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RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

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RICiFa 2014

3rd International Meeting on Pharmaceutical Sciences
18-19 september, 2014
Córdoba, Argentine

Organized by:

FCQ Facultad de Ciencias Químicas

UNC Universidad Nacional de Córdoba

Co-organized by:

FCBF Facultad de Ciencias Bioquímicas y Farmacéuticas

Universidad Nacional de Rosario

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Cámara de Farmacias de la Provincia de Córdoba – Trabajando juntos, progresamos todos

JENCK

BRIEF REPORT ABOUT THE MEETING

The "3rd International Meeting of Pharmaceutical Sciences (RICiFa 2014)" was held in Cordoba, Argentina, on 18 and 19 September 2014. This meeting was organized by professors and researchers from the National University of Córdoba, Córdoba (Argentina), in collaboration with members of the National University of Rosario (Rosario, Santa Fe, Argentina).

The main objective of this meeting was to promote a space for diffusion of scientific knowledge encouraging the integration of the participants in an academic and social framework.

The activities covered all areas of Pharmaceutical Sciences: Clinical Pharmacy, Drug Quality Control, Healthcare Pharmacy, Medicinal Chemistry, Pharmaceutical Biotechnology, Pharmaceutical Education, Pharmaceutical Microbiology, Pharmaceutical Technology, Pharmacobotany, Pharmacognosy and Pharmacology.

More than 240 scientific works (mode: poster) were presented at this event and prestigious foreign scientists and leading researchers of our country spoke at this event covering all the above mentioned areas.

RICiFa2014 was sponsored by the National Council for Scientific and Technological Research (CONICET), the National University of Córdoba and the National University of Rosario. The auspices mentioned added to the economic contribution of different companies have made possible the realization of RICiFa 2014.

Together with RICiFa2014 the the 1st Workshop on Pharmaceutical Professional Services were conducted, activity organized as part of celebrations commemorating the 50th anniversary of the creation of the College of Pharmacists of Córdoba (CFC).

The Organizing Committee thanks all the participants of the event and look forward to your presence in RICiFa2016.

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SCIENTIFIC PROGRAME

Thursday September 18, 2014

8:00 am	Registration.
9:30 am -10:00 am	Inaugural function.
10:00 am-10:30 am	Speaker: Dr. Daniel Allemandi, SCIENTIFIC COMMITTEE-RICiFa2014
10:30 am - 11:15am	Topic: **Mucus-penetrating nanoparticles for the oral administration of anticancer drugs** Speaker: Dr. Juan Manuel Irache, UNIVERSIDAD DE NAVARRA- SPAIN Chairperson: Dr. Daniel Allemandi, UNC
11:15 am - 11:45 am	Topic: **Responsibilities and strategies in pharmacovigilance. The experience of "Laboratorio de Hemoderivados – UNC** Speaker: Dr. Daniela Fontana, LABORATORIO DE HEMODERIVADOS-UNC ARGENTINE Chairperson: Dr. Fabiana Alovero, UNC
11:45 am - 12:45	**Free paper sesión I** Chairpersons: Dr. Susana Lavaselli - UNR, Dr. Daniela Quinteros - UNC
12:45 -1:45 pm	**Lunch**
1:45 - 4:00 pm	**Simposium I.** Chairperson: Dr. Fabiana Alovero, UNC
1:45 - 2:30 pm	Topic: **Phototherapeutic agents based in porphyrins to inactivate microbes** Speaker: Dr. Edgardo Durantini, UNIVERSIDAD NACIONAL DE RÍO CUARTO-ARGENTINE
2:30 -3:15 pm	Topic: ***New trends in antihelmintic drug therapy for dogs and cats*** Speaker: Dr. Sergio Sanchez Bruni, UNIVERSIDAD NACIONAL DEL CENTRO DE LA PROVINCIA DE BUENOS AIRES, TANDIL-ARGENTINE
3:15 -4:00pm	Topic: **Influence of gender, aging and circadian rythms on drug pharmacokinetics** Speaker: Dr. Martha Vazquez, UNIVERSIDAD DE LA REPUBLICA-URUGUAY
4:00 – 5:00 pm	**Free paper sesión II** Chairpersons: Dr. Ariana Zoppi, UNC,Dr. Dario Lonardi, UNR
(coffe break)	
5:00- 7:15 pm	**Simposium II.** Chairperson: Dr. María Celina Lamas, UNR
5:00 - 5:45 pm	Topic: **Pharmaceutical co-crystals: a new strategy to improve medicines** Speaker: Dr. Alejandro Ayala- UNIVERSIDADE FEDERAL DO CEARÁ-BRAZIL.
5:45 -6:30 pm	Topic: **Chemometric approaches to the study of pharmaceutical dissolution** Speaker: Dr. Teodoro Kaufman, UNIVERSIDAD NACIONAL DE ROSARIO-ARGENTINE
6:30 -1:15 pm	Topic: **Pharmaceutical Salts: the case of Diclofenac** Speaker: Dr. Adamo Fini, UNIVERSIDAD DE BOLOGNA – ITALY

Friday September 19, 2014

9:00 am-11:15 am	**Simposium III.** Chairperson: Dr. Alvaro jimenez Kairuz, UNC
9:00 - 9:45 am	Topic:**Research and development of bioproducts** Speaker: Dr. Edemilson Cardoso da Conceição, UNIVERSIDADE FEDERAL DE GOIÁS-BRAZIL
9:45 -10:30 am	Topic: **Importance of administration context in benzodiazepines dependence expression: Molecular mechanism involved** Speaker: Dr.Mariela Perez, UNIVERSIDAD NACIONAL DE CORDOBA-ARGENTINE
10:30 -11:15 am	Topic: **Expression, function and regulation of drug transporters. Pharmacotherapeutic and diagnostic implications** Speaker: Dr.Adriana Torres, UNIVERSIDAD NACIONAL DE ROSARIO-ARGENTINE
11:15 -12:15	**Free paper sesión III** Chairpersons: Dr. Gladys Granero, UNC, Dr. Renee Calafato, UNR
12:15– 1:00 pm	Topic: **Enabling formulations for chronic wound healing** Speaker: Dr. Carla Caramella, UNIVERSIDAD DE PAVIA-ITALY Chairperson: Dr. Claudio Salomon, UNR
1:00 -2:30pm	**Lunch**
2:30 – 3:15 pm	Topic: **Development of an oral vaccine platform based on the protective properties of surface proteins of the intestinal parasite Giardia lamblia** Speaker: Dr. Hugo Luján, UNIVERSIDAD UNIVERSIDAD CATÓLICA DE CÓRDOBA-ARGENTINE Chairperson: Dr. Santiago Palma, UNC
3:15 – 4:00 pm	Topic:**Towards a more rational and efficient utilization of drugs** Speaker: Dr. Antomio Rabasco Alvarez, UNIVERSIDAD DE SEVILLA-SPAIN Chairperson: Dr. María E. Olivera, UNC
4:00 -5:00 pm	**Free paper sesión IV** Chairpersons: Dr. Marcela Longhi, UNC, Dr. María Laura Guzman, UNC
(coffe break)	
5:00 – 5:45 pm	Topic: **Current challenges on pharmacological treatment of Parkinson's disease** Speaker: Rui Daniel Prediger, UNIVERSIDADE FEDERAL DE SANTA CATARINA UFSC-BRAZIL Chairperson: Dr. Mariela Perez, UNC
5:45 – 6:30 pm	**Closing Conference** Speaker: Dr. Jaime Lazosvky. Secretary of Health Relations and Health Research Health Research Commission, MINISTRY OF HEALTH OF THE NATION-ARGENTINE
6:45 pm	**Closing Ceremony:** Award RICiFA-2014 and Special Mentions Chairpersons: Dr. Santiago Palma, Dr. Fabiana Alovero and Dr. Claudio Salomon.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

INVITED LECTURE

Mucus-penetrating nanoparticles for the oral administration of anticancer drugs

Juan M. Irache
Departamento Farmacia y Tecnología Farmacéutica, University of Navarra, C/Irunlarea 1, 31080 Pamplona, Spain.

Oral chemotherapy is attractive because of its convenience and ease of administration, particularly in the palliative setting. It is also especially appropriate where prolonged drug exposure is desirable. At present, many current anti-cancer therapies are cytostatic in nature and thus are optimally effective when given chronically for a continuous tumour exposure. However, this mechanism of action virtually requires oral daily and prolonged therapies. Unfortunately, a number of anticancer drugs show limited solubility properties and low permeability capabilities and, as a result, they show a poor oral bioavailability. Paclitaxel (PTX) and other taxanes are typical examples of such a drug suffering from these drawbacks.

One possible strategy to solve these drawbacks would be the use of slippery nanoparticles with mucus-penetrating properties. Using paclitaxel, these nanoparticles displayed a size of about 180 nm and a drug loading close to 15% by weight. The pharmacokinetic study in rat and mice has shown that these nanoparticles were capable to offer therapeutic plasma levels of paclitaxel up to 72 hours. In addition, the oral relative bioavailability of paclitaxel when loaded in these nanoparticles was found to be close to 85%. Antitumor efficacy was evaluated in a subcutaneous tumour model in mice. Nanoparticles orally administered (1 dose every 3 days, 9 days) presented a slow tumour growth rate and were capable of maintaining similar tumour sizes than the conventional treatment intravenously administered (Taxol®, 1 daily dose, 9 days).

In summary, these devices would be capable of reaching the surface of the enterocytes after passing through the mucus layer. Then, the intimate contact between nanoparticles and the cell membrane would facilitate the drug absorption in a continuous way during prolonged periods of time.

Responsibilities and strategies in pharmacovigilance. The experience of "Laboratorio de Hemoderivados – UNC"

Daniela Fontana
Dpto de Farmacoepidemiología e Información Científica. Dirección de Marketing y Comercialización. Laboratorio de Hemoderivados. Universidad Nacional de Córdoba. Córdoba, Argentina.

The "Laboratorio de Hemoderivados-UNC" is a not for profit pharmaceutical industry that produces plasma products, processes bone tissue for therapeutic purposes and produces injectables medicines. Since 2001, there is an area that deals specifically with pharmacovigilance of its marketed products.

Pharmacovigilance strategies follow local and international regulations, WHO and PAHO guidelines, including different methodologies and actions to complement each other. In 2013,the pharmacovigilance activities were adapted as set up with ANMAT Disposition 5358/2012 about Good Pharmacovigilance Practices, which is mandatory for pharmaceutical industry in Argentina. Our social role is reinforced taking an active role by implementing the following activities:

Passive pharmacovigilance

-Voluntary notification: reception, registration and delivery to peripheral effector of reports prepared by professionals who use the pharmacovigilance official form of ANMAT.

Active pharmacovigilance

-Stimulated notification: design and deliver of specific forms to collect information about effectiveness and safety. This activity reinforces working with patients receiving products by donation so that both they and the professionals complete these pharmacovigilance records.

-Pharmacoepidemiological studies: design and implementation of national and international, retrospective and prospective studies using different qualitative and quantitative methodologies to collect data related to effectiveness and safety.

Diffusion: preparation and distribution of letters and brochures about pharmacovigilance plan of Laboratory. Participation in scientific activities.

Training: conducting internal and external educational activities.

Implementation of Risk Management Plan (RMP): strengthening active and passive pharmacovigilance activities for products that require RMP, by performing routine actions (risk identification and specific mention in leaflets and brochures) and additional actions (educational material for health professionals and patients through leaflets, videos and user's manual guidelines). Trainings for reconstitution and administration of medicines aimed at health professionals and patients. Follow up and impact assessment of the activities by defining the expected standards.

Preparation and submission of Periodic Safety Update Reports (PSUR): the PSUR are assembled and shipped according to the requirements and deadlines set by the Health Authority regulations.

Phototherapeutic agents based in porphyrins to inactivate microbes

Edgardo N. Durantini (edurantini@exa.unrc.edu.ar)

Departamento de Química, Facultad de CienciasExactasFísico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina.

In recent years, new approaches to the treatment of microbial infections have become necessary. In this sense, photodynamic inactivation of microorganisms is based on the administration of a photosensitizer that is accumulated in the microbial cells. The irradiation with visible light, in the presence of oxygen, produces cell inactivation.Two oxidative mechanisms can occur after photoactivation of the photosensitizer. In the type I photochemical reaction, the photosensitizer interacts with a biomolecule to produce free radicals, while in the type II mechanism, singlet molecular oxygen, $O_2(^1\Delta_g)$, is produced as the main species responsible for cell inactivation.

According with their photochemical properties, porphyrin derivatives have been used as efficient agents to mediate PDI of various classes of microbial cells. In previous studies, we have investigated the photodynamic activity of porphyrins with different number of cationic charges as agents to eradicate microorganisms. Cationic porphyrins induce direct inactivation of Gram-negative bacteria without the presence of an additional permeabilization agent.The presence of cationic groups appears to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the Gram-negative bacteria, increasing the efficiency of the photodynamic activity. On the other hand, photodynamic action mechanism mediated by these cationic porphyrinsmainly involves the intermediacy of $O_2(^1\Delta_g)$. The results indicate that amphiphilic cationic fullerenes have potential as agent to the photoinactivation of

microbial cells. Also, novel porphyrin-fullerene C_{60} dyads have been evaluated as efficient photosensitizers.

The mainly advantages of PDI are that bacteria can be eradicated in very short time, resistance development in the target bacteria is improbable and damage to adjacent host tissues and disruption of normal microflora can be avoided. This approach is useful to photoinactivate bacteria in a liquid medium and also immobilized on a surface, which allows establishing conditions for the treatment of pathogenic microorganisms growing as localized foci of infection.

New trends in antihelmintic drug therapy for dogs and cats

Sergio F. Sánchez Bruni (sbruni@vet.unicen.edu.ar)
Laboratory of Pharmacology, Faculty of Veterinary Medicine, UNCPBA - Tandil Veterinary Research Center (CIVETAN) -CONICET, Tandil (7000)-Argentina.

Parasitic diseases are an important health concern to small animal veterinarians worldwide, and their zoonotic potential is also of relevance to human medicine. The treatment and control of such conditions relies heavily on pharmaceutical intervention using a range of antiparasitic drugs and/or their biologically active metabolites.

From the new millennium to the present new pharmacological strategies are being investigated in order to optimise the posology and efficacy of antiparasitic drugs in dogs and cats. The international trend about this topic is mainly based in: a) to short the therapeutic, seeking single dose administration in repeated therapy of Benzimidazoles (BZD) formulations – based and b) to simplify the administration (from clinical practice view) prioritizing the topic via for endocides and endectocides molecules, which traditionally were given orally or injectable.

In this context a novel formulation based on the active metabolite albendazole sulphoxide (ABZSO) or Ricobendazole has been developed for using in dogs. In a single dose comparative pharmacokinetic (PK) study with 2 albendazole parent drug formulations (conventional tablets and poloxamer- solid dispersion), that formulation showed a significant higher PK profile with increase in AUC and Cmax values of 500 and 487%, respectively. The later correlated with higher efficacy (>90%) against gastrointestinal nematodes, giving it a protection period of at least 30 days post –treatment. Other sort of formulations like ABZ nanocristals and RBZ gelucire, are being investigated in preclinical and clinical studies, respectively.

Endocides and endectocides antiparasitic formulations given using the topic via (Spot on) require a more sophisticated pharmacotechnic development., since the different histophysiology of the skin between dogs and cats would affect the absorption of the active ingredients, with consequent low efficacy. Formulations based in moxidectin, selamectin (dogs) and emodepside (cats), or broad spectrum combinations using praziquantel are available on the veterinary pharmaceutical market. This new trend is a big challenge for pharmacotechnicists since the future improvement of these formulations should be based in decrease the proven inter-specie variability, including the significant PK differences found between male and females.

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Influence of gender, aging and circadian rythms on drug pharmacokinetics

Marta Vázquez
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The main goal of clinical pharmacokinetics is to enhance drug efficacy and decrease drug toxicity of a patient's therapy. The understanding of pharmacokinetics faces many problems. Circadian rhythms, gender and age among others can affect drug absorption and disposition. The interplay of these factors determines drug concentrations over time and the effect at the action site. Too little drug exposure leads to ineffective regimens, whereas too much creates the risk of adverse effects.

Sex-related differences have important implications for drug activity, including pharmacokinetics and pharmacodynamics. For example, men and women have different gastrointestinal physiology. Stomach pH under fasting conditions is more acid in men whereas women show delayed gastric emptying and slower intestinal transit. The expression of cytochrome P450 enzymes and efflux transporters is also different. Additionally, women are 50 to 75 percent more likely than men to experience adverse drug reactions.

The ageing process and circadian variations also impact in gastric acid secretion and pH, intestinal motility, gastric emptying time, gastrointestinal, hepatic and renal blood flow and drug protein binding among others.

Regarding cardiac output distribution, it is well known the important role it plays in pharmacokinetics. Aging and circadian rhythms present similar cardiac output distribution pattern. There is an increase blood flow delivery to the extra-splanchnic region in the elderly and in the morning but with a decreased cardiac output in aged subjects and an increased one during the day. Sex-related differences in cardiac output can also be mentioned. Due to a larger muscle mass, men have a higher fraction of cardiac output destined to this region in detriment of renal and splanchnic regions in comparison to women.

No doubt pharmacokinetics represents a valuable contribution for ensuring medication safety, however, only a correct interpretation of all these factors can turn it into a useful tool.

Pharmaceutical co-crystals: a new strategy to improve medicines

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Drug molecules with limited aqueous solubility are one of the main problems in the development of new pharmaceutical formulations. It is estimated that about 40% of marketed drugs have low solubility, whereas 80 % of the drug candidates in the R&D pipeline could fail due to solubility problems. In the last years, the crystal engineering of pharmaceutical co-crystals has emerged as a promising research field to enhance the physicochemical propertiesof active pharmaceutical ingredients, such as, the dissolution rate (and hence bioavailability),hygroscopicity, physical/chemical stability, etc. The rational design of multicomponent solids by selecting adequate synthons gives rise to a new solid form: the pharmaceutical co-crystals. Pharmaceutical co-crystals represent an attractive and broad ranging alternative to the traditional forms of API's: polymorphs, solvates and salts. Co-crystals are at least

partially based on a design involving non-covalent interactions, such as hydrogen or halogen bonds. These multicomponent forms offer several benefits over the traditional salt approach because even molecules without ionizable functional groups can co-crystallized with a large number of coformers with GRAS status (General Recognized As Safe) to tune their physicochemical properties. This novel approach also represent a challenge for regulatory and intellectual properties agencies.From the experimental point of view, the new crystal engineering strategy involve standard and non-conventional crystallization methods, as well as, the state-of-the-art of solid state characterization techniques. These methodologies will be discussed in this presentation in the context of recent applications to several pharmaceutical active ingredients.

Chemometric approaches to the study of pharmaceutical dissolution

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Análisis de Medicamentos, Fac. Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario and Instituto de Química Rosario (IQUIR, CONICET-UNR), Rosario, Argentina

In vitro dissolution testing has become a relevant strategy for assessing the performance of solid oral dosage forms. Construction of dissolution profiles of pharmaceutical formulations is currently an established operation, contained in modern pharmaceutical regulations. The dissolution profiles gain significance because of their suitability for estimating the availability of the active ingredients, being critical means of assessing similarity between innovator and generic products for exchangeability purposes. They are also useful guides during the development of new formulations, allowing control of lot-to-lot consistency during the manufacturing process, and serving to confirm its reproducibility after changes in location, equipment, lot size and other key manufacturing parameters.

Fixed-dose pharmaceutical associations are a special case of combination products, which are advantageous in terms of better therapeutic efficiency, reduced adverse effects, convenience of dose and improved patient compliance. However, their dissolution profiling for quality control purposes faces some practical challenges, including the need of carrying out the simultaneous quantification of their active principles in several samples, in a wide range of drug concentrations and under cost-effective conditions. Furthermore, performing these determinations without physical separation steps entail additional hurdles, due to the possibility of mutual interference of the formulation ingredients.

The development of simple and efficient Ultraviolet spectroscopy/chemometrics-assisted approaches as alternatives for monitoring the pharmaceutical dissolution of drug associations will be presented. Emphasis will be placed in the use of the Partial Least Squares regression (PLS) and Multiple Curve Resolution with Alternating Least Squares (MCR-ALS) as chemometrics methods. On-line and off-line data acquisition procedures will be detailed, as well as on-line dilution as a strategy to solve cases when the amounts of the dissolved drugs saturate the detector. Several real examples will be analyzed and validation of the proposed methods will be discussed.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Pharmaceutical salts: the case of diclofenac

A. Fini, C. Cavallari (adamo.fini@unibo.it)
University of Bologna, Italy

The term *pharmaceutical salt* indicates salts of acidic or basic drugs independently of the counterion nature. The formation of a salt is usually related to improve solubility of the drug and, since it must undergo a complete and expensive pre-formulative study, more interesting would appear the research of counterions for a given drug suitable to obtain singular aspects of the solid state or behavior in solution for the final compound, enabling improvement of the behavior much more than a simple salt.

Diclofenac was selected as a model drug for this research for the presence in its molecule of the imino group, known to form complexes with numerous transition metal ions with potentially interesting anti-inflammatory activity. This prompted our research to experiment numerous hydroxyalkyl amines as salt-forming agents capable to interact with the imino group of diclofenac to prepare salts having the form of a complex and displaying specific aspects in the solid state and in solution different than those of a generic salt.

The resulting diclofenac salts exist as ion-pair complexes, in the solid state and in aqueous solution, stabilized by hydrogen bonds; polymorphs are encountered when the base molecule is relatively small; hydrates are formed in few cases.

This systematic research highlighted the interesting properties of the complex between diclofenac andN-(2-hydroxyethyl) pyrrolidine. It exists in three forms: A, B and C. B is the di-hydrate form of C; A and C are polymorphs.

The form A is very soluble in water (about 230 mM) and, starting from 35 mM, micelle-like aggregates of diclofenac anions are formed able to solubilize hydrophobic molecules; below this value anion/cation pairs are present of improved partition and absorption ability. This salt, due to these excellent properties, was selected to prepare a marketed transdermal delivery **patch** for anti-inflammatory local therapy.

