30–35 g, were used in the experiments. Test groups were orally treated with the decoction for 21 following days, once a day.

Blood samples were withdrawn by cardiac puncture and then the mice were sacrificed. Plasma obtained was aliquoted for biochemical analysis using *A25 BioSystems* autoanalyzer. Following blood collection, animals were sacrificed and liver and kidney of all animals were removed. The macroscopic appearance of the organs was noted and their weights were recorded. Liver of each mice was stored in 10% formaldehyde and prepared for routine histology.

Results are expressed as mean±SD (*n*=10) and statistical significance was determined by ANOVA followed by Newman-Keuls test. The level of significance was set at *p*<0.05

**Results**

The analysis of the biochemical parameters of the test group showed alterations only in the serum albumin level, when compared with that of the control group. Macroscopic examination and weights of kidneys and livers failed to reveal any differences between groups. The hepatocytes from the treated group are histologically similar to those of the control group, but exists increase of hepatic vascularization, with sinusoidal dilatation.

**Discussion and Conclusions**

Determination of plasma albumin can act as a criterion for assessing synthetic capacity of the liver, and *M. spinosum* decreased albumin level. Therefore, it is suggested that neneo causes liver function changes. Many drugs and compounds derived from herbal medicines are largely detoxified via hepatic metabolism, endangering the functional status of hepatic detoxification system. The mild sinusoidal dilatation observed in the liver of the treated animals can be related to this effect. However, the increase of hepatic vascularization and decrease serum albumin in the treated group may suggest an initial stage of hepatic alteration, but further studies are required to evaluate the effects of *M. spinosum* on the liver.

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**Table 1:** Biochemical parameters of animals treated with extract of *Mulinum spinosum*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>M.spinosum 1g%</th>
<th>M.spinosum 10g%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>105.75±7.95</td>
<td>120.77±7.26</td>
<td>132.63±9.36</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>114.83±5.12</td>
<td>111.33±6.01</td>
<td>125.23±7.39</td>
</tr>
<tr>
<td>Aspartate aminotransferase (UI/L)</td>
<td>217.12±22.95</td>
<td>235.62±33.42</td>
<td>225.08±33.24</td>
</tr>
<tr>
<td>Alanine aminotransferase (UI/L)</td>
<td>60.87±6.81</td>
<td>70.22±6.87</td>
<td>74.89±9.93</td>
</tr>
<tr>
<td>Albumin (gr/dl)</td>
<td>4.81±0.02</td>
<td>2.87±0.05</td>
<td>1.07±0.06*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>54.71±2.12</td>
<td>51.33±2.61</td>
<td>55.08±3.89</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.82±0.04</td>
<td>1.09±0.07</td>
<td>1.16±0.07</td>
</tr>
</tbody>
</table>

*Significantly different from the control, p <0.05*
PHYTOCHEMICAL STUDIES AND EVALUATION OF *Baccharis* (*Asteraceae*) SPECIES USING *Artemia salina* BIOASSAY

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Introduction

*Baccharis* is one of the largest genus of Asteraceae family and it is found in South America. *Baccharis* species are known in Brazil as “carquejas” and are used in folk medicine as diuretic, for digestive disorders and for curing wounds or local infections. The literature also describes the anti-inflammatory, antioxidant, and antimicrobial activities to them(1).

People uses plants as medicines and this use has great importance, because plants can provide drugs to widen the therapeutic arsenal. However, many plants are known to be toxic. As an example, we have the toxicity caused by the trichothecenes present in *Baccharis coridifolia* and *Baccharis artemisioides* in livestock (equine, ovine and bovine) that is very common in Argentina(2).

*Artemia salina* L. (Artemiidae), the brine shrimp larva, is an invertebrate component of the saline aquatic and marine ecosystems fauna. It has good correlation with “*in vivo*” test and it is used as alternative method to determine toxicity of chemical and natural products. This method, which determines the LC₅₀ value of the active compounds and extracts in saline medium in µg/mL(3), has been used in research on medicinal plants carried out in different countries in order to evaluate toxicity as a screening method mainly for products of plant origin. In toxicity evaluation of plant extracts by brine shrimp bioassay, an LC₅₀ value lower than 1000 µg/ml is considered bioactive(4).

The present study describes the phytochemical study and toxicity assay of *B. articulata*, *B. trimera*, *B. spicata*, *B. usterii* and *B. anomala* forward to *Artemia salina* LEACH.

Materials and methods

Aqueous extracts from five *Baccharis* species were prepared, separately, by decoction of plant material. Phytochemical profiles of *Baccharis* species were established by thin-layer chromatography according to De Oliveira(5).

For every plant extract, seven concentrations from 1330 to 33 µg/ml (in triplicate) were tested in order to determine the dose-response relationship, and a control group was set with the artificial salt water and tween solution. Each test tube with sample contained 10 brine shrimp larvae, including the control group(6). After 24 hours, live larvae were counted and the LC₅₀ value was estimated using the statistical method of Probits(7).

Results

Flavonoids, together with diterpenes, are the major compounds found in *Baccharis* and they are described as good chemical markers to Asteraceae family(8). Phytochemical studies have shown the presence of phenolic and terpenoid compounds as the main constituents of the studied species. The aqueous extract from *B. articulata* showed LC₅₀ = 433.42 µg/mL ± 1.04 and the aqueous extracts from *B. trimera*, *B. spicata*, *B. usterii* and *B. anomala* displayed LC₅₀ > 1330 µg/mL.

Conclusions

Five aqueous extracts from *Baccharis* genus were prepared and tested in the brine shrimp bioassay and only *B. articulata* displayed toxicity (LC₅₀ < 1000 µg/mL). These results are very important not only considering the popular use of these species, but the activities previously reported to them.
Further studies are needed to evaluate which compound present in *B. articulata* extract is responsible for its toxicity.

**Acknowledgements**

The authors thank to CNPq, CAPES and FAPERGS and Programa de Pós-Graduação em Ciências Farmacêuticas/UFRGS for scholarships and financial support.

**References**


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**α-Glucosidase Inhibitory Activity of Smallanthus Sonchifolius (Yacon) Roots**

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**Introduction**

α-glucosidase in the small intestine plays a physiologically important role for the digestion process of dietary carbohydrate. The inhibitors of this enzyme, in consequence, are known to reduce the post-prandial hyperglycemia and may contribute to the optimal glycemic control in the management of obesity and diabetes. *Smallanthus sonchifolius* [Poeppl. & Endl.] H.Robinson or yacon is a native specie of South America belonging to the Asteraceae family. In recent work we have demonstrated that the administration of syrup obtained from yacon roots produced beneficial health effects on obese women with insulin resistance (1). In the present study we have investigated the effect of yacon root flour to control the post-prandial hyperglycemia and also determinated its possible mechanism of action through studies in Wistar rats and "in vitro" α-glucosidase inhibition assay.

**Materials and Methods**

Tolerance Glucose and Sucrose Tests: adult male Wistar rats weighting 200-220g were selected for all experiments. The animals were divided as follow: a) treated daily with yacon roots (0,95g dw/Kg bw and 10,5g dw/Kg bw), before eating, during a 30 days period, b) animals without treatment. Oral Tolerance Glucose and Sucrose were carried out in all animal groups.

"In vitro" Inhibition assay for the α-glucosidase activity: experiment was carried out by the method described by Li et al., 2004 (2). Acarbose was used as positive control. Released glucose was quantified with a commercial glucose oxidase-based assay (Sigma®) and was read at 540nm. Inhibitory activity was reported as mg of free glucose.

Maceration, decoction, infusion and syrup (4, 16, 64µg/ml) was obtained from yacon roots and used for inhibitory α-glucosidase assay.

Statistical analysis: The statistical significance was assayed using analysis of variance (ANOVA). A p value ≤0,05 was considered statistically significant.

**Results**

Oral Glucose Tolerance Test: The oral administration of yacon roots caused a rapid decreased in the hyperglycemic peak after glucose loading in both animal groups (with and without treatment).

Oral Sucrose Tolerance Test: Animals underwent sucrose tolerance curves 15 min after water, acarbose or yacon roots. After 30 min, yacon roots decreased the glycemic peak, compared with the control. This effect was dose-dependent being 10,5g dw/Kg bw the most effective dose. The rats treated with yacon roots during 30 days showed a better response to oral sucrose.

Inhibition of α-glucosidase activity: Maceration, decoction, infusion and syrup obtained from yacon roots inhibited α-glucosidase activity. This inhibition was significant in relation with negative control and was even lower than with acarbose, a therapeutic drug used as positive control.

**Discussion**

Our results indicate that the control of post-prandial glucose level showed by yacon roots might involve an anti-hyperglycemic effect, mediated by the inhibition of carbohydrate digestion. This could be possible by retarding the postprandial glucose level by inhibition of intestinal α-glucosidase as shown by Shim et al. (3).

**References**

PSYCHOPHARMACOLOGICAL EFFECTS OF Artemisia copa AQUEOUS EXTRACTS IN MICE

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Introduction
Artemisia copa (A. copa) Phil. (Compositae), commonly known as “copa-copa”, is a small and very branched bush that grows in the northwest of Argentina and north of Chile. The infusion of the aerial parts is used in popular medicine as antitussive, digestive, febrifuge, for the treatment of pulmonary diseases, and hypertension (1). The leaves, macerated in alcohol, are also used locally to rub off rheumatic pains.

A previous pharmacological study of the plant revealed that the aqueous extract of aerial parts of A. copa, possess analgesic and antiinflammatory activity (2).

The aim of this study was to carry out a psychopharmacological screening of Artemisia copa in different experimental models in mice.

Materials and Methods

The aerial parts of Artemisia copa were collected in Antofagasta de la Sierra, Catamarca Province, Argentina. The aqueous extract was prepared by macerating 50 g of powdered plant material for 20 min using 500 ml of boiling water.

The aqueous extract from aerial parts of A. copa (AC) administered p.o., was evaluated for its psychopharmacological activities in several experimental models using female Swiss albino mice: hypnogenic activity, Marble-burying test (3), studies on spontaneous motor activity, hole-board test (4), and pentylene tetrazol induced seizures.

The statistical test were used one –way analysis of variance (ANOVA) folowed by Dunnet t-test.

Results

A. copa at doses up to 1.5 g/kg produces a dose dependent sleep induction and potentiation of subhypnotic and hypnotic doses of pentobarbital (PB30: 0.5 ± 0.35 min, A. copa 1.5 g/kg + PB30: 18.24 ± 3.88 min, P<0.01. PB40: 34.83 ± 5.90 min, AC 1.5 g/kg + PB40: 51.33 ± 3.22 min, P<0.03, respectively). The extract also produced a dose dependent increase and decrease in the spontaneous motor activity (0.5-1.5 g/kg) and no modification or a decrease on exploratory (holeboard) behavioral profiles (1.5 g/kg). A. copa displayed dose-related anxiolitic-like activity as indicated by increases in the percent of marbles they left uncovered in the marble-burying test. In addition the extract (1.5 g/kg) produced a significative decrease in the latency time and a decrease in the duration of seizures and mortality induced by PTZ 75 mg/kg.

Discussion

These results suggest that the aqueous extract of A. copa may contain sedative principles with potential anxiolytic and anticonvulsant activities.

References

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POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN *Lippia alba* (MILL.) N. E. BROWN EXTRACT


Carrera Farmacia, Universidad Nacional del Chaco Austral. 2Universidad Nacional de Buenos Aires.


**Introduction**

Many plant species belonging to the genus *Lippia* and in particular their fragrant essential oils present economic potential in the industry. Moreover, polyphenols and flavonoids are natural components of plants. Antioxidant activity and antialergic, antiviral, vasodilator, antimicrobial and anti-inflammatory properties are attributed to these compounds.

The aim of the present study was the evaluation of polyphenolic composition and the antioxidant activity of fluid extract of *Lippia alba* (Mill.).

**Materials and methods**

The fluid extract was prepared from percolation of dried leaves powder (particles of 250 - 710 µm) with ethanol 70°. It was carried out its phytochemical screening through secondary metabolites identification reactions and a thin layer chromatography for flavonoids exploration.

The total polyphenolic content was performed according to Singleton et al method, with Folin-Ciocalteu reagent, using a Beckman DU 640 spectrophotometer. Total flavonoids content was determined by Lock et al technique. In vitro antioxidant activity was tested in accordance with the Brand-Williams’ modified method through the free radical scavenging activity upon DPPH reagent. IC$_{50}$ was calculated by plotting the DPPH remanent percentage at the steady state (10 min) against various concentrations of phenols in extract (7.1 a 35.5 µg). An autographical test in TLC was carried out for checking biological activity in situ (with DPPH reagent) using two running systems and revealed with ammonia vapor and UV-Vis.

All the precedent measurements were for triplicate and expressed as average (n=3) and standard deviation.

**Results**

The secondary metabolites found were phenols/tannins, flavonoids and terpenes. The total phenols content test revealed a value of 70.8 ± 0.104 mg of AG equivalent /mL ($R^2 = 0.9946$ from the calibration curve), in the meantime total flavonoids content showed a value of 6.92 ± 0.203 mg of quercetin equivalent/mL ($R^2 = 0.9988$). The registered values of antioxidant activity were 2.18 ± 0.005 mg of gallic acid equivalent /mL and 0.59 ± 0.009 mg of quercetin equivalent /mL.

The autographical test revealed two components with more intense antioxidant activity and two with low activity, all of them with solvent system of medium polarity.

**Conclusions**

The extract’s phenolic and flavonoid composition would probably justify its antioxidant activity. The total phenolic content was different from the reported in another papers in relation to *Lippia alba* (1,2).

The differences in the chemical composition could be due to geographical, climatic and/or extraction method’s factors. The radical scavenging activity of extract could be related to the nature of phenolics compound. The flavonoids detected would be of medium polarity.

**References**


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CARBOHYDRATES AND PHENOLICS COMPOUNDS FROM LEAVES OF
Hydrocotyle bonariensis LAM. (Apiaceae)

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²Farmacognosia y ³Química Biológica II, Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Km 4 (9000) Comodoro Rivadavia, Chubut, Argentina.

Introduction
Hydrocotyle bonariensis Lam. (Apiaceae) it is an herbaceous plant of wide distribution in South America. In this work we are presented advances at the study of the carbohydrates and phenolic compounds of the leaves, considering the possible relationship that can have between this substances and the biological properties of this vegetable specie.

Materials and Methods
The leaves of H. bonariensis were collected in April of 2006 in Punta Lara. 10 g of the milled leaves were extracted with distilled water recently boiled¹. The extracted was separated by filtration and was recovered by freeze-drying (I). One portion of the I was suspended into distilled water and dialyzed in closed system. The dialyzed fraction (Id) and the waters dialysis (Iwd), were concentrated at reduced pressure in rotational evaporator and dried off in vacuum oven.

Total carbohydrates were determined by the phenol–H₂SO₄ method ²,³. The percentages of sulfate were measured by turbidimetry, after hydrolysis with 1 M HCl³. Uronic acids were determined by Filisetti-Cozzi and Carpita method ³. Hydrolysis of the polysaccharides was carried out with 2 M CF₃COOH³.

Total aqueous extract were analyzed in their composition of carbohydrates without and with previous hydrolysis, obtaining the profiles of them by planar chromatography and HPLC⁴. For the analysis of phenolic compounds was obtained the chromatography profile with cellulose ⁵.

Results
Yields of the I, Id and Iwd were important (23.9; 14.0 and 85.0 %, respectively). Id was constituted exclusively of carbohydrates (100 %). The uronics acids represented 5 %.

The principal phenols derivates were rutin, quercetin, chlorogenic acid and the other not yet identified.

The sulfate groups were distributed among Id and Iwd.

Discussion
The constituent carbohydrates of the infuse, their partially sulfate nature and the phenolic derivate, they keep narrow relationship with the traditional uses as diuretic, anti-inflammatory and antiseptic.

References

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FLAVONOIDS FROM Dalea boliviana AS TYROSINASE INHIBITORS

Peralta MA, Santi MD, Agnese MA, Ortega MG, Cabrera JL.


Introduction
The melanin formation is one of the most important factors to determine the mammalian skin colour. Tyrosinase is a mixed function oxidase enzyme which catalyzes two different reactions in the synthesis of melanin: the formation of 3, 4-dihydroxyphenylalanine (L-DOPA) from tyrosine and further oxidizes L-DOPA to dopaquinone. Tyrosinase inhibitors may be of important to treat abnormal pigmentation disorders and to use as skin whitening agents in cosmetics.(1) Our research group began the chemical and pharmacological study of Dalea boliviana. We previously report the isolation and structural characterization of three new prenylated flavanones from the hexanes extract of D. boliviana roots: (2S) 5, 7, 2' trihydroxy-5'-(1''',1'''-dimethylallyl)-8-prenylflavanone (1), (2S) - 5, 7, 2' - trihydroxy-8, 3' - diprenylflavanone (2), and (2S) - 5, 2' - dihydroxy-6', 6''- dimethylchromeno-(7,8,2',3'') - 3' - prenylflavanone (3), together with a known chromeno (dimethylpyrano) flavanone, obovatin (4). Taking into account that similar compounds have been related to inhibition of tyrosinase activity, we propose to evaluate compounds 1-4 as tyrosinase inhibitors.

Materials and methods
Plant material
D. boliviana Britton was collected in February 2007, near Iturbe in Humahuaca Department, Jujuy province, Argentina. A voucher specimen is on deposit at the Botanical Museum - UNC as CORD 1066. The plant material was dried at room temperature and the roots (60 g) were separated from the aerial parts, powdered and extracted with hexanes (250 ml) at room temperature for 24 h. From 3g of hexane crude extract, by the application of chromatographic techniques (column and TLC), (2S) 5, 7, 2' trihydroxy-5'-(1''',1'''-dimethylallyl)-8-prenylflavanone (1), (2S) - 5, 7, 2' - trihydroxy-8, 3' - diprenylflavanone (2), and (2S) - 5, 2' - dihydroxy-6', 6''- dimethylchromeno-(7,8,2',3'') - 3' - prenylflavanone (3), together with a known chromeno (dimethylpyrano) flavanone, obovatin (4), were isolated.(4)

Tyrosinase activity assay
It was performed according to the method of Rahman et al. (8) adapted to work conditions. L- tyrosine (1.7mM) was used as a substrate of the enzyme and tyrosinase from mushroom (250U/mL) was the enzyme source. Activity was measured spectrophotometrically at 475nm and 25 °C. Different concentrations of compounds 1, 2, 3 or 4 (25-100 µM) were evaluated. Results were expressed as percentages of inhibition using the following equation: % inhibition = [(Abscontrol - Abssample)/Abscontrol] × 100, where Abscontrol is the absorbance of the control and Abssample is the absorbance of the experimental sample. The IC50 values were estimated by using non-linear fitting of concentration-response data.

Results
As a result, 1 and 2 showed inhibitory activity with IC50 of 27.1 µM and 68.5 µM, respectively. On the other hand, 3 and 4 demonstrated a very low activity on tyrosinase enzyme even at 100 µM. Kojic acid(1) was used as positive control (IC50 = 10.2 µM).

Conclusions
The present work reports the effect on tyrosinase activity of compounds 1-4, isolated from the hexanes extract of Dalea boliviana roots. They exhibited different capacities being 1 and 2 much better inhibitors than 3 and 4. It is important to highlight that the first two compounds present a 4-substituted resorcinol moiety at A ring, which has been demonstrated to be essential for inhibitory activity against tyrosinase activity(1). On contrary, 3 and 4 were weak inhibitors probably as a consequence.

References
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SEARCH OF ANTIOXIDANTS COMPOUNDS FROM Dalea SPECIES

Peralta MA, Del Gaudio MP, Cabrera JL, Ortega MG


Introduction
Reactive oxygen (ROS) and nitrogen species (RNS) include free radicals and molecules derived from oxygen and nitrogen, respectively. There are increasing suggestions by considerable evidence that free radicals induce oxidative damage to biomolecules, causing eventually atherosclerosis, ageing, cancer, inflammation, and degenerative diseases in humans. There is an increasing interest in natural antioxidants, present in plants, which might help to prevent oxidative damage. Plant phenolic compounds are secondary metabolites with interesting antioxidant activity. Flavonoids are naturally occurring polyphenolic compounds found in the vegetal kingdom that have effect on a great variety of enzymatic systems, enclosing important pharmacological and biochemical activities such as antioxidant, antitumoral and anti-inflammatory ones.

Dalea is an exclusively American genus with more than 250 species. Different species of this genus were studied, and the isolation of compounds, including flavonoids, has been reported. There are four species described for Argentina, namely Dalea elegans Gillies ex Hook. & Arn., D. boliviana Britton, D. leporina (Aiton) Bullock, and D. elegans var. onobrychioides (Griseb.) Barneby. The first phytochemical and bioactivity studies on Argentinean Dalea species were made by our research group starting with D. elegans and D. boliviana. In the present work, three crude extracts of Dalea species were studied (D. elegans, D. boliviana and D. leporina) regarding to their total phenols and flavonoids content and antioxidant activity. The results could impact in the research of new antioxidants compounds from autochthons species.

Materials and methods

Total phenols content
Total phenols content were estimated by Folin-Ciocalteau reagent. The results were expressed as mg of gallic acid equivalents/g of extract (mgEqAG).

Total flavonoids concentration
The flavonoid concentration was estimated by the AlCl₃ method. The results were expressed as mg of quercetin equivalents/g of extract (mgEqQ).

Antioxidant potential assay
The antioxidant potencial of the extracts was assessed with the phospomolybdenum reduction assay (FM). The reducing capacity of the extracts were expressed as ascorbic acid equivalents/ mg of extract analyzed.

Determination of ABTS radical-scavenging activity (TEAC)
The ABTS radical-scavenging activity values were estimated by the Trolox equivalent antioxidant capacity (TEAC) test. The results were expressed as µg of Trolox equivalents/ of mg of analyzed extract.

Results

Results are shown in the Table 1.