Research and development of bioproducts

Edemilson Cardoso da Conceição
Universidade Federal de Goiás – Brasil

Bioproducts are products que are made from biomass. Our conference will focus on the related research and development of bioproducts with several applications in the pharmaceutical, cosmetics, food and agriculturework areas. In the pharmaceutical area will focus on the development of herbal medicines; In the cosmeticsarea will focus on developing phytocosmetic; In the food industry, we focus on the development of functionalfood applications in poultry and in agriculture we will focus on the development of fungicides and pesticides.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Importance of administration context in benzodiazepines dependence expression: Molecular mechanism involved

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Universidad Nacional de Córdoba, Córdoba. Argentina

Benzodiazepines are commonly prescribed for therapy of disorders such as anxiety and sleep disturbances, among others. However,prolonged treatment may lead to dependence and/or addiction, with evident withdrawal syndromein many patients. It is generally accepted that long-lasting neuroadaptations resulting from repeated drug exposure involve an associative learning process. These learning and cognitive aspects of addiction suggest the existence of common neurobiological mechanisms mediating drug addiction and memory. It is known that contextual memories recruit the hippocampus, an important structure for processing the associations between the environmental context and unconditioned stimuli, such as drugs of abuse. Moreover, at cellular level, protein kinase M zeta (PKMζ) is critical for the maintenance of hippocampal long-term potentiation (LTP) and spatial conditioned long-term memories. Also, a link between activity-regulated cytoskeleton-associated protein (Arc), PKMζ and LTP maintenance has been proposed. In our laboratory, we have evaluated an anxiety-like behavior as expression of a mild discontinuation symptom of chronic (18 days) diazepam (DZ) administration in rats. The anxiety-like behavior and an increased hippocampal LTP were present only up to five days of withdrawal. After fifteen days of withdrawal, re-exposure to the withdrawal environment was able to evoke anxiety, without drug exposure, and the increased hippocampal LTP was restored. Furthermore, changes in contextual cues during re-exposure to the withdrawal environment as well as intrahippocampal administration of PKMζ inhibitor, previous to re-exposure, not only prevented the expression of the anxiety-like behavior, but also the associated enhanced hippocampal LTP and the increase in Arc expression. These results support the relevance of hippocampal synaptic plasticity in the maintenance of the memory trace during DZ withdrawal, adding new evidences for common mechanisms between memory and drug addiction that can be therapeutic target for treatment or prevention of DZ dependence and/or abuse.

Expression, function and regulation of drug transporters. Pharmacotherapeutic and diagnostic implications

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Membrane transporters play an important role in drug disposition, therapeutic efficacy, and adverse drug reactions. The research on the expression, function and regulation of drug transporters will lead to the development of new safer and more effective drugs.

The transporters of organic anions are subdivided into three major gene families: organic anion transporter (OAT), organic anion transporting peptide (OATP) and multidrug resistance-associated protein (MRP). The OAT family mediates the body disposition of clinically important drugs, including anti-HIV therapeutics, antitumor drugs, antibiotics, anti-hypertensives and anti-inflamatories Therefore, understanding the regulation of these transporters has profound clinical significance. Several OAT

members have been cloned. These OATs are expressed in distinct tissues and cell membranes. In the kidney, organic anion transporter 1 (Oat1), 3 (Oat3) and 5 (Oat5) are organic anion/dicarboxylate antiporters. Oat1 and Oat3 are involved in the energetically linked basolateral entry of organic anions into the proximal tubule cells of the kidneys. The organic anion transporter 5 (Oat5) is only expressed in the apical membranes of proximal tubule cells and our group was pioneering in detecting Oat5 in urine.

The topics to be exposed in this conference include:

- an introduction related to the relevance of drug transporters in pharmacokinetics, pharmacotherapeutic and also in diagnosis,
- the importance of evaluating the expression, function and regulation of Oat1 and Oat3 in different pathological states (renal and extra-renal diseases) for the design and optimization of rationale dosage regimens,
- the latest results obtained after the therapeutic manipulation of drug transporters in the treatment of different pathologies,
- the studies performed to validate the urinary excretion of Oat5 as an early biomarker of acute kidney injury induced by ischemia, mercury, cisplatin and methotrexate.

Enabling formulations for chronic wound healing.

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There are still unmet needsand suboptimal situationsin the therapeutic area of wound healing. Besides accidental injuries, chronic skin ulcers of various etiology such as decubitus sores,vascular and neuropathic ulcersrepresent a major health care burden, likely to increase as the population ages and becomes affected by chronic diseases. One practical reason why the treatment of these pathologies is still not optimal is the lack of standard protocols that allow the medical doctors, the nurses and the decision makers inside the health institutions to evaluate the functionality of the plethora of medications available on the market. This very often leads to wrong decisions and unsuccessful treatments. On the other hand, the research-based therapeutic scenario is evolving and the treatment of epithelial and cutaneous lesions is progressively moving from a symptomatic palliative approach, based on the use of medical devices and traditional drugs, towards a more modern approach, so-called reparative medicine. Within this frame platelet growth factors and hemoderivatives thereof such as platelet rich plasma preparations (PRP) and platelet lysate (PL), are increasingly proposed in the clinical practice for various reparative treatments. Given the peculiar nature of these factors and the need to combine them with antioxidants and/or antinfectives, which may require different release rates, the development of so-called enabling formulations is of paramount importance for adapting drug release to repairing requirements. The presentation aims at demonstrating how pharmaceutical technology and nanotechnology can assist in the development of new therapeutic platforms and, at the same time, how the classical paradigm of technological testing need to be changed when working in this area.

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Development of an oral vaccine platform based on the protective properties of surface proteins of the intestinal parasite *Giardia lamblia*

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Despite the impact of world-wide vaccination programs, there is still a great necessity to develop novel, cheap, and safe vaccination strategies. Since most infectious agents invade the organism via mucosal surfaces, adaptive mucosal immunity plays a central role in protecting the host against infections. Oral administration of vaccines represents an attractive option because it is not invasive and suitable for mass vaccination. However, the main impediment for oral vaccine development has been that orally administered antigens are easily destroyed by the gastrointestinal tract. The intestinal parasitic protozoan *Giardia lamblia* expresses at its surface variant-specific surface proteins (VSPs) that are extremely resistant to the low pH of the stomach as well as to intestinal proteases, allowing the parasite to survive in the harsh environmental conditions of the small intestine. These VSPs are able to induce potent mucosal and systemic immunity against this diarrhea-causing parasite upon immunization via the oral route. On the other hand, it has been reported that virus-like particles (VLPs) given by injection are efficient immunogens to induce both cellular and humoral responses. We thus hypothesized that expression onto VLPs of *Giardia* VSPs should shield these particles for oral administration. To obtain a proof of principle and, simultaneously, to develop a potential vaccine candidate, we used Influenza Hemagglutinin (HA) as a vaccinal antigen, for which we have already established all the procedures to monitor cellular and humoral anti-HA immune responses, including challenge experiments with live virus. We produced our vaccines composed of VSP-HA chimeric proteins as control or HA-expressing VLPs covered with VSPs and HA. Our results clearly demonstrated that *Giardia* VSP can protect vaccinal antigens in the gastrointestinal track, generating strong T and B cell-mediated protective responses. The development of this universal platform for oral delivery of vaccines should have a broad application to different infectious diseases.

Towards a more rational and efficient utilization of drugs

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Over the last decades, the average life of the population is rising gradually. This fact provides a significant increase of patients who require treatment of their frequent chronic diseases. And besides that, polymedicated elderly patients who need a treatment composed by numerous medicines (sometimes even more than a dozen) are also getting frequent.

This has led to an increase in drug expenses by the health system in many countries; the economic crisis suffered by many of them has forced the health administrations to take wide-ranging measures, designed to reduce the spending on medicines. Moreover, for quite some time there have been evidences of drugs that do not produce beneficial effects in patients, but sometimes even adverse effects that end up with their hospitalization. These drugs represent a significant economic cost, totally wasted. And what is it possible to do? To use drugs in a much more rational way in order to optimize their therapeutic

response and expenses. The idea is that every euro spent on medicines becomes a real benefit for the patient. And which are our tools? Different types of performances could be established:

1) Adopting measures in order to prevent disease.
2) Enhancing health education to patients and caregivers.
3) Taking the drug prescriptions in a much more personalized way, focusing on each patient.
4) Minimizing all the causes that could produce variability in drug response.

Current challenges on pharmacological treatment of Parkinson's disease

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Parkinson's disease (PD) is the most prevalent motor neurodegenerative disease, with higher incidence in the elderly population. Some important features of this disease, such as its progressive nature, the presence of non-motor symptoms that largely do not respond to antiparkinsonian drugs, and the efficacy loss and the emergence of serious side effects after chronic treatment with dopaminergic drugs represent major challenges to clinical and academic communities. The present presentation attempts to discuss recent findings from our group and others indicating the potential of new agents (e.g., agmatine, caffeine, atorvastin and physical exercise) to manage non-motor symptoms of Parkinson's disease as well as to improve the current pharmacological treatment with levodopa.

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POSTERS PRESENTATION

CLINICAL PHARMACY- HEALTHCARE PHARMACY

Increased risk of cardiac adverse effects associated with the use of domperidone in adult population: a sistematic review

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Keywords: domperidone, ventricular arrhythmia, QT prolongation

Domperidone is a peripheral dopamine D_2 receptor antagonist widely prescribed in children and adults with gastrointestinal disorders. Events of QT interval prolongation, ventricular arrhythmia (VA) and sudden cardiac death (SCD) have been lately reported, which resulted in international alerts and the withdrawal from the american market. In Argentina, domperidone is commercially available for oral and intravenous administration with scant concerns about its safety profile.
Our aim was to determine if there is an increased risk of cardiac adverse effects associated with the use of domperidone in adult population through a systematic review of the scientific literature.
The electronic databases MEDLINE, LILACS, ScIELO and The Cochrane Library were consulted without date or language restrictions. The search strategy consisted of combining free and indexed text words in titles and abstracts. The inclusion criteria were studies in human adults treated with domperidona, which considered the occurrence of QT interval prolongation, VA or SCD.
The original search produced a total of 73 articles. Of these, 11 met inclusion criteria: 6 case reports of cardiac adverse effects in patients with cancer treated with intravenous domperidone; 1 double-blind randomized controlled trial showing QT interval prolongation in healthy volunteers after oral administration of domperidone; 4 case – control studies showing an increased risk related with the use of oral domperidone estimated by odds ratio adjusted by several confounding factors.
Evidence that the use of domperidone increases the risk of occurrence of cardiac adverse effects in adult patients was found. However, multicenter studies should be organized in order to evaluate the risk of each reported adverse effect according to prescribed dose, administration route and age group. Meanwhile, it is recommended to administer domperidone cautiously followed by a continuous monitoring of the cardiac function throughout treatment.

Prescription of cisplatin in a Public Outpatient Oncology Center of the city of Rosario, Argentina.

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Keywords: Oncology medicines - clinical practice guidelines - pharmacoepidemiology

The patterns of physician prescribing and patient drug utilization often cannot be predicted prior to marketing, although have importance on definition of drug treatments. In oncology there is a high variability in treatments that include several and costly drugs. In this clinical practice, clinical practice guidelines (CPG) are widely used to optimize prescription of medicines. The aim was to assess the appropriateness of cisplatin prescriptions to the recommendations of CPG, in adult outpatients treated at a Public Outpatient Oncology Center (CEMAR- Rosario).

Drug utilization study. Adult outpatients treated at CEMAR diagnosed with tumors (neoplasms–ICD10) and with cancer pharmacotherapy (ATC Code L01 and L02), between January-June 2012. Source of information: medical record patients and records of Pharmacy and Oncology Services. Appropriateness of drug use compared with local, national, and international reference guidelines published by recognized scientific societies.

133 patients were diagnosed with: tumors of breast (27,8%), colon cancer (10,5%), cervical cancer (9,0%), lung cancer (7,5%). 33 different oncology medicines were prescribed, the most prescribed: cisplatin, fluorouracil, paclitaxel.

Cisplatin (L01XA01) prescribed for 30 patients. The appropriateness of drug use with CGP for each indication of treatment was:

- Cervical cancer: 91% supported by local and international CPG.
- Testicular cancer: all supported by national and international CPG.
- Oropharynx cancer: not recognized in local CPG.
- Gastric cancer: not supported by local or national CPG but supported by international ones.
- Melanoma: all supported by CPG considered.
- Hard palate cancer: no specific CPG but included in “oral cavity” or “head and neck” guidelines.

Although most of prescriptions of cisplatin are supported by international guidelines, local ones do not. This may be because local guides need a more frequent review.

Thus, a consensus on the application of these guides could allow accessibility to the best option of treatment for each patient.

Pharmacovigilance: analysis of adverse drug reactions reported in the Chaco province during the 2010-2013 period

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Keywords: Pharmacovigilance, Adverse Drug Reactions, Chaco-Argentina

The aim of this study was to analyze reports of Adverse Drug Reactions (ADR) from year 2010 to 2013. Reports were made to Chaco's Peripheral Reporter for the National Pharmacovigilance System, part of the National Administration of Drugs, Food and Medical Technology (ANMAT). Chaco's Peripheral Reporter operates at the National University of the Chaco Austral. A retrospective descriptive study was conducted to analyze reports of suspected ADR, using the data collected from ANMAT's Yellow Cards. The World Health Organization Adverse Reaction Terminology (WHO-ART) Dictionary and the Anatomical Therapeutic Chemical (ATC) Classification System were used for their evaluation. Causality was assessed using the Naranjo Algorithm. Regarding severity, three categories were established (WHO):

mild, moderate and severe. From a total of 275 analyzed reports, according to the ATC code, the groups most frequently reported were the Anti-infectives for systemic use (J, 22%), Cardiovascular system (C, 22%), Nervous system (N, 14%), Musculo-skeletal system (M, 12%), Respiratory system (R, 7%) and Alimentary Tract and Metabolism (A, 6%). The remaining 17% was shared among other groups. In terms of causality, they were probable (65%), possible (26%), certain (8%) and unlikely (1%). Regarding severity, they were moderate (66%), mild (24%) and severe (10%). Reported ADR affected the Gastrointestinal System (24%), Disorders of Skin and Appendages (23%), Respiratory System Disorders (15%), Central and Peripheral Nervous System Disorders (14%), Body as a Whole-General Disorders (11%), Psychiatric Disorders (4%), Heart Rate and Rhythm Disorders (4%), Vision Disorders (4%) and General Cardiovascular Disorders (1%). The distribution by sex revealed females (59%) and males (41%). The most frequently reported age group was the 45-59 years old group (23%). The most commonly reported drug group was β-Lactam antibiotics. The ADR were mostly probable and moderate, involving the Gastrointestinal System and Disorders of Skin. The obtained results broaden the knowledge of ADR in the Chaco Province.

Analysis of the use of medicinal plants in the province of Chaco

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Keywords: Medicinal Plants, ADRs filled reports.

Worldwide there are medicinal plants, and throughout history man has used them as a healing remedy. Currently, it is intended to justify such use based on scientific knowledge derived from pharmacological study and clinical experimentation. The aim of this study was to analyze the filled reports on Communication of Adverse Events by use of Phytotherapeutical Medicines, Plant Products and/or preparations using Vegetables Drugs in the province of Chaco, during the period 2010-2013. Filled reports, which were distributed in pharmacies and health centers, were analyzed using the Herbal ATC classification. Of a total of 78 filled reports, 86% involved the use of a single medicinal plant and 14% the mixture of two and three of them. In terms of age distribution, the most reported group was between 0-16 years old with 45%. In accordance to the origin of the plant material, 29% was collected material, while 71% corresponded to purchased material, from which 33% was acquired from pharmacies, 24% from health food stores and 14% from street vendors. Regarding the severity of Adverse Drug Reactions 55% were severe, 34% were moderate and 11% were mild. According to the HATC classification, 73% corresponded to the HA group, Alimentary tract and metabolism; 10% to the HN group, Nervous System; 9% to the HP group, Antiparasitic Products; 6% to the HG group, Genito urinary system and sex hormones; and 1% to the HC Cardiovascular and HR Respiratory system groups. Based upon the analysis of filled reports, there is a predominating use of a single medicinal plant such as *Chenopodium ambrosioides* L.; *Illicium verum* Hook. f.; *Pimpinella anisum* L. and *Matricaria chamomilla* L. and adverse drug reactions such as abdominal pain, vomiting, cramps, diarrhea, convulsions and nausea, prevailing the pediatric age group.

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Over the counter non-steroidal anti-inflammatory drugs: Characteristics of dispensation and use in a pharmacy of Córdoba (Argentina)

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Key words: dispensation, pharmacy, non-steroidal antiinflammatory drug (NSAID), over-the-counter (OTC).

Non-steroidal anti-inflammatory drugs (NSAID) are extensively used, and complications may arise due to their inappropriate use. The characteristics of dispensation to patients using over-the-counter NSAID were analyzed. A descriptive, retrospective and cross-sectional study was used and we worked with a normalized procedure using a registry table including gender, age, stated reason of purchase and NSAID dispensed. Data was processed in an Excel database, and it was analyzed using the SPSS statistical software. Sample comprised 472 medicine dispensations in 15 community pharmacies in the city of Córdoba (Argentina).

Women comprised 63% of the users, and 45% of the total sample were between the ages of 25-44 years. There was a concentration of 84% of the medicine in only four active ingredients: Ibuprofen, Acetaminophen (Paracetamol), Aspirin and Diclofenac, with Ibuprofen being the most dispensed (40%). Of the dispensations of aspirin, 43% were due to its antiplatelet effect. It was observed that with increasing age of the patients in the sample, the consumption of Ibuprofen decreased, while the proportion of consumption of other NSAID increased. The relationship between the consumption of NSAID and the "reason for the consultation / purchase" was observed. In the case of Ibuprofen, the purchase was mainly related to menstrual pain and in two pharmaceutical equivalents, manufactured and marketed by the same drug company, that have been named "Ibuprofen Quick Action" and "Ibuprofen Woman Strong Quick Action", significant differences in sex and the reason for the consultation / purchase consumption were observed (Chi-square test). These differences were attributable to the influence of advertising rather than to its own therapeutical properties.

Those results agree with current publications of Latin America and Europe especially in the proportion of different NSAID dispensed due to the occurrence of non-specific symptoms.

These studies about medicines utilization allowed evaluation of diseases and behaviors in a community context, where the pharmacist could offer drug information that contributes to knowledge and control of diseases.

Drug-related problems as a cause of hospital admissions to the on-duty medical Unit, 2012, Córdoba Capital.

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Keywords: health-related problems, drug-related problems, emergency service, pharmacoepidemiology.

Drug-related problems (DRPs) are health problems brought about as a result of the patient's pharmacotherapy failures that interfere with expected health outcomes. The World Health Organization (WHO), on Fact sheet Nº 293 October 2008, stated that unexpected and harmful reactions to drugs are among the ten main causes of death worldwide.

Our aim is to know the percentage of patients being admitted to the both on-duty service due to drug-related problems, 2012 Córdoba, confirm which types of (DRPs) produce more hospital admissions, and determine some of the factors associated to their occurrence.

It was used for the data collection questionnaire Baena et al 2001,with modifications adapted to this study and recertified again, and drug-related problems classification was carried out with the Dader method.

It is a descriptive, prospective, cross-sectional, randomized study which aims at detecting drug-related problems.

280 patients were interviewed along a period of 10 months, in two on-duty medical units by pharmacists and students of degree thesis. Pregnant patients, occupational accidents, and children under 16 were excluded from the survey.

Of the 280 patients interviewed, 20% came to the emergency service due to drug-related problems. Of this percentage, 6% came for necessity; 10% for effectiveness; and 4%, for safety.

Only 5 patients stayed admitted. Among the factors associated to admission through the on-call service, age is one of most important, being 68-77 the age group with the greatest percentage of drug related problems, which represents 25%.

Antibiotics caused 4% of the admissions; antihypertensives, 3%; anti-inflammatory drugs, 2%; and corticoids, 1%.

The fact that 20% of hospital admissions is caused by drug-related problems accounts for the extent of the problem.

Prescription and use of human albumin in two private institutions belonging to the third level of care in Cordoba, Argentina

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Keywords: albumin, use, third level of care

Introduction. Albumin is a restricted use medicine that represents a problem in hospitals due to its low availability, high prescription and high cost. Also, its biological origin and the increasing clinical use has been object of debate and controversy.

Objective

- ✓ To evaluate and compare the indications and dispensations of intravenous albumin in Sanatorio Aconcagua (SA) and Clinica Sucre (CS), since they both have a dispensing protocol based on the albuminemia value but not on the pathologies for which it is prescribed.
- ✓ To evaluate the compliance with such protocol in both institutions.

Material and Methods. An observational and cross-sectional study was carried out for 4 months (December 2013 - March 2014). Data for use and indications were obtained from the patients' medical records, prescriptions and dispensing records in the Pharmacy Service. Distribution by clinical services was also analyzed.

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Results. In the study period 33 patients of each institution (45% male and 55% female) received vials of human albumin and 100% of the prescriptions were in accordance with the approved protocol.
In SA 273 vials (mean = 8.27 vials / patient) were distributed and used as follows: ICU (Intensive Care Unit) 66.7%, Medical Clinic 24.2% and Renal Transplantation 9.1%.
In the CS 207 vials (mean = 6.3 vials / patient) were distributed and used as follows: ICU 60.6%, Medical Clinic 33.3% and General Surgery 6.1%.
The pathologies with greater consumption of albumin were:
SA: septic shock and bowel/colon cancer, with 34% and 15% of total consumption, respectively.
CS: septic shock and ascites, with 42.5% and 28.5% of total consumption, respectively.
Conclusions. Although the institutions have not a clinical protocol by pathologies, the use of albumin in the SA and CS was similar. The information obtained allowed us to confirm compliance with the protocol of prescription and dispensing by the Hospital Pharmacy Services in both institutions.
We propose to improve the existing protocols for use of albumin with indications per pathologies, to assess the appropriateness of the prescription.

Pharmacoepidemiological study on the consumption and use of Human Albumin 20% in patients admitted to Intensive Care Services (SCI) in a public hospital in Córdoba.

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Keywords: pharmacoepidemiological. Human Albumin. Medicines of Restrained Use.

The National Clinical Hospital (HNC) has a therapeutic form, among others, includes medicines of restrained use (MUR). Being such medicines understood as the ones dispensed by the Central Pharmacy after a participatory, multidisciplinary and representative procedure in which medical examiners, those who based on existing protocols as well as scientific evidence, evaluate the particular clinical situation of each patient on whether or not to authorize the dispensing. Human albumin 20% (AH) is found among those drugs whose indication usually cause controversy and even more since there is no protocol at corporate level of dispensing. According to literature (bibliography), the diagnoses where it´s indicated its use are: paracentesis ascites, and in the treatment of Syndrome hepatorenal Nephrotic Syndrome and Spontaneous Bacterial Peritonitis. The aim of this paper is to analyze and evaluate the diagnoses that required dispensing of AH in patients hospitalized in intensive care services (SCI) at the HNC between May to October 2013, to further evaluate the institutional need of the corresponding protocol.

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Study of consumption and utilization of Jugular Puncture Set in patients admitted to the National University Hospital

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Keywords: central venous catheter set jugular puncture.

Introduction. The Jugular Puncture Set (SPY) is a catheter. It is inserted into the large venous vessels in the chest or in the right cardiac cavities. SPY is used for diagnostic or therapeutic purposes. Catheter placement is an invasive medical procedure commonly performed in hospitals due to increasing ill patients requiring intravenous therapy or for a long. (1-2)
The National Clinics Hospital (HNC) is a public teaching hospital, and it has 165 inpatient beds. In this hospital different Medical Products (MP) are used for inpatient care such as SPY are used. In 2012 254 different varieties of PM were used in the institution but the SPY ranked fifth of the PM generating more spending and consumption that progressively increases (100 units per month).
Therefore, the objective of this work is to follow up the use of SPY during a month in patients admitted to the HNC.
Methodology. Prospective e and descriptive study to evaluate the use and indication of SPY in HNC patients admitted to the study. Study period: September 2013.Report to the authorities and professional placement indicating that an audit system was implemented from the Central Pharmacy to monitor the use of SPY before start the study. It was to supervise each dispensed SPY: if it was placed, which drugs were administered by the same and the number of days remaining in place. Forms were designed to record the patients of the study period and which ones were dispensed one SPY.
Results. • Of the patients admitted during the month under study (694), 49 (7.06%) were fitted with SPY.
• The main reasons for the placement of SPY were intravenous polypharmacy, mechanically ventilated, underwent complex surgery and / or peripheral venous access is difficult. The average age between 60 and 80 years.
• For the average 10 SPY drug, corresponding to the greatest number was given: Heparin, ranitidine, metoclopramide, amiodarone, furosemide, magnesium, hydrocortisone, antibiotics, and parenteral nutrition.
• The number of patients had have placed SPY were between 2 and 8.

Study on consumption and use of drugs in a public hospital in the province of Córdoba

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Keywords: drug utilization study, consumption study.

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In health institutions is important to know how medications are used to promote and achieve rational use and detect deviations of consumption. The Hospital Nacional de Clínicas (HNC) is a high complexity public hospital with 165 inpatient beds. The pharmacy service dispenses medications for inpatients.
The aim of this work is to analyze the consumption of drugs in the HNC during 2012 and 2013.
Retrospective, descriptive, observational study on the use of medicines of patients admitted to the HNC from January 2012 to December 2013. Drugs were classified into therapeutic groups, based on the hospital formulary. To evaluate the cost increase caused by differences in consumption, the current price of drugs was considered.
Costs due to drugs dispensed from the pharmacy service were $ 6,367,026.22 in 2012 and $ 7,886,469.53 in 2013. Among a total of 280 different medications, the 50 % increase in cost was due to Ampicillin-sulbactam, Vancomycin, Imipenem, Piperacillin-Tazobactam, Sodium Heparin, low molecular weight heparin, hydrocortisone and Parenteral Feeding. The average bed occupancy/day was 124.4 in 2012 and 129.2 in 2013; generating a cost per bed/day of $ 140.22 and $ 167.24, respectively. From a total of 20 groups of drugs, in both periods, the 80% of total expenditure was represented by: solutions for electrolyte imbalance correction, anti-infective drugs, hematological agents, psychotropic drugs and anesthetics.
In 2013 there was a 24% increase in drug costs compared to 2012, leading to increased expenditure bed / day of 20%. The bed occupancy rate increased only 3%. Only 8 medications were responsible for 50% of the increases in 2013.
Therefore, it is important to protocolize the use of drugs that do not have a protocol in the institution and to implement audit system for those drugs that represent the 5 groups responsible for the deviations of consumption.

Assessment of adherence to the surgery antibiotic prophylaxis protocol in a public hospital

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Key words: Prophylaxis- Antibiotic- Protocol of Surgery- Pharmacoepidemiology

The Antibiotic Prophylaxis (AP) is the administration of antibiotics before, during and a little time after the surgical intervention with the objective of reducing the quantity of germs under the critical level needed to produce an infection. The rational uses of AP minimize the bacterial resistance and reduce the hospital stay.
The antibiotics used in PA must be correctly stated, basing their selection on the type of surgery and its classification according to the possibility of bacterial contamination.
The Hospital Nacional de Clínicas (HNC) is a high-complexity, polyvalent University hospital with 165 beds. The surgeries are performed on two general surgical wards (39 beds) and the services of Gynecology, Orthopedics and Urology (28 beds).
The aim of this study was to assess the compliance with the Surgery Antibiotic Prophylaxis Protocol (SAPP) of the HNC, which wasdeveloped in 2011 by the Infectious Disease Committeeand is reviewed and updated annually.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

For this purpose a prospective, observational study involving hospitalized patients undergoing surgery in HNC during May 2013 was performed. The pharmacist in charge of the rooms made a return where were registered for each patient the indicated antibiotic, its dose and length of treatment and the type of surgery was recorded in a spreadsheet, using as a data base the clinical records and the daily indications sheet.
In the period studied, 67 surgeries were registered (16 gynecological, 16 orthopedic, 7 urologic, 7 hernioplastic, 12 biliary, 4 colorectal and 5 various) were recorded. Of these, 33 met with SAPP (8 bile, 8 trauma 3 hernioplasties 11 gynecological and 3 colorectal). From the remaining 34, 16 lasted PA for more than 48 h, 5 used other doses than those suggested by the protocol and in 13 the antibiotics not included in the SAPP were used.
The reasons for non-compliance were not justified in the medical records.
The use of protocols for AP is an important practice to achieve a rational use of medicines and to prevent microbial resistance generated by improper use. The results show poor compliance of SAPP and suggest the need for revision to establish educational (improving the dissemination of the existence of PAPC) and/or corrective (audit requirements by the pharmacy service, modification of the protocol) measures required to improve adherence by medical professionals of the institution.

Medication errors in an emergency department of a teaching hospital in Chile

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Keywords: Medication errors, department emergency, medication use process, patient safety.

Emergency departments (ED) are one of the most vulnerable areas to medication errors (MEs) occurrence in a hospital. The number of patients attended and the stressful situation therein lived increase the risk of MEs and their severity.
The purpose of this study was to determine the frequency and characteristics of MEs in an adult ED.
A prospective observational study was conducted in a representative and randomized sample of adult patients attended in an ED of a teaching hospital in Chile between August and September 2013. The ED has 14 attentions` box and receives 37.000 patients annually approximately. Direct observation was used to detect ME. The data were collected daily by independent and trained observers (independent pharmacists), who registered the each stage of the medication use process (MUP) i.e. prescriptions, dispensing, preparations and drug administration. The prescription order was compared with registration nursing, on the other hand, was observed as the drug was dispensed, prepared and administered to the patient. MEs were defined and characterized according to the National Coordinating Council for Medication Error Reporting and Prevention. This study was approved by the institutional ethic committee.

A total of 364 patients and 646 drugs (medical indications) were evaluated. The 61% of patients were women; the mean age of the sample was 45.3±19 years, using 2.2 ± 1.0 drugs per patient. MEs were detected in 107 (29.4%) patients. There were 153 ME in the MUP, mainly during prescription (42.4%) and preparation stage (37.9%). The frequency of MEs was higher among patients receiving 2 o more drugs at the same time.

Identifying the most vulnerable stages (prescription and preparation) allow to suggest strategies to timely detect and prevent MEs, improving safety and quality of patients care.

Benzodiazepine treatment management in older inpatients

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Keywords: Older people, benzodiazepines, inappropriate medication.

Some clinical indication of benzodiazepines (BZD) in older people may be inappropriate, for instance, BZD use for long periods of time are associated with falls, cognitive impairment, frailty, among others adverse outcomes. On the other hand, the abrupt discontinuation of the BZD is associated with craving and delirium, both of them underestimated in the daily clinical practice.

The purpose of this study was to describe BZD treatments management in Medicine Department among older inpatients.

A retrospective cohort study was conducted during 6 months in a Medicine Service of a teaching hospital in Chile. BZD chronic use was defined for use longer than 3 months or a medical record indicating as BZD chronic user. Information related to discontinuation during hospitalization, change of therapy and delirium was collected from each patient medical record. Additionally, BZD new users` information was also collected and described.

A total of 283 patients were studied; 161 (57%) were female. The mean age was 73±9 years. Thirty one patients (11%) were chronic users, 52% of them kept receiving BZD during hospitalization period. The 13% of patients who BZD was suspended, experienced craving or delirium, mainly treated with quetiapine. On the other hand, 14 patients (5%) were BZD new users during the hospitalization, being clonazepam (86%) the most common BZD prescribed, mainly as hypnotic.

In conclusion BZD utilization among older people requires to be used under protocol, defining clearly therapeutic alternatives for older patients, duration of treatment and strategies for discontinuation to prevent adverse outcomes because the inappropriate use.

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Analysis of the need for extemporaneous compounding of medicines in a pediatric hospital in Santiago (Chile)

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Keywords: pediatrics; extemporaneous compounding; pediatric formulation; ambulatory patients.

Appropriate pediatric drug formulations are the basis of an efficient drug therapy for children. The lack of commercially available pediatric dosage forms of drugs is of concern for health professionals and caregivers. The re-formulation of medicines into a suitable dosage form is referred to as 'extemporaneous compounding'. In many occasions, the re-formulation of dosage forms is the only option for administering a drug that does not exist in the market.

In the hospital Dr. Exequiel Gonzalez Cortés, pharmacy service cares the ambulatory pediatric population in the south of Santiago city. By the foregoing, is necessary to know the medicines commonly prescribed for children that are candidates for re-formulation.

The purpose is to identify the medicines whose dosage form was necessary to re-formulate during the year 2013, for use in pediatrics.

An observational study was conducted and the prescriptions dispensed to ambulatory patients in 2013 were recorded.

Data collected: Number of prescriptions, number of medicines in need of re-formulation, age, sex and diagnosis of the patients. Patients' confidentiality was assured using codes to identify them.

A total of 36,301 prescriptions, were reviewed. In 708 prescriptions (1.9%), corresponding to 179 children (86 males, 93 females), there were 764 drugs which re-formulation was needed. The active pharmaceutical ingredients were 27 and all were solid dosage forms. The drug most prescribed was hydrocortisone (404 times). The second drug most prescribed was captopril (142 times). The most common diagnose was congenital suprarenal hyperplasia (297 prescriptions).

In 2% of the drugs dispensed there was, at least, one which dosage form was modified to suit the prescription. From de 27 drugs identified, hydrocortisone was the most required in doses different to the marketed products and was used to treat suprarenal hyperplasia. These results are important to address the problem and to propose strategies to overcome it.

Medicines used in pediatrics prior to the emergency room visit: identification of off-label situations

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Keywords: pediatrics; off-label drug use; approved labeling; leaflets for health professionals.

Children are routinely given drugs that lack specific pediatric information. The term "off-label" refers to use of a drug that is not included in the package insert (approved labeling) for that drug. The aim of this

study was to identify the use of medicines in children prior to the emergency room (ER) visit and assess which of them has information in the approved labeling.
Observational and descriptive study (June-August 2013) was conducted. Parents or caregivers were asked to mention the medicines used before the visit to the ER. The names obtained were grouped by active pharmaceutical ingredient (API). To identify the off-label use, the information leaflets for health professionals available in the Chilean Health Authority website were consulted. Also, Summaries of Products Characteristics (SPC) were reviewed in the Spanish Agency of Medicines (AEMPS) website. Finally, they were checked as essentials according to the list provided by the WHO.
From a total of 325 children, 203 children received at least one medicine prior to the ER visit. A total of 71 medicines were identified corresponding to 41 APIs. The most frequent drugs used were acetaminophen (20.3%) and ibuprofen (12.6%).
According to the leaflets, of the 41 APIs in only one there is information for use in pediatrics, 11 could be used with certain age or weight restrictions. For 26, information of marketed products was not available. In the AEMPS website, information for 12 suitable APIs for children was found, 20 could be used with restrictions. For 7, information was not found. Finally, WHO considers 13 as essentials drugs.
In 203 children, 71 different products were used, corresponding to 41 APIs. Analgesics and antipyretics were the most used drugs. Information obtained from the brochures was scarce and for 63% of the drugs, the data was not found.

Pharmacy interventions to prevent medication errors in pediatrics: a review

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Keywords: pediatrics, medication errors, prevention, pharmacy intervention

The medication errors (MEs) are inherent to clinical practice; it could be increased in emergency departments and are common in pediatric population. Risks factors that predispose children to develop adverse drug events could be inherent to physiological age´s. Pharmacists could perform a very important role intercepting and preventing MEs. Interventions which have demonstrated to be effective to reduce EMs are:

1. Technology interventions
2. Individualized system drug delivery
3. Education
4. Clinical pharmacist

The purpose is to search and recover bibliographic information referred to pharmacy interventions to prevent medication errors in pediatrics.
In April-May 2014, a databases search and recover of information was done. Different keywords referred to pediatrics, medication errors, pharmacy intervention and prevention, were used. Also, the search was filtered by age and year. PubMed and Scielo were consulted as free-databases while Scopus was a restricted one. Original articles were recovered in English and Spanish. If the review of articles generated an interesting reference, it was recovered even though the interventions were not for pediatrics.

Using the keywords combination, 20 papers were recovered from PubMed, but there was one which was specific related to interventions. Meanwhile, in Scopus 23 papers were recovered, two were considered interesting for this work, and one was coincident. Eleven articles were obtained from references, 6 of them were in included in both databases, but they did not use the selected keywords. From 13 papers recovered, 4 were referred to technology interventions, 1 to individualized system drug delivery, 6 to education, and 12 mentioned the work of a clinical pharmacist. Only 4 papers specifically measured a decrease in the number of MEs with interventions.

Combination of keywords restricted the search and the number of papers recovered was low. All the papers mentioned the presence of the pharmacist as a need to prevent MEs.

Benzodiazepines prescription as mono-drugs in a pharmacy of San Luis, Argentina

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Key words: Benzodiazepines, chronic treatment, dependence, drug rational use

Benzodiazepines are among the most prescribed drugs in the world; they are used mainly as anxiolytics and hypnotics, and also have myorelaxant and anticonvulsant actions. Their main side effects are psychomotor activity reduction and development of dependence; also, they present various interactions with other drugs. The aim of this study was analyze the benzodiazepines prescription in a centric pharmacy of San Luis during one year (July 2013-June 2014) and to determine its relationship with the patient age and sex. A retrospective study was carried out; the data about the consumption were obtained from the prescriptions. The acquisition of more than five medicines during the study period, and during a time major at 3 months without disruptions, was considered as chronic treatment. Benzodiazepines were indicated in 1,490 prescriptions (1,346 as mono-drugs) Clonazepam 46% (n=620; Sex: M=27.8% F=72.2%; Age: ≤60=39.2% and >60=60.8%), Alprazolam 35% (n=473; Sex: M=32% F=68%; Age: ≤60=40% and >60=60%), Bromazepam 5% (n=64; Sex: M=30% F=70%; Age: ≤60=13.5% and >60=86.5%), Lorazepam 4% (n=52; Sex: M=26.9% F=73.1%; Age: ≤60=40.4% and >60=59.6%), another 10% (n=137; Sex: M=34.1% F=65.9%; Age: ≤60=42.4% and >60=57.6%). Chronic treatment: (22.5%) Clonazepam 54.5% (Sex: M=26.7% F=73.3%; Age: ≤60=31.5% and >60=68.5%), Alprazolam 35.5% (Sex: M=33% F=67%; Age: ≤60=44.6% and >60=55.4%), Bromazepam 5.5% (Sex: M=27.3% F=72.7%; Age: ≤60=12.5% and >60=87.5%), Lorazepam 4.5% (Sex: M=35% F=65%; Age: ≤60=35% and >60=65%), another 12%. The indications for anxiety and insomnia were predominant. An important chronic use of benzodiazepines and a great difference between the proportion of male and female users were observed. The older patients received more benzodiazepines than younger patients, despite the known morbidity associated with their use in this population. The daily dose and the time of continuous use of benzodiazepines are important factors for the installation of dependence. Our results indicate an inadequate use of these drugs.

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Contribution of the central sterilization service to the patiente safety. A public hospital experience

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The National Ministry of Health regulates the organization and operation of the Central Sterilization Services (organizational and functional structure dedicated to the reception, cleaning, reconditioning, sterilization and dispensing of sterile elements), adding them to the National Program of Quality Guarantee of Medical Attention.

Working on Patient Safety means to apply the best knowledge to generate the best practice with the available resources and the context within the attention is providing to generate actions preventing unnecessary damages.

The aim of this work is to generate actions of quality improvement that have effects on the patient safety at the Municipal Hospital of Emergency.

A qualitative methodology was outlined, introducing group discussion techniques and brainstorming to generate proposals. Afterwards, those were added to a prioritization matrix, where measure impact, economical, technical and administrative feasibility, need perceived by the user and regulation aspect were evaluated. The best scored were implemented then.

Two meetings with each shift of the service were carried out in November 2012 and March 2013. Participation was voluntary. All the agents (100%) attended to theses meetings.

As a result of the discussions and subsequent prioritization, the best scored proposals were implemented then:

- ✓ Replacement of sulfite paper with medical grade paper.
- ✓ Thermosetting envelopes incorporation.
- ✓ Dosing of nitrofurazone in droppers.
- ✓ Development of a protocol for nitrofurazone use.
- ✓ Extra-large surgical drapes incorporation for central line.
- ✓ Manufacture and supplying of dressing for Neurosurgery.
- ✓ Elimination of aspiration catheter cleaning flasks.
- ✓ Containment bags incorporation.

We can conclude that the taken actions were welcome by the users (doctors and nurses). The staff of the Central Sterilization Service was driven due to these actions and stated its commitment to develop further interventions.

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Analysis of the structure and operation of the Sterilization Central, according to regulation. Parameters to be evaluated to project changes that optimize the service

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Health institutions try to decrease the incidence of nosocomial infections. The Unit sterilization takes center stage in solving this problem. The aim of this study was to evaluate the activity, structure and operation of the Sterilization Unit of a high complexity hospital (level III) located in the city of Santa Fe, according to regulations. An evaluation of organizational aspects was conducted through a checklist on the supervisor in charge of the plant. The percentage of compliance parameters was analyzed. The sterilization plant is close to the surgery room does not have a unique area where sterilization procedures are carried out, covers 110 m^2 total area, each area is defined. The equipment consists of a steam autoclave with electronics and programmer, two drying ovens with forced air circulation and other no. The sterilization staff consists nine auxiliary, in two shifts, suitable in various activities and one staff supervisor who is in charge. The adequacy of the regulatory framework and the quality of health services are primordial purposes for this Institution. In conclusion, this work highlights which are the priority to make changes in terms of physical conditions, equipment and personnel of the sterilization unit.
To close it is added that, considering that it has begun construction of a new building for the hospital, all the highlights in this study will be useful for planning future Sterilization Unit to establish science-based objectives service and its functions so to design it according to the needs of the institution.

Designing a Quality Management System for the Sterilization Service within a Health Care Organization in Córdoba.

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Key words: Sterilization Service, Quality Assurance, ISO9001-2008, Process, Instructional.

Healthcare organizations are nowadays forced to provide proof of a safe and high quality service delivery. Every single department within a hospital, specially the Sterilization Service (SS), must align with a set of rules and specifications that lead to a continual improvement of the services they provide.
The Standard ISO9001 implementations not only leads to a better understanding of the current service level, but also allows the identification of potential changes, and fulfil legal and technical requirements as

well as customer expectations. This implementation permits re-classify existing procedures and create new procedures focused on the Process Approach to Management defined on ISO9001.
This paper aims to design a Quality Management System (QMS) to be implemented in the SSof a healthcare organization in Córdoba.
To carry out the development of the QMS the following tasks were performed:

- Review of existing processes and activities within the SS, classifying them and identifying critical activities.
- Writing of documents.
- Definition of work instructions along with detailed activities and records for Key, Support and Management processes.
- Design of the Quality Manual (QM) based on ISO9001 requirements.
- Design of the necessary documents to fulfil the standard ISO9001.

This work involved the design of the documentary base of a QMS, the elaborated documents are:

- 1QM
- 8 Required Procedures.
- 27 Work Instructions.
- 3 Job Descriptions.
- 8 Process forms
- 10 Product forms.
- 65 Records.

Among the 27 Work Instructions, the followings are important to highlight:

1. Sanitary Conditions Assessment (Sanitary Profile).
2. Hazard analysis and critical control points
3. Integrated Pest Control.
4. Control Panel.
5. Pharmacoeconomics Assessment.

The 115 documents designed and prepared are the basis for an integrated management system that ensures the quality of the products and services offered by the SS.
The adoption of a QMS lead to a differentiation from other traditional services by systematically organizing what is "intuitively" done.

Replacement of mercury-containing products campaign as a community pharmacy service.

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Keywords: Mercury, Mercury-Containing Products, Community Pharmacy Services.

Mercury fever thermometers and other devices can cause a serious health threat if they break, spill, or leak. Mercury is also found in lab chemicals, fluorescent light bulbs, and other products commonly used.

At ambient temperature, it is a liquid which evaporates exposing people to high level of gas. A careful waste management is required to avoid the toxicity of organic mercury by environment pollution.
To protect the health of communities worldwide, Health Care Without Harm and the WHO are co-leading a global partnership to achieve virtual elimination of mercury-based medical devices over the next decade. The Córdoba Pharmacists' Association has joined to this initiative in 2014.
The aim of this work is to display the Association's activities for training and informing professionals about replacement of mercury-containing products.
A self-administered questionnaire related to management of small spills of mercury was adapted from the web site of the international initiative. The survey was conducted before a conference with 3 different consecutive speakers about the impact of mercury in health and environment, and the role of health professionals.
A campaign was designed for replacement of mercury-containing products, focused on general population and based on community pharmacies.
The conference took place on May 9, and 46 questionnaires were completed, but the audience was about 60 people. Most of the attendees were pharmacists (72%), and 65% were working in community pharmacies. Only 6 facilities had a protocol for managing small spills of mercury and 33% of surveyed persons checked they do nothing if a mercury thermometer breaks.
Most hospitals and pharmacies should not be using or selling mercury-containing products.
To disseminate information among health professionals and public is urgently needed.
A general information brochure was designed for the campaign, which is ready to be launched on September-October.
All efforts for awaking and being conscious of health threat and environmental pollution by mercury have to be carried out.

Periodic health team training on sexually transmitted infections (STIs) is necessary to improve the detection, recording and treatment of these diseases.