Conclusions

The aim of the present work was to quantify and compare total phenols and flavonoids content in the ethanol/ H₂O extracts of three Argentinean species from Dalea genus: D. elegans, D. boliviana and D. leporina as well as to assess their in vitro potential as new natural antioxidants source. Antioxidant activity was analyzed in two different test systems. According to the results, it can be concluded that all the tested extracts demonstrated important antioxidant potential and free radical scavenging activity.
compared with the positive control Quercetin, which could be due to their phenols and flavonoids content. The results show correlation between concentration of total phenolic and flavonoids compounds and antioxidant capacity of the extracts. Therefore, these values in Dalea extracts are relevant indicators of their antioxidant power. Further studies would focus on the isolation, purification, structural characterization and evaluation of new antioxidant substances from this genus.

Table 1.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenols (a)</th>
<th>Total flavonoids (b)</th>
<th>Antioxidant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>D. elegans</strong></td>
<td>55.26±0.06</td>
<td>46.9±0.05</td>
<td>25.29±0.04</td>
</tr>
<tr>
<td><strong>D. boliviana</strong></td>
<td>48±2</td>
<td>42.1±0.7</td>
<td>36.2±0.9</td>
</tr>
<tr>
<td><strong>D. leporina</strong></td>
<td>17.2±0.2</td>
<td>9.1±0.2</td>
<td>13.7±0.2</td>
</tr>
</tbody>
</table>

FM (c) = 25.29±0.04 (100%)
TEAC (d) = 6.13±0.03

References

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EFFECTS OF A NATURAL PRODUCT ON *Fusarium semitectum* A CAUSATIVE AGENT OF FUSARIOsis

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Introduction

*Fusarium* species have emerged as major opportunistic fungal agents that cause a broad spectrum of infections in humans, including superficial, locally invasive, and disseminated infections¹. The clinical form of fusariosis depends largely on the immune status of the host and the portal of entry, with superficial and localized disease occurring mostly in immunocompetent patients and invasive and disseminated disease affecting immunocompromised patients². There has recently been great concern about the development of *Fusarium* fungal keratitis among people wearing soft contact lenses. This serious infection appears to be associated in many cases with the use of contact lens solution³. In 2005 and 2006, outbreaks of *Fusarium* keratitis associated with soft contact lens use occurred in multiple U.S. states and Puerto Rico⁴. Fertile areas of chemical investigation for many years have been the plant secondary metabolites research. A growing number of natural compounds are useful as biochemical tools and / or pharmacological to elucidate biological processes relevant. The latter approach is proportionally gave the best results⁵. In recent years there has been an upsurge in interest from other disciplines, related to use of secondary metabolites that could have a considerable impact on human health⁶. The aim of this study was to evaluate in vitro the antifungal effect of the 2RB(B)B6Bhidroxitremetona isolated from *Xenophillum poposum* (Compositae) on *Fusarium semitectum* (Deuteromycetes) causing keratomycosis.

Methods and Materials

The bioassay was conducted to evaluate tremetone effect at different doses (25, 50, 75,100, 150 and 200 mg / L) on the mycelial growth of *F. semitectum*. A portion of APG medium to which were added 2.5 ml of solution of tremetone at mentioned concentrations, were placed in several Petri dishes. A colony of *F. semitectum* (5 mm Ø) that was removed of the growth zone was sown in the middle of the dishes. The trial was conducted under a completely randomized design with three replications for each dose. The assays were kept in a culture stove under controlled conditions (22 ° ± 2º C). The diameter of mycelial growth was measured at 72 and 120 hours. The results are reported as mean ± SEM. The differences in the mean values were evaluated by analysis of variance (ANOVA). The LSD test was used for all pair wise multiple comparisons of groups. In all statistical analysis, P > 0.05 were considered not significant (Statistix 7.1 2000).

Results

The results were: 72 hours: control, 4.87 ± 0.17 cm, 25 mg / L: 3.21 ± 0.87 cm, 50 mg / L: 4.00 ± 0.37 cm, 75 mg / L: 3.82 ± 0.08 cm, 100 mg / L : 3.61 ± 0.25, 150 mg / L : 2.97 ± 0.26 cm, 200 mg / L: 0.92 ± 0.59 cm, and 120 hours: control, 7.72 ± 0.45 cm, 25 mg / L: 4.81 ± 0.43 cm, 50 mg / L: 6.68 ± 0.40 cm, 75 mg / L: 6.76 ± 0.27 cm, 100 mg / L:6.30 ± 0.48, 150 mg / L: 5.25 ± 0.81cm, 200 mg / L: 1.72 ± 2.27 cm. At the highest dose tested (200mg/L) the substance produce marked inhibition rates (over 80%) at 72 hrs and over 70% at 120 hrs.

Conclusions

This substance had a significant inhibition on mycelial growth at the dose 200 mg/L. We report previously that tremetone inhibited the mycelial growth of *Fusarium* sp, and now we inform, for the first time, the antifungal effects of 2R(-)-6-hidroxitremetona on *F. semitectum*.. These results lead to future studies to evaluate an antifungal product from natural origin.

Acknowledgments

Work was supported by Research Council of the National University of Tucumán (CIUNT) of Argentina and National University of Chilecito.
References


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ESSENTIAL OILS OF THE NATIVE SPECIES OF Verbena OF THE PROVINCE OF BUENOS AIRES, ARGENTINA

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Introduction
In the last years the use of natural products in therapeutics has come back (1). In the family Verbenaceae there are numerous species used in traditional medicine. Particularly, the species of the Verbena genus present properties hepatoprotective, digestive, anti-inflammatory, healing, anti-diarrheal, antibacterial and antifungal, among others (2)(3). Many of these properties are due to the presence of essential oils in the representatives of the genre. In Buenos Aires province (Argentina) six native species exist: V. montevidensis Spreng., V. rigida Spreng., V. bonariensis L., V. intermedia Gillies & Hook., V. litoralis HBK and V. gracilesens (Cham.)Verter (4). The aim of this work consists of determining the components of the essential oils of the native species of the genus Verbena of the province of Buenos Aires, in order to establish resemblances and differences between them and their potential application to the phytotherapeutical medicine.

Materials and methods
The material of study consisted of flowers of V. bonariensis, V. litoralis, V. intermedia, V. rigida, V. montevidensis and V. gracilesens collected from natural populations and experimental plots in the National University of Luján in two consecutive years. The essential oils were extracted through a process of hydrodistillation and analyzed by GC-MS.

Results
The essential oil yield of all species was low. From the material collected in 2008/09 period were obtained essential oils with a high percentage of 1-Hepten-3-ol, 2-Pentadecanone-6,10,14-trimethyl and phytol with respect to the rest of the components (Table 1). This relationship was not maintained during 2009/10 period. However, phytol and 1-Hepten-3-ol were more abundant substances in V. litoralis, V. bonariensis, V. rigida and V. gracilesens, while in V. intermedia showed more phytol and Hexacosane (Table 2). All species showed similarity in the number and type of components during both periods. Despite this, there were differences in the percentages of the components of essential oils according to the species analyzed, the source of the material and date of collection (Tables 1 y 2).

Discussion and Conclusions
These species of Verbena exhibited different composition in its essence with respect to other Verbenaceae representatives (5). The main components found did not match with those determined for other authors in the Verbena genus (6). The potential phytotherapeutic use of Verbena native species from the province of Buenos Aires could be attributed to phytol, 1-Hepten-3-ol, 2-Pentadecanone-6,10,14-trimethyl and Hexacosane.

Acknowledgments
Bioq. Mónica Hourcade, CG/EM Laboratory. FCBY-UNR.

References.
Primera Reunión Internacional de Ciencias Farmacéuticas – RICI Fa 2010


**Tabla 1.** Composición de los aceites esenciales de *Verbena* nativas especies de la provincia de Buenos Aires. Período 2008/09.  
1° Lugar y fecha de recolección: 1 y 3, Alberti; 2, Zárate; 4, Luján; 5, Solís; a, 18/2/09; b, 3/3/09; c, 11/2/09; d, 31/3/09; e, 24/2/09; f, 10/3/09; g, 25/3/09.

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**Tabla 2.** Composición de los aceites esenciales de *Verbena* nativas especies de la provincia de Buenos Aires. Período 2009/10.  
2° Lugar y fecha de recolección: 1, Cortinez; 2, 3 y 4, Experimental plots UNLu; 5, Luján; 6, Solís; 7, Zárate; 8, Alberti. a, 24/11/09; b, 12/1/10; c, 14/1/10; d, 15/1/10; e, 22/12/09; f, 23/12/09; g, 16/2/10; h, 29/12/09; i, 7/1/10; j, 18/2/10.

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IN VITRO ANTIOXIDANT PROPERTIES OF HYDROALCOHOLIC EXTRACT FROM FERMENTED SOYBEANS

Romero AM, Sturla MA, Doval MM y Judis MA

Departamento de Tecnología, Universidad Nacional del Chaco Austral

Introduction

Oxygen-free radicals and other reactive oxygen species can be formed both in the human body and in the food system. In addition to inducing lipid peroxidation that causes the deterioration of foods, these radicals may also cause oxidative damage by oxidizing biomolecules leading to cell death and tissue damage (1).

An interesting application of solid-state fermentation is the production of foods enriched in non-enzymatic antioxidants. It has been proven that tempeh fermentation of legume seeds may increase the concentration of antioxidant phenols capable of scavenging free radicals and chelating metal ions (2).

The purpose of this study was to evaluate the antioxidant activity of the fermented extract from soybeans with Saccharomyces cerevisiae on lipid emulsion. Moreover, reducing power, total phenolic and flavonoid contents, scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl (DPPH·), 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS·+) and superoxide (O2·-) radicals were also investigated.

Materials and methods

The fermented extract was prepared from soybeans (MUNASQA®), which were sterilized, inoculated with Saccharomyces cerevisiae (ATCC 32052) and then incubated at 30°C for 24 h. The fermented suspension was extracted with ethanol 96º and dried under vacuum. The antioxidative activity of dry extract at different concentrations (0 – 0.5 and 1.0 mg ml⁻¹ ethanol:water) was evaluated on linoleic acid emulsions (Yen et al, 2003) following hydroperoxide formation (FILBIDF,1991) at 37ºC during 24 h. Butylated hydroxyanisole (BHA) was used as control. The results were expressed as reduction percentage of peroxidation (RP%) and as EC₅₀ value (mg extract ml⁻¹). The antioxidant reactivity of the extract was measured through reducing power (Oyaizu, 1986); phenolic content (Slinkard and Singleton, 1977), flavonoid content (Kim et al, 2003); and scavenging ability on DPPH· (Siddhuraju et al, 2002), ABTS·⁺ and superoxide (O₂⁻) radicals (Bamforth, 1983).

Results

The extract exhibited a significant antioxidant activity on linoleic acid emulsion for both concentrations assayed with RP% 84.10 and 95.62 being the effect of the higher concentration comparable to BHA (97.04%). EC₅₀ value was 0.45 mg extract ml⁻¹.

The reducing power of extract enhances with increased concentrations reaching 91.18% at 8 mg ml⁻¹, while that EC₅₀ value at which the absorbance was 0.5 was 3.95 mg extract ml⁻¹.

Phenolic and flavonoid contents are highly associated with antioxidant properties and in 1g of extract were detected 77.97 mg gallic acid equivalent of phenols and 16.90 mg quercetin equivalent of flavonoids. The scavenging activity of extract againsts ABTS⁺ and DPPH· radicals expressed as EC₅₀ were 0.11 mg extract ml⁻¹ and 0.13 mg extract ml⁻¹ respectively. The equivalent SOD units of the fermented soybeans extract was 4876 U g⁻¹.

Conclusion

The hydroalcoholic fermented soybeans extract with Saccharomyces cerevisiae showed antioxidant activity comparable to BHA. This behaviour, in addition the antioxidant properties assessed indicate their potential use in cosmetic and pharmaceutical fields.

References


Corresponding author: Tel-Fax 54-03732-420137; e-mail: judis@uncaus.edu.ar

25
GASTROINTESTINAL EFFECTS OF *Mikania micrantha* Kunth and *Mikania cordifolia* (L. F.) Willd (*Asteraceae*) ON ISOLATED RAT ILEUM

*Colares, M.*¹; *Muguerza, A.*²; *Debenedetti, S.*² ; *Spegazzini, E.*³; *Rosella, M.*²; *Consolini, A.E.¹* #

Cátedras de Farmacología¹ y Farmacognosia²-LABRAM³, Área Farmacia, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP)

47 y 115, La Plata, Argentina, CP: 1900

**Introduction**

The genus *Mikania* Willd (*Asteraceae*) has about 430 species of plants. *Mikania micrantha* Kunth. and *Mikania cordifolia* (L. f.) Willd., “guaco”, are perennial vines growing in Buenos Aires province. The aerial parts of *M. micrantha* have medicinal use as antibacterial and antifungic (1). In Brazil *M. cordifolia* is used as antispasmodic, antiparasitic and anti-inflammatory (2). In both species there were found terpenes, coumarins and flavonoids (3). Their popular use as eupeptic has not pharmacological evidences yet, so we studied the antispasmodic effect on isolated rat ileons.

**Materials and methods**

Aerial parts of *M. micrantha* and *M. cordifolia* were collected in Punta Lara and Martín García Island (sample deposited in Herbario LPAG). Both extracts were assayed with Shinoda reagents and ethanol solutions of Cl₃Al, Cl₃Fe, boric acid and KOH. TLC was performed on silica gel 60 F 254 with solvent EthylAcetate/MeOH/H₂O 100:13:10 and EthylAcetate/FormicAcid/AcOH/H₂O 100:11:11:26 (upper stage), using 1% methanol solutions of rutoside, quercetin and luteolin as standard. Developing was performed by UV254 and 366 observation and Natural Products reagent. For biological activity the aqueous crude extract was prepared by decoction at 20% and lyophilized. Ileons were isolated from anesthetized Sprague-Dawley rats after 24 h fasting and mounted in organ baths with Tyrode solution (37°C, pH 8.2). Contraction was measured by isometric force transducers (WPI) and A/D converted. Dose-response curves (DRC) of acetylcholine (Ach) were done in the absence and presence of one dose of the extract (0.3, 1 and 3 mg/ml). Calcium-DRC (Ca-DRC) were done by adding cumulative doses of CaCl₂ on a 40 mM KCl-0 mM Ca²⁺-Tyrode, in the presence and absence of *M. micrantha* DRC were statistically analyzed by Prisma 4.0.

**Results**

The tests made it possible to verify the presence of flavonoids. We also observed in *M. micrantha* extract a spot with blue fluorescence (UV366) in the presence of KOH. These results were confirmed through TLC. The DRC of Ach (pD2:: 6.6 ± 0.3) was non-competitively inhibited by *M. micrantha*, with an inhibitory concentration of 50% (IC50) of 0.54 ± 0.05 mg/ml, n=6. *M. micrantha* also inhibited the Ca-DRC (pD2: 6.0 ± 0.2) in a non-competitive way (IC50: 0.5 ± 0.05 mg/ml, n=4). Contrarily, *M. cordifolia* produced a dualism with Ach-DRC (agonism at low and antagonism at high [Ach]). When a DRC of *M. cordifolia* was done, the effective concentration 50% was EC50 = 3.6 ± 0.3 mg/ml (n=4). The potency of *M. cordifolia* was 1/45 of Ach, but the effect was also completely inhibited by atropine.

**Conclusions**

The intestinal effects of the aerial parts of *M. micrantha* and *M. cordifolia* are different: *M. micrantha* has antispasmodic effects associated to non-competitive blockade of calcium influx, and *M. cordifolia* has stimulant effects associated to stimulation of muscarinic receptors. The chemical tests allow us to infer that in both extracts there are flavonoids probably present as glycosides, and probably coumarin derivatives from caffeic acid in *M. micrantha*.

**Acknowledgments**

¹ Grant X-513-UNLP; # Maestría en Plantas Medicinales, Fac. Cs. Exactas, UNLP; ² Farmacia student

**References.**

(2) Carollo, CA, Lopes NP, Camilo Rodrigues de Oliveira D. Identificação dos Compostos Polares de *Mikania cordifolia* por HPLC-DAD-MS. Presented at: 30a Reunião Anual da Sociedade Brasileira de Química; 2007 May 31-June 3; Águas de Lindóia-SP, Brazil.

ENTRAL AND GASTROINTESTINAL EFFECTS OF Brugmansia arborea AND Verbascum thapsus IN MICE AND RATS

Di Ianni, M.1,a, Ruiz A.1,a, Querini, P.1,a, Spegazzini, E.D3; Rosella, M.A.2; Consolini, A.E1 #

Cátedras de Farmacología1 y Farmacognosia2-LABRAM3, Área Farmacia, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata 47 y 115, La Plata, Argentina, CP: 1900

Introduction

Some plants are popularly used to modify emotions but their knowledge is useful to prevent abuse or find an application. In our region grows the genus Brugmansia (Solanaceae) whose flowers are known as "floripondio" and used by indigenous in rituals and externally as analgesic and descongestive. Verbascum thapsus (Escrofulariácea) is known as “gordolobo” or “tabaco indio” and originary from Europe and Asia but grows in dry regions as Patagonia. Its leaves are used for respiratory illnesses as demulcent (1). There are reports of intoxication with both plants (1,2,3,4), which could be related to the presence of tropanic alkaloids in Brugmansia. We studied the central effects of both plants and the gastrointestinal mechanism of Brugmansia.

Materials and methods

Aerial parts from Brugmansia arborea were collected in our region, and from Verbascum thapsus were collected in Gualjaina, Chubut. Respective vouchers were deposited in the “Carlos Spegazzini” Museum (LPE). The respective aqueous crude extracts (a.c.e) were prepared by decoction and lyophilized.

Central activity: was assessed in groups of Swiss mice in the open-field test by injecting i.p. doses of 30 and 100 mg/kg B. arborea, 100 and 300 mg/kg V. thapsus, amphetamine 5 mg/kg as positive control and saline (SF). Spontaneous locomotion and exploration were measured each 30 min in each mice. Two-way ANOVA was applied with a-posteriori tests (n=8 by group).

Gastrointestinal effects: ileons were isolated from guinea-pigs after 24 h fasting and mounted in organ baths with Tyrode solution (37º C, pH 8.2). Contraction was measured by isometric force transducers and A/D converted. Dose-response curves (DRC) of acethylcholine (Ach) were done in the absence and presence of a dose of B. arborea a.c.e (3, 10, 30 and 100 µg/ml).

TLC was performed on silica gel 60 F 254 with solvent tholuene/ethylacetate/diethylamide 70:20:10, using NH3-ether extracted of B. arborea and hiosciamine as standard.

Results

Central activity: mice spontaneous locomotion in the open-field was significantly decreased by V. thapsus from 80±15 (SF) to 30±17 and 33±12 lines/5 min (respectively at 100 and 300 mg/kg after 2 h), but not by B. arborea. The exploratory activity was also decreased by V. thapsus from 13±5 (SF) to 7±4 and 7±5 rearings/5 min (respectively at 100 and 300 mg/kg after 2 h), and by B. arborea to 6±3 and 7±4 at 30 and 100 mg/kg, respectively.

Gastrointestinal effects: the DRC of Ach (pD2: 6.9±0.1) was competitively inhibited by B. arborea, with an inhibitory concentration of 50% (IC50) of 9.6±4.5 mg/ml, n=10.

The B. arborea extract run similarly to hiosciamine in the TLC.

Conclusions

Both plants induced sedation, which was more important for V. thapsus and could explain its utility in asthma. B. arborea decreased more the exploration than locomotion, and it could be due to the presence of atropinic alkaloids as hiosciamine. These compounds may be responsible for the antispasmodic effect seen in ileons and caracterizated as a competitive muscarinic antagonism.

Acknowledgments

1Grant X-513-UNLP; 2Farmacia students

References.

# Corresponding author. Tel +54-221-423-5333 INT 42, e-mail: dinamia@biol.unlp.edu.ar
ACUTE ORAL TOXICITY STUDY OF CLADODES AND FRUIT FLOUR OF *Opuntia salagria* IN RATS

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\(^{a}\)INIBIBB (UNS-CONICET), C.C.857, Bahía Blanca
\(^{b}\)INSIBIO (UNT-CONICET), Chacabuco 461, Tucumán

**Introduction**

The cactus *Opuntia* appears to be one of the most promising sources of plant-derived diabetes mellitus active suppressants (1). We have demonstrated *O. salagria* hypolipidemic and hypoglycemic effects on normal and streptozotocine-induced diabetic rats (2, 3). *Opuntia* toxicity has not been scientifically studied to date. The aim of this work is therefore to analyze the acute toxicological effects of stem (cladodes) and fruit powders of *Opuntia salagria* on rats.

**Materials and Methods**

Plant materials include stems and fruits of *Opuntia salagria* collected in the area of Bahía Blanca, Argentina. A specimen of reference of this species (Villamil 8829) is deposited in the herbarium of the Universidad Nacional del Sur. After removing the spines, the stems and fruits were sliced, dried and milled. The flours thus obtained were stored at 4°C until required.

The experimental animals include female Wistar rats (13 weeks old) fed on a standard laboratory diet and kept under controlled laboratory conditions until the beginning of experiments.

The experience was performed according to the OECD Guidelines (4). Animals were divided into different groups according to a randomized block design (N = 24). Briefly, cladodes, seeds and fruit pulp flours were orally administered (2000 mg/kg body weight) to the rats at the onset of the experiment. General clinical observations were made daily. After 14 days, animals were subjected to euthanasia using CO\(_2\) and blood for hematology, coagulation and clinical chemistry studies was collected by cardiac puncture. The organs were weighed, measured, and preserved for future histopathological studies.

Data represent mean values ± S.E.M. and they were subjected to analysis of variance in blocks. Mean values were compared using Bonferroni t statistics. Letters (a-b) indicate significant differences (\(p\)<0.05) among groups.

**Results**

Plant oral administration produced neither mortality nor changes in behavior and physiological activities. Necropsy study revealed no abnormal signs, and body and organs weights were not significantly affected by the assayed treatments. Hematological analysis (erythrocytes, hemoglobin, platelets, leucocytes, and coagulation time) were similar both in control and treated rats. Serum levels of glucose, urea, creatinine, total proteins, albumin, triglycerides and hepatic enzymes evidenced no changes after *Opuntia* administration. On the other hand, pulp fruit consumption induced a mild increase in serum total cholesterol but LDL-c and HDL-c levels and the aterogenic risk remained constant.