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Key words: sexually transmitted diseases, hospital pharmacy, pharmaceutical intervention, public health

The National Program for Human Retroviruses, AIDS and STIs (NPHRAS) was implemented in the Hospital Arturo Umberto Illia in 2009. Using the information available in the Pharmacy Service on patients with syndromic treatment of sexually transmitted infections (STIs) became evident underreporting of patients in the NPHRAS. This situation leads to insufficient use of medicines and resources provided by the NPHRAS at the expense of own funds of the Pharmacy.
STIs training activities were implemented during the second half of 2011 by Infectious Diseases Committee encouraged from the Pharmacy and a significant increase was observed in the number of patients included in the Program in 2012.

The objective of the study is to analyze the evolution of the patient records in NPHRAS 2 years after the training sessions on STIs. A descriptive cross-sectional study was conducted to determine the status of records, analyze interventions from the Pharmacy and contribute to the knowledge of epidemiological trends. Data from the first half of 2014 were compared with the same period in 2011 and 2012.

Patients registered in the NPHRAS in 2014 decreased by 24.5% compared to 2012, while it increased the total number of patients attending the Hospital and in Pharmacy Department. Nevertheless, the number remained above those before the training sessions.

There was no change in the proportions of patients by gender but increased the patients over 50 years under program.

The high percentage of trichomoniasis registered in 2012 and syphilis cases did not exhibit significant changes.

The pharmaceutical interventions were increased in 2014 requiring several dose changes (8.8% *versus* 4.7%). There was 33% reduction in the proportion of Program records submitted with incomplete personal and epidemiological information.

Patients who had treatment of sexual partners in 2014 were reduced by 21%. Also decreased dispensing condoms supplied by NPHRAS. This implies a reversal of previously obtained achievements regarding actions to reduce the transmission of these infections.

Decreased registration in NPHRAS detected in 2014 highlights the need to conduct regular workshops and training on STIs, with emphasis in periods in which new medical professionals join the Hospital. Improve and maintain adequate records in NPHRAS leads to optimizing the use of resources and helps reduce the transmission of STIs. This contributes to the effectiveness of treatments and knowledge of epidemiological trends, providing guidance for the implementation of new prevention efforts.

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DRUG QUALITY CONTROL

An alternative methodology to evaluate dissolution similarity. Intra-batch variation rule for f_2.

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Dissolution test is an useful tool to ensure the quality of a drug product during the stages of development and production of solid dosage forms.
Difference (f_1) and similarity (f_2) factors proposed by Moore and Flanner are an example of model independent methods for the comparison of dissolution behavior; to this end, f_2 is the most used approach. The f_2 factor is a mathematical transformation of the difference between dissolution profiles (mean of 12 curves) of test and reference formulation. However, f_2 has several disadvantages; among them it ignores variability of the individual dissolution curves within each batch, since it is calculated from the dissolution profile.
For this reason we propose an improvement of the f_2 methodology, where each curve is used for comparison. The fact of taking into account the data distribution within batch is an emerging advantage over the traditional method.
The strategy proposed for the evaluation of the dissolution of two batches can be divided in two stages. In a first instance, the f_2 is calculated among all reference curves. Using these values, a lower limit of acceptance for f_2 (LLAF) is set, given by the condition that 97.5% of the calculated f_2 values are above this limit. In a second instance, the f_2 values are calculated among all reference curves and all test curves. This set of f_2 values are individually compared with the LLAF calculated in the first stage. The rule of acceptance is that at least 80% of the reference-test f_2 values must be above the LLAF.
The proposed methodology was validated with dissolution data of two batches each of four commercial brands (totalling 8 batches) of hydrochlorothiazide tablets. Comparing the traditional f_2-based determination with the proposed method, few discordant datapoints were found.

Similarity study of metoclopramide tablets.

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Key words: Biowaiver, Dissolution efficiency, Metoclopramide

Active substances are categorized into four groups, within the Biophamaceutics Classification System (BCS), based upon their solubility and permeability. EMA and WHO consider Class I and III drugs as eligible for a BCS based biowaiver. Therefore multisource formulations containing such a drug may be exempted from proving bioequivalence with the reference product. In lieu of in vivo data, dissolution profiles at recommended pH should be provided for multisource and reference formulations.

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Metoclopramide, a class I/III drug, is used for the treatment gastro-esophageal reflux, nausea and vomiting. Our research attempted to compare dissolution profiles of multisource metoclopramide tablets with the reference product in three different media.

Samples (A-E) of metoclopramide tablets (10mg) were purchased from pharmacies in Bahía Blanca. Dissolution profiles were performed using USP Apparatus 1 at 50 rpm, in 900 mL of USP buffer solutions (pH 1.2, 4.5 and 6.8). Drug concentration was determined by UV-Vis spectrophotometry at 308.1nm. Analysis of variance was used to evaluate dissolution similarity, in terms of Dissolution Efficiency (DE%).

Formulations B, C, D and E were very rapidly dissolving in the three media, whereas sample A was only very rapidly dissolving at pH 6.8, and rapidly dissolving at pH 1.2 and 4.5. The highest dissolution rate and DE% values corresponded to the sample E at pH 4.5 and 6.8, although at pH 1.2 corresponded to sample B. Sample A had the lowest DE% values in the three media. In comparison with the reference (D), samples B and C had higher DE% values in all media, whereas sample E had lower results at pH 1,2. Statistical differences were found between DE% values of samples A, B, E and the reference.

Despite the statistical differences found, formulations B, C and E could be considered similar to the reference based on its "very rapid dissolving" status.

Dissolution stability of furosemide tablets

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Keywords: Furosemide, Dissolution Stability, Tablets

Dissolution Stability is a critical biopharmaceutical parameter that may be influenced by the manufacturing process, physicochemical properties of the active substance and excipients and possible interactions between them, storage conditions and packaging. Considering the possible impact it may have on the bioavailability of the product, it is essential that the dissolution characteristics of a formulation remain unchanged throughout its shelf life. Furosemide, a class IV drug in the Biopharmaceutics Classification System, is a loop diuretic used in the treatment of edematous states and hypertension. Our research attempted to evaluate the influence of natural aging conditions on the chemical and dissolution stability of different furosemide formulations available in Argentina, during one year of storage.

Samples (A–I, reference and multisource products) of furosemide tablets (40 and 50mg) were purchased from pharmacies in Bahía Blanca. All dissolution studies were performed using USP Apparatus 2 at 50 rpm, in 900 mL of USP phosphate buffer pH 5.8 and drug concentration was determined by UV-spectrophotometry (276.1nm). Spectrophotometric assay was performed in NaOH 0.1N (271nm). The formulations were stored for twelve months under ICH natural conditions (25°C-60% R.H.). Analysis of variance (ANOVA) was used to evaluate chemical and dissolution stability, in terms of Dissolution Efficiency (DE%).

At time zero and after one year of storage, product I failed to comply with assay and uniformity of dosage units test criteria. Furthermore, this product did not meet neither USP nor FA dissolution test specifications, and presented a considerably lower dissolution performance compared to other products. Significant differences in DE% results were found between A, F, H and I respect to the reference, both at time zero and after one year of storage. Moreover, the ANOVA results indicate that there were significant differences throughout the dissolution stability study for samples D, H and I, whereas only F was not chemically stable. Further research is required to establish the causes of these differences.
Natural aging conditions clearly affected dissolution and chemical stability of some furosemide tablets.

A new UV spectrophotometric method of ertapenem sodium.

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Keywords: Ertapenem Sodium, Analytical Methods, Quality control, Spectrophotometry.

Ertapenem sodium (ERTM), a synthetic antimicrobial agent of the class of carbapenems, shows action against Gram-negative, Gram-positive, aerobic and anaerobic microorganisms. This work proposes the development of methodologies for the quantification of ertapem sodium, giving priority for ease of implementation, reduced analysis time and reducing the use of organic solvents, since the sustainability has been discussed and encouraged globally. The sample was weighed from a *pool* of three vials the samples. The equipment used was Spectrophotometer UV Shimadzu^{TM}, quartz cells, aqueous solutions of ERTM at concentrations of 12, 15, 18, 21, 24 and 27 µg mL^{-1}, each concentration was prepared in triplicate. A standard calibration curve of ERTM 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. In the spectrophotometer, the absorption was obtained at 297 nm. The linearity of the method was proven by the correlation coefficient that was 0.9999. The content of ERTM in the samples analyzed was 108.26 %. The accuracy of the method was proven by the recovery test, with an average of 102.61 % with RSD 1.211 %. The robustness was assessed at 296 nm and 298 nm, as well as using two different equipment and different sources to obtain water used as solvent. The content results for robustness were in wavelength 296 nm 106.702 %, in wavelength 297 nm 107.433 % and in wavelength 298 nm 108.589 % with RSD 0.884 %. The content results for robustness between equipments were 109.913 % and 109.909 % with RSD 0.002 %. The content results for different fonts of obtaining water were 109.596 % and 110.205 % with RSD 0.391 %. The precision for repeatability presents RSD 0.824 %. The intermediate precision presents RSD 0.427 % for different days and RSD 0.993 % for different analysts. The ERTM showed a linear relation between absorbance and concentration, in addition the method developed shown to be appropriate and simple. Thus, the results demonstrated that the spectrophotometric method could be applied for the analysis of the pharmaceutical formulations assuring the quality and efficacy of the ERTM, helping to improve the quality control of the drug marketed.

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Development and validation of a HPLC-UV method for determination of Levofloxacin in lung tissue. Application to the quantification of the drug after pulmonary administration into biopolymeric microparticles.

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Keywords: HPLC - Levofloxacin – Lung – Microparticles – Validation

A HPLC-UV method for determination of Levofloxacin (LFX) in mouse lung tissue was developed and validated, in order to quantify the drug after pulmonary administration into biopolymeric microparticles, as a targeted delivery strategy useful for the management of pulmonary infections in patients with cystic fibrosis.

Conditions: C18 column, acetonitrile/KH2PO4 30 mM pH 2.80 (22/78) mobile phase, 1.0 ml/min and 300 nm detection. Enrofloxacin (EFX) was used as internal standard (IS). For sample preparation, excised mouse lungs were homogenized, spiked with IS and diluted in 2 ml of physiologic solution. After 24 hs at 4 °C, two 0.9 ml-aliquots were extracted with 2 ml of dichloromethane and centrifuged. The separated organic layer was evaporated to dryness, and the residue resuspended with 100 µl of mobile phase prior to injection.

In an initial validation stage, samples were prepared in blank homogenized lung tissue by the addition of known concentrations of LFX and EFX in methanolic solution. The response was linear between 0.02 (LQ) – 15.00 µg/ml, precision RSD values were < 4.0% and accuracy was 90–100%. The method was specific to the biological matrix, and the drugs were stable in the matrix, methanolic solution and prepared samples. Recovery was around 70% due to the drug loss in the tissue, since the extraction procedure had a 95-100% recovery.

Microparticles were prepared by co-precipitation of CaCO3 in presence of the alginate biopolymer. The obtained hybrid microparticles showed narrow size dispersion around 5 µm, ideal for pulmonary delivery. LFX was incorporated by absorption, reaching a loading of 40.0 µg/mg of matrix. Therefore, in a second validation stage, validation parameters were re-assessed using these microparticles instead of the methanolic solution, with similar results.

Finally, the method was successfully applied to the quantification of LFX in lungs of mice sacrificed after receiving pulmonary administration of the microparticles.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Development and validation of cefazolin sodium by Infrared Spectroscopy method.

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Keywords: Infrared, Quality Control, Method Validation.

Cefazolin sodium (CFZ), a first generation cephalosporin, is often used as therapeutic agent and for perioperative prophylaxis. This work proposes the development and validation of an analytical method for the quantification of cefazolin sodium in powder for injection preparation using Fourier-transform infrared (FT-IR) transmission spectroscopy, giving priority for ease of implementation and reduced analysis time. Spectra of KBr pellets containing CFZ were obtained with spectrophotometer Shimadzu™ FTIR, model IR Prestige-21. The method involved absorbance measurements of the band correspondig to carbonyls of molecule, centered in the region between 1820.802 and 1722.433 cm^{-1}. This method was validated over a concentration range from 0.4 to 1.7 mg. The linearity of the method was proven by the correlation coefficient that was 0.9994; the content of CFZ in the samples analyzed was 100.64 %. The accuracy of the method was proven by the recovery test, with was 100.50 % with RSD 0.25 %. The robustness results for KBr brand Synth™ presents RSD 0.54 % and Shimadzu™ 2.25 %, in time of compression for 10 min presents RSD 1.25 % and 15 min 3.49 % and in compression pressure with 100 kN presents 3.15 % and with 90 kN 3.53 %. The precision for repeatability presents RSD 0.74 %. The intermediate precision presents RSD 1.82 % for different days and RSD 1.96 % for different analysts. The CFZ showed a linear relation between absorbance and concentration in the region mentioned above. A simple, fast and reproducible infrared spectroscopy method was developed and validated for quantification of CFZ in powder for injection preparation. The technique could allow the characterization and quantitation of CFZ, with the advantages does not use organic solvents, so contributes to minimize the generation of organic solvent waste by the chemical and pharmaceutical industry and thereby reduces the impact of its activities on the environment. Therefore, the results could be applied for the analysis of the pharmaceutical formulations assuring the quality and efficacy of CFZ.

A new, safer and easier thin-layer chromatographic method for cefepime hydrochloride identification.

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Keywords: Cefepime hydrochloride, Analytical Methods, Quality control, Thin-layer chromatography.

Cefepime hydrochloride, a fourth generation cephalosporin antibiotic for parenteral use, is a semi-synthetic product which has activity against several Gram-positive and Gram-negative aerobic bacteria. The Thin-Layer Chromatography (TLC) is among some of the methods described for the identification of cefepime hydrochloride. It is a common method for drug's identification, because it is simple, fast, visual and relatively inexpensive. However, the TLC method described for cefepime hydrochloride in the main pharmacopoeia is complex and the solvents used as mobile phase are potentially toxic to the operators, for

example a mixture of *n*-propyl alcohol, water, and ammonium hydroxide (7:5:4). The aim of this work was to develop an easier, safer and more economical TLC identification method for cefepime hydrochloride. The mobile phase used was a mixture of absolute ethyl alcohol and water (40:60 v/v) and the stationary phase used was silica gel impregnated in aluminum. The standard and sample solutions of cefepime hydrochloride were prepared in purified water at concentration 2 mg mL^{-1} (w/v) and it was applied 10 µL of both solutions on the plate. After the mobile phase's elution, the spots were detected by exposing the dry plate to iodine vapor during ten minutes. The result of chromatogram showed a spot and Retention factor (Rf) of 0.53 for each solution, demonstrating that the test solution was similar in position, color and size to the reference solution spot. The TLC method proposed in this study showed to be easier, safer and more economical than the methods described in the literature and can be used instead of those for cefepime hydrochloride identification, in the routine analysis of quality control.

Specificity evaluation of chromatographic method for determination of cefepime hydrochloride in powder for injection solution.

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Keywords: Cefepime hydrochloride, RP-HPLC, Stress study, Quality control.

Cefepime hydrochloride (CFP) is an antibiotic for parenteral use which is included among the class of antibiotics called fourth generation cephalosporin and is a semi-synthetic product. It has broad spectrum of action against Gram-positive and Gram-negative aerobic bacteria. It is mechanism of action is similar to other β-lactams, it means, it inhibits bacterial cell wall synthesis by binding to one or more Penicillin-Binding Proteins - PBPs. In this work, the aim was to develop and evaluate the specificity of the method of RP-HPLC for the determination of cefepime hydrochloride and possible degradation products in pharmaceuticals products. The mobile phase used was composed by water:absolute ethanol (45:55, v/v) and the flow rate was 0.5 mL min^{-1}. For the stationary phase used was a C_{18}-column (250 x 4.6mm) and the UV detection was performed in the wavelength at 258 nm. The retention time observed for CFP was 4.9 min. The stress degradation studies were conducted under conditions of hydrolysis (acid, basic, oxidative and neutral) and photolysis. The hydrolysis conditions were carried out at 60°C. The results showed for all hydrolysis conditions, there were a substantial decrease of the peak area of CFP, also was observed the appearance of new peaks in the chromatograms and exhibited first-order reactions with rate constants (k) of 0.08 h^{-1} (basic), 0.05 h^{-1} (acid), 0.02 h^{-1} (neutral) and 0.66 h^{-1} (oxidative). The drug was more stable on photolytic condition, and the maximum degradation was observed on oxidative hydrolysis with retention time at 4.6 min. Thus, the forced degradation studies demonstrated the ability of stability of the proposed method that can be applied to the quantitative analysis of cefepime hydrochloride in pharmaceutical dosage forms, which contributes to improving the quality control and to ensure the therapeutic efficacy. Furthermore, this study contributed to the knowledge of the behavior of the drug under conditions of stress.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Potentiometric titrations: some tips when working in hydroalcoholic media.

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Key words: Potentiometric titrations- Hydroalcoholic media- APIs

Many acid-base titrations are difficult to accomplish using a visual indicator for several reasons. There may not be a suitable color change or the solutions may be opaque or turbid near the end point. In such situations, potentiometric titrations using a hydrogen glass electrode with a silver/silver chloride reference electrode may be advantageous.
British Pharmacopoeia (BP) 2013 specified titration assays in hydroalcoholic media for 158 active pharmaceutical ingredients (APIs) determining the end point potentiometrically, due to weak acid-base strength or poor aqueous solubility characteristics.
Due to some inconsistencies of the results that we obtained in the titration of Bromhexine hydrochloride (BROM), Ambroxol hydrochloride (AMB), Pseudoephedrine hydrochloride (PSE) and Clopidogrel bisulfate (CPG) following BP 2013 specifications, we assayed different conditions that may be taken into account for titration of these APIs.
We compared different type of glass electrodes from several brands and applications and also test the effect of performing warming or sonication some minutes during sample solutions preparation.
Experiments were performed in quintuplicate. APIs were pharmaceutical grade. We used Cole Parmer ®, Sartorius ® and Mettler Toledo SG2 SevenGo ™ potentiometers. Glass-silver/silver chloride electrode combination recommended for aqueous media PY-P11 Sartorius and Mettler Toledo Inlab SG/2m 413 electrodes and non aqueous media Mettler Toledo Hanna InLab Science and Hanna FC200 electrodes.
The results showed that all electrodes are suitable for potentiometric titrations of these APIs, however repeatability of the assays was better when non aqueous specific electrodes were used.
We suggested some practical advices for potentiometric titrations in hydroalcoholic media: use non aqueous electrodes with fast response time, sonicated or warming is the same during sample preparation and be carefully with adding constant and little aliquots of titrant near to end point.

Design and physicochemical stability of Glyburide pediatric oral liquid formulations for the treatment of Permanent Neonatal Diabetes.

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Keywords: Glyburide, Glibenclamide, stability, permanent neonatal diabetes mellitus

Introduction: Permanent neonatal diabetes mellitus (PNDM) is a rare disease, affecting less than 1% of all cases of diabetes. Several small clinical studies and case reports have demonstrated Glyburide's efficacy in the treatment of this disease. Since this rare condition is often diagnosed at birth or small age, oral

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liquid formulations are needed for treatment. Until now, there have been no reports of development and stability studies for Glyburide liquid formulations

Purpose: To study the chemical and microbiological stability of Glyburide formulations, used as therapeutic agent in treatment of PNDM, in two liquid formulations developed: one suspension (2.5 mg/ml) using Glyburide tablets and another suspension (2.5 mg/ml) using Glyburide raw material, both for pediatric use. Furthermore, to optimize and validate a stability-indicating HPLC method for the analysis of Glyburide in the studied formulations.

Methods: Samples were stored at 4°C, 25°C and 40°C. Glyburide content of each formulation was analyzed in triplicate using high performance liquid chromatography (HPLC) at 0, 7, 14, 28, 56 and 84 days. The chromatographic system consisted of a C18 column (150mm, 4.6mm; 5 µm), a mobile phase of acetonitrile:KH_2PO_4 1.36% w/v pH=3 (47:53) with a flow of 1.5 ml/min, temperature of 25ºC and ultraviolet detection at 300 nm.

Results: All formulations stayed stable at 4°C and 25°C during the 84 days of the study. However, the raw material formulations stored at 40°C only stayed stable during 56 days. Microbiological stability was guaranteed for both formulations during the whole study.

Conclusion: Glyburide formulations were developed and can be stored for at least 84 days at 25 °C or less. These formulations are perfectly suitable for pediatric patients who are usually not able to swallow tablets. The proposed analytical method was suitable for the study of stability of different formulations.

Forced degradation study of daptomycin in powder for injectable solution.

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Keywords: daptomycin, stress study, HPLC

Daptomycin is an antimicrobial agent for parenteral use and was the first approved member from a new class of antimicrobials, the cyclic lipopeptides. Considering the importance of this drug for the global scene, the aim of this work was to perform a forced degradation study of daptomycin in powder for injectable solution and to evaluate the selectivity of an HPLC method that was prior developed. Sample solutions of daptomycin were subjected to forced degradation by acidic, basic, aqueous, oxidative and photolytic conditions and were evaluated by HPLC. Photolytic degradation was performed at room temperature and the others at 60 ^{o}C. The HPLC methodology employed as mobile phase ethanol and water (55:45, v/v) with pH adjusted to 4.5 with glacial acetic acid, pumped at a flow rate of 0.6 mL min^{-1}. A C_{18} column was used as stationary phase and the UV detection was performed at 221 nm. Daptomycin showed to be more stable on photolytic condition and the maximum degradation was observed on alkaline hydrolysis. Alkaline and oxidative degradations exhibited first-order reactions with rate constants (*k*) of 0.07 h^{-1} and 0.10 h^{-1}, respectively. Acidic and aqueous degradations presented second-order reactions, with *k* values of 6.0 x 10^{-4} μg^{-1} mL h^{-1} and 3.0 x 10^{-4} μg^{-1} mL h^{-1}, respectively. Due to the low degradation of daptomycin under photolytic conditions, it was not possible to estimate the kinetics of the reaction in this case. Four degradation products were detected, at 6.6 min (basic and aqueous), 4.8 min (acidic and oxidative), 5.2 min (oxidative) and 3.2 min (photolytic). The retention time of daptomycin

was 5.7 minutes. The stress study of daptomycin showed the adequate selectivity of the HPLC method, since it was possible to quantify the drug in the presence of its degradation products. The HPLC method also showed its stability indicating capability.

Development and validation of a rapid turbidimetric assay to determine the potency of daptomycin in powder for injectable solution.

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Keywords: daptomycin, turbidimetric assay, microbiological method, quality control

Daptomycin is an antimicrobial agent recently included in the market and used in many parts of the world. It has great importance for the clinical practice nowadays, mainly because this antimicrobial presents selective action against Gram-positive bacteria, including methicillin and vancomycin resistant strains. There are few studies in the literature regarding the development of analytical methodologies for the analysis of this cyclic lipopeptide, so researches in this area are highly relevant to optimize the analysis of this drug in the industry and ensure the quality of the marketed product. The development of microbiological methods for the analysis of antimicrobials has gained strength in recent years and has been highlighted in relation to physicochemical methods, especially because they make possible to determine the bioactivity of the drug against a microorganism. In this context, the aim of this work was the validation of a microbiological method for quantitative analysis of daptomycin in powder for injectable solution by turbidimetric assay. For performing the method, *Staphylococcus aureus* ATCC 6538 IAL 2082 was used as the test microorganism and the culture medium chosen was the brain heart infusion. The method was validated according to ICH guidelines, showing to be linear, precise, robust, accurate and selective, over a concentration range from 8.0 to 18.0 µg mL^{-1}. Thus, the validated method is able to quantify daptomycin in powder for injectable solution, while being an economical, rapid and environmentally friendly alternative for its routine analysis in quality control.

Development of dissolution test for fenbendazole and praziquantel tablets for veterinary use.

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Fenbendazole (FEN) and Praziquantel (PRA) association is widely used in veterinary treatment of parasitic diseases. However, this association is not included in any renowned Pharmacopoeia.
Consequently, the aim of this work was to develop a dissolution test for this association. A batch of capsules containing 500 mg of FEN and 50 mg of PRA were prepared in our laboratory as a reference

formulation. A published HPLC method was revalidated and used to determine analytes during dissolution experiments. (G. Morovján et al, *J Chromatogr A*, 797, 1998).
All the tests and assays were carried out according to the USP 35 Pharmacopoeia.
In the screening phase, two factors, HCl concentration and ethanol proportion, were found to be significant in the dissolution of analytes. In the optimization phase a full factorial 3^2 experimental design was carried out.
The optimal conditions for the dissolution test were: 900 mL dissolution medium composed of a mixture of 300 mL of ethanol and 600 mL 0.5 N HCl, USP apparatus 2, 75 rpm in a bath thermostated to 37.0 ± 0.5 °C.
Two brands of commercial tablets were evaluated and the optimized conditions were used to build the dissolution profiles of both brands. The amount of drug dissolved for brand 1 was greater than 80 % ($Q_{proposed}$) for both analytes after an hour of dissolution. However, brand 2 failed the dissolution test.
Other quality parameter tablets were also evaluated. Brand 1 complied with the disintegration test, while Brand 2 did not pass this test, which agrees with the results obtained in the dissolution test. In addition, hardness obtained for both brands shows some differences, brand 1, 12 Kg (RSD 7) and brand 2, 15 Kg (RSD 14).
On the other hand, the brands met the general pharmacopoeial requirements of uniformity of dosage units, dose control and friability, showing the need to carry out dissolution test to detect failures in formulation.

Simultaneous separation and determination of Avermectins by MEEKC previous solid-phase extraction/pre-concentration.

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Keywords: Ivermectin, Moxidectin, Capillary Electrophoresis, Microemulsion Electrokinetic Chromatography.

Ivermectin (IVM) and Moxidectin (MXD) are macrocyclic lactones belonging to Avermectin families, structurally related compounds. These were the first macrocyclic lactones (MLs) developed as antiparasitic agentin production animals and antifilarial treatment in humans. Due to their wild use, they have being identified as potential contaminant for the aquatic sediment ecosystem, it is therefore of particular interest to monitor the presence of these veterinary drugs in surface waters at low concentrations. Several analytical methods have been developed for the detection and quantification of Avermectin in different matrices such as HPLC, CE, LC–MS/MS among others.
The aim of this study is to develop method for determine IVM and MXD at low concentrations in surface waters. For this purpose, non-polar XAD-4 resin is used for solid-phase extraction as pre-concentration step previous to separation by MEEKC (Microemulsion Electrokinetic Chromatography). The studied Avermectins in aqueous solution were retained by polimeric resin due to theirs hydrophobic property. The adsorbates were then eluted with ethanol and recollected for further separation. The microemulsion buffer used for MEEKC was prepared with phosphate buffer at pH 2.5, n-octane as oil droplets, sodium dodecylsulphate as surfactant, 1-butanol as co-surfactant and appropriate types and amounts of organic co-solvent.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Under optimal experimental conditions, a complete electrophoretic separation has been achieved within 12 min. The sensitivity of this methodology is adequate for separation and trace determination of IVM and MXD in environmental samples. Moreover, the proposed methodology avoids the use of highly pollutant and toxic solvent commonly employed for this purpose, which could be considered as green chemistry.

Effect of MWCNT as SPE packing material for the separation of chiral emerging contaminants.

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Keywords: Chiral drug, MWCNT (multiwalled carbon nanotubes), SPE (solid phase extraction).

Nowadays the emerging pollutants have a great concern, and between them the most studied are pharmaceutical drugs. Thesechiral compound often exhibit different effects in pharmacological activity, transport mechanism, metabolism pathway and toxicity. Specifically, β-blockers are sold and administered as a racemic mixture, but the (S) enantiomer is pharmacologically active one. Paradoxically, the (R) enantiomer is the one which has negative and toxic effects. Carboxylated multiwalled carbon nanotubes (cMWCNT) have shown interesting characteristics as enantioselective packing or stationary phase material in separative methods.

The aim of this work was develop a method able to separate a racemic mixture of emerging pollutants. The studied analytes were β-blockers (pindolol, propranolol and labetalol) commonly used in the treatment of heart rhythm disorders.

We performed a separation procedure using carboxylated and non-carboxylated multiwalled carbon nanotubes (ncMWCNT) as SPE packing material. For the assembly of the columns, 2.7 to 12 mg of MWCNT was weighed and the elution solution was water. On the back of the column a 0.45 µ filter was placed to retain MWCNT. The analytes were determined using an HPLC system with a C18 column and the mobile phase used was Acetinitrilo/NH_4HCO_3(50:50), pH 10 with a PDA detector.

The results showed that using ncMWCNT there were not separation for any of the studied analytes. In the case of cMWCNT the results for the three studied analytes were different. In the case of labetalol there was not separation, however for propranolol the separation was not so evident, and the result may be improved with some simple changes in the eluent. But, for pindolol the separation was very clear.

The preliminary conclusions of the work were that cMWCNT could be an interesting and economic alternative for the separation of chiral drugs. The next step is the molecular study of the interaction the three drugs with the cMWCNT.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Uncertainty of measurements in pharmaceutical analysis. Quality assurance in the laboratory control: Internal validation of volumetric flasks.

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Keywords: Calibration, Quality Assurance, Analytical Control

Uncertainty is a useful tool in the assessment of compliance or noncompliance in the process of the production of medicines, therefore this assessment in analytical processes requires a detailed study of all possible causes.

The sources of uncertainty are present in every aspect of the measurement process, causing results with random or systematic errors.

In the preparation of the solutions used for quantitative determinations there are two processes that are fundamental sources of uncertainty: weighing and correct reading of the volume.

In this context, along with UPM's laboratory staff, we have started the validation of analytical control processes, starting with the evaluation of the process of preparing solutions. Systematic errors associated with the equipment were tested with periodic checks of linearity of scales and the implementation of an internal protocol for the existing volumetric flask contrasting with the certified material. The uncertainty generated by the generated random errors associated with the operators is evaluated on intra and inter-operator measurements by statistical analysis of the values obtained on different days, four people, in triplicate.

The activities prior to assessing the uncertainty were:

. Designing a protocol to determine the specific content of each flask by the method called "Adjustment to In".

Staff training in the use and control of volumetric flasks (involved two senior students of Pharmacy with two laboratory technicians).

The work was carried out with flasks 10, 25, 50 and 100ml, a single batch of distilled water, temperature and humidity were tested during the experience and the scales were recently checked/calibrated.

Of the 52 non-certified laboratory flasks, 14 were discarded for not meeting the acceptance range. It is relevant to note that these have a significant deviation of up to 0.33%.

Moving forward in these procedures will allow us an internal certification of the volumetric flask and a high reliability in our results. We have scheduled the process pipettes by the "Adjustment to Ex" method.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Related compounds: atorvastatin calcium.

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Key words: Atorvastatin Calcium – Working Standards – Related compounds

Standards related compounds are routinely used for quality assesment of raw materials of active pharmaceutical ingredients (APIs). They are very expensive and are sold by a few miligrams by United States Pharmacopoeia (USP).

According to USP Atorvastatin Calcium has several related compounds (RC): desfluoro (related compound A); 3S,5R isomer (B); difluoro (C); epoxide (D); 3S,5S enantiomer (E); diamino; 3-deoxyhept-2-enoic acid; lactone (H); epoxide tetrahydrofuran analog; ethyl ester and acetonide (I). These compounds have an acceptance criteria no more than (NMT) 0,1-0,3% and other minor impurities (NMT 0,1%) depending on the synthetic route.

The aim of this work was obtain by synthesis or degradation of Atorvastatin calcium working standards of related compounds for quality control. We have synthetized lactona (related compound H), α,β-unsaturated lactone, methyl ester of Atorvastatin and Atorvastatin acid. Lactone is a synthetic precursor of calcium salt, and therefore is an impurity of synthesis and a degradation product of Atorvastatin calcium, as it is easily formed in weakly acidic media. Atorvastatin methyl ester is a synthetic impurity. The α,β-unsaturated lactone, is a impurity of acidic degradation. These compounds were purified by column chromatography and recristalization. They were characterized by NMR (1H-NMR, 13C-NMR, and homo and heteronuclear correlation spectroscopy), FTIR and HPLC. Bruker NMR (200MHz) spectrometer, Shimadzu FTIR, Waters HPLC were used.

Lactone was succesfully obtained with a good yield, purified, identified and used as working standard for quality control. The methyl ester compound was characterized but it was not the major product of the synthesis. The α,β-unsaturated lactone was obtained in drastic acidic condition. Atorvastatin acid were also identified by NMR and FTIR.

We concluded that it is possible to obtain some Atorvastatin impurities in laboratory by simple and low expensive procedures.

Electrochemical determination of chlortetracycline in water samples.

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Keywords: Tetracyclines, Environmental pollutant, Electrochemistry, Water samples

Tetracyclines are an important group of antibiotics commonly used in human and veterinary medicine, aquaculture and animal husbandry. Owing to their extensive uses, these compounds are one of the pharmaceutical products most widely distributed as environmental pollutant. The presence of low levels of antibiotics and their metabolites in the environment could provide adverse effects like bacterial resistance and disruption ofcritical cycles toaquatic ecology.

Therefore, it is important to develop an analysis tool for the determination of tetracycline antibiotics in water samples. The purpose of this work was the electrochemical determination of chlortetracycline (CTC) in synthetic water samples.

Electrochemical experiments were performed using a BAS 100B/W electrochemical analyzer. Cyclic and square-wave voltammograms were obtained using a three electrodes system: glassy carbon working electrode modified with multi-walled carbon nanotubes (MWCNT), an Ag|AgCl|3M NaCl reference electrode and a Pt wire counter electrode. For the electrode modification 1 milligram of MWCNT was dispersed with the aid of ultrasonic stirring for 45 min in methanol/water (50:50, v/v) in an aqueous 0.1% Nafi on solution. Then, 5µL of this dispersion was dropped on the working electrode and the solvent was evaporated under an infrared heat lamp. CTC synthetic water samples were prepared by diluting CTC stock solution of 119 mg L^{-1} in purified deionized water. The CTC quantification was carried out by Square Wave Voltammetry (SWV) in 10 mL of 0.01 M phosphate buffer.

The CTC calibration plot was performed using SWV in a concentration range of 2 to 119 mg L^{-1}. The calibration equation was $\Delta I\ (\mu A) = 0.1026 + 0.0074\ C_{CTC}$ with a regression coefficient of 0.997. Based on the results, the proposed approach was successfully used to analyze the CTC content in water samples.

Electrochemical monitoring of ethinylestradiol oxidation by the horseradish peroxidase enzyme in water.

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Keywords: Ethinylestradiol, Environment, Horseradish peroxidase enzyme, Square-wave voltammetry

Ethinylestradiol (EE2) is a synthetic estrogen used in contraceptive therapy. The presence of this compound in the environment has been attributed to their incomplete removal in wastewater treatment plant processes.

The purpose of this research was to optimize the process catalyzed by Horseradish peroxidase (HRP) enzyme for removal of EE2 from synthetic water (purified deionized water) and the monitoring of the enzymatic process by square-wave voltammetry (SWV).

In this study, HRP catalyzes the oxidation of EE2 in presence of H_2O_2 and the products formed are polymerized through a non-enzymatic process which leads to the formation of high molecular weight polymers. These low-solubility polymers can be removed from wastewater by co-precipitation, sorption to solids, sedimentation or filtration. The reaction mixture was prepared by diluting stock solution of a EE2 to a concentration of 0.5 µM, in 10 mL of 0.01 M citrate buffer pH 5.0 containing HRP 0.02 U mL^{-1}. The reaction was initiated by adding 1 mM of H_2O_2. Then, after stirring for 10 min, the sample was centrifugated and EE2 was quantified by SWV. The enzymatic process was monitored by SWV using a three electrodes system: glassy carbon working electrode, Ag|AgCl|3M NaCl reference electrode and Pt counter electrode.

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For the measurement of EE2 by SWV a calibration equation was ΔI (µA) = 1.436 + 2.382 C_{EE2} with a correlation coefficient of 0.998, over the concentration range of 0.3–50 µM with a limit of detection of 0.11µM.

Our analysis demonstrated that EE2 disappeared almost completely in the reaction mixture after 10 min treatment. These results strongly suggest that HRP is effective in removing the estrogenic activities of EE2 in water samples.

Molecular imprinting applied to the analysis of nanoencapsuled Coenzyme Q10.

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Keywords: Coenzyme Q10, Molecular imprinting, Nanoparticles.

Coenzyme Q10 (CoQ10) is an essential cofactor in mitochondrial oxidative phosphorylation and a powerful antioxidant. It is used as a therapeutic agent in certain conditions like cancer, Parkinson, Alzheimer, cardiovascular, mitochondrial, neurological and muscular diseases. However, due to its high hydrophobicity and low permeability, CoQ10 bioavailbility is extremely reduced. That is why it was decided to employ a polymer-based nanoparticle approach to improve it.

CoQ10 is currently analyzed by HPLC in pharmaceutical and biological matrices.

In our experience, HPLC analysis of nanoencapsulated drugs, implies an intrinsic challenge due to the potential presence of chromatographic interferences and reduced lifetime in the chromatographic column produced by the polymer and surfactants which are dissolved with the drug in the sample.

In order to minimize these problems, it was decided to apply a clean up technique based on a molecularly imprinted solid phase extraction (MISPE), previously developed by this group, which have demonstrated a high specificity for CoQ10. The MISPE extract was analysed by a micro-HPLC system developed by our group.

CoQ10 was nanoencapsulated using an Eudragit® E PO copolymer, via a nanoprecipitation technique. HPLC direct injection of nanoencapsuled CoQ10 was compared to the sample previously purified by the MISPE procedure before chromatographic injection. The recovery assay using MISPE was carried out by spiking nanoparticles with CoQ10 at three different levels (120, 100 and 80%) by triplicate.

It was observed an excellent clean-up of the sample using the MISPE procedure with respect to the direct injection showing the complete absence of interferences with recoveries higher than 99%.

This method provides a practical way to analyze CoQ10 nanoparticles, being useful in the analytical monitoring during the development of this type of highly complex matrices.

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Study for thermal characterization of pentacyclic triterpenes: α, β amirin.

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Keywords: Amirin, anti-inflammatory, thermal analysis, thermogravimetry

Pentacyclic triterpenes belong to the group of terpenes and have been the subject of a large scientific interest because their various pharmacological activities such as hepatoprotective, antioxidant, anti-inflammatory and antitumor. TG–DTA/DSC is the quick and convenient techniques and has been widely used to characterize the changes of mass and enthalpy of materials during the thermal decomposition. Studies about thermal behavior of pentacyclic triterpenes using thermal techniques are scarce in the literature. In the present study, the thermal characterization of the α, β amirin, a pentacyclic triterpenes substance, was studied through differential scanning calorimetry (DSC) and thermogravimetry/differential thermoanalysis (TG/DTA). The α, β amirin curves were obtained on a SHIMADZU thermobalance and calorimeter, at heating speed of 2.5, 5 and 10ºC/ min^{-1}, on a temperature interval of 25 – 500ºC, under an atmosphere of nitrogen at 50 mL/ min^{-1}. Sample weights were around 4 mg and 2 mg, respectively. DTA and DSC curves of amirin, present two events and TG curve only one thermo decomposition processes. Thermal data of the pentacyclic triterpenes supply important information on evaluation of stability of the drugs, becoming an important tool on quality control and pharmaceutical technology on the development of products. TG/DTA and DSC study of α, β amirin show your thermal stability, and yours thermal parameters was determined to be used in the development and characterization of pharmaceutical products.

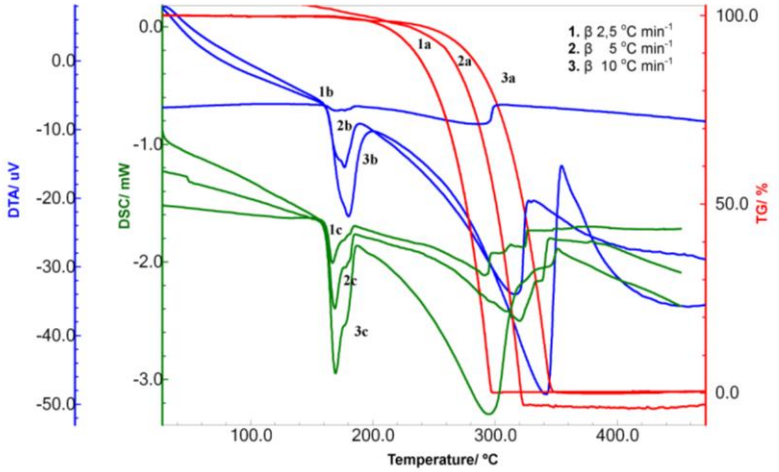

Figure 1. Thermal analysis by DSC, TG/DTA in different heating speed (2,5ºC min^{-1}, 5ºC min^{-1} e 10ºC min^{-1}).

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Enzimatic determination of glucose in total parenteral nutrition: Method validation.

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Key words: Glucose –Total parenteral nutrition– Enzimatic method validation.

Parenteral nutrition is widely administered to patients directly into the bloodstream when gastrointestinal tract is non functional. It supplies the main nutrients that the human body needs such as amino acids, carbohydrates, lipids, electrolytes, vitamins and essential elements in aqueous solution. The composition of total parenteral nutrition (TPN) solutions is formulated according to each patient, depending on age, disease etc.

The pharmacist is responsible for the preparation, quality control of process and final product.

Glucose (GLU), the most important hydrocarbon component of TPN, may be determined by glucose oxidase method (GOM). This method is based on GLU oxidation to gluconate, by the action of glucose oxidase enzymes and formation of hydrogen peroxide, which oxidizes 4-aminoantipyrine to form a red-stained compound with a maximal absorbance at 505 nm the color intensity is directly proportional to the glucose concentration. It is used for determining GLU in whole blood, serum, plasma, orine or cephalorraquid liquid samples.

In this work, specificity, linearity, accuracy, precision (repeatability and intermediate precision between days and analysts) have been established for quantify GLU in TPN by GOM. GTLab reactive and Anhydrous GLU Sigma-Aldrich were used. Perkin Elmer Lamda 25 Spectrophotometer was employed.

The method resulted specific to determine GLU in TPN. Linearity calibration curve shows linear response over the range of concentrations between 6 and 11%. The recovery assays, at five concentration levels showed percentages between 98-101%. Satisfactory results of precision data were obtained.

A specific, simple, easy and rapid method was developed for the quantification of GLU in TPN. It is particularly useful for quality control and process validation.

Comparative study of dissolution profiles of multisource atorvastatin tablets.

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Key words: atorvastatin tablets, dissolution profile.

Atorvastatin Calcium (ATV) is an antilipemic drug of great interest to the pharmaceutical industry. According to the Biopharmaceutics Classification System, ATV is a class II drug (low solubility, high permeability). For such drugs, dissolution testing is a very important tool to assess the lot-to-lot quality of a drug product, and it is often use as a prerequisite for bioequivalence studies.

In recent years a few studies on the dissolution of ATV tablets have been carried out. In 2004, the FDA published a condition under which the dissolution test can be performed for quality control of tablets (using 0.05 M potassium phosphate buffer pH 6.8 as medium). The only official dissolution test for ATV

tablets, in Japanese Pharmacopeia (JP), employed water as dissolution medium. Additionally, there is diversity in chromatographic analytical methods to quantification the ATV dissolved.
In this context, the aim of the present work was to compare chromatographic conditions, dissolution mediums, and release profiles of four brands of 20 mg ATV tablets.
In all experiments, 4 ml of dissolution sample was withdrawn at 2.5, 5, 10, 15, 20, 30 and 40 min without reposition of medium. The samples were assayed in two HPLC conditions.
The dissolution profiles of the innovator (RP) and drug multisource products (identified A to C) were compared using water and phosphate buffer pH 6.8 as dissolution mediums (900 ml, paddle method, at 37ºC and 75 rpm). In addition, Similarity Factor (*f2*) was determined.
The results showed not significant statistic difference between the two chromatographic conditions for each brand.
The dissolution release was similar for brands A and B in both mediums, whereas in buffer was greater than in water medium for RP and brand C. With respect to the f2 factor, all the multisource products are not similar to the RP in both mediums.

Caffeine quantification in dietary supplements by fluorescence using bovine serum albumin.

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Keywords: Caffeine monitoring; Bovine serum albumin; Fluorescent quenching.

Caffeine (1,3,7-trimethylxanthine; CF), is a substance found naturally in the leaves, beans and fruits of a variety of plants. It is regularly consumed by a large percentage of adults, although in Argentina in recent time, young people have been added to this group. Attending to the health risks, many countries have started action to establish the regulatory boundaries around CF.
A new methodology for the determination of caffeine based on the fluorescence quenching of Bovine serum albumin (BSA) is proposed. When the alkaloid CF is presents (quencher), the protein's fluorescence emission is diminished. The diminution of the signal is caffeine concentration proportional. The effects of experimental parameters were investigated by univariation assays, including buffer nature and pH, surfactants nature and its concentration. Under optimum experimental conditions, a detection and quantification limits of 1.9 and 6.4 mg l^{-1} were obtained, respectively. A linear range was achieved varying from concentrations of 6.4 to 94 mg l^{-1} (r^2 = 0.997).
Satisfactory recovery values (≥ 95%) were obtained using the method of standard addition, confirming the feasibility of this method for caffeine determination in energizing dietary supplements and energy drinks. In order to obtain the known advantages of on-line procedure, a flow injection manifold will be sketched and quality analytical parameter will be evaluated.