**Conclusions**

Data indicate that cladodes and fruit seed and pulp powder of *Opuntia salagria* have no toxicological effects on the clinical chemistry and haematological parameters analyzed in rats, thus supporting their potential medicinal use.

**References**


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NON-TOXIC EFFECTS OF Smallanthus sonchifolius LEAFS AND ITS MAIN ACTIVE COMPOUND, ENHYDRIN.

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Introduction
Smallanthus sonchifolius [Poepp. & Endl.] H. Robinson or yacon is a native specie of South America belonging to the Asteraceae family. In recent work we have demonstrated that leaves decoction and enhydrin, the major sesquiterpene lactone of yacon leaves, was effective to reduce post-prandial glucose and useful in the treatment of diabetic animals (1). For this reason, in order to continue assessing their potential antidiabetic use, it is necessary to investigate their safety through toxicity studies. We evaluated the toxicity both, “in vivo” using Wistar rats and “in vitro” with cultured cells.

Materials and Methods
Preparation of decoction and isolation and purification of enhydrin: was carried out as described previously Aybar et al (2) and Genta et al.(1)

“In vitro” toxicity assay: Cell proliferation rates were determined by citotoxicity test based on MTT assay (3) using CHO-K1, HEP-G2 and COS cells culture. The results were expressed as a percentage of the control.

“In vivo” toxicity assay: adult Wistar rats of both sexes weighting 200–220 g were selected for all the experiments. The animals were divided into groups of 6 rats each and were given daily, distilled water, 10% yacon decoction (140 mg/Kg) or enhydrin (0.8 and 8 mg/kg b.w.), using an intragastric tube during a 60 days period. General conditions, biochemistry parameters and histopathology examination of the main organs were performed.

Statistical analysis: The statistical significance was assayed using analysis of variance (ANOVA). A \( p \) value <0.05 was considered statistically significant.

Results
Cells culture toxicity Studies: The citotoxicity on the three cell lines was dose-dependent. The concentration at which the number of viable cells (CHO-K1, HEP-G2 and COS cells) was reduced to 50% of the control (IC\(_{50}\)) was 50±5, 160±12 and 200±13 \( \mu \)g/ml for yacon decoction and 0.75±0.08, 0.15±0.02 and 1.5 ±0.12 \( \mu \)g/ml for enhydrin.

Rats toxicity Studies: The administration of 10% yacon decoction or enhydrin did not cause mortality in any rats group. There were no abnormal clinical signs and haematological and biochemical parameters during the experimental period. Body organs weights in treated animals had no difference with the control group. The weight, size, shape or histological characteristics of various organs (liver, kidney, and gastrointestinal tract) showed no significant difference between treated and control animals. The results presented clearly demonstrated that yacon decoction and enhydrin was safe without any toxicity and side effects at the doses used.

Discussion
In our laboratory we found that enhydrin, the high yielding chemical constituent of yacon leaves, decreases blood glucose levels at minimun dose of 0.8 mg/kg body weight. “In vitro” citotoxicity assays were used as screening test. The disadvantage of this test is that the homeostatic mechanisms and pathways found in animals are not present. “In vivo” studies in rats demonstrated that enhydrin is safe in the therapeutic dosage range (0.8 and 8 mg/kg/day).

References
ANTIOXIDANT ACTIVITY AND ACUTE TOXICITY OF CHAÑAR FRUITS (Geoffroea decorticans)

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Introduction
Studies of the medical folklore of Northwestern Argentina are of great ethnomedicine value. The fruits of Geoffroea decorticans (Fabaceae) are used for both culinary and medical purpose (1). The aim of the present work was determined the total phenolic and flavonoid contents and antioxidant activities of extracts of chañar fruits. It was investigated the possible toxic effect on rats.

Materials and methods
Geoffroea decorticans fruits are collected in Santiago del Estero. The fruits were soaked in ethanol (70%) and water to prepared the respectively extracts.

Phenolic content (Folin-Ciocalteau method) (2) and flavonoids content (Ciou Jhih-Ying et al method) (3) were determined. Antioxidant activity by β carotene bleaching method, free radical scavenging activity by 1,1 diphenyl 2 picrylhydrazil (DPPH) assay.

The acute toxicity was evaluated in Wistar rats. The animals were divided into six groups (n=5). The controls groups received orally water and ethanol (70%) respectively. The other groups received 1800 and 3600 mg/kg body weight of test extracts equivalent to 100 times the human use. The animals were observed for 1, 2, 4, 6, 24, 48 hours up to 14 days.

Results
The phenolic content of the ethanol extract was 6,78 mg/g and the acqueous extract was 20,61 mg/g, all as mg gallic acid/100 g dry weight respectively.
The flavonoid content of the ethanol extract was 11,15 mg/g and the acqueous extract was 26,23 mg/g, all as mg Quercetin/100 g dry weight respectively.
The scavenging effect of acqueous and ethanol extracts on DPPH radicals were 94,59 %, 94,71 % at a concentration of 10 mg/ml, respectively, indicating that both extracts showed similar DPPH radical scavenging activity. The ethanol (76,60 %) and acqueous (83,20 %) extracts (10 mg/ml) were effective in inhibiting the oxidation of linoleic acid and subsequent bleaching of β carotene.

No toxic symptoms or death were observed in the animals after oral administration of different doses of the extracts. No changes were observed in body weight, food and water intake. When the emotionally parameters such us the number of grooming and faecal boluses were analyzed, no significant differences between the control and the experimental group was detected. Pathological examinations of the tissues on a gross basis showed no detectable abnormalities.

Conclusions
Fruits acqueous and ethanol extracts showed good antioxidant activity. No toxic symptoms or mortality was observed in 14 days of study in rats. The results support the traditional culinary and medicine uses.

References

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COMPOSITION AND ANTI-INFLAMMATORY EFFECT OF *Catasetum macroglossum* BY THE CARRAGEENIN-TEST

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**Introduction**

*Catasetum macroglossum* is originary from Ecuador, growing in its four natural regions. The pseudo-bulbs of *C. macroglossum* are topically used as anti-inflammatory and antirheumatic. Different polysaccharides, phenanthrene derivatives, triterpenes and flavonoids were isolated from the Family Orchidaceae (1). There are no reports about the composition of *C. macroglossum* and there is no pharmacological evidence to support the traditional use. We studied the anti-inflammatory activity of extracts of *C. macroglossum* with the carrageenin-edema test in rats.

**Materials and methods**

Aerial parts of *C. macroglossum* were collected in Los Ríos, Ecuador. A voucher is in “Carlos Spegazzini” Museum (LPE). Aqueous crude extract (a.c.e) was prepared by decoction of pseudo-bulbs of *C. macroglossum* and lyophilized. Aqueous solution from lyophilized decoction was hydrolyzed in HCl and both extracts were assayed with Fehling reagent, Shinoda, and ethanol solutions of Cl₃Al, Cl₃Fe, boric acid and KOH, aimed at determining the presence of reducing sugars and flavonoids. Both extracts were assayed by TLC on silica gel and cellulose plates with some eluent mixtures. Standards: 1% solutions of glucose, mannose, xylose, rhamnose, galactose, sucrose and fructose. Developer: benzidine reagent or SO₄H₂ 10% (hydro alcoholic).

Anti-inflammatory activity was assessed by inhibition of rat paw edema induced by injecting 0.1 mL of 1% carrageenin into the sub-plantar region of the hind paw (2). Wistar rats were divided into 4 groups, which received i.p. the a.c.e (30 and 90 mg lyophilized/kg), saline or indomethacin (5 mg/kg) 30 min before the injection of carrageenin, and the respective paw volume was measured as control. The respective paw volumes were measured after 0.5, 1, 2, 3 and 4 h by using a plethysmometer and edema was calculated by the difference with control value and expressed as % of it.

**Results**

After 2 to 4 h, indomethacine completely inhibited the edema (0.3±7.9%, n=4, vs. 27.6±5% at 2 h and 41.7±6.3% at 4 h in saline group n=12). The extract significantly inhibited the edema during the same period in a dose-dependent way at 30 mg/kg (19.2±7% p<0.05, at 3 h and 19.3±7.9% p<0.05, at 4 h, n=8) and at 90 mg/kg (4.7 ± 3.5% p<0.01, at 2 h and 17.5 ± 5.7% p<0.01, at 4 h, n=8).

The chemical tests made it possible to verify the presence of reducing substances after hydrolysis, and trace amounts of flavonoids. The TLC results (eluent: BuOH/AcOH/H₂O 10:1:1) determined the presence of glucose and probably mannose in aqueous extract after hydrolysis.

**Conclusions**

Since the carrageenin-edema is mediated by prostaglandins in the second phase, inhibited by indomethacin, results demonstrate that *C. macroglossum* has anti-inflammatory effect associated to inhibition of prostaglandin synthesis. Based on tests carried out, the aqueous extract contains mainly a polysaccharide of glucose and mannose (glucomanan).

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References.
BIOCONVERSION OF INHOFFEN-LYTHGOE’S DIACETATE BY *Fusarium verticillioides*

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Introduction

Biotransformations are a good example of ability of microorganisms to change one of several functional groups with similar reaction properties or to functionalize low reactive positions in organic compounds through enzymatic reactions, allowing to obtain novel compounds with potential biological activity. During last years both in industry and in pharmaceutical chemistry innovation and productivity of drugs is one of the most important processes, which is done by chemical synthesis, semi-synthesis or by harmless biotechnological procedures known as biotransformation or bioconversion that involve the use of microorganisms. This technique include oxidation-reduction modifications, rearrangement, opening or ring closure, C-C bond formation, addition-elimination, racemic mixture resolution, among others. Besides microorganisms can be used as a model of mammalian metabolism, allowing to predict and prepare metabolites related to drugs administered to animals and humans.

This methodology has permitted to obtain a wide variety of products of high value added so the aim of this work is about biotransformation the substrate, the diol of Inhoffen-Lythgoe which is related to some portion of vitamin D and has two rings.

Nowadays biotechnology is applied to several areas. Looking for the best biocatalysts and using new technologies to keep competitive and innovative pharmaceutical industries obtain active drugs economically attractive so that quiral intermediates are an important task of fine chemical market, showed by the increased claim of these products.

Materials and methods

Biotransformation was carried out using the filamentous fungus *Fusarium verticillioides*, which has the capability to hydroxylate several compounds.\textsuperscript{2,3} This process was done using liquid culture medium and appropriate conditions for microorganism growth, followed by preparative scale under the same conditions as preliminary screening.

Results

Several metabolites were obtained and two of them in higher amounts which were isolated by chromatographic techniques. This derivatives as well as starting material were identified using besides classical spectroscopic methods, NMR techniques like homo and heteronuclear COSY, DEPT and IR. Those spectra were compared with substrate spectrum and hydroxyl group signals in different positions were found.

Conclusions

In structures of metabolites close related to calcitriol has been found that modifications of the cyclopentane ring side chain have incidence on the selectivity to biological response of physiological functions related to calcium and phosphorous homeostasis processes.\textsuperscript{3} So it is expected this compounds shows a higher biological activity as a consequence of changes on chemical structure.

References


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EFFECT OF D-GLUCOSAMINE AND N-ACETYLGUCOSAMINE ON OXIDATIVE METABOLISM AND BIOFILM FORMATION OF Staphylococcus epidermidis

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Introduction
Coagulase-negative staphylococci are important causes of infections associated with diverse biomaterials; biofilm-producing strains have emerged as important pathogens especially in patients with implanted devices (1). The formation of biofilm is not only an adhesive medium; it also affects the virulence of strains and enhances the resistance of bacteria against antibiotic treatments (2). The adherence to biomaterials and formation of biofilms is associated to less sensitivity to the stress caused by diverse agents (3) and it was proved that microorganisms suffer less oxidative stress in biofilms than planktonic bacteria during antibiotics treatment (4). The present study was designed to address the issue of S. epidermidis adhesion and inhibition of biofilm formation in relation to the generation of oxidative stress by D-Glucosamine (D-Glu) and N-acetylglucosamine (NAG) in order to find conditions that reduce the adhesion of the bacteria and consequently the biofilm formation.

Materials and methods
Three clinical strains (848, 1569, 2786) were isolated from infected catheters, in Hospital Tránsito Cáceres de Allende, (Córdoba) to investigate the influence of glucose (Glu), D-Glu and NAG on bacterial adhesion by optical microscopy, quantification of biofilm on glass surfaces stained with crystal violet, and production of reactive oxygen species (ROS) by chemiluminiscence assay and by nitro blue tetrazolium reduction.

Results
There was correlation between reduction of biofilm formation and oxidative stress. Glu incremented the adhesion whereas reduced the oxidative stress of all the strains. Inversely, D-Glu inhibited the adhesion and generated an increase of ROS at 0.05 mM in the three strains while NAG decreased the adhesion in less extent and required more concentration to cause oxidative stress, except in the strain that was more sensitive (2786). Similar results were obtained during the biofilm quantification specifically; Glu incremented the biofilm formation while D-Glu and NAG provoked decrease of biofilm.

Conclusions
Since Glu reduced the production of ROS and favoured the biofilm formation, while D-Glu and NAG caused oxidative stress and decreased biofilm, therefore, it would be interesting to apply these hexosamines to enhance the antibacterial action of antibiotics by exploiting their ability to reduce adhesion and biofilm formation of S. epidermidis.

Acknowledgments
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References

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ANTIBACTERIAL ACTIVITY OF TRIARYLMETHANE DERIVATIVES ON Staphylococcus aureus.

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Introduction

Photodynamic therapy (PDT) is based on the concept that certain photosensitizers (PS) can be localized in neoplastic tissue and subsequently be activated with the appropriate wavelength of light to generate active molecular species that produce toxicity in cells and tissues (1, 2).

Antimicrobial PDT research has increased in the last 20 years because of concerns resulting from the emergence of antibiotic-resistant bacterial strains. The control of infections by chemotherapeutic agents is often jeopardized by the spreading of bacterial strains resistant to many conventional antibiotics. As PDT is a multi-target process, it is unlikely to induce resistance in microorganisms (3).

In the present work we have studied the antimicrobial activity of New Fuchsin (NF+) against S. aureus.

Materials and methods

Staphylococcus aureus ATCC 29213 was grown aerobically in trypticase soy broth.

The antimicrobial activity of the compounds was evaluated by using the standard tube dilution method on Mueller Hinton Broth. Bacterial growth was observed at 18 h of incubation, following the indications of the Clinical and Laboratory Standards Institute.

A macrodilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

Bacterial suspensions incubated with NF+ were irradiated at 0, 1, 6, 18 and 24 h and incubated for 18 h to 37 °C. Reactive oxygen species (ROS) were investigated by reduction of Nitro blue Tetrazolium.

Crystal Violet (CV+) was obtained from Anedra, Methyl Green (MG+) was obtained from British Drug Houses LTD and NF was obtained from Sigma.

Results

S. aureus was found to be susceptible to NF+ with a MIC and MBC of 1 µg/mL. MG− showed the highest activity with a MIC of 0.002µg/mL and a MBC of 0.063µg/mL while CV− showed a MIC of 0.098µg/mL and a MBC of 0.39µg/mL.

When S. aureus cultures (10⁶ CFU/mL), without NF+, were illuminated for 24 h, they did not decrease the number of viable cells. In the presence of NF+ and light treatment, the percentages of survivors after 1, 6, 18 and 24 h were reduced by 1.4 %, 1.5%, 93.2% and 93.9%, respectively. Thus, increasing the time of the contact between the PS and the bacterial cells has improved the performance of the PS against the mentioned bacteria.

An efficient photoinactivation of S. aureus was obtained from a light exposure time of 6 h. Shorter irradiation times did not further affect the percentage of photoinactivated bacteria.

The results obtained for ROS generation at all concentrations and times assayed showed similar values to the controls.

Conclusions

The photodynamic therapy with NF+ may be an effective bactericidal method against S. aureus and potentially against other bacterial pathogens. The ROS are not involved in the mechanism of bacterial killing. We conclude that the most important species involved in the mechanism of bacterial killing is the triarylmethane (TAM+) radical principally from the TAM+ triplet state.

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BIOFILM FORMATION BY *Escherichia coli* O157:H7 UNDER REDUCING CONDITIONS

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**Introduction**

*Escherichia coli* O157:H7 was first identified as being responsible for causing outbreaks of enteric infection in 1982. Symptoms include diarrhoea, haemorrhagic colitis, and haemolytic uraemic syndrome (1).

The ability of *E. coli* O157:H7 to form biofilm has been described. A biofilm can be defined as a sessile bacterial community of cells that live attached to each other and to surfaces (2).

**Objective**

This study evaluated the capability to form biofilms of *E. coli* O157 strains under reduction conditions and their relationship to bacterial stress.

**Materials and methods**

In this study, the biofilm formation of the reference strain of *E. coli* EDL 933 and eight isolates were tested using thioglycollate as a culture medium with and without 0.5% glucose.

The biofilm-forming ability was measured by adhesion to biofilms on polystyrene microtiter plates and stained with crystal violet (3). The Biofilm Biomass Unit (BBU) was arbitrarily defined with OD$_{595}$ 0.1 being equal to 1 BBU.

The extracellular production of Reactive Oxygen Species (ROS) was detected in 48 h cultures by the reduction of nitro blue tetrazolium to nitroblue diformazan. The reaction was proportional to the ROS generated in biofilm and was measured by OD at 540 nm.

The Nitric oxide (NO) was evaluated as nitrite by a microplate assay method using the Griess reagent. 50 µl aliquots were mixed with 100 µl of Griess reagent. Absorbance was measured at 540 nm in a microplate reader and results were expressed as µM.

**Results**

There was a significant increase in biofilm formation and a significant decrease in ROS and nitrite in all strains tested using thioglycollate as a culture medium supplemented with 0.5% glucose.

**Conclusions**

In conclusion, we suggest that biofilm formations in reduction conditions are influenced by cellular stress. However, improved knowledge of ROS and RNI regulation may help in clarifying the relevance of biofilm formation in the pathogenesis of infections, and furthermore could also be of great value in the development of better preventive and therapeutic measures.

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**References**


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**Candida albicans** BIOFILM ON ARTIFICIAL SURFACE IN CONTACT WITH INNATE IMMUNE CELLS

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**Introduction**

It has been documented that at least 65% of all microbial infections are related to formation of biofilms. The formation of these organizations by *Candida* primarily begin with adherence and colonization of a biotic host or an artificial surface as for example medical devices in current use, including stents, shunts, prostheses (voice box, heart valves, dentures, etc.), implants (lens, breast, etc.), and various types of catheters (1). We previously developed a model of *Candida* biofilms attached to polystyrene microtiter plates, where the oxidative metabolism under different culture conditions was assayed (2,3). In the present study, the effect of macrophages (Mø), a crucial innate immune cells, on the production of reactive oxygen species (ROS), the liberation of nitric oxide (NO), and the activity of superoxide dismutase (SOD) in these cultures was evaluated.

**Materials and methods**

*Candida albicans* was incubated in a 96-well polypropylene microtiter plate to form mature biofilms. Then, mouse Mø cell line (RAW) or medium alone, was added to the plate for 1h, centrifuged and supernatants were collected.

The biofilms-forming ability was measured by determination of the adhesion to polystyrene microtiter plates (4). The Biofilms Biomass Unit (BBU) was arbitrarily defined with 0.1 OD$_{595}$ equal to 1 BBU (5).

In the supernatant, the extracellular ROS (eROS) production was detected by the reduction of nitro blue tetrazolium (NBT) to nitroblue diformazan, the NO production was evaluated as nitrite by a microplate assay method using the Griess reagent, and total SOD activity was assessed photochemically based on the inhibition of NBT reduction (6).

**Results**

The addition of Mø to the cultures results in a decrease in the biomass of mature biofilms with respect to control cultures (medium alone). Besides, we were not able to detect the production of eROS and NO by *Candida* biofilms subjected to Mø. On the other hand, the SOD activity of biofilms co-cultured with Mø was higher than that found in control cultures.

**Conclusions**

Our results show that Mø are able to reduce the biomass of mature biofilms from a polymeric surface. Furthermore, the presence of these cells also induces an antioxidative profile in our model, indicated by the high SOD activity found in this system. In this way, this antioxidant power could be the reason why the production of eROS and NO was not detectable. Therefore, in this *in vitro* model, Mø can interact with biofilms already formed, diminishing their biomass and altering its oxidative balance, however, futures studies are necessary to explain the mechanism by which these cells induced the biofilms detachment in our system.

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Reference


RELATIONSHIP BETWEEN TWO TECHNIQUES FOR BIOFILM RESEARCH

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Introduction
Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. They can cause significant problems in many areas, both in medical devices (e.g., persistent and recurrent infections, device-related infections) and in non-medical ones (1), therefore its detection and quantification is very important. Two methods used to biofilms evaluation are crystal violet (CV) and confocal scanning laser microscopy (CSLM); however, a correlation between these two methods has not previously been reported. In this work, a comparative study on correlation between them, evaluated under different culture conditions of biofilms growth previously assayed in our laboratory (2), was realized.

Materials and methods
In this study, the biofilms formation of the reference strain of _Staphylococcus aureus_ (ATCC 29213) was evaluated by CV and CSLM.

Quantification by CV
The biofilms-forming ability was measured by determination of the adhesion to polystyrene microtiter plates (3), which is based on the ability of bacteria to form biofilms on solid surfaces and uses CV to stain biofilms. Briefly, a final cell concentration of approximately $1 \times 10^8$ cfu/ml of _S. aureus_ was put with Tryptic Soy Broth (TSB) into each well of flat-bottomed microtiter plates, with/without supplements and incubated at 37°C for 24 h. A quantitative assessment of the biofilms formation was obtained by extracting the CV with a bleaching solution. The Biofilms Biomass Unit (BBU) was arbitrarily defined with 0.1 OD$_{595}$ equal to 1 BBU.

Biofilms Research by CSLM
Biofilms were observed by CSLM. Prior to imaging, the samples were rinsed with sterile potassium phosphate buffer for 10 min and were then stained with propidium iodide to detect bacterial cells. After being washed in PBS, the sections were incubated with fluorescein isothiocyanate-concanavalin A conjugated to stain the glycocalyx matrix. Then, images of the vertical (X/Z) sections from three randomly selected positions were obtained and analyzed using an Olympus Fluoview FV 1000 (4).

Results
Previous studies in our laboratory have demonstrated that the capacity of _S. aureus_ to form biofilms changed under different culture conditions. With the information obtained from the two methods employed (table), a high correlation between them ($R^2 = 0.940$), calculated by linear regression, was found.

Conclusions
Our results showed a good correlation between the two techniques, which were assayed at different culture conditions of biofilms formation. The quantification by CSLM was able to detect change in the biofilms formation of _S. aureus_, for example, high levels of growth were observed in presence of Glucose or NaCl&Glucose. In addition, this method allows us to obtain data on biofilms structure (3D visualization) and the differentiation between live and dead bacterial cells. However, training personnel and confocal microscope are necessary for the development of this technique. In the same way, although it is not possible to obtain biofilms structure information by CV staining, this assay, simple and cheap, also was sufficiently sensitive to evaluate the ability of this bacterium to form biofilms. Therefore, since similar results were obtained by these two methods, they could be indistinctly used for the study of biofilms formation on polymeric surface of medical devices.

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Acknowledgments

The authors wish to thank Drs. C.Mas and C.Sampedro. This work was supported by the following Grants: FONCyT, CONICET and SECyT.

References

HEMOLYSIN FROM *Escherichia coli* INDUCES OXIDATIVE STRESS IN BLOOD

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**Introduction**

Oxidative stress is caused by an imbalance between the production of oxidants and the levels of antioxidants present in the biological system. In this situation, the overproduction of reactive oxygen species (ROS) can lead to the damage of cellular components including lipids, protein, and DNA. If this damage is not repaired, mutagenesis and cellular death can occur, and participate in the pathogenesis of different diseases such as Chagas, meningitis and illnesses associated with *Escherichia coli* infections such as hemolytic uremic syndrome and pyelonephritis (1). *E coli* is an important food-borne pathogen in Argentina and other parts of the world. During the infection, this bacterium secretes different products, including lipopolysaccharide, Shiga toxin and Hemolysin (HlyA). The latter is a pore-forming toxin and numerous effects on different cellular populations have been attributed to sublytic concentrations of this toxin, including secretion of ROS and nitric oxide (2,3).

Therefore, considering that oxidative stress has been linked to *E. coli* infections and that HlyA is a very important virulence factor of this bacterium, the objective of this study was to analyze the capacity of this toxin to induce oxidative stress in whole blood cultures (WBCs), which could contribute to the pathogenesis of the infection by this pathogen.

**Materials and methods**

The capability of HlyA, purified from a clinical isolate of *E. coli* by gel chromatography, to generate ROS was examined in WBCs using luminol sensitized chemiluminescence. In addition, to determine whether ROS production by HlyA resulted in oxidative damage of plasma proteins and lipids, whole blood were incubated with an equal volume of HlyA (0.4 or 0.2 hemolytic activity (HU)/ml) or PBS like negative control for 4 h at 37°C. After incubation, the blood samples were centrifuged and the plasma obtained was assayed for different oxidative stress biomarkers, such as malonyldialdehyde (MDA), carbonyl residues and advanced oxidation protein products (AOPP). The antioxidant system also was evaluated through the determination of total antioxidant capacity of plasma by the Ferric Reducing Antioxidant Power (FRAP) assay and the activity of superoxide dismutase (SOD) assayed photochemically based on the inhibition of nitroblue tetrazolium reduction.

**Results**

We found that HlyA increased the level of free radicals detected by chemiluminescence assay. Moreover, plasma MDA levels, as an index of lipid peroxidation, were significantly increased in cultures treated with HlyA in comparison with those found in control cultures. In addition, biomarkers of protein damage such as carbonyl residues and AOPP also were elevated after treatment of blood with HlyA. On the other hand, a decrease in total antioxidant capacity of plasma and in the activity SOD was observed in plasma from blood treated with HlyA.

**Conclusions**

Collectively, our data demonstrate that low concentrations of *E. coli* hemolysin induce oxidative stress in WBCs. This oxidative imbalance produced by HlyA may have an important role in the pathogenesis of infections caused by *E. coli* strains that produce this toxin.

**References**


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ANTIBACTERIAL ACTIVITY OF NATURAL ANTHRAQUINONES

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Introduction and objectives
Substances with photosensitizing characteristics have become particularly important in the field of pharmacology due to their potential applications (1). In relation to this, research oriented towards photodynamic antimicrobial chemotherapy is in progress with promising results. This therapeutic treatment consists in the application of ultraviolet or visible radiation (UV-Vis) on infected tissue, which is previously associated to the photosensitizer (PS), which is activated by light in order to produce reactive oxygen species (superoxide anion radical and/or singlet molecular oxygen). These species act on microorganisms, generating microbial inactivation (2). Previously, we have demonstrated that three majority anthraquinones (AQs): rubiadin, soranjidiol and rubiadin 1-methyl ether, isolated from stems and leaves of Heterophyllaea pustulata, showed photosensitizing properties by generation of superoxide anion radical and/or singlet molecular oxygen under irradiation conditions (3). On the other hand, in previous microbiological studies we have established that two of the tested AQs (rubiadin and soranjidiol), have in vitro photodynamic antimicrobial activity against Gram (+) bacteria (Staphylococcus aureus ATCC 25923) (4).

In the present work, the objective was to evaluate the antibacterial activity of these AQs against another microorganism that belong to Gram (+) genus: Staphylococcus.

Materials and methods
Measurement of the Minimum Inhibitory Concentration (MIC) for each AQ was carried out in Staphylococcus epidermidis ATCC 12228, by means of broth macrodilution method, in accordance with international standards of the Clinical and Laboratory Standards Institute (CLSI) (5).

Results
The results of dilution in broth were satisfactory for the three assayed AQs. Thus, S. epidermidis showed susceptibility with a MIC of 125 µg/mL for rubiadin 1-methyl ether, and 250 µg/mL for rubiadin and soranjidiol.

Discussion
Previous results showed that rubiadin and soranjidiol had a MIC for S. aureus between 32-64 µg/mL, and the MIC for rubiadin 1-methyl ether was higher than 250 µg/mL (4). However, this methylated derivative exhibited a higher antimicrobial activity against S. epidermidis than rubiadin and soranjidiol. Therefore, these results are remarkably promising because each one of these AQs derivatives has demonstrated to possess a selective effect against one or the other of the two assayed species of Staphylococcus. Thus, this encourages us to carry out further test in order to study in depth the photodynamic antimicrobial activity of these metabolites.

References

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ANTIFUNGAL ACTIVITY OF POLYGODIAL ISOLATED FROM Polygonum acuminatum AND OF THE BIOTRANSFORMATION PRODUCT

Derita, MG., # Álvarez, SL., Zacchino, SA.


Introduction

The current interest in the development of new antifungal agents can partially be due to the dramatic rise in the number of patients with suppression of their immune system. Otherwise, the emergence of fungal resistance to available antifungal agents, leads to an increasing need for new and effective structures.\(^{(1)}\)

Polygonum acuminatum (Polygonaceae) is used to heal infected wounds in the traditional Argentinean medicine.\(^{(2)}\) This study was carried out to evaluate the antifungal properties of this plant to give support to its ethnopharmacological use, and to isolate the compound(s) responsible for its activity. In addition, a fungal biotransformation of the main compound was performed.

Materials and methods

Aerial parts of \(P.\) acuminatum were collected at Puerto Gaboto (Argentina) and once dried, they were powdered. 100 g of the vegetal material was successively extracted by maceration with petroleum ether (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and methanol (MeOH). After filtration and evaporation 1.3, 2.0, 1.2 and 2.7 g of each extract were obtained respectively.

For antifungal evaluation, strains from the American Type Culture Collection (ATCC) and Centro de Referencia en Micología (CEREMIC) were used. Minimum Inhibitory Concentration (MIC) of each extract or compound was determined by using broth microdilution techniques according to the guidelines of the CLSI\(^{(3)}\) for yeasts (M27-B2) and for filamentous fungi (M38-A).

For biotransformation experiments, suspensions of conidia (5x10\(^6\) CFU/mL) of Aspergillus fumigatus ATCC 26934 were used to inoculate flasks containing Czapek medium (250 mL). The cultures were incubated at 30 ºC for 72 h. The substrate (70 mg) in DMSO (3 mL) was poured into flasks containing the fungal biomass and the reaction mixtures were incubated. The mixtures were filtered, and the aqueous phases were extracted with ethyl acetate. The organic phases were dried and analyzed by TLC and GC.

Results

The four extracts of \(P.\) acuminatum were evaluated against a panel of human opportunistic and pathogenic fungi. Results showed that all yeasts and dermatophytes, but not \(A.\) fumigatus spp., were sensitive to \(P.\) acuminatum extracts. DCM extract displayed the broadest spectrum of action, inhibiting 6/9 fungi tested (MICs: 31.25 - 500 µg/mL). Hex and EtOAc extracts inhibited 4/9 fungi (MICs: 125 - 500 µg/mL) and MeOH extract was inactive (MIC > 500 µg/mL).

The bioguided fractionation of the DCM extract led to the isolation of polygodial (1), which have been previously isolated from another natural sources.\(^{(2,4)}\) Biotransformation of (1) by \(A.\) fumigatus led to the obtainment of decahydroantonotofuran-1-ol (2) known as isodrimeninol.\(^{(5)}\) Both compounds were evaluated for antifungal activity and results showed that (1) possessed the strongest antifungal activity (MICs: 3.9 - 62.5 µg/mL) meanwhile (2) was less active (MICs: 31.2 - 250 µg/mL).

Conclusions

DCM extract of \(P.\) acuminatum possessed antifungal activity against yeasts and dermatophytes, giving support to its ethnopharmacological use in Argentine.\(^{(6)}\) The sesquiterpene polygodial was the main compound responsible for this activity. Its biotransformation major product (isodrimeninol) possessed lower activity, suggesting that \(A.\) fumigatus modifies (1) to the antifungal compound (2), with lower antifungal capacity, as a defense mechanism.

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Acknowledgments
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References
(2) Natural Products Alert (NaPrAlert). Program for Collaborative Research in the Pharmaceutical Sciences. College of Pharmacy, University of Illinois. Chicago, III, USA.

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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF Satureja parvifolia (PHIL.) ELING. WITH GALLS

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Introduction
Satureja parvifolia (Lamiaceae) is mainly distributed in Perú, Bolivia, center and northwestern of Argentine(1). Medicinal plant, is traditionally used as a stimulant, stomachic, carminative, and aphrodisiac(2). Literature review showed variation between chemical composition of different Satureja species oils(3). S. parvifolia that grows in El Mollar, Tucumán, Argentina is infested by Dasineura sp., a parasitoid that frequently produced galls in stem branches. Present work aims to provide data of essential oil of Satureja parvifolia infested by Dasineura sp. and its antibacterial activity.

Materials and methods
Aerial parts of infested S. parvifolia were collected at flowering stage, in January 2009, from El Mollar, Tafi del Valle, Tucumán, Argentina (1800 m osl). Dried aerial parts, were subjected to the hydrodistillation of 3 h, using an all glass Clevenger-type apparatus to produce oil. The oil was dried over anhydrous sodium sulfate. The GC-MS analysis of essential oils was carried out on a GC-HP 6890 with mass selective detector (quadrupole) HP 5973, source 70eV, fitted with a HP-5MS column (5% phenyl methyl siloxane, 30 m x 0.25 mm i.d., film thickness 0.25 µm) with helium as carrier gas at 1.0 ml/min. Quantitative data were obtained from automatic of area percent data (FID) without the use of an internal standard or response factors. Identification of components of the oil was based on comparison of their mass spectra with those found in the literature (4) and mass spectrometry data bank (NBS75K, NIST, WILEY) and computer search Wiley library. Essential oil was tested in vitro using agar diffusion method against Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and Staphylococcus aureus clinical isolated. Suspensions of microorganisms were prepared to contain approximately 10^8 UFC / ml (0,5 Mac Farland’s Standard). The diffusion method was performed, putting 50 µl of different concentrations of essential oils in cups to make on plates containing Müeller Hinton Agar and previously inoculated with microorganisms. Zones of growth inhibition around the cups were measured after 24 h of incubation at 37ºC. Gentamicine (0,5 mg/ml) was used as standard. Minimum Inhibitory Concentrations (MIC) was determined by the agar dilution method (5). All the tests were repeated in duplicates.

Results
Thirty-eight components were identified representing 88.8% of the oil. The main components were pulegone (61.6%), neoiso-isopulegylacetate (6.1 %) and piperitone oxide (3.7 %) . The oil exhibited antimicrobial activity against Staphylococcus aureus ATCC 25923 and Staphylococcus aureus clinical isolated.

Conclusions
Chemical composition of essential oil of S. parvifolia infested by Dasineura sp. showed qualitative and quantitative differences with literature reported by S. parvifolia non infested. Main compounds of S. parvifolia oil from Argentina, province of Jujuy are piperitone and piperitone oxide(6) and from province of San Juan, piperitone and piperitenone(7). Differences in the composition of essential oil of S. parvifolia from El Mollar are probably due to existence of infestation by Dasineura sp. or for being a different chemotype.

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Acknowledgments
We wish to thank Lic Susana Popich of National University of Chilcito (La Rioja, Argentina) for
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financial support.

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5- Jorgensen JH, Turnidge JD and Washington JA. Antibacterial susceptibility tests: dilution and disk
7- Viturro CI, Molina A, Guy I, Charles B, Guinaudeau H and Fournet A. Essential oils of Satureja
boliviana and Satureja parvifolia growing in the region of Jujuy, Argentina. Flavour and Fragrance

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SYNTHETIC BACTERIOCIN ANALOGS AS THERAPEUTICS PEPTIDES. EFFECTS OF FATTY ACID CONJUGATION ON THEIR ANTIMICROBIAL PROPERTIES

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Introduction
The increasing bacterial resistance against conventional antibiotics has led to the search for new antimicrobial drugs with different modes of action. Cationic antimicrobial peptides (AMPs) are promising candidates to treat infections since they act on bacterial membranes causing rapid destruction of sensitive bacteria. Previous studies have demonstrated that conjugation of AMP with saturated fatty acids increased their antimicrobial properties (1).

The aim of this study was to design short antimicrobial peptides based on Plantaricin 149a sequence and to evaluate the effect of conjugation with fatty acids (C8 and C12) on their antimicrobial and conformational properties.

Material and Methods
Plantaricin 149a (YSLQMGATAIKQVKKLFKKKGG), CT (GATAIKQVKKLFKKKGG), CTc (IKQVKKLFKKK), and N-terminal fatty acid conjugated analogs C8-CT, C12-CT and C8-CTc, were synthesized by Fmoc-SPPS as C-terminal carboxamides. Peptides were characterized by HPLC and Maldi-Tof-MS.

Minimal inhibitory concentration (MIC) was determined by the microtiter dilution assay and the following bacterial strains were used: *Listeria monocytogenes* DBFIQ LM 3 and *Staphylococcus aureus* DBFIQ S 21. The hemolytic activity of the peptides against human red blood cells was determined by measurement the release of haemoglobin spectrofotometrically at 405 nm.

The Circular Dichroism spectra of the peptides were recorded on a Jasco J-810 CD Spectropolarimeter from 190 to 250 nm at 25ºC. Peptides were diluted in MiliQ water (0.20 mg/mL) and in 20-40-80% of trifluoroethanol/water (v/v).

Results
Pln149a and CT exhibits the lowest antimicrobial activity against *S. aureus* strain (MIC: Pln149a, 66.0 µM; CT, 88.8 µM). Conjugation of CT with fatty acids significantly increase its antimicrobial activity (MIC: C8-CT, 10.3 µM; C12-CT, 20.0 µM) against this strain. Moreover, CTc was also found to be very active against this strain (MIC: CTc, 15.9 µM), but in this case conjugation with n-octanoic acid had no favorable effect on the antimicrobial activity (MIC: C8-CTc, 28.6 µM).

On the other hand, Pln149a was the most active peptide against the studied strain of *L. monocytogenes* (MIC: 8.3 µM) compared with CT and CTc (MIC: 177.7 and 63.6 µM, respectively) and their conjugated analogs (C8-CT, 164.7 µM; C12-CT, 40.0 µM; C8-CTc, 57.0 µM).

According to these results, CTc analog was shown to be more active than CT against the two studied strains, suggesting region 11 to 19 of Pln149a is crucial for antimicrobial activity against Gram (+) bacteria.

The conjugation of the peptides increased their hemolytic activity, at 100 µM, C8-CT (20.5%), C12-CT (21.3%) and C8-CTc (46%). In contrast, Pln149a, CT and CTc were non-hemolytic.

CD spectra analysis evidenced that fatty acid conjugation of the analogs increases their tendency to adopt alpha-helical conformation in the presence of TFE. Then, the stabilization of the secondary structure may improve the interaction of the lipopeptides with the bacterial membranes.

Conclusions
Based on these results, synthetic bacteriocin analogs may be considered as novel peptide therapeutics for treatment of infections produced by Gram (+) bacteria.

References

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SYNERGISTIC EFFECT OF THE ANTIBACTERIAL COMPOUND 23-METHYL-6-O-DESMETHYLAURICEPYRONE ISOLATED FROM ACHYROCLINE SATUREIOIDES.

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Introduction

Staphylococcus aureus causes a wide range of infectious diseases in humans (1) from relatively mild skin infections such as folliculitis and furunculosis to life-threatening conditions, including sepsis, deep abscesses, pneumonia, osteomyelitis, and infective endocarditis (2). As a result of a study of the antibacterial activity of Achyrocline satureioides a bioactive compound was identified. A remarkable inhibitory effect of this compound on the mentioned pathogenic bacterium was determined. Then, the antibacterial activity of combinations of this active principle with subinhibitory concentrations of commercial antibiotics was investigated.

Materials and methods

The structural elucidation of the isolated compound was achieved through different spectroscopic techniques. The MIC, MBC and synergistic studies were carried out by micro dilution test in agar according to CLSI (3) against Staphylococcus aureus (ATCC 6538) used as reference.

Results

The most active compound present in A. satureioides extract was identified as 23-methyl-6-O-desmethylauricepyrone (1). The MIC and MBC values of this compound and of commercial antibiotics are shown in Table 1. Table 2 and 3 show the results obtained in synergistic assays with erythromycin and gentamicin, respectively.

Table 1. Antibacterial effect of 23-methyl-6-O-desmethylauricepyrone

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (MBC) µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-methyl-6-O-desmethylauricepyrone</td>
<td>2 (8)</td>
</tr>
<tr>
<td>erythromycin</td>
<td>0.6 (60)</td>
</tr>
<tr>
<td>gentamicin</td>
<td>8 (10)</td>
</tr>
</tbody>
</table>

Table 2. Synergism between 23-methyl-6-O-desmethylauricepyrone and erythromycin

<table>
<thead>
<tr>
<th>23-methyl-6-O-desmethylauricepyrone</th>
<th>Erythromycin Times below MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

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Table 3. Synergism between 23-methyl-6-O-desethylauricepyrone and gentamicin

| 23-methyl-6-O-  | Gentamicin. Times below MIC |
| desethylauricepyrone | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 |  |
| Times below MIC | - | - | - | - | - | - | + | + | + | + |
| 2 | - | + | + | + | + | + | + | + | + | + |
| 4 | - | + | + | + | + | + | + | + | + | + |

(-) growth inhibition; (+) growth.

Conclusions
Despite 23-methyl-6-O-desethylauricepyrone has already been described in *A. satureioides* (4), this is the first time that any biological activity has been attributed to it. The compound demonstrated a strong antibacterial activity against *S. aureus* with MIC and MBC values of 2 and 8 µg/mL, respectively. These results are very encouraging; this active principle exceeded the activity shown by the commercial agents used as reference. The activity of 23-methyl-6-O-desethylauricepyrone is comparable to that of other commercial antibiotics like vancomycin (MIC = 0.5-2 µg/mL) or chloramphenicol (MIC = 2-8 µg/mL) (5). When the compound was combined at 1/2 MIC (1 µg/mL) with erythromycin and gentamicin, bacterial growth was still inhibited even when the concentration of the two last was 256 and 128 times under their MIC value, respectively. Even at 16 times below its MIC, compound 1 exhibited antibacterial activity in combination with erythromycin at 16 times below its MIC. According to the obtained results, compound 1 could arise as a new antibacterial agent against staphylococcal infections, not only because of its effectiveness but also because of the synergistic effect with conventional antibiotics. These facts take relevance considering that most of the antibacterial agents used today have many side effects (6). Therefore, reducing their doses could contribute to a healthier lifestyle.

References
ANTIMICROBIAL RESISTANCE IN COMMERCIAL FARMS OF BUENOS AIRES FROM FAECAL SAMPLES USING Escherichia coli AS AN INDICATOR

Marchetti, M.L., Huber B., Quintero, M., Mestorino, N., Errecalde J.
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Introduction:
Bacterial antimicrobial resistance has become a serious problem worldwide. Mechanisms of resistance have been described for almost all known antimicrobials. Genetic elements involved in antibiotic resistance are able to be transferred among intestinal microorganisms. Escherichia coli represents a major group among intestinal microflora. The surveillance of its resistance is an important tool to control the non prudent use of antimicrobials. The objective of this research was to evaluate in vitro commensal E. coli resistance in strains isolated from four commercial farms of Bs As to compare resistance profiles.

Materials and methods:
A survey protocol to obtain information about the use of antimicrobial products in different farms during the last year was designed. Samples were collected from Tandil, Luján, San Vicente and Trenque Lauquen commercial farms in the Province of Buenos Aires, Argentina.