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Determination of thimerosal in pharmaceutical industry effluents by High Performance Liquid Chromatographic with post-column derivatization coupled to Atomic Fluorescence Spectrometry

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Key words: Thimerosal traces, HPLC- CV-AFS, Pharmaceutical industry effluents

Thimerosal (THM) has been employed as antimicrobial agent in a variety of products including topical anti-septic solutions, cosmetics, cleaning solutions for contact lenses, vaccines and other injectable biological products. THM is decomposed by oxidation to 2,2´-dithiosalicylic acid, thiosalicylic acid, EtHg and elemental mercury. Due to THM is now considered as a potential emerging contaminant. The aim of this work was to develop a hyphenated methods for simultaneous determination of THM, EtHg(II) and Hg(II) in pharmaceutical industry effluents.

Separations were performed with a Series 200, Perkin-Elmer binary pump. The column used was Zorbax SB-Aq C18-RP Agilent Technologies. Mercury fluorescence measurements were carried out with an atomic fluorescence spectrometry (AFS), AI 3300, Aurora Instruments. A standard solution of THM was obtained dissolving 1000 mg L^{-1} of sodium ethylmercurythiosalicyclate from Sigma Aldrich. A 10 mg L^{-1} standard solution of inorganic mercury was obtained from Perkin-Elmer. A 1000 mg L^{-1} standard solution of ethylmercury chloride in water were obtained from Fluka. All other reagents and solvents were of analytical grade.

Mercury cold vapor formation from THM and EtHg in the mobile phase was evaluated in a univariate approach. THM eluted quickly without decomposition using formic acid 0.5% mobile phase, whilst both Hg(II) and EtHg(II) did not elute even at elution times as long as 30 min. Hg(II) and EtHg(II) species eluted well, however, in a mobile phase composed by 0.5% of formic acid and 0.1% of β-mercaptoethanol (β-ME). Under optimized condition THM eluted in 0.5% formic acid with retention time of 3.9 min, and EtHg(II) and Hg eluted at 8.3 and 12.5 min respectively after switching to a mobile phase with 0.5% formic acid and 0.1% β-ME.

This procedure methodology is fast and simple becoming adequate for screening procedures for the determination of Hg(II), EtHg(II), and thimerosal in pharmaceutic industrial effluents.

Validation of isocratic reverse-phase HPLC method for benznidazole determination in human plasma.

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Keywords: Chagas disease, bioanalytical methods, benznidazole

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Benznidazole (BZN) is the first choice medicine for Chagas disease treatment denoting a clinical efficacy higher than 80%. However, few studies of bioavailability/bioequivalence (BD/BE) have been published despite high incidence of toxic and adverse events, more often in young and adult patients, reported. The aim of this work was the validation of a simple HPLC methodology for assay BZN in human plasma, for be used in Phase-4 Clinical Trials of new BNZ delivery systems in the framework of the BD/BE Program of UNC (1,2).

The BNZ was assayed using reverse-phase isocratic HPLC method. Chromatographic analysis was performed on a 250x4.6-mm (5-µm) C18-column. Acetonitrile:water (6:4) was used in the mobile phase at flow rate of 0.9-µL.min^{-1}. The UV-detector was set at 324-nm. The procedure was fully validated to meet the requirements of FDA and GLP Guidelines for Industry (3) in terms of: calibration curve, selectivity, accuracy, precision and recovery, sensitivity, reproducibility and stability. Benzocaine and metronidazole were used as internal standard and analogue structure (4).

In assayed conditions, BZN and benzocaine showed t_R between 6.3-7.0 and 11.0-12.0 min, respectively. Adequate selectivity was demonstrated using metronidazole. Calibration curve showed linearity (r^2*=0.9987, N=3*) in the BNZ concentration range between 1.6-100.0 µg/mL (LLOQ-High) with acceptable sensitivity. Accuracy and precision showed main values of 98% (*CV≥10%*) and 94% (*CV≥5%*) for inter-/intra-day experiments, respectively (*N=3*). Reinjection (*N=5*) denoted acceptable reproducibility. Recovery of analyte was close 100%. Analyte stability in mobile phase (stored for 24-h) and after freeze and thaw cycles (*N=3*) comply with requirements (*CV≥10%*).

A simple, robust and selective HPLC-method was validated for benznidazole determination in human plasma meeting the bioanalytical method validation requirements and may be used in clinical trials.

Stability of pellets formulation from powder obtained by spray-drier infusion and phytomedicines based on *Melissa officinalis*

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Keywords: *Melissa officinalis L*, Rosmarinic Acid, stability

Melissa officinalis L., 'melisa', is rich in terpenoids, flavonoids and cinnamic, acid derivatives, with rosmarinic acid (RA) as the main component. In order to establish a quality control assay to evaluate this phenolic acid, factors such as stability and sensitivity to high temperature, exposure to UV radiation or air oxygen, and metal ions should be considered. Thus the aim of this work was to establish a rapid and sensitive method for the analysis of RA in *M.officinalis* derivatives considering the RA stability.

The samples consisted of teas and tinctures prepared from dried ground plant material, obtained from both cultivated herbs and others available in the local herbal shops; spray dried extracts; freeze dried extracts; pellets, coated pellets, and Melissa-based commercial products. Water extracts were both analyzed directly and mechanically dried on a Mini-spray BÜCHI Dryer 290, at inlet temperatures

ranging between 90 and 140 °C, with Aerosil® as carrier, and subsequently reconstituted. For photostability studies, samples were exposed to daylight at room temperature and were stored in a refrigerator under darkness conditions; for extreme thermal stability studies, one part of each sample was placed in an oven configured at 140 ◦C and 90% of humidity for a period of time.

The analytical 2-minutes run LC-MS/MS methodology was validated and applied to the above-mentioned samples. In terms of stability, spry dried extracts stored from different years did show no aging variation. However, under extreme temperature and humidity conditions, the RA concentrations drop off abruptly. In addition, greater degradation was observed in pellets without coating. On the other hand, many of the commercially available *M.officinalis* products did not contain the specified RA.

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MEDICINAL CHEMISTRY

Characterization of the inclusion complex formed by the antihelmintic drug Mebendazole and β-cyclodextrin. Molecular modeling and FT-IR analysis

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Keywords: Mebendazole; Cyclodextrins;FT-IR; Molecular Modeling.

Mebendazole (MBZ) is commonly used as a broad spectrum antihelmintic drug, commercialized as a tablet formulation or as a suspension. However, this drug is associated with a low aqueous solubility and therefore, a poor absorption from the intestinal tract. Different methods can be used to increase the aqueous solubility of a drug, being the formation of inclusion complexes with cyclodextrins one of the most employed. A molecular modeling analysis and FT-IR spectroscopy measurements were performed in order to elucidate the intermolecular interactions between MBZ and β-cyclodextrin (βCD). The inclusion process between neutral and protonated MBZ (MBZ-H^+) with βCD was simulated with the PM3 semiempirical method, and two possible orientations were considered for each complex, Head up and Head Down, following a previously published report. The more stable complexes were further optimized with a two-layer ONIOM (B3LYP/6-31G(d):PM3) method and the interactions between the guest drug and βCD were quantified with a NBO population analysis. The FT-IR spectra of MBZ, βCD, the inclusion complex obtained by freeze dried and the physical mixture were registered in a Shimadzu IR Affinity-1 spectrophotometer, with a spectral resolution of 4 cm^{-1}.The PM3 and ONIOM calculations indicated that the Head Down orientation is the preferred one for MBZ and MBZ-H+ inclusion complexes, by 5 and 9 kJ/mol, respectively. The benzimidazolic moiety was completely embedded in the βCD cavity in both cases. The NBO results showed an intermolecular H-bond between the imidazolic N of MBZ and one OH group from βCD, with a bond length of 2.61 Å and with stabilization energy of 7.9 kJ/mol. This interaction was further confirmed by FT-IR spectroscopy, where the MBZ stretching C=N band is located at 1645 cm^{-1} in the free drug and at 1653 cm^{-1} in the freeze dried MBZ:βCD complex.

Antiparasitic and cytotoxicity assays of benzenesulfonyl derivatives of heterocycles against *Trypanosoma cruzi*

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Keywords: benzenesulfonyl derivatives, chagas disease, drug design

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A chemical focused library of benzenesulfonyl derivatives of heterocycles, BSHet, was designed and synthetized in our laboratories. Preliminary screening of some of them showed interesting activity against protozoarian parasites.[1,2,4] At present, 146 compounds with drug-like molecular properties were included in the library[1-3]. Because of the need of new antiparasitic agents against Chagas disease, the aim of this work was to carry out a careful screening on *in vitro* activity against epimastigotes of *Trypanosoma cruzi* (*Tc*) and on cytotoxicity.

The BSHet were synthesised by methods previously reported.[1-4] Currently, 116 derivatives were screened by method described by Sánchez Moreno et al.[5] Epimastigotes *Tc* (cultured *in vitro* in MTL medium plus 10% inactivated foetal bovine serum medium) were incubated with the BSHet at 1, 10, 25 and 50 µM. The number of epimastigotes was determined at 72 h. by using a Neubauer hemocytometric chamber in triplicate. The unspecific cytotoxicity assays were performed against Vero cells (cultured in RPMI medium plus 10% inactivated foetal bovine serum medium) incubated with the BSHet at same concentrations. After 72 h., the cell viability was determined by flow cytometry. Both determinations were expressed as IC_{50} and determined in comparison to the control culture. Besides, the selectivity index, SI, was calculated as the ratio between cytotoxicity and activity index.

Results showed 19 derivatives with SI lower than benznidazole (reference compound) and qualified[6] to enter to the next step, the *in vitro* screening against amastigotes and trypomastigotes forms.

The screening of 116 derivatives of a focused in house library of BSHet showed high *in vitro* activity against epimastigotes *Tc* and low cytotoxicity over Vero cells. 19 out of 116 compounds could be selected as hits[7] and also will be screened over amastigotes and trypomastigotes forms, the infective forms at the human host.

Aggregation effect of Thiazine dyes over Singlet Oxygen quantum yield

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Keywords: Photosensitizer; Azure B; monobrominated Azure B; photochemical reactivity

Thiazine dyes are used for coloring substrates in biological and medical studies and are promising compounds for photosensitizing applications. Recent identification, in our laboratory, of the monomeric species of these dyes showed that the monomer described in literature corresponds to different dimers. Due to the aggregation of photosensitizers reduces the production of singlet oxygen ($^1O_2$), the objective of this study was to evaluate the $^1O_2$ quantum yield (Φ_Δ) of the monomeric and dimeric species of two Thiazines: Azure B (AzB) and monobrominated Azure B (AzBBr).

Phosphorescence decay of $^1O_2$ and photooxidation of 9,10-dimethylanthracene (DMA) were used as methodology. Monomeric and dimeric forms were analyzed in *N*,*N*-dimethylformamide (DMF) and Methanol (MeOH) employing Rose Bengal (RB) and Methylene Blue (MB) as references.

Monomeric species of AzBBr and AzB presented high Φ_Δ by luminescence (1.0 and 0.72, respectively) and low values by photooxidation of DMA (0.09 and 0.29, respectively). Moreover, the dimeric species showed similar values of Φ_Δ by both methodologies (AzBBr: 0.7 and AzB: 0.3, approximately). Furthermore, the bromination increased Φ_Δ of the compound.

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To understand the differences obtained, scavenging effect of AzBBr monomer was evaluated by photooxidation of DMA using RB as photosensitizer. These results indicated that AzBBr monomer could act as physical quencher due to Φ_Δ decreased 20% in presence of the dye. This behavior could be associated to the presence of NN-dimethylaniline moiety as part of its chemical structure. In addition, dimeric species of AzBBr and AzB presented high Φ_Δ because the aromatic amine is involving in different interaction forces that stabilize their aggregates. For this reason, the amines could be less available for the formation of exciplex with $^1O_2$ which trigger the deactivation of excited molecule.
The dimers of AzB and AzBBr will be the optimal photosensitizers, due to these species presented highest Φ_Δ than its respective monomer.

Aggregation study of Triarylmethane dyes

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Keywords: Photosensitizer; New Fuchsin; di-iodinated New Fuchsin; Photodynamic therapy

Photodynamic therapy (PDT) is a non-invasive medical technology that involves three key components: photosensitizer (PS), light and tissue oxygen to generate reactive oxygen species, which cause cells death. Numerous studies in bibliography report that many PS tend to aggregate in aqueous solution. Due to these molecular assemblies present lower photochemical reactivity than the respective monomer, self-aggregation of New Fuchsin (NF) and its di-iodinated derivative (NFI_2) were evaluated by UV-Visible Spectroscopy.
Dye concentration effect in *N*,*N*-Dimethylformamide (DMF), DMF: water mixtures and aqueous solution were compared.
UV-Visible spectra obtained for NFI_2 in DMF showed a unique band with a maximum absorption at 460 nm. When water proportion was increased, a new band at 574 nm was observed and the dye presented maxima absorption at 558 nm and 525 nm in pure aqueous media. Moreover, absorption spectra of NF in DMF showed a single band with a maximum absorbance at 558 nm, while in aqueous solution an increase in absorbance of shoulder at 495 nm was observed along with increasing dye concentration.
Due to DMF is a monomerizing solvent, the unique absorption band observed for NFI_2 in this medium could be assigned to monomeric form. When water was added, this species began to form J-aggregates (574 nm). Moreover, high order assemblies were observed in aqueous solution (558 nm and 525 nm). Comparing these results with those obtained for NF, we can conclude that, under the experimental conditions, the monomeric species of NF was not observed.
In conclusion, the substitution by iodine in the structure of NF, destabilized the J-aggregate, which allowed identify the monomeric form in DMF as well as to larger aggregates in aqueous solution. Due to NFI_2 presented lower tendency to J-Aggregates formation than NF, it could present higher photochemical reactivity than its precursor and could be good candidate for use in PDT.

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Neutral red monobrominated as photoantimicrobial agent against *Staphylococcus aureus*

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Keywords: Neutral Red, Photosensitizer, Photodynamic Antimicrobial Chemotherapy

Photodynamic Antimicrobial Chemotherapy was proposed as an alternative treatment for localized infections in response to the worldwide problem of resistance because of its efficacy and to be less likely to induce resistance. Neutral Red monobrominated (NRBr) is a new azine compound synthesized in our laboratory derivated from Neutral Red (NR). In a previous work, NRBr showed physicochemical and photophysical properties appropriate for potential use as a photosensitizer (PS). In this study, the photoantimicrobial effect was evaluated in *S.aureus.* Simultaneously, NR was assayed since reports on effects against bacteria are limited.

S.aureus ATCC 25923 and methicillin- and fluoroquinolone-resistant *S.aureus* isolate (MRSA61) were used to determine effect of PS concentration (5-200 µM) and visible irradiation time (15, 30, 60 min). Aliquots serially diluted in phosphate buffered saline were subcultured on Müller-Hinton agar and incubated 18-24 h at 37°C for survival account. Three independent experiments were performed and data averaged. Means were compared by a two-tailed unpaired Student's t test.

Both PS were not toxic for *S.aureus* in the dark up to 200 µM. NRBr was significantly more phototoxic than NR toward these bacteria, displaying dependent effect of both time-exposure and concentration. NRBr 50 µM reduced >4 log survival of *S.aureus* ATCC 25923 after 30 min light exposure. No viable bacteria were detected after 60 min throughout the concentrations range.

Conversely, NR was unable to complete photoinactivation under assessed conditions. The greatest effect (~3.2 log decrease) was observed with 50 µM after 60 min, without significant changes by increasing the concentration.

NRBr was slightly less phototoxic against MRSA61; 25 and 50 µM led to 3.3 and 3.7 log killing, respectively, for multidrug-resistant isolate while 4.2 and 5.03 log were observed against sensitive strain.

Significant increases in photoantimicrobial action of NRBr compared to NR, suggesting that new azine derivative could be a promising PS for use in photodynamic inactivation of *S.aureus*.

Chemical hydrolysis of zidovudine prodrugs: correlation between experimental and theoretical studies

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Keywords: molecular dynamics simulations, AZT, AZT esters

The design of prodrugs of zidovudine (AZT) allows the optimization of its pharmacotherapeutic properties, among which acidic prodrugs encompasses the main potential to reach optimized biodistributions. Considering that an adequate chemical stability constitutes a critical property of a prodrug, in this work we present a structure-stability relationship for a serie of AZT prodrugs obtained by esterification with the following acids: oxalic (AZT-Ox), succinic (AZT-Suc), glutaric (AZT-Glu) and adipic (AZT-Adi). A relationship between experimental assays and molecular modeling techniques is established.

Experimental findings: the chemical hydrolysis was measured incubating AZT prodrugs in solutions at various pH, with analytical measurements being performed using an HPLC-UV technique. The rate of hydrolysis was dependent on the pH and molecular structure involved. Half-life times ($t_{1/2}$) under acidic conditions (pH=2) are: AZT-Ox (21.2 h) << AZT-Suc (stable) < AZT-Glu (stable) < AZT-Adi (stable). Under basic conditions (pH=10) the hydrolysis rate is considerably higher: AZT-Ox (10.9 min) << AZT-Adi (6.8 h) < AZT-Glu (7.7 h) < AZT-Suc (10.5 h). Marked stability differences were observed for AZT-Ox when compared to the rest of the prodrugs.

Theoretical findings: Applying molecular dynamics simulations it was observed that the carboxylate moieties and carbonyl oxygens of the ester functions establishes stable hydrogen bond interactions with solvent molecules, reducing the energetic barrier for the nucleophilic attack of the hydroxyl group to the ester under basic conditions. This effect is maximum for AZT-Ox, and is considerably lower for the rest of the prodrugs analyzed.

From the obtained results, it was concluded that the rate of hydrolysis of the studied prodrugs is dependent on the solvation shell surrounding the carboxylate moiety. Detailed molecular modeling techniques are able to account for the interplay between solvation and conformational properties, providing a powerful tool for the design of 5' OH prodrugs with rationalized reconversion rates.

Analysis of neuroprotective effects of propofol related compounds

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Keywords: Propofol; Gabaergic phenols; Neuroprotection; Cytotoxicity

$GABA_A$ receptor (GABA-R), a ligand-gated ion channel, constitutes the main inhibitory receptor of the Central Nervous System. The GABA-R possesses binding sites for drugs other than the neurotransmitter GABA which behave as allosteric modulators. The known anaesthetic agent propofol, as well as other phenols studied by our group, have been shown to act on this receptor as positive allosteric modulators. These phenolic compounds also demonstrated an important antioxidant activity.

Numerous investigative efforts have been promoted to identify pharmacologic agents that might reduce ischemic cerebral injury. Several compounds with GABAergic activity have shown neuroprotective effects attributed to their positive effects on GABA-R-mediated inhibition of synaptic transmission. Furthermore, it is important to remark that it was also found that antioxidant anesthetics, such as

propofol, directly scavenge reactive oxygen species and inhibit lipid peroxidation leading to a neuroprotective result.

In the present work, we investigated the neuroprotective effect of five phenolic compounds with gabaergic activity and with antioxidant properties (including propofol as reference compound), using primary cultures of cortical neurons which express functional GABA-R. Cell cultures were prepared from the cerebral cortices of 17-18 day-old rat fetuses and maintained in a DMEM supplemented with insulin, penicillin, and 10 % fetal calf serum. Following 6-7 days in vitro, the cells were exposed to different concentrations of each compound for 30 min or 24 h and cell viability was determined by lactate deshydrogenase release (LDH test). None compound demonstrated *per se* cytotoxic activity until 24 h of exposure. In addition, many of them exhibited partial protective effects against an injury model mediated by hydrogen peroxide. These results contribute to the understanding of the real neuroprotective mechanism exerted by anesthetics, involving pharmacological activity, an antioxidant effect or both actions mutually applied.

Synthesis and cytotoxicity evaluation of hydroquinazolinone derivatives

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Keywords: Cyclohexene-fused quinazolinones; Inhibitory activity; Cancer.

In recent years, quinazolinones and their partially saturated analogs have come into the focus of interest because of their noteworthy pharmacological activity, as nucleoside analogs with potential analgesic and antiinflammatory effects or thymidylate synthase inhibitor activity.[1]

Quinazolinones present tautomerism;[2] therefore, the understanding of their equilibrium properties and energetics is relevant for the study of molecular processes leading to their pharmacological activities. Besides the existence of tautomers, there are other important structural features, especially for *cis*-quinazolinones, as for instance the coexistence of two distinct conformers derived from the precursors in their synthesis.

We present here the synthesis, and the structural determination (measured by NMR 1-, 2-D) of ten hydroquinazolinone derivatives. Furthermore, the cytotoxic activity was evaluated using three cancerous cell lineages for different carcinomas (HEP-2, larynx carcinoma cells; NCI1-H, lung carcinoma cells and MCF-7, breast carcinoma cells).

The *cis/trans*-2-thioxoquinazolin-4-ones and their 2,4-dione analogs were obtained by modification of previous methodology.[1] The starting material for both isomers was cis-1,2,3,6-tetrahydrophtalic anhydride that was transformed first to an amino acid and then to a methyl ester, which then reacted with an aryl iso(thio)cyanate. In order to obtain the *trans* counterpart, we isomerized the starting material.

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The measurement of the inhibitory concentration at 50% cell growth (IC_{50}) was the procedure chosen for the determination of the cytotoxicity activity together with a positive control (doxorubicin 2 mg/mL). The only compound showing values above 25 µg/mL is *N*-Phenyl-hexahydro-2*H*-benzo[*d*][1,3]oxazin-2-imine, (IC_{50}= 84,5 µg/mL) acting on larynx carcinoma cells.

The results show that all the studied compounds have inhibitory activity irrespective of the tumor cells they act on. We then conclude that the inhibitory activities measured are promising since for all, but one specimen, the values are around one order of magnitude below that of the control.

Preferential solvation of norfloxacin and ciprofloxacin with sulfanilamide or sulfametoxazole in mixed binary solvent

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Keywords: preferential solvation, binary solvent, fluorquinolones, antibacterial sulfamides.