Sample collection, isolation and tipification: Strains were isolated from the faeces of healthy animals (dairy cattle, calves, cats and dogs). They were collected rectally from individual animals by using culturette swabs. Samples of human fecal material and other excreta were collected from septic tanks in sterilized bottles. Water samples were obtained from different sources in each commercial farm. Faecal samples were cultured in plates with EMB agar, coloured by Gram and typified by biochemical tests. Water samples were tested by the Most Probable Numbers method.

Qualitative susceptibility test: The standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial agent sensitivity profiles for eight antimicrobial agents (Table 1) chosen based on surveys results. Isolations were classified as resistant or non resistant (1). E. coli ATCC 25922 was used for quality control.

Results and conclusions:
The prevalence and resistance profiles are expressed in table I and II. Resistant and multirresistant strains were isolated from different kinds of samples. There were high level of tetracycline and ampicillin resistance which agreed with reports from other authors (2-3-4). In every multirresistance profile, resistance to tetracycline was expressed. It may suggest that tetracycline resistant strains are probably able to gain resistance mechanisms and become multirresistant.

References:
1- Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing 2009; M100-S19, Wayne, PA.

Table I: Percentage of antimicrobial resistance in E. coli isolates from commercial farms of Bs As

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Tandil (n=206)</th>
<th>Lujan (n=224)</th>
<th>San Vicente (n=71)</th>
<th>T. Lauquen (n=256)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>CIP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FLR</td>
<td>0</td>
<td>0</td>
<td>0,4</td>
<td>1</td>
</tr>
<tr>
<td>SXT</td>
<td>0</td>
<td>0</td>
<td>1,3</td>
<td>3</td>
</tr>
<tr>
<td>TIO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AMP</td>
<td>1,5</td>
<td>3</td>
<td>4</td>
<td>9</td>
</tr>
</tbody>
</table>
### Table II. Resistance profile of *E. coli* strains isolates in each sampled farm

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Number of strains</th>
<th>Resistance profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tandil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>septic tank</td>
<td>AMP  AMC</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>calve</td>
<td>AMP  TCY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>dairy cattle</td>
<td>AMP</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>dairy cattle</td>
<td>TCY</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>calve</td>
<td>TCY</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>San Vicente</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dairy cattle</td>
<td>CIP  SXT  AMP  TCY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>dairy cattle</td>
<td>FLR  SXT  TCY</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>calve</td>
<td>AMP</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>septic tank</td>
<td>AMP</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>dairy cattle</td>
<td>TCY</td>
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<td></td>
</tr>
<tr>
<td>calve</td>
<td>TCY</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><strong>Lujan</strong></td>
<td></td>
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</tr>
<tr>
<td>dog</td>
<td>FLR  SXT  AMP  TCY</td>
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<td>dog</td>
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<tr>
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</tr>
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<td>perro</td>
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</table>

Corresponding author.  mlaurnarchetti@yahoo.com.ar  – noram@fcv.unlp.edu.ar
EVALUATION OF 1-(1-NAPHTHYLMETHYL)-PIPERAZINE EFFECT ON ANTIMICROBIAL DRUG SUSCEPTIBILITY OF MULTIDRUG RESISTANT 
*Escherichia coli* ISOLATED FROM ANIMALS AND ISOGENIC STRAINS

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*Department of Pharmacology. Faculty of Veterinary Science and Faculty of Medical Science. UNLP, 60 y 118, CC 296, 1900. La Plata, Bs.As, Argentina*

**Introduction:**
Bacterial multidrug resistance (MDR) has become a serious problem in human and veterinary medicine. Active efflux by pumps is a widespread mechanism for MDR bacteria. Inhibition of efflux pumps appears to be a promising strategy for restoring the activity of these drugs. The objective of this research was to evaluate the interaction of the efflux pump inhibitor 1-(1-naphthylmethyl)-piperazine (NMP) with florfenicol and ampicillin in resistant mutants of *E. coli* with differing expression of acrAB efflux pumps and multidrug resistant *Escherichia coli* strains isolated from commercial farms.

**Materials and methods:**
Laboratory isogenic strains included a pump deficient (AG100A), an overexpressing acrAB efflux pump (AG112) and a normal expressing efflux pump (AG100) strains. Ten MDR *E. coli* strains collected from healthy animal of four commercial farms of Buenos Aires (as resistance “indicator”) were also studied. Bacterial cells were grown in LB agar at 37º C for 24 hs. Minimal inhibitory concentration, (MIC) to florfenicol and ampicillin were studied by a two fold standard broth microdilution method (CLSI 2009) in LB broth in presence or absence of NMP at five different concentrations.

**Results and conclusions:**
Based on a four-fold or greater MIC reduction as a significant result, the effect of NMP with ampicillin in every strain studied was limited (Table II). Otherwise, NMP exerted a highly selective action on the reduction of florfenicol’s MIC mainly at higher concentrations of the efflux inhibitor (50-100 mg/L) clearly shown in mutant resistant strains’s CIM(Table I). On the other hand, the reduction of florfenicol MIC with NMP in field strains isolations was no significant (Table III). We conclude that NMP can partially reverse multidrug resistance in *E. coli* because of the combination of different resistant mechanisms in these ones, which agreed to other authors (2-3).

**Reference**
1-Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing 2009; M100-S19, Wayne, PA.

**Tabla I: Synergic in vitro activity of NMP combined with florfenicol (FLF) against E. Coli ATCC 25922 and mutant resistant strains.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Expression of acrAB</th>
<th>FLF MIC (µg/ml) in the presence of concentrations of NMP</th>
<th>NMP MIC (µg/ml)</th>
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</thead>
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<td></td>
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<td>normal</td>
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<td>400</td>
</tr>
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<td>AG100ΔacrAB</td>
<td>deletion</td>
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</tr>
<tr>
<td>AG112</td>
<td>AG100 marR</td>
<td>overexpression</td>
<td>32 32 16 16 4/8 1/2</td>
<td>400</td>
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<tr>
<td><em>E.coli</em> ATCC 25922</td>
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<td>normal</td>
<td>8 8 4 4 2 2</td>
<td>800</td>
</tr>
</tbody>
</table>
### Tabla II: Synergic in vitro activity of NMP combined with ampicillin (AMP) against E. Coli ATCC 25922 and mutant resistant strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Expression of acrAB efflux pump</th>
<th>AMP MIC (µg/ml) in the presence of concentrations of NMP (µg/ml)</th>
<th>NMP MIC (µg/ml)</th>
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### Tabla III: MIC in the presence of concentration of NMP in commercial farm isolations comparing the results of the different resistant profiles.

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<th>NMP MIC (µg/ml)</th>
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<tr>
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IN-VIVO DETERMINATION OF DANOFLOXACIN POST-ANTIBIOTIC EFFECT AGAINST Staphylococcus aureus ATCC 29213 STRAIN.

Moncada Cárdenas, L.A.; Daniele, M.; Haag, G.; Dadé, M.; Errecalde, J.O.; Mestorino, N.

Department of Pharmacology, Faculty of Veterinary Science, Faculty of Medical Science, UNLP, 60 y 118 CC 296, 1900 La Plata.

Introduction
The post-antibiotic effect (PAE) (1) is considered as the period of time needed by abacterial population to overcome the suppression imposed to its growth after a short exposure to an antimicrobial agent (2). The duration of the PAE is influenced by the bacterial species, the nature of the antibiotic and its concentration, and by environmental factors (temperature, pH, pO2, growth medium, the kind of body fluid) (3). The clinical significance of the PAE pertains primarily to the impact it may have on the design of antimicrobial dosing regimens in clinical practice (5).

The purpose of the present study was to investigate the in vivo PAE of danofloxacin, using the thigh infection model in neutropenic mice (6), against standard strains of Staphylococcus aureus ATCC (29213).

Materials and methods
Microorganism. S. aureus ATCC 29213
Antimicrobial agents. Danofloxacin mesylate (DAN), obtained from Pfizer Inc. N.Y.
Culture medium: Mueller-Hinton broth, Mueller-Hinton agar.
Animals: Female and male C57/h3 mice weighing 28 to 32 g were rendered neutropenic by intraperitoneal injection of cyclophosphamide.
In vivo PAE. The in vivo PAE was determined according to the experimental procedure of Craig et al. (4).

Results
The determination of danofloxacin in vivo PAE against S. aureus, using the methodology described by Craig in 1996, showed a duration of 2 hours (Fig 1).

Conclusions
The present study shows a significant PAE of DAN against S aureus. This finding is of major importance in the clinical use of this quinolone because it could be administered at longer intervals without losing effectiveness in this infection.

References

a Corresponding author: e-mail: noram@fcv.unlp.edu.ar
ANTIMICROBIAL ACTIVITY OF CHROMIUM(III) COMPLEXES ON Staphylococcus aureus AND Escherichia coli

Páez PL(1,2), Bongiovanni ME(2), Bazán CM(1), Albesa I(2), Becerra MC(2) and Argüello GA(1).

(1)INFIQC-CONICET, Dpto. de Fisicoquímica; (2)Dpto. de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria. 5000 Córdoba. República Argentina.

Introduction
Since their discovery, antibiotics have played an important role in health care. However, the increasing emergence of antibiotic resistance among a variety of microbial pathogens stimulates intensive research efforts with the aim to identify alternative therapeutic approaches (1). Macrocyclic complexes have attracted attention because of their pharmacological properties, i.e. toxicity against bacterial and fungal growth. It was reported that complexes of chromium(III) would have a role in the microbiological application to reduce the virulence and antibiotic resistance (2). In the present work we report the biological activities of chromium(III) complexes against Escherichia coli and Staphylococcus aureus.

Materials and methods
Two test bacterial strains viz S. aureus ATCC 29213 and E.coli ATCC 25922 were considered for the determination of Minimum Inhibitory Concentration (MIC) of the synthesized complexes by the photochemistry group of INFIQC-CONICET, Dpto. de Fisicoquímica (3). The MIC of the compounds was evaluated by using the standard tube dilution method on Mueller Hinton broth. Bacterial growth was observed at 18 h of incubation, following the indications of the Clinical and Laboratory Standards Institute (CLSI) (4). The MIC of the compounds were ranged from 0.004 to 2 µg/mL. In addition, the MIC values for the ligands 1, 10-phenanthroline (phen) and dipyrido [3,2-a:2´,3´-c]-phenazine (dppz) was determined. Dilutions of ciprofloxacin (0.008 to 4 µg/mL) were used to compare the activity. The lowest concentration of the compound that prevented bacterial growth was considered to be the MIC.

Results
Two chemically macrocyclic complexes and their ligands were screened for their in vitro antibacterial activity against S.aureus and E.coli. [Cr(phen)2(dppz)]3+ was found to be the most potent. MIC values obtained for the chromium complexes were in the range of those obtained with ciprofloxacin. E.coli was found to be more susceptible to [Cr(phen)2(dppz)]3+ than S.aureus, with a MIC for E.coli of 0.125 µg/mL and a MIC for S.aureus of 0.5 µg/mL while the MIC for E.coli was 0.25 µg/mL and for S.aureus 1 µg/mL for [Cr(phen)3]3+. The phen and dppz ligands did not show activity against the two bacterial strains.

Conclusions
The work provides the first evidence of antimicrobial activity of the synthetized metallomolecules [Cr(phen)3]3+ and [Cr(phen)2(dppz)]3+. The synthesized metal complexes of chromium(III) exhibited higher antimicrobial activities against Escherichia coli and S. aureus than the ligands alone. Our results indicate that biologically active chromium(III) complexes have biological toxic activity against Gram negative and Gram positive bacteria.

Acknowledgments
CONICET, SeCyT – UNC, ANPCYT.

References

Corresponding author. Tel +54 351 4334163, fax +54 351 4334127; e-mail: plpaez@fcq.unc.edu.ar
IMPROVED BACTERICIDA PROPERTIES OF OFLOXACIN LOADED ON BIOADHESIVE HYDROGELS AGAINST FLUOROQUINOLONE-RESISTANT 

Pseudomonas aeruginosa (FQR-P)

Romero VL, Rosset CI, Manzo RH, Alovero FL
Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.
Laboratorio 210. Edificio de Ciencias II. Haya de la Torre esquina medina Allende. Ciudad Universitaria. Córdoba

Introduction
Bacterial resistance to fluoroquinolones is a major challenge for researchers who seek to preserve the usefulness of these valuable antibiotics used to treat a large variety of infections. In particular, P. aeruginosa infections are difficult to overcome owing to the high intrinsic resistance to antibiotics. Development of new strategies for drug delivery using the concept of mucoadhesion shown to prolong the residence time of dosage forms in the mucosa, allowing sustained drug release remained at a particular target site. That condition allows an intimate contact with the membrane absorption, providing the basis for a high concentration gradient as a driving force for passive drug absorption. Carbomers are synthetic polymer of acrylic acid cross-linked with allylsucrose well established as a good bioadhesive and extensively investigated in the pharmaceutical field. Three carbomers were selected in this work to prepare colloidal aqueous dispersions (hydrogels) with different viscosities and used as carriers of ofloxacin. The aim of this work is to evaluate the effect of the bioadhesive carrier system on antimicrobial properties of ofloxacin by determining MIC values, the bactericidal profiles and bacterial uptake exhibited by hydrogels against fluoroquinolone-sensitive and -resistant P. aeruginosa.

Materials and methods
Hydrogels of carbomer 971, 934 y 940 partially neutralized with ofloxacin (C-OFL) were prepared as previously reported (1). P. aeruginosa ATCC 27853 and 3 clinical isolates of Fluoroquinolone-resistant P. aeruginosa were used. The MICs of the ofloxacin-containing hydrogels were carried out by standard two-fold macrodilution technique according to CLSI. The bactericidal activity was determined in Mueller-Hinton broth in the presence of a wide range of drug concentrations. Bactericidal indexes (BI) were calculated using data from the bactericidal activity assay (2). The accumulation of Ofloxacin from C-OFL was performed by a fluorometric method (3).

Results
The MICs of the ofloxacin-containing hydrogels against P. aeruginosa are similar to or two times lower than those of free drug. No differences were observed between the MICs of the hydrogels prepared with different carbomers. C-OFL exhibit enhanced bactericidal action against FQ-R P. aeruginosa achieving bacterial eradication in drug concentrations that did not do it ofloxacin. In addition, hydrogels exhibited prolonged effect as compared to that of free drug. These properties were confirmed by the highest Bactericidal Index calculated from bactericidal profiles.

The uptake of ofloxacin from hydrogels was higher than that yielded with ofloxacin solution in FQ-R and –S P. aeruginosa. Additionally, the trend of enhanced intracellular accumulation of ofloxacin in the FQ-R strain correlated with the viscosity of the hydrogels as three of them were compared.

Conclusions
The enhanced bactericidal performance of the hydrogels could be important against highly resistant isolates and the prolonged activity could contribute to reduce the development of persistent cells. Moreover, these polyelectrolyte-fluoroquinolone reversible complexes promote a higher uptake of ofloxacin. That behavior could be ascribed to bioadhesive interactions between the complex polyelectrolyte-fluoroquinolone and the envelopment of bacterial cells. Rheological and bioadhesive properties of the hydrogels result in prolonged contact time in the site of administration compared with a solution (5), allowing a sustained drug release at the target site. Hence, hydrogels could be used in the development of a more effective formulation for the topical administration of older Fluoroquinolones. An improved performance of an old antibiotic can preserve the use of fluoroquinolones of new generation.
References.
ANTIMICROBIAL PEPTIDES FROM *Leptodactylus latrans* SKINS INHIBIT THE GROWTH OF *Mycobacterium tuberculosis* H37Rv STRAIN.


*Laboratorio de Péptidos Bioactivos, Departamento de Química Orgánica, Cátedra de Ecotoxicología, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Instituto Nacional de Enfermedades Respiratorias “Dr. E. Coni”. Santa Fe, Argentina.*

**Introduction**

In response to stress or predator attack, amphibians secrete a complex chemical cocktail from highly specialized skin structures, namely the venom or granular glands (1). Although efforts done over decades around the world to determine the structural and biological properties of peptides from amphibian skin, the vast majority of species remain unstudied. In this context, Argentina, and especially the provinces of Santa Fe and Entre Ríos, have a great diversity of anuran amphibians that generate a number of opportunities in this field of study. Most antimicrobial peptides are cationic and kill microbes via mechanisms that predominantly involve interactions between positive charged residues and anionic components of target cell membranes. Nevertheless, anionic antimicrobial peptides have also been established as an important part of the innate immune systems of vertebrates, invertebrates and plants, but their mechanisms of action are unclear or have not been elucidated (2).

Our work focused on the isolation and purification of peptides from an extract of *Leptodactylus latrans* (Anura: Leptodactylidae) skins which inhibited the growth of a strain of *Mycobacterium tuberculosis* (MT).

**Materials and methods**

Adult specimens of *Leptodactylus latrans* were collected in rural zones of Paraná (Entre Ríos, Argentina) during summer months. After euthanizing each specimen, the skin was removed, tritutrated and extracted with ethanol /water (60:40) (v/v) in acid medium. After centrifugation, samples were lyophilizated and conserved at -20°C. Fractioning of the complete extract was done first by dialysis and then by HPLC on a C18 semi-preparative column.

The inhibitory activity of the samples against *Mycobacterium tuberculosis* H37Rv strain was performed using the micromethod described by Leite et al. (3).

**Results**

The complete extract of *L. Latrans* inhibited the growth of MT H37Rv strain (MIC 187mg/mL). After dialysis, it was shown that only samples obtained by means of membranes with cut off: 1 kDa were actives (MIC: 78 mg/ml). This sample was purified by RP-HPLC and seven fractions were separated, but only Fraction 3 inhibited the growth of MT strain. Further analysis by MALDI-TOF-MS showed the presence of four peptide components with MW in the range of 1200 - 1900 Da. The major constituent (MW: 1578 Da) was sequenced by LC-MS-MS and the identified sequence was: DEMKLDGFNMHLE.

**Conclusions**

In this work we have found that skins of *Leptodactylus latrans* contain peptides that inhibit the growth of a strain of *Mycobacterium tuberculosis* and the principal component of the active fraction is an anionic peptide. These results may possess potential for medical and biotechnological applications.

**Acknowledgments**

This work was supported by grants from Universidad Nacional del Litoral, Santa Fe, Argentina.

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References.


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Antifungal Effects of Combination of Fluconazole and 2',4'-Dihydroxy-Chalcone Isolated from Zuccagnia punctata

Postigo A, Raimondi MP, Petenatti, EM, Zacchino SA, Sortino MA.


Introduction
Fungal infections, particularly invasive candidiasis, are important causes of morbidity and mortality in immunocompromised patients (1) which are usually treated with Fluconazole (FCZ). There has been a change of paradigm in antimicrobial chemotherapy that involved a transition from mono-substance therapy, which required high dosages for efficacy which often produce bioavailability problems and undesirable effects, towards to multidrugs therapy. Drugs combinations are used in order to increase efficacy or reduce toxicity.

Plants provide unlimited opportunities for the isolation of new antifungal compounds because of the unmatched availability of chemical diversity (2-3). Here we use a High-Throughput Synergy Screening method (HTSS) based in the inhibition of fungal growth by plant extracts in combination with FCZ (4). In our previous work we studied with the HTSS method several Argentinean plants and found that DCM extract of aerial parts from Zuccagnia punctata in combination with FCZ (both at sub-inhibitory concentrations) inhibit the fungal growth of Candida tropicalis. Herein we report the isolation and identification of 2',4'-dihydroxy-chalcone, responsible of the improvement of FCZ activity.

Materials and methods
Extracts preparation and isolation of the chalcone
Z. punctata was collected in Ayacucho, San Luis Province (Argentina) and a voucher specimen was deposited (L.A. Del Vitto & E. Petenatti #9230). Air-dried, powdered plant material was successively extracted with hexane, dichloromethane and methanol to give the respective extracts. A sample of the DCM extract was chromatographed on Silica Gel 60H, each fraction was assayed with HTSS. Repeated column chromatography of the active fractions led to the isolation of 2',4'-dihydroxy-chalcone.

Antifungal susceptibility testing
C. tropicalis strain used for the biological evaluation was purchased to the Centro de Referencia en Micología. Minimal Inhibitory Concentration (MIC) was determined by using broth microdilution techniques according to the guidelines of the M27-A2 (5).

HTSS
To perform the assay, the following wells were prepared: (i) FCZ at inhibitory concentration; (ii) FCZ at non-inhibitory concentration; (iii) Vegetal extract or isolated compound at non-inhibitory concentration; (iv) a combination of (ii) and (iii) (4). A fungal inoculum identical to the used for antifungal susceptibility was added to all wells.

Determination of the type of interaction
Interactions of FCZ with selected extracts and isolated compound when acting against C. tropicalis were determined by using the microbroth dilution chequerboard method. Fractional Inhibitory Concentration (FIC) = MICcombination/MICalone of FCZ (FICFCZ) and extract and compound (FICEC) were determined. The Fractional Inhibitory Concentration Index (FICI) was calculated using the following formula: FICI= FICFCZ+FICEC and interpreted as: IFIC≤0,5 (synergism); 0,5<IFIC<4 (additivism) and IFIC>4 (antagonism)

Results
The guided-fractionation of the extracts showed that the combination of 2',4'-dihydroxy-chalcone with FCZ inhibit C. tropicalis growth, although each component of the mixture are inactives at those concentrations The MIC of FCZ and 2',4'-dihydroxy-chalcone alone against C. tropicalis are 16µg/mL and 62.5µg/mL, respectively. FCZ’s MIC decreases to 8µg/mL in the presence of 31.25µg/mL of the

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chalcone. The chequerboard method indicated that the type of interaction between them is additivism (IFIC=1).

**Conclusions**
HTSS demonstrated to be a useful method for the rapid detection of active combinations of compounds. An amount of 31.25µg/mL of 2’,4’-dihydroxy-chalcone, isolated from *Z. punctata*, reduces to the half FCZ’s MIC against *C. tropicalis* and, concomitantly their undesirable effects.

**Acknowledgments**
MS acknowledges CONICET for a post doctoral fellowship; AP, MS and SZ thank to Fundación Banco de Santa Fe for a fellowship to AP. Authors are greatly acknowledged to ANPCyT, UNR and UNSL.

**References.**
1) Pfaller MA, Diekema DJ. Clinic Microb Rev. 2007; 20: 133-163

### # Sortino MA. Tel/fax +54 341 4375315; e-mail: szaabgil@citynet.net.ar
ANTIFUNGAL ACTIVITY OF R- AND S-4-(4'-HIDROXYPHENYL) BUTAN-2-OLS OBTAINED THROUGH FUNGAL BIOTRANSFORMATIONS

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Introduction
Drug enantiomers many times differ in pharmacological effects, potency, or toxicity. One example of the different pharmacological response of two enantiomers is propanolol whose S enantiomer possesses antihypertensive and antiarrythmic activities while the R-enantiomer acts as a contraceptive.\(^1,2\) Biocatalysis has become an increasingly valuable tool for the easy preparation of chiral compounds, having great potential because of their sustainable methodology for the production of chemicals, that is, green chemistry. Although the potential of fungi as biocatalysts has been demonstrated in some previous works,\(^3\) new studies exploring the potentiality of fungal species as new routes to obtain chiral compounds are highly welcome. In this work, the fungal biotransformation of 4-(4'-hidroxyphenyl)-3-buten-2-one (1) is reported along with the evaluation of the antifungal activity of the chiral products. In addition, the racemic mixture of the product was tested too.

Materials and methods

Biotransformation
Fungi were grown on plates with agarized Czapek [for Aspergillus fumigatus (Af)] or Sabouraud [for Trichosporon cutaneum (Tc)] culture media for 3 days at 30 °C until well sporulated. Compound 1 was prepared by Claisen-Schmidt condensation of 4-hidroxybenzaldehyde with acetone\(^4\) and submitted to biotransformation as follows: Suspensions of fungal conidia (2-5 x 10^8 CFU/mL) were used to inoculate 1L erlenmeyer flasks containing Czapek or Sabouraud broth medium (500 mL) for Af and Tc respectively. The cultures were incubated at 30 °C for 72 h on an orbital shaker (150 rpm; Innova 4000, New Jersey, USA). The substrates (100 mg) in DMSO (1 mL) were poured into the flasks containing the fungal biomass and the reaction mixtures were incubated at 30 °C for 72 h on an orbital shaker (150 rpm). After purification, the conversion percentages were analyzed by TLC and GC. The enantiomeric purity of the products was determined by ^1H NMR with Eu(hfc).\(^6\)

Antifungal Activity
The minimum inhibitory concentration (MIC) of each compound was determined by using broth microdilution techniques according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). Strains from the American Type Culture Collection (ATCC) and CEREMIC (C), Centro de Referencia en Micología, were used. Inocula of cell or spore suspensions were obtained according to reported procedures and adjusted to 1-5 x10^3 cells/spores with colony-forming units (CFU) mL\(^{-1}\).\(^5\) Ketoconazole (Ket), terbinafine (Terb), and amphotericin B (Amp) were used as positive controls.

Results
The C-C double bond of 1 was hydrogenated and the C=O was enantioselectively reduced to alcohol by both fungal strains to afford 4-(4'-hidroxyphenyl) butan-2-ol. A. fumigatus has the ability of generate S- (+)-4-(4'-hidroxifenil) butan-2-ol (2a) with ee= 99% and T. cutaneum, R(-)-4-(4'-hidroxifenil) butan-2-ol (2b) with ee= 99%. Regarding the antifungal activity, Table 1 show the MICs of compounds 2a, 2b and the racemic mixture (2).

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Table 1. MICs of (±)-4-(4’-hydroxyphenyl) butan-2-ol (2); S-(+)-4-( hydroxyphenyl) butan-2-ol (2a) and R-(−)-4-(4’- hydroxyphenyl) butan-2-ol (2b); i: >1000 µg/mL.

<table>
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<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
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<td>i</td>
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<tr>
<td>2a</td>
<td>250</td>
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<td>250</td>
<td>i</td>
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<tr>
<td>2b</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
<td>i</td>
</tr>
</tbody>
</table>

Amp.  0.25  0.50  0.50  0.50  0.50  0.50  0.12  0.07  0.07
Ket.   0.25  0.50  0.12  0.50  0.12  0.50  0.25  0.04  0.02  0.02
Terb.  0.04  0.01  0.04

Conclusions
We have found that Af and Tc are efficient biocatalysts for the enantioselective C–C double bond and C=O reductions of 1 into 2a and 2b respectively, leading to the introduction of one chiral center, with high enantioselectivity, into an achiral structure.

Although the activity of the R-enantiomer (2a) against the yeasts is moderate (MIC 250 µg/mL), it is at least four times higher than the S-enantiomer (2b) and the racemic (2) (both MIC > 1000 µg/mL). This work provides a new evidence that fungal biocatalysis is useful to prepare chiral compounds by an environmentally viable alternative, that could be new alternatives in the search of bioactive compounds.

Acknowledgments
LS acknowledges ANPCyT (PICT 995) for a doctoral fellowship.

References.

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ANTIMICROBIAL ACTIVITY OF TERNARY COMBINATIONS OF DIHYDROXYLATED CHALCONES WITH CONVENTIONAL ANTIBIOTICS

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Introduction
The increased resistance to antimicrobial agents of some microorganisms makes of great interest the study of combinations of different compounds with conventional antibiotics. In the present study the antimicrobial actions of ternary combinations of flavonoids, a family widely distributed in nature (1), and conventional antibiotics, oxacillin for Gram + and acid nalidixic for Gram – microorganisms (2), were assayed. A kinetic-turbidimetric method (3) was used to determine the existence of synergism. The ternary combinations used were: 2',4-dihydroxychalcone - 2',3-dihydroxychalcone - oxacillin against *Staphylococcus aureus* ATCC 43 300 (MRSA) and 2',4-dihydroxychalcone - 2',3-dihydroxychalcone - nalidixic against *Escherichia coli* ATCC 25 922.

Materials and methods

**Compounds:** oxacillin, sodium salt monohydrate and nalidixic acid were purchased from Sigma-Aldrich. 2',3-dihydroxychalcone and 2',4-dihydroxychalcone were synthesized in our laboratory by Claisen-Schmidt condensation (4) and identified by chromatographic and spectroscopic techniques (TLC, UV-Vis, IR, RMN). Oxacillin, nalidixic acid and different chalcones solutions were prepared in absolute ethanol and diluted for antimicrobial assays.

**Bacterial strains.** *Staphylococcus aureus* ATCC 43 300 (MRSA) strain and *Escherichia coli* ATCC 25 922 (purchased from American Type Culture Collection), maintained by successive subcultures in trypticase soy agar BBL (Becton Dickinson) at 4 ºC, were used.

**Culture media.** Broth and agar nutritive and broth and agar Müller-Hinton (Oxoid) were used.

**Kinetic-turbidimetric assays.** In order to determine quantitatively the sensitivity of *S. aureus* and *E. coli* to oxacillin and nalidixic acid in combination with dihydroxylated chalcones, a previously developed kinetic-turbidimetric method was employed (3).

Antimicrobial activity of ternary combination against *S. aureus* was determined using an inoculum prepared from a 24 h culture of *S. aureus* ATCC 43 300 in agar slant, transferred to 30 mL of Müller-Hinton broth and incubated 18 h at 35 ºC with permanent stirring. Every kinetic experiments of microbial growth was performed in Erlenmeyer flasks containing 100 mL of Müller-Hinton broth, 2',3-dihydroxychalcone and oxacillin in constant concentration (6 µg.mL⁻¹) and increasing concentrations of 2',4-dihydroxychalcone. The kinetic started after the addition of 2 mL of previously prepared inoculum. Subsequently, Erlenmeyer flasks were incubated in a Rosi 1000 culture chamber (35 ºC, 180 rpm). Aliquots were extracted at 20 min intervals for 5 h and the transmittances were measured at 720 nm. A flask without antibiotic and other containing 2',3-dihydroxychalcone and oxacillin (6 µg.mL⁻¹) were used as controls.

On the other hand, antimicrobial activity against *E. coli* ATCC 25 922 was determined performing a similar experiment in presence of the corresponding ternary combination.

The following equations:

\[
\ln N_t = 27.4 - 10.3 \cdot T \\
\ln N_t = 27.1 - 8.56 \cdot T
\]

where the transmittance (T) values at 720 nm were related to the number CFU/mL (Nₜ) (3), allowed us to evaluate ln Nₜ.

**Results**

The microbial growth can be expressed by the equation:

\[
\ln N_t = \ln N_0 + \mu \cdot t
\]

where t is time in min, N₀ is CFU/mL at t = 0, Nₜ is CFU/mL at t = t and μ is specific growth rate (min⁻¹).

Specific growth rates values in media containing 2',3-dihydroxychalcone and oxacillin constant concentration and increasing 2',4-dihydroxychalcone concentration were obtained from the exponential phase of ln Nₜ vs. t plots for *S. aureus*. Table I exhibits the specific growth rates of *S. aureus* obtained in...
presence of this ternary combination and minimum inhibitory concentration (MIC) value. In Table II the results obtained for the ternary combination against the Gram (-) microorganism are informed. All the results are in accordance with the bacteriostatic inhibition mechanism previously proposed (3), where the specific growth rate ($\mu$) varies with the drug concentration in a linear relation leading to the following equation:

$$\mu = \mu_T - k_I \times C$$  \hspace{1cm} (4)

where $\mu_T$: specific growth rate without drug (min$^{-1}$) (control), $k_I$: specific inhibition rate (mL.$\mu$g.$^{-1}$min$^{-1}$) and C: drug concentration (mL.$\mu$g$^{-1}$).

MIC was determined in previous experiments for a binary mixture where oxacillin concentration was kept constant and 2’, 4-dihydroxychalcone concentration was varied. The obtained value was 22.4 µg.mL$^{-1}$. The corresponding experiment against *E.coli* using 2’,4-dihydroxychalcone-nalidixic acid combination showed a MIC value of 66.5 µg.mL$^{-1}$ (5).

**Conclusions**
It is very well known that the specific growth rate values of the microorganisms used decrease in presence of oxacillin (for *S. aureus*) or nalidixic acid (for *E. coli*). The results obtained in this work show that there is synergism for both combinations assayed and they were more effective than binary combinations (MIC$_{S. aureus}$ = 22.4 µg.mL$^{-1}$ and MIC$_{E. coli}$ = 66.5 µg.mL$^{-1}$). These results are very interesting in further investigations to obtain a greater antimicrobial effect against Gram (+) and Gram (-) microorganisms.

**Acknowledgments**
This work was supported by San Luis National University, Argentina.

**References.**

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# Corresponding author: e-mail: jmtalia@unsl.edu.ar

### Table I. Specific growth rates for *S. aureus* ATCC 43 300 (MRSA) in presence of ternary combination.

<table>
<thead>
<tr>
<th>C</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
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<tr>
<td>$\mu$</td>
<td>0.0240</td>
<td>0.0214</td>
<td>0.0167</td>
<td>0.0154</td>
<td>0.0129</td>
<td>0.00687</td>
<td>0.00258</td>
<td>0</td>
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</table>

C: 2’,4-dihydroxychalcone concentration (µg.mL$^{-1}$); $\mu$: specific growth rate (min$^{-1}$). MIC: 17.5 µg.mL$^{-1}$.

### Table II. Specific growth rates for *E. coli* ATCC 25 922 in presence of ternary combination.

<table>
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<tr>
<th>C</th>
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<tr>
<td>$\mu$</td>
<td>0.0246</td>
<td>0.0222</td>
<td>0.0205</td>
<td>0.0188</td>
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</table>

C: 2’,4-dihydroxychalcone concentration (µg.mL$^{-1}$); $\mu$: specific growth rate (min$^{-1}$). MIC: 56.9 µg.mL$^{-1}$.
IN VITRO INHIBITION OF Trypanosoma cruzi BY TRIFLURALIN

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Introduction
Zaidenberg et al. demonstrated the efficacy of trifluralin in an experimental model for Chagas disease [1,2] of mice infected with highly virulent trypomastigote Trypanosoma cruzi (H510 C8 C3 clone) [1]. However, in vitro studies were performed from a low-pathogenic clone (Bra C15 C2) [2,3]. The study is aimed at comparing the efficacy of in vitro trifluralin on two T. cruzi epimastigote clones (H510 and Bra C15 C2).

Materials and methods
We evaluated the activity of variable final trifluralin concentrations against epimastigotes (H510 C8 C3 and Bra C15 C2 clones) cultured in F-29 at 27º C [4]. After 72-h incubation, parasites were stained with Wright-Giemsa stain and counted in a Neubauer chamber. Benznidazole and Allopurinol were used as reference drugs.

Results
The 50% inhibitory concentration (IC50) for clones H510 C8 C3 and Bra C15 C2 was 2.26 and 3.3 µg/ml, respectively, whereas the 90% inhibitory concentration (IC90) was 8.86 and 10.9 µg/ml, respectively. On the other hand, benznidazole IC50 and IC90 for clone Bra C15 C2 were 3.2 and 48.3 µg/ml, respectively.

Conclusions
The inhibitory activity of trifluralin was higher for clone H510 C8 C3 compared to clone Bra C15 C2. With these results, and the drug’s kinetics in mice [5], it would be possible to predict a favorable prognosis in the search for an adequate therapy for Chagas disease.

References
### Table 1.
Trifluralin inhibition against clone H510 C2 C3 epimastigotes

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Number of parasites</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>(DMSO)</td>
<td>2466 667</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>230 000</td>
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<tr>
<td>20</td>
<td>25 000</td>
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<td>30</td>
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<tr>
<td>100</td>
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<tr>
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<tr>
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### Table 2.
Trifluralin inhibition against clone Bra C15 C2 epimastigotes

<table>
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<th>% inhibition</th>
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<tr>
<td>46</td>
<td>352 500</td>
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<td>272 500</td>
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<tr>
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<td>Lisis</td>
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</table>

Villagra S’e-mail: sergionicvillagra@yahoo.com.ar
PRELIMINARY ANTIFUNGAL AND PHYTOCHEMICAL STUDY OF *Minthostachys verticillata* EXTRACTS

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**Introduction**

*Minthostachys verticillata* is an aromatic shrubs found in the region of South America Andes of great ethnobotanical interest in Argentina. There have been many studies to determine the biological activities of *M. verticillata*, but little is known about its antifungal activity (1).

**Materials and methods**

The plant was collected in Córdoba province, Argentina in May 2008 and was identified by Dr. Marta Ojeda, professor in the Universidad Nacional de Córdoba (voucher number MSO_32). The vegetable was powdered and n-Hexane (HE), Chloroform (CE), Methanol (ME), Cold Aqueous (CAE) and Warm Aqueous (WAE) extract were obtained. Antifungal activity was determined by microbroth dilution method against *Fusarium graminearum*, *Fusarium solani*, *Fusarium verticillioides* and *Macrophomina phaseolina* (2), phytochemical analysis was made on thin layer chromatography to identify the major constituents (3). Hexane and CE were submitted to flash chromatography and the fractions obtained were evaluated against the phytopathogenic strains.

**Results**

Antifungal assays found inhibitory activity mainly in HE and CE of *M. verticillata* against *F. graminearum* and *M. phaseolina* with Minimum Inhibitory Concentration (MIC) values between 500 and 1000 µg ml⁻¹ (Table 1). Phytochemical analysis indicates the presence of terpenoids and steroids in both active extracts. CE and HE were fractionated by flash chromatography, eleven and nine fractions were obtained respectively. Fraction C of HE was the most active, and *F. graminearum* and *M. phaseolina* (CIM 250 µg ml⁻¹) were the most sensitive species. This fraction was rich in a triterpene compound.

**Conclusions**

These results indicate that the hexane and chloroform extract of *M. verticillata* have inhibitory activity of fungal growth. Flash chromatography and preliminary phytochemical analysis helped to isolate a Fraction rich in a triterpene compound of nonpolar nature with high antifungal activity against *F. graminearum* and *M. phaseolina*, both important plant pathogens of crops.

**Acknowledgments**

The authors would like to thank CONICET, Universidad Nacional de Río Cuarto and PICTOR program, BID 1728 /OC-AR, for financial support.

**References.**


# Corresponding author. E-mail: vvogt@exa.unrc.edu.ar
<table>
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<tr>
<th>Extract, Fraction</th>
<th>( F. ) graminearum</th>
<th>( F. ) solani</th>
<th>( F. ) verticillioides</th>
<th>( M. ) phaseolina</th>
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<td>500</td>
<td>&gt;2000</td>
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</tr>
<tr>
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<td>1000</td>
<td>&gt;2000</td>
<td>&gt;2000</td>
<td>1000</td>
</tr>
<tr>
<td>Fraction C (HE)</td>
<td>250</td>
<td>2000</td>
<td>1000</td>
<td>250</td>
</tr>
</tbody>
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EVALUACIÓN DE LA PRESCRIPCIÓN DE OXIGENO EN UN HOSPITAL PÚBLICO DE RIO CUARTO-CÓRDOBA

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¹Alumnas de la Carrera de Especialización en Farmacia Hospitalaria

Introducción
La oxigenoterapia es una medida terapéutica utilizada en Servicios de Internación General y Cuidados Intensivos de adultos, pediátricos y neonatos en Instituciones de Salud, Públicas y Privadas. El oxígeno es un medicamento que requiere indicación y monitoreo. El objetivo del estudio fue evaluar si el Personal Médico cumple con las pautas de prescripción requeridas para la oxigenoterapia, con el fin de efectuar intervenciones farmacéuticas encaminadas a controlar/mejorar la gestión y utilización de este medicamento esencial que impacta en la salud de los pacientes así como en los gastos hospitalarios.

Materiales y Métodos
Este trabajo se realizó en el Hospital de Río Cuarto, Polivalente, III Nivel de Complejidad, 212 camas. El estudio fue observacional, descriptivo, transversal y prospectivo, basado en observación directa del paciente y examen de hojas de indicación en Historias Clínicas de pacientes adultos, pediátricos y neonatos no intubados, que se encuentran internados en el Hospital. Se relevó la prescripción de oxigenoterapia, utilizando un cuestionario (Anexo 1) que fue completado por observación directa del paciente y examen de hojas de indicación en Historias Clínicas. Los pacientes fueron clasificados en adultos, pediátricos y neonatos. La prescripción se consideró correcta cuando se explicitaban los siguientes parámetros: (1) la concentración de oxígeno de la mezcla y flujo suministrado al paciente; (2) si se encontraba indicado el monitoreo.

Resultados y discusión
La demanda de oxigenoterapia en pacientes hospitalizados, que no se encuentran intubados, corresponde al 38%. Las Historias Clínicas evaluadas correspondieron: 57% a Pacientes adultos, 28% a Pediátricos y 15% a Neonatos. (Tabla 1). Se evidencio que en el total de la muestra un 46,8% tenía escrito en la Historia Clínica la indicación y el monitoreo. De las prescripciones correctas un 84,4% se observó en las UTIs mientras que solo el 9,4% en las áreas de Internación General (Tabla 2). El 53,2% del total de las prescripciones no contaba con la indicación correcta, observándose indicaciones de O2 - S.O.S o administración por indicaciones verbales, sin fines terapéuticos o para efecto placebo. De las prescripciones incorrectas, un 15,6% fue observada en las UTIs y un 90,6% en Internación General. De la valoración de estos datos surge que en las UTIs hay mayor adhesión a las correctas normas de prescripción que en las salas de internación general.

Conclusiones
El 53,2% de las prescripciones del oxígeno no son correctas. Los resultados ponen de manifiesto la falta de conocimiento en la prescripción y uso racional del gas medicinal por parte del personal médico. Se propone la intervención del farmacéutico a través de actividades educativas dirigidas al personal del hospital y a pacientes que reciben oxigenoterapia que puedan redundar en un mejor uso y control de las terapias con oxígeno.

ANEXO 1

<table>
<thead>
<tr>
<th></th>
<th>SI</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se encuentra indicada la oxigenoterapia en la Historia Clínica?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>En la indicación se coloca la concentración y el flujo?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>El monitoreo está indicado?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

393
Tabla 1: Pacientes con oxigenoterapia relevados vs. ocupación hospitalaria.

<table>
<thead>
<tr>
<th>PACIENTES</th>
<th>Servicio</th>
<th>Nº casos relevados</th>
<th>% relativo n=232</th>
<th>dia cama</th>
<th>% relativo ocupación por servicio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADULTOS</td>
<td>Uti/Uco</td>
<td>23</td>
<td>9,9%</td>
<td>71</td>
<td>32,4%</td>
</tr>
<tr>
<td></td>
<td>Clinica Medica</td>
<td>61</td>
<td>26%</td>
<td>188</td>
<td>32,5%</td>
</tr>
<tr>
<td></td>
<td>Clin. Quirurgica</td>
<td>48</td>
<td>21%</td>
<td>191</td>
<td>25%</td>
</tr>
<tr>
<td>PEDIATRICOS</td>
<td>Uti Pediatria</td>
<td>12</td>
<td>5,2%</td>
<td>18</td>
<td>66%</td>
</tr>
<tr>
<td></td>
<td>Pediatria</td>
<td>55</td>
<td>22,8%</td>
<td>155</td>
<td>35,5%</td>
</tr>
<tr>
<td>NEONATOS</td>
<td>Uti/Uci Neo</td>
<td>35</td>
<td>15%</td>
<td>96</td>
<td>36,5%</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>232</td>
<td>100%</td>
<td>719</td>
<td>38%</td>
</tr>
</tbody>
</table>

Tabla 2: Prescripción de la oxigenoterapia por personal médico

<table>
<thead>
<tr>
<th>SERVICIO</th>
<th>% INDICACION CORRECTA y MONITOREO</th>
<th>% INDICACION INCORRECTA O SIN INDICACION</th>
<th>% INDICACION CORRECTA POR COMPLEJIDAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTI/UCO ADULTOS</td>
<td>83%</td>
<td>17%</td>
<td>84,4%</td>
</tr>
<tr>
<td>UTI PEDIATRIA</td>
<td>73%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>UTI/UCI NEO</td>
<td>97%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>CLINICA MEDICA</td>
<td>8%</td>
<td>92%</td>
<td>9,4%</td>
</tr>
<tr>
<td>CLINICA QUIRURGICA</td>
<td>5%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>PEDIATRIA</td>
<td>15%</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>PROMEDIO</td>
<td>46,8%</td>
<td>53,2%</td>
<td></td>
</tr>
</tbody>
</table>

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ACTIVIDAD PRÁCTICA PARA ANALIZAR Y DISCUTIR EL CONCEPTO DE USO RACIONAL DE MEDICAMENTOS (URM) CON FARMACÉUTICOS, APLICADO EN ÁMBITOS ASISTENCIALES ESPECÍFICOS.