Fluoroquinolones and sulfamides are antibacterial drugs with low solubility. The solute-solvent interactions allow understand the absorption of drugs and the interactions involved in the molecular recognition. Solute may induce changes in the solvation sphere with respect of the bulk, this phenomenon is denominated preferential solvation (PS). In a mixed solvent system the maximum energy of absorption of the solute at UV-Vis spectra, depends on the composition of solvent mixture in the solvation shell.

In this work, we studied the solvation of norfloxacin (NOR), ciprofloxacin (CIP), sulfanilamide (SNA), sulfamethoxazol (SMX) and equimolecular combinations of NOR-SNA, NOR-SMX, CIP-SNA and CIP-SMX in two different binary mixtures of solvents, aqueous buffer (amphiprotic):MeOH (protic) and aqueous buffer:ACN (aprotic). The solvent was buffer pH 7.4 and increasing mole fractions of MeOH or ACN. Concentrations of the solute were 5.0 x 10^{-5} M. The UV spectra were recorded in the range 200-400 nm. The results were analyzed by the Taft and Kamlet's linear free energy relationship method.

In buffer:ACN, all the samples showed PS by ACN, except CIP. When buffer:MeOH was used, it was not observed PS with NOR, SNA and CIP; SMX, SNA-NOR and SMX-NOR exhibited PS by buffer and SNA-CIP and SMX-CIP showed PS by MeOH.

In all the samples, except for SMX-CIP in buffer:ACN, the dipole moment of the solutes decrease upon excitation. SMX, CIP and SNA-CIP are sensitive to the hydrogen bond donor ability of buffer:MeOH, and SMX, CIP, SNA-NOR and SMX-CIP are to the mixture buffer:ACN. CIP in both mixtures and SMX and SMX-CIP in buffer:ACN are affected by the hydrogen bond acceptor ability of the solvent.

The molecular characteristics of these drugs produce multiple interactions with the solvent system. This methodology allows as inferring the interactions involved in the molecular recognition.

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Antimicrobial peptides and lipopeptides containing unnatural amino acids. Biological effects and potential therapeutic properties.

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Keywords: antimicrobial peptides, lipopeptides, enzymatic stability

Antimicrobial peptides and lipopeptides are promising candidates to treat infections, and many of them interact with bacterial membranes causing rapid destruction of sensitive bacteria. However, these compounds present low stability against proteases. The aim of this study was to design analogs of the oligomer $(KLFK)_3$ (TA4) and of the lipopeptide decanoic acid-IKQVKKLFKK (C10:0-A2), in order to evaluate the effect of substitution by N-methyl- and D-amino acids on their biological properties and enzymatic stability.

For this, C10:0-A2(5-NMeLys), C10:0-A2(6-NMeLys), C10:0-A2(9-NMeLys), C10:0-A2(8-NMePhe), C10:0-A2(2,5,6,8,9-dK), TA4(3,7-NMePhe) and TA4(1,4,5,8,9,12-dK) were chemically synthesized. The Minimal Inhibitory Concentration (MIC) was determined against the following bacterial strains: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25929, *Staphylococcus aureus* Methicillin Resistant SAMR1. The therapeutic index (TI) was calculated as the ratio between the minimal hemolytic concentration that produces 100% of hemolysis and the MIC. The enzymatic stability was determined against trypsin.

Analogs of C10:0-A2 containing N-methyl-amino acids, TA4(3,7-NMePhe) and TA4(1,4,5,8,9,12-dK) were active against all bacterial strains tested (MIC= 0.7 to 40 µM), while C10:0-A2(2,5,6,8,9-dK) only showed antimicrobial activity against Gram (-) bacteria (MIC=1.6 to 22.4 µM). The substituted analogs were not hemolytic and showed good TIs, ranging from 727.3 to 66.1 for Gram (-) and from 216.2 to 43.6 for Gram (+) bacteria, with the exception of C10:0-A2(2,5,6,8,9-dK) that presented a very low TI against Gram (+) bacteria.

Substitution by D-lysine allowed to obtain analogs with improved enzymatic stability against trypsin. Whereas, substitution by N-methyl-amino acids did not improve the enzymatic stability of the analogs.

According to these results, the peptides containing D-amino acids were the most interesting as potencial antimicrobial therapeutic compounds. Current studies are focused to determine the plasma stability of these substituted analogs.

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Strategies to improve the cholinesterase inhibitory activity of a novel peptide isolated from the skin secretions of *Hysiboas pulchellus* (Anura: Hylidae)

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Keywords: Cholinesterase Inhibitors, Alzheimer Disease

The butyrylcholinesterase (BChE) may represent an important therapeutic target for Alzheimer Disease (AD), and the search for new dual AChE (acetylcholinesterase) and BChE inhibitors is mandatory to find new alternative treatments for AD patients that do not respond to selective AChE inhibitors.

P1-Hp-1971 (TKPTLLGLPLGAGPAAGPGKR) is a proline-glycine rich peptide which was identified in the skin secretions of *Hypsiboas pulchellus*. It was found that this peptide inhibited BChE and AChE enzymes (35% and 20% respectively at 400 µM).

In this work, molecular docking calculations were carried out on BChE protein model (pdb-1P0M) to get better insights about the interaction peptide-enzyme, in order to delineate smaller bioactive analogs. According to these results five shorter sequences showed the highest *in silico* interactions, and were further synthesized by Fmoc (9-Fluorenylmethoxycarbonyl) chemistry.

The cholinesterase inhibitory activity of the different peptides was determined by Ellman's method. Hemolysis assay was performed using human red blood cells (hRBCs) and following previously described protocols.

Low hemolytic activity was found for all the analogs in the whole range of concentrations tested (50–400 µM) and the one covering region 5-21 was the most hemolytic (22% at 200 µM). Analogs 7-21 and 5-21 presented the highest inhibitory effect against both enzymes (over 45% at 100 µM) while the shortest peptides (8-16 and 11-16) showed similar inhibitory activity like P1-Hp-1971.

The results suggest that this strategy was useful for designing shorter peptides with improved inhibitory activity against AChE and BChE, and with low cytotoxicity against eukaryotic cells.

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PHARMACEUTICAL BIOTECHNOLOGY- PHARMACEUTICAL TECHNOLOGY

Evaluation of the use of ascorbyl derivatives as membrane liposomes stabilizers

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Keywords: Liposomes, ascorbyl decanoate (Asc_{10}), ascorbyl palmitate (Asc_{16}), generalized polarization, DLVO theory.

The aim of this work was to compare the biophysical properties of liposomes of DMPC containing Asc_{16} with those containing Asc_{10} and also to predict by DLVO theory their properties when other drugs are added to the membrane.
The ascorbyl derivatives up to 30% did not disturb the membrane order of DMPC liposomes, as evaluated by the calculation of the Generalized Polarization (GP) using the fluorescence probe Laurdan, neither did they modify the DMPC liposome size (~ 150 nm), as calculated by DLVO theory. Thus, all the studied liposomes were stable. Their presence conducted to more negative zeta potential (ζ) values: Asc_{16} developed a lower ζ than Asc_{10} (-16.7 mV and - 9.98 mV respectively). Considering the calculated DLVO potential, the stabilization of DMPC liposomes by addition of Asc_{10} is predictably lower than Asc_{16}, and hence, more Asc_{10} will be needed to stabilize the membrane.
The addition of Asc_{10} or Asc_{16} to liposomes of DMPC does not disturb the phases of the membrane. However, the stabilization of DMPC liposomes by addition of Asc_{10} is lower than Asc_{16}.
It is important to consider that under experimental conditions (pH ~ 6), both derivatives were negatively charged. Thus, it is expected that if a positively charged drug is inserted into the liposome membrane, a less negative ζ will occur and, consequently, the destabilization of the system will be higher. Therefore, we suggest that the incorporation of active molecules that could reduce the DLVO potential of DMPC liposomes could be accompanied by either Asc_{10} or Asc_{16}. However, considering these results, the Asc_{16} will be better than Asc_{10} to stabilize the liposome membrane.

Synthesis and characterization of micro-spheres using modified xanthan gum

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Keywords: Xanthan gum, micro-encapsulation

Xanthan gum is a biopolymer used as a food additive and rheology modifier, commonly employed for thickening salad dressings and for stabilizing cosmetic products. It is produced by fermentation of glucose, sucrose or lactose by the *Xanthomonas campestris* bacterium. One of the most remarkable properties of this biodegradable polymer is its ability to produce a large increase in the viscosity of liquids

by adding a very small gum quantity, on the order of 1-2%. In cosmetics, xanthan gum is used to prepare water gels; it is also used in oil-in-water emulsions to help stabilize the oil droplets against coalescence. In pharmaceutical sciences, this hydrophilic biopolymer slowly forms thick gels, which retain integrity of formulations and promotes drug release.

The aim of this work is the synthesis and characterization of micro-spheres using a modified xanthan gum, through micro-encapsulation techniques.

The micro-spheres were obtained by ionic gelation technique, adding a 1:1 mixture of xanthan gum:paracetamol dropwise to a 0.5% (wt.) aqueous solution of iron(III) chloride, used as complexation agent, under continuously stirring.

The characterization of the obtained micro-spheres was performed by spectroscopic (FTIR, UV-Visible), thermal (TGA, DTA, DSC) and optical (SEM) techniques. The quantitative composition was determined by microanalysis.

Drug encapsulation efficiency under the experimental conditions was found in the range of 40-50% determined by UV-Visible spectroscopy.

According to the obtained results, it can be concluded that modified xanthan gum is highly efficient for the encapsulation of paracetamol and therefore, it can be considered as promising for its applications with other drugs in order to improve their vehiculization.

Matrix formulations for indomethacin colonic delivery

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Keywords: Colonic delivery, indomethacin, alginate, guar gum.

The aim of this work was to improve the performance of uncoated matrix tablets for indomethacin colonic release based on pH-dependent formulations. These systems are also susceptible to enzymatic degradation.

Sodium alginate (55-60%), guar gum (9.5-13.5%), indomethacin (12.5%), microcrystaline cellulose (14.5-16.5%), colloidal silica or micronized amorphous silica (2%) and magnesium stearate (1%). The tablets were obtained by direct compression. A 2^3 full factorial design was used to study formulation variables: amount of matrix former excipients, alginate-guar gum ratio and type of glidant. The responses were: swelling (SW) and erosion (ER) at pH 1.2 and 6.8, tensile strength (TS), weight relative standard deviation (rsdW), friability (FR), moisture content (MC), and drug release (DR) at pH 1.2, 4.5, 6.8 and 7.4. Analysis of variance (ANOVA) followed by Tukey's test, principal component analysis (PCA) and cluster analysis (CA) were used for data analysis.

The first two dimensions of PCA explained 82% of the variance of the experimental data. The formulations were located in different clusters. The pursued product attributes were found in the cluster which grouped formulations with low values of rsdW, low percentage of DR at pH 1.2 and high TS values. Moreover, all formulations in this cluster fully released indomethacin at colonic pH. Significantly different DR results at pH 1.2 were used to select the formulations better suited for gastric transit within the cluster. The best formulations were those containing micronized amorphous silica and high alginate percentage.

The uncoated tablets showed sufficient mechanical strength, weight uniformity and release profiles suitable for pH-dependent indomethacin colonic delivery systems. In future works, effect of enzymes on release will be also studied.

The experimental design demonstrated that alginate-guar gum ratio and type of glidant were the most relevant formulation variables. High alginate-guar gum ratio and micronized amorphous silica as glidant yielded the best tablet attributes.

Permeation in artificial membrane of allopurinol derivatives with potential anti-*T.cruzi* activity

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Keywords: Allopurinol derivatives; Trypanocidal activity, *in vitro* permeability.

Chagas disease is caused by *Trypanosoma cruzi* (T. cruzi), and is a major health problem in Central and South America that affects nearly 8-10 million people. Allopurinol (Allop) acts inhibiting the parasite, but it presents unfavorable physicochemical properties causing versatile responses in patients with Chagas.

In an attempt to improve its performance, we have developed twelve derivatives of Allop by chemical modification, resulting in active anti-*T cruzi* agents *per se* or prodrugs compounds.[1] The present study deals with the permeability of Allop and its derivatives (like an additional study in drug development) by using artificial membranes as an *in vitro* technique.

The studies were conducted using Franz horizontal diffusion cells with an artificial membrane constructed by lipids and *n*-octanol on a hydrophilic membrane support and cells pH 5.5_{donor} – pH $7.4_{acceptor}$ at 37 °C.[2] To obtain each apparent permeability coefficient (Papp), each experiment was performed in triplicate and 14 samples were taken over a period of 4 h and quantified by Micellar Liquid Chromatography.

All Allop derivatives increased the Paap with respect to Allop (up to 9 times), which provides an important pharmacokinetic advantage.

We observed that there are different physicochemical properties (previously determined), not only lipophilicity, that play an important role in the Papp such as, polar surface area, molecular rigidity, H-bond donor and H-bond acceptor.

Determination of ivermectin solubility in different stabilizer solutions and temperatures

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Keywords: Ivermectin, Nanosuspension, Solubility.

Ivermectin (IVM), a widely used antihelmintic agent, presents a very low aqueous solubility, which lead to an erratic bioavailability and great intra-individual variations. One of the possibilities for improving its dissolution rate is to develop nanosuspensions, through high pressure homogeneization. Some critical points of this technique are the proper selection of the stabilizers and the concentration of the drug. Furthermore, the size and stability of nanosuspensions are dependent on the drug solubility in stabilizer solutions. Therefore, the aim of the present study was to determine the solubility of IVM in different stabilizer solutions, in order to assure supersaturation conditions in the subsequent nanosuspension development.

Surfactant (polysorbate 80 and SLS-sodium lauryl sulfate) and polymeric (polyvinylpyrrolidone and poloxamer 188) agents were selected as stabilizers. Stabilizer solutions were prepared in triple distilled water, at two concentrations and different binary mixtures, combining a surfactant with a polymer (final concentration 1% and 2% w/v). The solubility of IVM in water and aqueous stabilizer solutions was determined by addition of an excess of the drug to the solvent (n=3). The mixture was stirred in a thermostatic bath (at 25° and 40° C) for 72 hours, centrifugated, filtered and assayed by UV-spectrophotometry (245nm).

IVM solubility in triple distilled water was around 1 and 3 µg/ml, at 25° and 40°C, respectively. Solubility values were increased by 1000 to 2000 times when polysorbate was used as the surfactant component of the stabilizer binary mixture, with a significant temperature influence. When SLS was used, in combination with polymers, solubility values were increased by 5000 to 10000 times, regardless of the temperature. Furthermore, the IVM solubilities were exactly doubled when SLS concentration was duplicated.

Formulation of IVM nanosuspensions at concentrations of 1% or 2% (w/v), in a 1:1 relationship with the stabilizer, assures a supersaturated system for nanosuspension development.

In vitro dissolution evaluation of ivermectin nanosuspensions

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Keywords: Dissolution rate, Ivermectin, Nanosuspension, Particle size

The dissolution properties of a drug contained in a dosage form, have a great impact on its bioavailability. The dissolution rate is a function of drug intrinsic solubility and particle size. Ivermectin (IVM), a widely used antihelmintic agent, is a very poorly soluble drug; therefore its bioavailability could be compromised. Nanosizing the drug substance is one promising strategy to increase surface area and thus enhance dissolution rate and bioavailability. The aim of the present study was to evaluate in vitro

dissolution rate of IVM nanosuspensions (NSs), developed by high pressure homogenization (HPH), and compare them with bulk IVM.
The NSs consisted of IVM (1% w/v) and different stabilizer mixtures (Tween 80-Poloxamer 188; Tween 80-PVP; SLS-Poloxamer 188), at the same total concentration. NSs were obtained by HPH, at 800bar and 40 cycles of homogenization. Particle size analysis was carried out using photon correlation spectroscopy and laser diffraction. Dissolution studies were performed using apparatus 2, at 50rpm, with 900ml of USP phosphate buffer pH 5.8 (with 0.5% SLS). Samples were filtered (0.22µm filter) and analyzed by HPLC.
The Z-size of NSs containing Tween 80 were between 174.6-218.1nm with a polidispersity index (PDI) of about 0.25-0.36; meanwhile for NSs prepared with SLS, the Z-size was 918.4nm (PDI 0.31). Bulk IVM showed a mean particle size of 233µm. Different dissolution profiles were recorded for NSs and IVM powder, with significant differences in terms of dissolution rate and Dissolution Efficiency (43.87% for bulk drug, and values between 67.18% and 90.88% for NSs). Besides, the NS formulated with SLS-Poloxamer 188 showed low dissolution percentages, because of IVM chemical degradation (confirmed by the appearance of degradation products in the chromatogram).
The enhanced dissolution rate of IVM nanocrystals, particularly in the presence of Tween 80-PVP as stabilizer mixture, could be a promising approach for IVM oral delivery.

Development of liposomes for the delivery of poorly water-soluble drugs

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Keywords: liposomes; poorly-soluble drugs; integrity; entrapment.

The objective of this work was the development and characterization of liposomes for the delivery of poorly water-soluble drugs, Sulfamerazine (SMR) and Indomethacin (INM). Liposomes are colloidal vesicles of nanometric size composed of lipid bilayers surrounding aqueous compartments. Hydrophobic drugs can be incorporated in their lipid bilayer.
Drug load and unload liposomes composed by phosphatidylcholine (PC) and cholesterol (CHO) were prepared by the thin film hydration method: dispersion of lipids and drugs, evaporation, exposition to a stream of nitrogen and hydration with a Phosphate Buffer solution (PBS).The particle size, polidispersity and zeta potential were determined at 25^{o}C. The interaction of the drugs with the liposomes components was determined by NMR-H^{1} spectroscopy.The integrity was evaluated by determining the retention (%) of calcein in the vesicles at 37^{o}C for 48 h by fluorescence intensity measurement.The molar ratio of drug encapsulated over the total lipid concentration (D/L) was determined by UV-spectroscopy and a phospholipid colorimetric assay (Stewart, 1980) was applied.
The particle size of all liposomes were in the range of 31.1-115 nm, the values of polidispersity were around 0.2 indicating that monodispersed systems were obtained and the zeta potential values of unload, SMR load and INM load liposomes were (-0.34 to -0.03 mV), (-2.9 to -0.07 mV) and (-0.41 to -0.09 mV), respectively. The NMR studies suggested the incorporation of the drug inside the lipid bilayer.The retention of unload, SMR load and INM load vesicles were 80, 80 and 95%, respectively, indicating the

high stability of the liposomes. The higher entrapment values of SMR and INM were 308.98 and 10.14 mmol/mol, respectively.
In conclusion, it was possible to obtain and characterize liposomes, presenting small size, high stability and high drug entrapment, suggesting being a promising strategy for the delivery of SMR and INM that may improve the bioavalability of these drugs.

Chitosan dressings as therapeutic wound healing device

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Key words: chitosan, wound dressing, biomaterials

Despite the many advances in wound treatment, this topic remains a difficult problem. At present, there are three categories of wound dressing: biologic, synthetic and biologic-synthetic which are all used frequently in the clinical setting but none is without disadvantages.
Chitin and its derivative, chitosan, are biocompatible, biodegradable, nontoxic, anti-microbial and hydrating agents that have been employed as a biomaterial for many uses. Dressings of these polymers are higher cost products and at the present are not commercially available in the country. Therefore, the local development of similar products would offer an available lower cost alternative. Here, we present the results of the development of chitosan bandages for wound treatment.
Chitosan powder, previously purificated by standard procedures, was disolved in acetic acid and the solution was neutralized with NaOH. Resulting solution was poured in square plates and lyophilized to obtain chitosan wound dressings. Dressings were packed and sterilized by gamma radiation (25KGy).
Studies in vitro were done to assess the eventual cytotoxicity of dressings, for this propose, cells monolayers plated 24 h before were incubated with pre-wetted chitosan dressings during 72 h. Tetrazolium salt MTT method was used to evaluate cells viability and the results were expressed by percentage respect to the control (cells without chitosan dressing). The results showed not citotoxic effect of the dressings on the cells.
In summary, we developed a chitosan bandages that showed promising in vitro results, further in vivo studies are needed to complete the validation of the product.

Use of consumer studies in the development of antiaging face creams with olive oil

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Keywords: antiaging creams, sensory characterization, consumers

During product development it is essential to have information on the sensory attributes that determine whether the consumers like or dislike a given product. The aim of this study was to use consumer studies in the development of antiaging face creams with olive oil.

Two prototypes were developed and compared with three well positioned creams on the market. The five samples were assessed by a total of 112 female consumers. For each sample, consumers had to score their overall liking using a nine-point hedonic scale and to answer a check-all-that-apply (CATA) question comprised of 39 words or phrases related to the sensory properties of the products, the sensation on the skin, the indications of use, price and quality.

Based on the liking scores, a variance analysis was conducted considering sample as fixed source of variation. Based on the CATA results, a frequency analysis, a Cochran's Q test, Marascuilo's multiple comparisons test and Multiple Factorial Analysis were conducted.

The score of the best positioned prototype (6.7) did not present significative difference against the market leader (6.6). Also both profiles were similar. This prototype was considered adequate and moved on to the following development stages, such as efficacy evaluation. The profiles of the two prototypes did not present the defects that were seen in previous studies as characteristics to avoid in antiaging creams with olive oil such as strong smell, food-like smell, oily, etc.

The use of consumer studies allowed to obtain a profile of the product from the point of view of the consumer, making it possible to be used to evaluate samples during the development stage of the cosmetic product, and then, to compare it to its competitors.

Nanostructured lipid carrier: a pre-formulation

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Keywords: Nanostructured lipid carriers, factorial design, glyceryl monostearate, sesame oil.

Solid lipid nanoparticles (SLNs) were developed at the beginning of the 90s. However, potential problems associated with SLNs are solved or minimized by a new generation of lipid carriers called NLCs (nanostructured lipid carriers). Such systems are produced by mixing solid lipids with spatially incompatible lipids leading to special structures of lipid matrix. The aim of this work was to determine the best conditions for the production of a NLC. To produce the NLCs, the solid lipid (glyceryl monostearate) and the liquid lipid (sesame oil) were fused at 75 ± 5 °C with subsequent addition of surfactant solution (Pluronic F68) at the same temperature. Then, the mixture was subjected to ultrasonic stirring, followed by temperature reduction. Prior to establish the best parameters to NLC production, the concentration of lipids and the preparation time were evaluated. Then, a factorial design 2^2 without replications with five central points and four axial points was applied in order to analize the effect of proportion of liquid/solid lipid and the surfactant concentration on the particle size. After production, the

particles were stored at room temperature and observed for 24h. During the production of the NLC, some preparation conditions such as different lipid concentrations (2, 5 and 10%) and preparation time (2, 5 and 10 min) were evaluated. It was observed that the formulations containing 5 and 10% of lipids and prepared in less than 10 min were highly viscous in a period of up to 24h.Therefore, the optimal parameters of production were established as 2% of lipids in a stirring time of 10 min. The NLCs showed smaller particle size (64.4 nm) with a smaller proportion of liquid/solid lipid and lower surfactant concentration. Moreover, the preparation method using 2% of lipid portion (ratio of liquid/solid lipid 1:9), an aqueous solution with 3% of surfactant and with a preparation time 10 min was established.