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Departamento de Farmacia, Facultad de Ciencias Químicas (FCQ), Universidad Nacional de Córdoba (UNC), Argentina.

Introducción
El uso irracional de los medicamentos es un problema urgente y generalizado, con graves consecuencias para los pacientes, como reacciones adversas a los medicamentos, aumento de la resistencia a los antimicrobianos y despilfarro de recursos (1,2).

Definiciones de URM de la OMS (2-4):
✓ Los pacientes reciben la medicación adecuada a sus necesidades clínicas, en las dosis correspondientes a sus requisitos individuales, durante un periodo de tiempo adecuado y al menor costo posible para ellos y para la comunidad.
✓ Uso terapéuticamente justificado y costo-eficaz de los medicamentos entre los profesionales de la salud y los consumidores.

La noción de URM como “frase hecha” está muy difundida entre los profesionales del equipo de salud. Sin embargo, no existe certeza de que todos comprendan cabalmente el concepto asociado (1-6). Para analizarlo y discutirlo con farmacéuticos, se realizó un taller específico dentro del módulo de Atención Farmacéutica I de la carrera de Especialización en Farmacia Hospitalaria (EFH), FCQ-UNC.

Objetivo: presentar los resultados de un taller sobre URM dictado en el marco de la carrera de EFH.

Material y métodos
El taller de URM se realizó durante el primer cuatrimestre de la carrera EFH. La actividad consistía en un breve repaso teórico de los conceptos. Luego, se les indicaba a los participantes que, por grupos, identificaran situaciones asociadas al uso irracional del medicamento en sus lugares de trabajo. Cada situación debía vincularse claramente con un paso de la cadena terapéutica del medicamento (7). La siguiente consigna era señalar cuáles de las situaciones descriptas eran propias de la incumbencia profesional farmacéutica (8-10). Por último, debían proponer estrategias para revertir este uso irracional.

Las situaciones identificadas se presentaron en plenario para lograr el consenso al momento de aclararlas y unificarlas. Seguidamente, se agruparon según la etapa de utilización correspondiente.

El listado de estrategias fue unificado y agrupado con posterioridad al taller, por los docentes-investigadores.

Resultados
Participaron 22 farmacéuticos en 5 grupos.
Se identificaron 37 situaciones asociadas a los pasos de: prescripción, administración, dispensación y uso. De ellas, en 43,2% participa directamente el farmacéutico y en 91,9% podría/debería intervenir. Estas situaciones fueron agrupadas en 12 asociadas a la prescripción, 4 a la administración, 7 a la dispensación y 4 al uso. Total de estrategias propuestas: 46, que fueron posteriormente revisadas y unificadas en 14. A continuación, se reagruparon según si la iniciativa para el cambio puede o debe originarse en el SF (N=9) o si se trata de una estrategia inter-multidisciplinaria (N=5). Aquellas que asume el SF bajo su responsabilidad, corresponden a incumbencias del farmacéutico y 3 son exclusivas (8-10).

Conclusiones
La etapa de la cadena terapéutica del medicamento con más cantidad de situaciones de uso irracional fue la prescripción, seguida por la dispensación. En más del 90% del total de casos identificados el farmacéutico podría y/o debería intervenir.

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La mayoría de las estrategias propuestas en el taller consisten en iniciativas de cambio que deben ser implementadas por el SF, por razones de incumbencia profesional, dentro del contexto institucional y del trabajo en equipo (8-13).

Agradecimientos:
A los especializando que participaron del taller y a la Dra. María Eugenia Olivera, directora de la EFH.

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Introducción
El Laboratorio de Hemoderivados es una industria farmacéutica dependiente de la Universidad Nacional de Córdoba, productora de medicamentos derivados del plasma humano, inyectables genéricos y productos de tejido óseo humano. Desde hace 15 años ofrece un espacio para el desarrollo de las prácticas profesionales a los alumnos de la carrera de Farmacia.

Los lugares en el Laboratorio son muy solicitados, ya que los jóvenes son cada vez más conscientes de la necesidad de incorporarse al mundo laboral actual, caracterizado por ser altamente exigente. Hemoderivados brinda una experiencia inicial de formación y posibilita extender el proceso educativo más allá de los límites de aula, en el manejo de conceptos y herramientas específicas de calidad, que garantizan el correcto accionar en procesos de elaboración, control, almacenamiento, distribución e información médica de los medicamentos producidos, armonizando este aprendizaje con las necesidades académicas definidas por la Facultad de Ciencias Químicas.

Materiales y Métodos
El plan de trabajo diseñado por profesionales docentes que lleva a cabo la tarea de instructores (IPF), comprende:

- Rol del farmacéutico en la industria.
- Herramientas de calidad
- Buenas prácticas de fabricación y control
- Disposiciones y normativas nacionales e internacionales
- Teoría y práctica de normas de bioseguridad.
- Comprensión y ejecución de procedimientos operativos estándar.
- Registro e interpretación de datos y métodos estadísticos.
- Destreza en el manejo de instrumental de laboratorio.
- Desarrollo de habilidades analíticas y espíritu crítico.

Resultados:
Los alumnos reciben supervisión directa por parte del IPF y del Coordinador. El resultado de este monitoreo es la evaluación y calificación por el IPF. Se evalúa responsabilidad, relación alumno-IPF, interés, iniciativa, espíritu crítico y resolución de problemas.

¿Cuál ha sido la respuesta de los alumnos? En general, han mostrado interés y buena disposición que los han llevado a postularse o a ser seleccionados para cubrir puestos de trabajo en el Laboratorio. Actualmente 19 de 57 profesionales egresados de Cs. Químicas (33,3%) que trabajan en la planta, fueron seleccionados por su desempeño como practicantes. De esta manera podemos contar con una fuerza laboral joven, con ganas de aprender y con una energía que se transmite al medio en el que se desempeñan.

Conclusiones:
El espacio brindado al practicanto profesional de la carrera de Farmacia es enriquecedor para el alumno y la institución. Los alumnos tienen la oportunidad de acceder a:

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• Conocimiento de los requerimientos del mercado laboral y familiarización con el ámbito de trabajo.
• Aplicación de modelos teóricos desarrollados durante la carrera.
• Establecer relaciones profesionales y laborales.
• Enriquecer el Curriculum-Vitae
• Facilitar el acceso a opciones de formación para el trabajo, educación y mejorar la preparación para futuras opciones laborales
• Conocer el funcionamiento de una planta industrial única en Sudamérica, una empresa sin fines de lucro, con un marcado objetivo social dirigido a brindar a nuestra comunidad medicamentos de calidad, eficaces, seguros y económicamente accesibles.

A demás, el paso de los alumnos, le permite seleccionar jóvenes profesionales dispuestos a formar parte de nuestro proyecto, en estos casos es más conveniente ya que han sido capacitados para tareas complejas.
MINI-TRABAJOS DE CAMPO SOBRE SUSTANCIAS ORGÁNICAS MEDICAMENTOSAS. UN APORTÉ A LA EDUCACION FARMACEUTICA

Molina, M N

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Introducción

Los profesionales farmacéuticos son los encargados de aplicar hallazgos de investigaciones científicas y tecnológicas pertinentes en el ámbito de la salud, para beneficio y mejora de las condiciones de vida de la sociedad. Los desafíos educativos de la Ley de Educación Superior argentina, propenden a la conservación, producción y distribución del conocimiento socialmente significativo. La formación universitaria debe procurar alumnos capaces de operar con símbolos, ideas, representaciones, conceptos, desplegar habilidades comunicativas, tecnológicas y organizacionales, y además optar por conductas y valores éticos para desarrollarse a nivel personal y social (1-2-3).

La enseñanza de la química orgánica debe tender a un objetivo esencial de la educación científica: alentar a los estudiantes a convertirse en aprendientes autónomos, capaces de adquirir información de muchas fuentes, de sopesar alternativas y de arribar a conclusiones defendibles. Las estrategias didácticas deberán orientarse al autoaprendizaje y al desarrollo de habilidades metacognitivas. Una forma consiste en que el alumno ponga en práctica los conocimientos adquiridos de manera que pueda comprobar por su propia experiencia en la acción, el interés y la utilidad de esos aprendizajes. Al aplicarlos a otros contextos o situaciones, se enriquecen también sus construcciones cognitivas (4-5-6).

Se consideró útil un esquema cuyos propósitos son familiarizar a los alumnos con diversas técnicas de abordaje sobre algún tipo de referente de la realidad social y/o medioambiental que sea de interés investigar, procurar datos del entorno, organizarlos y formular hipótesis explicativas de lo observado (7-8).

La autor organizó una estrategia denominada mini-trabajo de campo de características acotadas. El tema elegido fue “Medicamentos derivados de ácidos carboxílicos aromáticos”.

Materiales y métodos

El trabajo se aplicó como prueba piloto a alumnos cursantes de Química Orgánica II (3° año) durante 2003 y 2004. Se los dividió en grupos pequeños con una Guía de tareas, bibliografía y consignas:

A- Medicamentos usados antiguamente, fuentes de obtención, fechas, descubridores. Drogas de síntesis actuales, estructura química, nomenclatura.
B - Visita a una farmacia y droguería cercanas para conocer las formas comecializables, farmacología, toxicidad, consumo por la comunidad, expendio.
C - Búsqueda similar en domicilios particulares.
D - Supuestos acerca de la fabricación, comercialización, usos.
E - Conclusión acerca del trabajo y opinión sobre la tarea grupal.

En clase teórica -semana siguiente- se expusieron los resultados del trabajo oral y escrito. La docente completó información sobre mecanismos de reacciones de obtención, propiedades químicas, físicas y espectroscópicas. En laboratorio, los alumnos realizaron las síntesis químicas de aspirina, salicilato de metilo y benzocaína.

Resultados

Las respuestas a las consignas, bibliografía, informe y participación fueron altamente positivas. Para todos los alumnos, el trabajo resultó muy satisfactorio y entusiasta, y los contenidos fueron mejor asimilados. Se sintieron implicados en su auto-aprendizaje y responsables; aportaron ideas nuevas e interés por temáticas relacionadas a la salud.

Conclusiones

El trabajo resultó una estrategia muy eficaz pues se relacionaron conocimientos teórico-prácticos de un modo innovador. Las actividades y contenidos estimularon el pensamiento científico. Se cumplió en gran parte, con los supuestos del auto y del meta-aprendizaje a partir de un tema de interés profesional.
Referencias
EVALUACIÓN FARMACOLÓGICA DE NUEVOS AGENTES ANTICONVULSIVOS.

Enrique A*, Samaja G, Pastore V, Bruno Blanch, L.
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Introducción
La epilepsia es una enfermedad neurológica que afecta al 0.5-1% de la población mundial según la WHO. A pesar del desarrollo logrado sobre nuevos agentes anticonvulsivantes, el tratamiento de la epilepsia sigue siendo insuficiente, por ello la búsqueda de agentes antiepilépticos continúa siendo un área de investigación muy importante en química medicinal.

La elección del modelo para determinar la actividad farmacológica es determinante en el descubrimiento de nuevos fármacos. El Máximal Electroshock Seizure (MES) y el test de pentilenetetrazol subcutáneo (ScPTZ) siguen siendo “normas de oro” para evaluar nuevos compuestos anticonvulsivos (1). Estos nos brindan una primera clasificación de la actividad de los fármacos, si bien no detectan la farmacorresistencia, y su validez está demostrada en que han sido capaces de seleccionar a todos los antiepilépticos usados en clínica, excepto el levetiracetam. Nuestro grupo de trabajo ha desarrollado tres líneas de síntesis de anticonvulsivos: 1) ésteres de myo-inositol, 2) compuestos heterocíclicos derivados de 1,2,3-oxatiazolidin-4-ona-2,2-dióxidos N-sustituidos y 3) sus α-hidroxiamidas intermediarias, compuestos que fueron evaluados mediante MES y ScPTZ. En la búsqueda de obtener mayor información del comportamiento farmacológico, se evaluaron algunos de ellos a una dosis de PTZ que permitiera el desarrollo de una convulsión completa, con tres fases bien definidas: 1) Mioclonías 2) Convulsión clónica generalizada 3) Extensión tónica o estatus epiléptico.

Materiales y métodos
Se utilizaron ratones Swiss (18–23 grs.) que fueron manipulados durante 6 días previos a la evaluación con el objeto de disminuir el stress. Se siguió el programa ADD del NIH (USA), en fase I. Además se administró PTZ en una dosis de 120 mg/kg registrándose la aparición y tiempo de latencia de cada una de las fases de la convulsión (2).

Resultados
Tanto los ésteres derivados del myo-inositol como los heterociclos miméticos de trimetadiona y fenitoína y sus α-hidroxiamidas intermediarias demostraron tener actividad anticonvulsiva a las dosis de 30 y/o 100 mg/kg frente a MES test. Al evaluarlos frente a PTZ 85 mg/kg, cinco compuestos del segundo y tercer grupo resultaron activos a las mismas dosis. En tres de las amidas se hallo actividad proconvulsivante a 30 mg/kg al registrarse una disminución de los tiempos de latencia de distintas fases de la convulsión frente al test PTZ 120 mg/kg. Además se observaron signos en el comportamiento luego de la administración, como sudoración, aumento de la frecuencia respiratoria y mioclonías a 100 mg/kg e incluso convulsión clónica a 300 mg/kg.
La totalidad de los animales superaron el test rotorod para neurotoxicidad a las dosis y tiempos evaluados.

Discusión
Los compuestos evaluados demostraron tener una significativa actividad anticonvulsiva, mostrando ser más activos que fármacos antiepilépticos usados actualmente, frente al mismo test. Los datos biológicos obtenidos nos permiten plantear cambios en las estructuras sintetizadas con el objetivo de optimizar su actividad.

Agradecimientos
CONICET/APCyT/U.N.L.P.

Referencias

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PATRÓN TEMPORAL DE RECUPERACIÓN LOMOYATORA DEL EFECTO SEDATIVO/ANESTÉSICO DEL PROPOFOL EN CODORNICES (Coturnix coturnix)

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Introducción
Desde hace unos años, el propofol es empleado tanto en medicina humana como en veterinaria para la inducción y mantenimiento de la anestesia y además posee efectos sedativos.

Últimamente se ha propuesto que el análisis de fluctuación con tendencias eliminadas (DFA) puede aportar información sobre la dinámica temporal del comportamiento adicional a la que usualmente se obtiene mediante los análisis tradicionales (1). En este trabajo se evalúa la organización y complejidad del patrón temporal de locomoción de codornices que fueron tratadas con dosis sedativas/anestésicas de propofol.

Materiales y Métodos
Ciento ochenta codornices juveniles (32-41 días de edad) fueron asignadas al azar a uno de los cinco tratamientos con droga (vehículo o propofol 10, 20, 40 u 80mg/kg). A los 10min de la administración i.p. de la droga, cada una de las aves fue individualmente alojada en una caja de campo abierto y su comportamiento de recuperación locomotora fue evaluado durante 45min. A intervalos de tiempo de 0,5s se registró si el ave se encontraba ambulando o permanecía inmóvil y la distancia que recorre en cada intervalo de tiempo.

Resultados
En la Tabla se observan los resultados del ANOVA sobre la latencia para iniciar la ambulación, la distancia ambulada y la tasa de ambulación.

Un ANOVA sobre el parámetro de autosimilitud (α, DFA) de las series que fueron monofractales (iguales propiedades fractales a todos los tamaños de ventanas) en la fase activa (una vez que el animal inició su ambulación) no mostró efectos significativos (P>0,05) de los factores evaluados (sexo y tratamiento) ni interacción entre ellos indicando que, una vez iniciada la ambulación, el patrón temporal de locomoción de los animales que fueron tratados con propofol es el mismo que el de las aves control y sugiriendo que se alcanzó una recuperación locomotora total.

Un análisis detallado de la distancia ambulada a intervalos de 5min mostró un efecto significativo del sexo (P=0,01), del factor tratamiento (P<0,0001), del intervalo de tiempo transcurrido desde la administración de la droga (P<0,0001), y una interacción entre el tratamiento y el tiempo transcurrido (P=0,009). Comparado con el vehículo, el tratamiento con propofol 40mg/kg redujo significativamente la distancia ambulada (P < 0,05) durante los primeros 30min de la prueba en las hembras y durante los primeros 20min y después de los 35min de la prueba en los machos.

Conclusiones
En codornices, el efecto sedativo-anestésico del propofol se observa para dosis iguales o mayores a 20mg/kg. Interesantemente, una vez que comienzan a ambular, las aves administradas con vehículo no diferencian en la locomoción de las tratadas con 20 o 40mg/kg. Esto implica una rápida capacidad del animal para recuperar un comportamiento ambulatorio normal luego de la administración de propofol y se puede explicar por las características farmacocinéticas de este compuesto (muy lipofílico, de rápida acción y de rápida eliminación). El presente trabajo es el primero que evalúa la dinámica temporal de la locomoción cuando el ave se está recuperando.

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Referencias.

Tabla. Análisis de la ambulación en un campo abierto de codornices hembras y machos administradas con vehículo (Tween 20%) o propofol 10, 20, 40 o 80 mg/kg.

<table>
<thead>
<tr>
<th>Sexo</th>
<th>Tratamiento</th>
<th>Lat. iniciar amb. (s)</th>
<th>Distancia amb. (m)</th>
<th>Tasa de amb. (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hembra</td>
<td>Vehículo</td>
<td>60,31 ± 25,37ª</td>
<td>53,31 ± 16,34b,c</td>
<td>0,021 ± 0,007a,c,e#</td>
</tr>
<tr>
<td>Hembra</td>
<td>Propofol 10</td>
<td>143,32 ± 48,34a,d</td>
<td>74,15 ± 16,63c,d</td>
<td>0,029 ± 0,007b,c</td>
</tr>
<tr>
<td>Hembra</td>
<td>Propofol 20</td>
<td>411,87 ± 130,91b,d</td>
<td>46,14 ± 10,25b,c</td>
<td>0,020 ± 0,004c,d</td>
</tr>
<tr>
<td>Hembra</td>
<td>Propofol 40</td>
<td>501,11 ± 178,16b,c</td>
<td>28,68 ± 9,94a,b</td>
<td>0,013 ± 0,004a,b</td>
</tr>
<tr>
<td>Hembra</td>
<td>Propofol 80</td>
<td>1130,56 ± 249,60c</td>
<td>14,47 ± 9,37a</td>
<td>0,007 ± 0,004a#</td>
</tr>
<tr>
<td>Macho</td>
<td>Vehículo</td>
<td>79,71 ± 34,70ª</td>
<td>101,81 ± 24,28c</td>
<td>0,039 ± 0,009b</td>
</tr>
<tr>
<td>Macho</td>
<td>Propofol 10</td>
<td>85,22 ± 37,76a,d</td>
<td>83,90 ± 14,92c</td>
<td>0,032 ± 0,006b,c</td>
</tr>
<tr>
<td>Macho</td>
<td>Propofol 20</td>
<td>166,42 ± 82,44b,d</td>
<td>104,61 ± 24,43c</td>
<td>0,039 ± 0,009b</td>
</tr>
<tr>
<td>Macho</td>
<td>Propofol 40</td>
<td>512,54 ± 226,97b,c</td>
<td>47,56 ± 12,86b,d</td>
<td>0,022 ± 0,006b,d,e</td>
</tr>
<tr>
<td>Macho</td>
<td>Propofol 80</td>
<td>705,00 ± 212,95c</td>
<td>9,72 ± 3,91a</td>
<td>0,006 ± 0,003a</td>
</tr>
</tbody>
</table>

Valor $P$:
- Sexo: 0,07, 0,02
- Tratamiento: 0,0004, <0,0001, <0,0001
- Sexo x Tratamiento: 0,44, 0,70, 0,38

Lat. Iniciar amb: latencia para iniciar la ambulación (s) es el tiempo desde el inicio de la prueba hasta que el animal inicia un periodo de al menos 2 s de ambulación continua; Dist. Amb.: distancia ambulada (m). Tasa de amb.: tasa de ambulación (m/s): distancia ambulada/ tiempo total de la prueba-latencia para iniciar la ambulación). Dentro de un mismo género, los tratamientos que no comparten una misma letra difieren entre sí ($P < 0,05$). Grupos que comparten # tienden a presentar diferencias entre sí ($P < 0,1$). Valor $P$: valor de probabilidad del ANOVA de doble vía para los factores sexo, tratamiento y de la interacción entre ambos factores (Sexo x Tratamiento).
CARACTERIZACIÓN ANÁTOMICA, QUÍMICA Y BIOQUÍMICA MEDIANTE ANÁLISIS DE PROCRUSTES GENERALIZADO DE ESPECIES DE Baccharis L.

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Introducción
La identificación de especies del género Baccharis L. es controvertida, especialmente la de las “carquejas”, utilizadas en medicina popular como hepatoprotectoras, diuréticas y colagogas. El objetivo del presente trabajo fue diferenciar a nueve de las mismas, B. articulata (Lam.) Pers., B. crispa Spreng., B. gaudichaudiana DC., B. microcephala (Less.) DC., B. phyteumoides (Less.) DC., B. penningtonii Heering., Baccharis sagittalis (Less.) DC., Baccharis triangularis Hauman y Baccharis trimera (Less) DC. (1) a través de la caracterización simultánea mediante tres tipos de datos (configuraciones): anatómicos y los obtenidos de perfiles proteicos y espectrofotométricos.

Materiales y métodos
El estudio de las diferentes poblaciones se efectuó mediante análisis de procrustes generalizado (APG) y agrupamiento jerárquico (UPGMA). Para confeccionar el APG, se utilizó como origen de las configuraciones que se deben consensuar, salidas de diferentes métodos de ordenación: análisis de componentes principales (ACP) para los datos anatómicos y espectrofotométricos y análisis de coordenadas principales (AcoorP) para datos de perfiles proteicos (2).

Resultados
En la evaluación por APG se observó que en el caso donde hay más de una población de la misma especie, éstas tienden a agruparse, y cuando las mismas están representadas por una única población, ocupan coordenadas específicas en el plano. El único par de especies para las cuales ésto no ocurrió fue el formado por B. articulata y B. penningtonii. Sin embargo, los resultados de las configuraciones individuales obtenidos del ACP y AcoorP usando únicamente los dos primeros componentes (R1 y R2) para datos anatómicos; el segundo y tercer componentes (R2 y R3) para datos espectrofotométricos y la primera y tercer coordenadas (C1 y C3) para perfiles proteicos, no lograron diferenciar adecuadamente a las especies. Por otro lado las correlaciones entre las configuraciones aisladas son bajas para el caso de datos anatómicos con datos espectrofotométricos (r = 0,1942, tabla 1) y de datos espectrofotométricos con datos electroforéticos (r = 0,2108, tabla 1). De lo anterior surge que las caracterizaciones individuales ofrecen información que puede ser considerada complementaria. En la tabla 1 también se puede observar que los tres tipos de configuraciones contribuyen distinto a la caracterización consenso dada por el APG, observándose una mayor influencia de los datos anatómicos (r = 0,5566) y una menor influencia de los datos espectrofotométricos (r = 0,2806), inclusive obteniéndose mejor correlación entre las matrices de distancia-similitud de datos anatómicos con datos electroforéticos (r = - 0,3903).

Conclusiones
De acuerdo a estos resultados concluimos que para caracterizar completamente a las nueve especies de Baccharis con tallos alados de Argentina, es necesario realizarles estudios endomorfológicos, espectrofotométricos y electroforéticos a la hora de identificarlas.

Referencias.
Tabla 1. Coeficiente de correlación ($r$) entre matrices de distancia y similitud de las caracterizaciones individuales y la caracterización conjunta (APG).

<table>
<thead>
<tr>
<th></th>
<th>Datos Anatómicos</th>
<th>Datos Espectrofotométricos</th>
<th>Datos electroforéticos</th>
<th>APG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Datos Anatómicos</strong></td>
<td>1</td>
<td>0,1942</td>
<td>-0,3903</td>
<td>0,5566</td>
</tr>
<tr>
<td><strong>Datos Espectrofotométricos</strong></td>
<td></td>
<td>1</td>
<td>-0,2108</td>
<td>0,2806</td>
</tr>
<tr>
<td><strong>Datos electroforéticos</strong></td>
<td></td>
<td></td>
<td>1</td>
<td>-0,3930</td>
</tr>
<tr>
<td><strong>APG</strong></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
ANTRAQUINONAS Y ACTIVIDAD TRIPANOCIDA EN Picramniaceae DE LA ARGENTINA

Travaini ML a, Betucci G a, Rodriguez MV a, Di Sapio O b, Nocito I b, Gattuso M a, Cortadi A a, Martinez ML b.


Introducción
Algunas especies de la familia Picramniaceae son empleadas tradicionalmente para el tratamiento de enfermedades parasitarias. Estudios fitoquímicos realizados en especies del género Picramnia llevaron a la identificación de antraquinonas con actividad contra distintos protozoos (1) justificando así su utilización popular. Recientemente se ha aislado la ácido 6-cloro-9,10-dihidro-4,5,7-trihidroxi-9,10-dioxo-2-antracenocarboxílico, que inhibe fuerte y específicamente a la trans-sialidasa (3) del Trypanosoma cruzi, agente causal del Mal de Chagas. También se han identificado hidroxi-antraquinonas naturales que presentan actividad contra el mismo. Por lo tanto, el objetivo de este trabajo fue realizar un estudio de la actividad tripanocida y su posible relación con las antraquinonas de Alvaradoa subovata Cronquist. (As), Picramnia sellowii Planch. (Ps) y Picramnia parvifolia Engl.(Pp) (Picramniaceae).

Materiales y métodos
La madera, corteza y hojas de las tres especies fueron extraídas con diclorometano a temperatura ambiente y concentradas bajo condiciones de presión reducida. Los extractos obtenidos fueron utilizados para ensayos antiparasitarios y fitoquímicos.

Ensayos antiparasitarios: Epimastigotes de T. cruzi fueron incubados con cantidades crecientes de cada extracto disuelto en DMSO, se utilizó Beznidazol como control positivo.

Ensayos fitoquímicos: Se realizaron cromatografía en capa delgada (CCD) en placas de Silica gel 60 F254, diferentes fases móviles y reveladores y Cromatografìa líquida de alta eficiencia (CLAE) para la que se utilizó una columna C18 y fase móvil: Metanol:Ac Acético 0,5% (85:15).

Resultados
Los resultados de la actividad antiparasitaria se observan en la Tabla 1. Los extractos más activos fueron los de madera de As y corteza de Ps, con un porcentaje de inhibición del crecimiento del epimastigote de T. cruzi, del 80% y 62% respectivamente. Cuando los extractos fueron analizados por CCD se observó que la mayor actividad tripanocida se correlacionaba con una mayor presencia de antraquinonas. Se realizó CLAE a los extractos más activos y se corroboró la presencia de crisofanol como compuesto mayoritario y emodina. En el extracto de hoja de Ps se detectó reína como compuesto mayoritario, sin embargo este extracto posee sólo un 35% de inhibición del crecimiento del parásito.

Conclusión
Los extractos de madera de As y corteza de Ps presentan una alta inhibición del crecimiento de T. cruzi. En estos extractos se encontró una mayor cantidad de antraquinonas, fundamentalmente crisofanol, con respecto al resto de los extractos ensayados.

References.

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Tabla 1. Porcentaje de inhibición del crecimiento de *Trypanosoma cruzi* en presencia de los extractos diclorometánicos de madera, corteza y hoja de *Alvaradoa subovata* (As), *Picramnia sellowii* (Ps) y *Picramnia parvifolia* (Pp)

<table>
<thead>
<tr>
<th>Extracto (100 µg/ml)</th>
<th>% de Inhibición</th>
</tr>
</thead>
<tbody>
<tr>
<td>As Corteza</td>
<td>25</td>
</tr>
<tr>
<td><strong>As Madera</strong></td>
<td><strong>80</strong></td>
</tr>
<tr>
<td>As Hoja</td>
<td>50</td>
</tr>
<tr>
<td>Ps Corteza</td>
<td>62</td>
</tr>
<tr>
<td>Ps Madera</td>
<td>46</td>
</tr>
<tr>
<td>Ps Hoja</td>
<td>35</td>
</tr>
<tr>
<td>Pp Corteza</td>
<td>14</td>
</tr>
<tr>
<td>Pp Madera</td>
<td>28</td>
</tr>
</tbody>
</table>

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LA FORMACIÓN FARMACÉUTICA DESDE LA INNOVACIÓN CURRICULAR: 
EXPERIENCIAS DEL TALLER DE PROBLEMÁTICA PROFESIONAL II EN LA 
FACULTAD DE CIENCIAS BIOQUÍMICAS Y FARMACÉUTICAS (UNR)

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Introducción
La experiencia del TPPII29 se inserta en las políticas de innovación curricular llevadas adelante por la
FCByF30 a partir del año 2006, principalmente a través de un nuevo Plan de Estudio para la carrera Farmacia. El mismo presenta un diseño curricular organizado a partir de dos ejes de formación: uno que
contempla los contenidos disciplinares y otro que incorpora elementos para el análisis de los aspectos
sociales y epistemológicos asociados al accionar profesional. Ambos ejes se articulan y confluyen en un
espacio curricular de Práctica Profesional, ubicado en el último año de la carrera.
Así, se incorporan espacios curriculares de Acercamiento a la Problemática Profesional31, cuyo propósito
es familiarizar a los estudiantes desde el inicio de su formación con el campo profesional específico,
integrar los conocimientos que van adquiriendo con la práctica profesional futura e implicarlos en una
práctica profesional ética y socialmente responsable que profundice el rol social y los valores éticos que
demanda la profesión. Estos espacios curriculares pretenden construir una base conceptual que,
profundizada luego en el ciclo de formación profesional, contribuirá a fortalecer el espacio de Práctica
Profesional.
Para concretar este proyecto se diseñan e instrumentan diversos dispositivos de formación para la Práctica
Profesional, entendidos como un conjunto interrelacionado de mecanismos, condiciones, espacios,
tiempos, recursos y personas.

Materiales y métodos
En el TPPII, y a partir de su implementación en el año 2009, se definió como dispositivo de formación la
observación de las actividades que se desarrollan en las oficinas de farmacia como uno de los ámbitos de
inserción profesional, con el objetivo de identificar las diversas variables que afectan el proceso de
dispensación para su posterior análisis.
Para la construcción de este dispositivo se abordaron temáticas y problemáticas propias del ejercicio
profesional farmacéutico: gestión de farmacias comunitarias e institucionales, comunicación profesional-
paciente, Buenas Prácticas de Dispensación, atención farmacéutica, nuevas tecnologías en la producción
de fármacos y en el diseño de medicamentos para el tratamiento de enfermedades huérfanas e
intervenciones profesionales. Como así también se contempló la participación de profesionales
especialistas para el desarrollo de dichas temáticas.

Resultados
Durante el año académico 2009 (primer año de implementación del TPPII), 229 estudiantes cursaron el
espacio, de los cuales 219 realizaron observaciones en 156 oficinas de farmacia, situadas en el
microcentro, macrocentro y en diferentes barrios de la ciudad de Rosario.

Conclusiones
La implementación del TPPII permitió a los estudiantes de farmacia un acercamiento temprano a los
fundamentos de la Práctica Profesional, con el objetivo de reconocer las funciones del farmacéutico
vinculadas a la atención farmacéutica, la industria farmacéutica, la gestión y administración de una

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29 Taller de Problemática Profesional II, espacio curricular perteneciente al 3º año de la carrera de Farmacia.
30 Facultad de Ciencias Bioquímicas y Farmacéuticas
31 Estos espacios comprenden: Seminario Introductorio a la Problemática Farmacéutica, Taller de Problemática
Profesional I y Taller de Problemática Profesional II, pertenecientes a 1º, 2º y 3º año de la carrera de Farmacia,
respectivamente.
farmacia comunitaria y/o institucional, como así también comprender la implicancia social y profesional de los avances tecnológicos en el desarrollo y producción de fármacos.

A partir de la reflexión crítica dada en el ámbito del TPPII se generaron disposiciones favorables a una visión social y ética de la Formación Farmacéutica.

**Referencias**


DOCKING APLICADO AL ESTUDIO DE INHIBIDORES DE C-MET QUINASA

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Introducción
Las Quinoxalinas son candidatos atractivos en química medicinal, debido a su capacidad de generar respuestas biológicas al interactuar con diversos objetivos biológicos. En particular, han revelado cierto potencial farmacológico como anticancerígenos, mediante su acción inhibitoria frente a c-Met quinasa. Utilizando metodologías de screening y posterior optimización estructural, Porter y colaboradores (1) detectaron una serie de derivados de quinoxalina capaces de inhibir selectivamente el sitio de unión al ATP de c-Met. Adicionalmente, estos investigadores pudieron cristalizar c-Met unida a una de las quinoxalinas estudiadas (1).

Con el objetivo de conocer o determinar las características estructurales necesarias para la interacción con el receptor, y de interpretar las diferencias de actividad encontradas para los distintos inhibidores, presentamos un estudio mediante Docking de las interacciones de las quinoxalinas con c-Met quinasa.

Materiales y métodos
Se utilizó el programa Autodock4.0. El receptor se preparó a partir de la estructura cristalográfica depositada (1). Las moléculas de agua, el ligando original (denominado “28”) y las γ-butilrolactonas cristalizadas fueron eliminadas de la estructura cristalográfica para el cálculo y los átomos de hidrogeno se agregaron utilizando el programa Amber 9.

Los parámetros para el cálculo fueron seleccionados de manera de poder reproducir la conformación experimental encontrada para “28”: Las cargas se calcularon por el método de Gasteiger y el Docking se realizó usando metodologías basadas en algoritmos genéticos (LGA). Se realizaron 50 corridas para cada ligando, considerando al receptor como una molécula rígida y a las quinoxalinas como flexibles. Los datos de actividad biológica fueron tomados de literatura (1).

Resultados
Se observó que Autodock es capaz de predecir la conformación activa del “28” con un error de RMSfit de 0.000 Å respecto a la estructura cristalográfica. Este resultado aumenta nuestra confianza en las conformaciones activas obtenidas para las otras quinoxalinas del set, de las cuales no se dispone de datos experimentales. En todos los casos se encuentra una interacción entre el N-4 de quinoxalinas y el residuo Met1160 dec-Met. Respecto a los valores de energías de unión predichas, se sabe que, en general los programas de Docking permiten una evaluación relativa de la energía de interacción, pero esto debe tomarse como una aproximación dado que estos cálculos suelen sobrepesar interacciones lipofílicas y, en este caso, no modelar la flexibilidad de la proteína. Sin embargo para este sistema se ha encontrado una correlación entre las energías de unión predichas y los valores de actividad experimentales para la mayoría de las moléculas, permitiendo la diferenciación de los ligandos promisorios.

Conclusiones
En el presente trabajo se aplicaron metodologías de docking para la simulación del reconocimiento molecular de quinoxalinas con c-Met. Esta metodología ha resultado exitosa para explicar de manera racional las diferencias en la actividad biológica, y para detectar las interacciones con el receptor que puedan estar influyendo en la acción inhibitoria.

Agradecimientos
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Referencias.

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EVALUACIÓN FARMACOLÓGICA DE NUEVOS AGENTES ANTICONVULSIVOS.

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Introducción
La epilepsia es una enfermedad neurológica que afecta al 0.5-1% de la población mundial según la WHO. A pesar del desarrollo logrado sobre nuevos agentes anticonvulsivantes, el tratamiento de la epilepsia sigue siendo insuficiente, por ello la búsqueda de agentes antiepilépticos continua siendo un área de investigación muy importante en química medicinal.

La elección del modelo para determinar la actividad farmacológica es determinante en el descubrimiento de nuevos fármacos. El Máximal Electroshock Seizure (MES) y el test de pentilenetetrazol subcutáneo (ScPTZ) siguen siendo "normas de oro" para evaluar nuevos compuestos anticonvulsivos. Estos nos brindan una primera clasificación de la actividad de los fármacos, si bien no detectan la farmacorresistencia, y su validez está demostrada en que han sido capaces de seleccionar a todos los antiepilépticos usados en clínica, excepto el leviteracetam. Nuestro grupo de trabajo ha desarrollado tres líneas de síntesis de anticonvulsivos: 1) ésteres de myo-inositol, 2) compuestos heterocíclicos derivados de 1,2,3-oxatiazolidin-4-ona-2,2-dióxidos N-sustituidos y 3) sus α-hidroxiamidas intermediarias, compuestos que fueron evaluados mediante MES y ScPTZ. En la búsqueda de obtener mayor información del comportamiento farmacológico, se evaluaron algunos de ellos a una dosis de PTZ que permitiera el desarrollo de una convulsión completa, con tres fases bien definidas: 1) Miclonías 2) Convulsión clónica generalizada 3) Extensión tónica o estatus epiléptico.

Materiales y métodos
Se utilizaron ratones Swiss (18–23 grs.) que fueron manipulados durante 6 días previos a la evaluación con el objeto de disminuir el estrés. Se siguió el programa ADD del NIH (USA), en fase I. Además se administró PTZ en una dosis de 120 mg/kg registrándose la aparición y tiempo de latencia de cada una de las fases de la convulsión.

Resultados
Tanto los ésteres derivados del myo-inositol como los heterociclos miméticos de trimetadiona y fenitoína y sus α-hidroxiamidas intermedias demostraron tener actividad anticonvulsiva a las dosis de 30 y/o 100 mg/kg frente a MES test. Al evaluarlos frente a PTZ 85 mg/kg, cinco compuestos del segundo y tercer grupo resultaron activos a las mismas dosis. En tres de las amidas se hallo actividad proconvulsivante a 30 mg/kg al registrarse una disminución de los tiempos de latencia de distintas fases de la convulsión frente al test PTZ 120 mg/kg. Además se observaron signos en el comportamiento luego de la administración, como sudoración, aumento de la frecuencia respiratoria y miclonías a 100 mg/kg e incluso convulsión clónica a 300 mg/kg.

La totalidad de los animales superaron el test rotorod para neurotoxicidad a las dosis y tiempos evaluados.

Discusión
Los compuestos evaluados demostraron tener una significativa actividad anticonvulsiva, mostrando ser más activos que fármacos antiepilépticos usados actualmente, frente al mismo test. Los datos bioquímicos obtenidos nos permiten plantear cambios en las estructuras sintetizadas con el objetivo de mejorar la actividad.

Agradecimientos
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**OBTENCIÓN DE MATERIALES NANOESTRUCTURADOS A PARTIR DE MATRICES POLIMÉRICAS CON FINES NEUROLÓGICOS.**

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**Introducción**
La encapsulación de principios activos es una tecnología ampliamente extendida[1], siendo uno de sus objetivos principales el control de la liberación de la droga, obteniéndose así una mayor efectividad y eficiencia en la administración de la misma. Los soportes usados en estos procesos de encapsulación pueden ser polímeros, hidrogeles, liposomas y materiales inorgánicos. El clorhidrato de fluoxetina\textsubscript{17}H\textsubscript{18}F\textsubscript{3}O, comercialmente conocido como Prozac\textsuperscript{®}, es un fármaco antidepresivo no heterocíclico usado ampliamente para el tratamiento de depresión, desorden obsesivo-compulsivo, bulimia nerviosa y pánico[2]. Investigaciones recientes sugieren que el mecanismo de acción de la fluoxetina es a través de la estimulación neurogénica del hipocampo[3,4,5].

En este trabajo se evaluó la obtención de materiales nanoestructurados de liberación controlada de fluoxetina a partir de un matriz polímérica y su posterior caracterización espectroscópica.

**Materiales y Métodos**
Los materiales nanoestructurados fueron obtenidos a partir de una matriz polimérica el Eudragit\textsuperscript{®} RL PO comercializados por la firma Evonik Röhm GmbH, por el método de doble emulsión modificada. Las nanopartículas (NPs) fueron obtenidas mediante un proceso de extracción/ evaporation del disolvente en el seno de una emulsión, donde una cantidad conocida fluoxetina fue disuelta en una solución etanólica. El sistema fue homogenizado usando un UltraTurrax\textsuperscript{®}. La dispersión obtenida en este primer paso fue emulsificada sobre una solución de NaOH que contiene el trietil citrato (20-40mg) a un rango de agitación de 12800-15000 rpm/min durante 15 min. Finalmente las NPs fueron centrifugadas y lavadas con pequeñas cantidades de agua secándose al vacío durante 8 horas. Este proceso fue realizado en diferentes condiciones experimentales como se puede ver en la tabla 1. Los análisis morfológicos fueron llevados a cabo por microscopia electrónica de barrido (JSM-5410LV) y por microscopia electrónica de transmisión (TEM-1200EXII). Los perfiles de liberación se midieron en un Espectrofotómetro U.V. (Modelo UV- Vis / Jasco V- 530) a 260 nm.

**Resultados**
Los mejores resultados fueron los obtenidos cuando se utilizó una agitación de 15000 rpm y 40mg de trietil citrato, a pesar de que el tamaño de las partículas obtenido fue algo mayor en este experimento (XII); sin embargo, la esféricidad y eficiencia de encapsulación fue mejor en comparación con el experimento (IX), lo cual indica un mejor control de la eficiencia de la encapsulación mediante los parámetros del proceso. (Tabla 2 y 3) sin afectar grandemente las características morfológicas de los materiales nanoestructurados.

**Conclusiones**
El control adecuado de los parámetros de síntesis, permitió obtener materiales nanoestructurados a partir de la matriz polímérica Eudragit\textsuperscript{®} RL PO. Se observó una morfología esférica en los materiales obtenidos por la técnica de microscopia TEM. El método de nanoencapsulación empleado permite incrementar el área superficial de las partículas, lo que aumentaría la solubilidad de la droga y permitiría favorecer la biodisponibilidad y efectividad de su liberación en un lugar específico. Con ello, existiría la posibilidad de eliminar o reducir los efectos colaterales que el fármaco puede producir y mejorar, en un futuro, su administración en pacientes.
Referencias

Tabla 1. Condiciones experimentales de los materiales nanoestructurados cargados con Fluoxetina.

<table>
<thead>
<tr>
<th>Experimento</th>
<th>Cantidad de principio activo (mg)</th>
<th>Cantidad de tensoactivo (mg)</th>
<th>Velocidad de agitación (rpm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>20</td>
<td>20</td>
<td>6600</td>
</tr>
<tr>
<td>XII</td>
<td>20</td>
<td>40</td>
<td>15000</td>
</tr>
<tr>
<td>XIV</td>
<td>-</td>
<td>40</td>
<td>15000</td>
</tr>
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</table>

Tabla 2: Determinación del diámetro de las materiales nanoestructurados.

<table>
<thead>
<tr>
<th>Experimento</th>
<th>Cantidad de partículas analizadas</th>
<th>Diámetro promedio (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>100</td>
<td>370.92</td>
</tr>
<tr>
<td>XII</td>
<td>100</td>
<td>472.02</td>
</tr>
<tr>
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<td>100</td>
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Tabla 3: Resultados obtenidos en el proceso de encapsulación.

<table>
<thead>
<tr>
<th>Experimento</th>
<th>Rendimiento de partículas de Fluoxetina (%)</th>
<th>Eficiencia de la encapsulación de Fluoxetina (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>64±0.4</td>
<td>37</td>
</tr>
<tr>
<td>XII</td>
<td>56±0.8</td>
<td>57</td>
</tr>
<tr>
<td>XIV</td>
<td>76±0.2</td>
<td>--</td>
</tr>
</tbody>
</table>

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