Nanoemulsion-loaded hydrogels containing a synthetic chalcone as an alternative for the treatment of cutaneous leishmaniasis

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Keywords: leishmaniasis, nanoemulsion, hydrogel, skin permeation

Our research group has investigated the feasibility of developing a nanoemulsion containing a synthetic chalcone ((E)-3-(3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl) prop-2-en-1-one) for topical delivery. Studies have ascribed to this synthetic chalcone a leishmanicidal activity. However, the low viscosity of nanoemulsions impairs its topical administration and the thickening these systems by its association to gelling agents has been described as a good alternative to improve the formulation skin adherence. The aim of this work was to obtain nanoemulsion-loaded hydrogels containing a synthetic chalcone (SC) for the treatment of cutaneous leishmaniasis. The nanoemulsion (NE) composed of 10.0% (w/w) medium chain triglycerides, 2.0% (w/w) soybean lecithin, 2.0% (w/w) polysorbate 20 and SC at 1.0 $mg.ml^{-1}$ was prepared by spontaneous emulsification. For the preparation of NE-loaded hydrogels, polymers (Aristoflex AVC®, 1.0 %, w/w, or Methocel®, 3.0 %, w/w) were directly dispersed into the nanoemulsion. The formulations were characterized with respect to SC content, droplet size, polydispersity index, zeta potential, pH and rheological properties. Furthermore, i*n vitro* skin permeation studies using dermal membranes as a model of skin damage were performed. The nanoemulsion presented nanometric droplet size (172.47 nm), low polydispersity index (0.14), negative zeta potential (-49.43 mV), pH 5.23 and drug content of 88.23%. Both hydrogels kept the nanoemulsions physicochemical characteristics (droplet size <181 nm, PDI <0.2, negative zeta potential <-48.7 mV) and SC content. The semisolid formulations exhibited non-newtonian pseudoplastic flow with pseudoplastic behavior. SC retention in dermis was 50.86 ± 10.72, 37.45 ± 7.50, 72.10 ± 10.81 $\mu g.g^{-1}$ for NE, NE-loaded Methocel® Hydrogel and NE-loaded Aristoflex AVC® Hydrogel, respectively. The incorporation of nanoemulsions into Aristoflex AVC® increased the SC retention in dermis, suggesting a penetration enhancer effect. In this context, nanoemulsion-loaded Aristoflex AVC® hydrogel seems to be an appropriate formulation for *in vivo* antileishmanial trials.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Characterization and skin permeation/retention of copaiba oil (*Copaifera multijuga* Hayne) cationic nanoemulsion and its respective hydrogel

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Key-words: copaiba oil, cationic nanoemulsion, hydrogel, skin permeation

β-caryophyllene is the main component of the volatile fraction of the oleoresin extracted from *Copaifera multijuga* Hayne and presents anti-inflammatory activity confirmed by earlier studies, as well as the oil itself . However, this oil has high lipophilicity, which imparts an unpleasant feel when used topically. An alternative is to formulate cationic nanoemulsions, since the small droplet size, the high contact surface and the positive charge of these systems can improve permeation of β-caryophyllene through the skin. The only inconvenience is its low viscosity, which requires thickening that can be achieved by its incorporation in a hydrogel. In this context, our research group developed a cationic nanoemulsion containing copaiba oil and its respective hydrogel in order to analyze its physicochemical characteristics and the assessment of β-caryophyllene permeation/retention profile in skin, comparing those formulations. The nanoemulsion was produced with copaiba oil (20%), medium chain triglycerides (10%), Span 80® (3%), Tween 20® (1%) and cetyltrimethylammonium chloride (2%). Afterwards, hydroxyethylcellulose was directly incorporated to the nanoemulsion at a final concentration of 3%. The formulations were submitted to physicochemical characterization regarding droplet size (DS), polydispersity index (PDI), zeta potential (ZP) and skin permeation/retention assay. The samples obtained from the permeation/retention assay were analyzed by gas chromatography. The results indicate that the nanoemulsion and the nanoemulsion based-hydrogel were successfully produced since they presented nanometric DS (165.90 ± 4.52 and 227.06± 6.27 nm), small PDI (0.117 ± 0.042 and 0.198 ± 0.020) and high positive ZP (44.43 ± 2.08 and 55.90 ± 1.90 mV). The concentrations of β-caryophyllene from the nanoemulsion and the hydrogel found in each layer of the skin were, respectively: (i) stratum corneum: 0.19 ± 0.08 and 0.11 ± 0.02 µg/mL; (ii) epidermis: 222.52 ± 30.05 and 614.00 ± 147.55 µg/g; (iii) dermis: 171.00 ± 40.54 and 193.62 ± 123.87 µg/g. Only with the hydrogel formulation it was found β-caryophyllene in the receptor fluid (0.13 ± 0.04 µg/mL), characterizing a permeation profile through the skin. In conclusion, the nanoemulsion and its respective hydrogel were successfully produced and the hydrogel containing the nanoemulsion could improve β-caryophyllene permeation through the skin compared to the nanoemulsion.

Different ways to incoportate amphotericin B in biocompatible microemulsions

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Keywords: Amphotericin B, Microemulsions, Drug Delivery Systems.

Amphothericin B (AmB), a macrocyclic polyene antibiotic isolated from Streptomyces *nodosus*, is used since the 50s to treat most systemic fungal infections, mainly those affecting immunocompromised patients. Despite their important chemotherapic characteristics, AmB is not an ideal molecule yet, since it shows low solubility and high toxicity. This fact has motivated the development of alternative vehicles to administer AmB. In order to decrease the potential adverse events and fix the problem regarding its low solubility, a microemulsion (ME) has been used as lipid nanocarriers. The aim of this work was to different methods for incorporation of the AmB into ME systems. To reach this goal, two methods were tested: i) AmB (1.5 mg/mL) was added to ME and subjected to magnetic stirring, at room temperature, for 72 hours; ii) AmB (1.5 mg/mL) was added to ME, sonicated for 20 minutes and then subjected to ultrasonic washing for 10 minutes; iii) AmB (1.5 mg/mL) was solubilized in a sodium hydroxide solution (NaOH) (1N), diluted on the ME at room temperature (25°C) and submitted to a temperature of 80°C; iv) AmB (1.5 mg/mL) was added to the ME under magnetic stirring. After 1 minute, the pH of the ME was increased by addition of NaOH solution (1N) until the total solubilization and incorporation of AmB to the system. Subsequently the pH was reduced to neutral using hydrochloric acid solution (HCl) (1N). After incorporation, the encapsulation rate was determined. The results revealed that the best method to incorporate AmB into ME systems was the last one, which showed an encapsulation rate of 70.20%.

Development of new hydrochlorotiazide:chitosan nanoparticles

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Keywords: Nanoparticles, chitosan, ionic gelation.

Nanoparticles (NPs) are frequently defined as solid, colloidal particles in the range 10 – 1000 nm. Chitosan (CHI) is a linear polysaccharide composed of D-glucosamine and N-acetyl-D-glucosamine. Low molecular weight (LMW) CHI shows better solubility, biocompatibility, bioactivity, biodegradability and even less toxicity than other kind of CHI. Hydrochlorotiazide (HCT) is a diuretic class of thiazides. This drug is poorly soluble in water and slightly permeable through intestinal mucosa.
NPs were prepared using ionic gelation method with sodium tripolyphosphate (TPP) as a cross-linking agent. This technique is based on the ionic interactions between the positive charge of CHI and the negative charge of TPP. NPs were characterized by size, zeta potential, mucoadhesive properties using "mucin method" and loading capacity was calculated by an indirect method with the following equation:

$$\text{Loading capacity}\ (\%) = \frac{\text{Amount of encapsulated drug}}{\text{Weight of nanoparticles}} \times 100.$$

The optimized NPs exhibited a mean hydrodynamic diameter of 220 nm when are empty and 338 nm when are loaded, with a polydispersity index (PDI) of 0.2 and 0.15, respectively. The increase in the size is due to the incorporation of the drug to the NPs. Zeta potential in all cases is above +30 mV, indicating that there are stables. Mucoadhesive studies revealed a reduction in the zeta potential values for CHI nanoparticles. This reduction could be attributed to the ionic interaction between negative charge of

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

mucin and positive charge of CHI. It resulted in the mucoadhesive properties of the CHI nanoparticles. These NP were loaded with HCT and the loading capacity was calculated of 80%.
CHI NPs represent an interesting delivery system for drugs due to their mucoadhesive properties that enable them to interact with mucosa.

Development of a micellar electrokinetic chromatography system applied to the quality control of ursodeoxycholic acid in oral liquid pediatric suspensions

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Keywords: ursodeoxycholic acid, impurities, capillary electrophoresis, suspensions

Introduction: Ursodeoxycholic acid (UDCA), also known as ursodiol, is a secondary bile acid which is synthesized in the liver. UDCA lowers the cholesterol content of bile by reducing hepatic synthesis and reabsorption by the gut. Bile acids are derivatives hydroxylated 5β-cholan-24-oic acid, which are characterized by the absence of chromophore groups in its structure, which results in low absorption in the UV-visible region.In UDCA raw material may be present as impurities other bile acids, some of them toxic, like lithocholic acid (LCA), and others associated with numerous side effects such as chenodeoxicholic acid (CDCA). The official USP monograph describes the determination of related compounds such as CDCA and LCA by thin layer chromatography (TLC), where each impurity limit should not be more than 1.5% and 0.05% respectively.
Purpose: the aim of this study was to optimize an analytical method by electrokinetic chromatography using cyclodextrins and micellar agents for determination of ursodeoxycholic acid and its impurities in pharmaceutical pediatric suspensions.
Method: the electrophoretic system consisted of 50 mM sodium dodecyl sulfate, 5mMβ-cyclodextrin, 5 mM hydroxypropyl-β-cyclodextrin, 5 mM borate/ 5 mM phosphate buffer, pH 7.0 with 10% acetonitrile. A capillary of 60 cm length (50 cm to detector) and 75 µmi.d. was used andthe applied voltagewas 28kV, a working temperature of40˚C and 195 nm were applied.
Results: different additives were optimized such as cyclodextrins, type and concentration, buffer solution and organic modifier. Instrumental parameters such as voltage, capillary length and temperature were evaluated. The final electrophoretic condition allowed the best resolution of the analytes. Content of UDCA in the suspensions was 98.9% (n=3; RSD1.8%), 99.0% (n=3; RSD 1.6%), 99.5% (n=3; RSD 1.7%) and the percentage of CDCA and LCA correspond with the specification.
Conclusion: the developed method is fast, simple and usefull for the determination of ursodeoxycholic acid and its impurities. Using this analytical method provides good resolution of the analytes, adecuate LOD and LOQ, making it suitable for the quality control of pharmaceutical formulations according to the requirements of regulatory agencies.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Ovalbumin nanoparticles as carriers of hydrophobic compounds

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Keywords: ovalbumin, nanoparticles, linoleic acid, retinol.

Currently, there is a great interest in increasing solubility and bioavailability of bioactive hydrophobic compounds for different applications. In this sense, different technologies have been developed, including protein-ligand nanocomplex formation. Proteins are amphiphilic compounds with high versatility, beside they are biocompatible and biodegradable. Some of them present binding ability and could act as carriers for hydrophobic molecules. However, there are not many studies about the interaction between ovalbumin (OVA) and hydrophobic ligands. OVA is the major egg white protein and possesses several functional properties. In the present work, OVA nanoparticles (OVA_n) were obtained by heat-treatment, characterized and evaluated as carriers of Linoleic Acid (LA) and Retinol, taken as model hydrophobic compounds. For preparing OVA_n, OVA solution (pH 7.5 and ionic strength 50 mM NaCl) was heat-treated in a water bath at 85°C for 5 min and immediately removed and placed in an ice bath. Dynamic Light Scattering results showed OVA_n sizes was around 69 nm. Fluorescence techniques reveled OVA_n surface hydrophobicity was 7 fold higher than native OVA values and there was a wavelength blue shift of 27 nm in maximum fluorescence intensity. These results indicate heat treatment caused an exposition of buried hydrophobic residues on protein. On the other hand, OVA_n intrinsic fluorescence as function of LA and retinol concentrations was performed. Fluorescence quenching phenomenon was recorded, showing higher LA and retinol binding ability for OVA_n than native OVA. However, OVA_n showed higher affinity for retinol than LA. With respect to OVA_n-LA interaction, when LA was added to OVA_n solution, initial turbidity (as a consequence of LA micelle formation) disappeared and a visually clear solution was formed suggesting the soluble OVA_n-LA nanocomplexes formation. In conclusion, OVA_n produced by simple heat-treatment showed a great ability to bind hydrophobic compounds and this would allow several applications as nanocarrier on pharmaceutical industry.

Wound healing test of Coumestrol/hydroxypropyl-β-cyclodextrin association

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Keywords: phytoestrogen, fibroblasts, coumestans

Coumestrol is a phytoestrogen which belongs to the class of coumestans. It is found in many species of *Fabaceae* family [1]. Coumestrol's chemical structure is similar to that of isoflavones. Coumestrol has aroused special pharmaceutical and cosmetic interest due to its antioxidant and estrogenic activity [2]. In

the present study the influence of hydroxypropyl-β-cyclodextrin (HPβCD) on the coumestrol wound healing effect is reported. The wound healing analysis was performed using human gingival fibroblasts. The cels were seeded into tissue culture plates in DMEM supplemented with 10% FBS and incubated for 24 h. After, an artificial wound was generated on the cell monolayer. Further, the treatments were added: dimethyl sulfoxide and PGDF as controls, coumestrol solubilized in DMSO and DMEM, coumestrol/HPβCD association, coumestrol/HPβCD physical mixture and HPβCD. The plates were incubated for 16 h. The quantification of the percentages of cell migration was performed using the CellC software [3]. The associtión coumestrol/HPβCD, at the concentration of at 50 µM and at 10 µM increased of cell number, similarly to the positive control PDGF. Slightly lower results were achieved by coumestrol dissolved in DMSO at 10 µM and at 50 µM. On the other hand, coumestrol dissolved in DMSO at 100 µM present negative effect might be due to its toxicity at this concentration. The present study demonstrated the wound healing properties of coumestrol and its HPβCD associations. Moreover, this HPβCD exhibited high performance for solubilizing coumestrol for the in vitro cell tests, a property useful as an alternative to DMSO use**.**

Improved *in vitro* cytotoxicity and cellular uptake of paclitaxel loaded nanoparticles made of TPGS-PCL *versus* MPEG-PCL and Abraxane®

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Keywords: nanotechnological platform, antineoplasic, MCF-7, MDA-MB-231

Paclitaxel (PTX) is an antineoplasic drug used to treat multiples cancers types. Because PTX has very low aqueous solubility, the conventional market formulation contains high concentration of Cremophor-EL® (CrEL) which is associated with a great number of side effects. Polymeric NPs may provide an alternative to the use of toxic excipients improving solubility and favoring a controlled release of chemotherapeutic drugs. Modification of NPs with polyethylene glycol (PEG) is the most widely used method to reduce the clearance of NPs from the circulation. However, PEGylation strongly inhibits cellular uptake which can negatively influence the NPs performance as drug carrier. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble form of vitamin E. NPs surface modifications with TPGS, increase system stability in biological fluids. In additton, TPGS is selectively cytotoxic to cancer cells so could be used for the delivery of anticancer drugs. In this sense, we have synthesized TPGS-poly(ε-caprolactone) (TPGS-PCL) and methoxy PEG-poly(ε-caprolactone) (mPEG-PCL) copolymers with the same hydrophobic–hydrophilic balance. TPGS-PCL and mPEG-PCL NPs were prepared by emulsion-solvent evaporation technique with a size value of 240 and 300 nm, respectively. The *in vitro* PTX release was slow and continuous from both NPs. Cellular uptake and *in vitro* anti-tumoral activity was assessed using two human breast cancer cell lines (MCF-7 and MDA-MB-231). PTX-loaded TPGS-PCL NPs exhibited better anti-cancer activity compared to PTX solution, PTX-loaded mPEG-PCL NPs and Abraxane®. The

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IC_{50} value for PTX-loaded PCL-TPGS NPs was 4 and 3.2 times lower than mPEG-PCL NPs after 72 h of incubation for MCF-7 and MDA-MB-231 cell lines, respectively. PCL-TPGS NPs exhibited an increased in cellular uptake for both cancer cells in comparison with the mPEG-PCL NPs. In conclusion, the novel NPs investigated might be an alternative nanotechnological platform for PTX delivery system in cancer chemotherapy.

Influence of particle size and hidrofobic coating on the release profile of gastrorrentive systems

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Keywords: gastrorretentive systems, Hydroxypropylmethylcellulose (HPMC), Eudragit

A modified release system with long residence time in the stomach, called gastrorretentive (floating tablets or pellets), has particular importance for drugs with local activity in the stomach, absorption window in the stomach or upper portions of the small intestine, instability to intestinal level or low solubility at high pH values.

The aim of this work was to study the influence of the size of the granules of gastrorretentive tablets in the release profile of the active ingredient. Moreover, show the retarding effect of pH independent anionic polymer coatings in the release of active ingredient from modified release pellets.

The following flow properties were evaluated for the different size of particles for the granules through sieve #10, #20 and #30: angle of repose, Carr & Hausner Index. The tablets were prepared by wet granulation and then compressed; uniformity of weight, hardness, friability, drug content and in vitro buoyancy were evaluated. The dissolution studies showed a modified release system after 24 hours for all formulations. The tablets exhibited a gelatinous film on the surface, and a central dry region near to 20 hours.

The three coated pellets lots were evaluated for angle of repose, Carr & Hausner Index and drug content.

All coated pellets and granules showed adequate rheological properties. The physical and chemical characteristics for all tablets were optimum.

It was observed that for the smallest particle size, 10 mg is released from the swellable matrix after 3 hours, which corresponds to the desirable minimum effective concentration for optimal bioavailability.

Better sustained drug release was observed at more layers of coated pellets, verifying the retarding effect of these coating.

Development, characterization and antibacterial activity of gels with extract of *Lippia turbinata* and *Lippia alba*

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

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Key words: plant extract, carbopol gels, antimicrobial activity, topical formulations.

Lippia alba and *L. turbinata* are claimed to possess antioxidant and antimicrobial activities. The aim of our work was to develop suitable formulations including ethanolic extract of these species. The plant extracts were obtained with ethanol 70% by percolation.
Gels with carbopol® 934 and 940 at concentrations of 0.5% and 1% (w/v) were prepared. The extracts were dried at 37°C. Then, they were dissolved in a mixture of water, ethanol and propylene glycol to be incorporated into carbopol® suspension. Finally, they were gelled with triethanolamine. Gels without extracts were developed and no preservative agent was added.
Physical evaluation (colour, odour, pH, appearance, viscosity, spreadability) of gels was tested. Determination of total phenolic content with the Folin-Ciocalteu reagent and antimicrobial activity by disc diffusion method were carried out. Hygienic control was performed according to Argentina Pharmacopeia 7° edition (FA7) for topical products.
The gels were homogeneous, brown like the extracts and with slightly cytral, herbaceous smell. The pH value was 7.6 ± 0.1 for gels containing extract, and 5.3 ± 0.5 for gels without extracts. Consistency varied from fluid gel to moderately firm gel depending on the concentration of gelling agent used. Spreadability was good being better in gels with extracts. Viscosity values were between 280 and 10,000 centipose (cP) for gels with extracts, and over 10,000 cP for gels without extracts. The total phenolic content was 4.28 ± 0.37 mg/g. All gels presented antimicrobial activity. Regarding hygienic control, all gels with extracts were within the specified requirements in FA7. These results suggest the feasibility of using partially soluble in water extracts by employing hydrophilic excipients.
These formulations would be a valid alternative for vehiculization of bioactive plant extracts considering that the bioactive compounds in gels retained the antimicrobial activity of extracts and they act as preservatives in gels.

Development and stability of formulations with herbal extract

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Keywords: hydrophilic gels, Lippia turbinata, phenols content

Lippia turbinata G., Verbenaceae is an aromatic herb widely recognized in folk medicine; its essential oil is used in the industry and in cosmetic. Many properties are attributed to it such as anti-inflammatory, digestive and carminative.

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This report present the comparative results of the different galenic formulations involving the ethanolic extract of *L. turbinata.*

The plants were cultivated under cover. The vegetal material was washed, dried for 5 to 7 days at room temperature; processed in a knives mill until a smaller particle size to 350 µm.

The powder obtained, was extracted with ethanol 70° by percolation (powder plant and extractive solvent, 1:1). Then this extract was filtered and stored at -20 °C.

Gels, as pharmaceutical form, have been chosen due to their low viscosity, high superficial area and quick thermal answer; useful in hairy zones. They were prepared by incorporating *Lippia turbinata* extract using polyethylene glycol, polyvinyl alcohol and polyacrylamide &C13-141 isoparaffin & laureth-7 (sepigel®) as gelling agents.

The chemical stability of the formulations was evaluated during one year by the quantification of phenols content transferred from the formulation. Physical parameters were tested for a year evaluating appearance, extensibility, pH and conductivity. The formulations and determinations were performed by triplicate.

Formulations based on polyethylene glycol and polyvinyl alcohol 10% contained *L. turbinata* extract up to 15,8%; whereas the formulation of sepigel® 4% included same extract at 5% only.

Formulated gels had acceptable physical parameters and demonstrated ability of vehiculization of ethanol extracts of *L. turbinate* and the gels tested could be compatible with the skin.

During twelve months gels shown acceptable physical stability parameters only the topical formulation based on polyethylene glycol and polyvinyl alcohol presented a variation between 90 and 110 % of the initial phenol content, demonstrating chemical stability.

Design of experiments applied to re-formulation of a dipyrone oral solution

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Keywords: Experiment design, oral solution, pharmaceutical development, formulations.

This work derives from a joint venture between a State Pharmaceutical Production Laboratory (Dorrego-ASSE) and the Universidad de la República through Instituto Polo Tecnológico de Pando applied to pharmaceutical development. The last version of the ICH Q8 standard recommends the use of statistical tools such as Design of Experiments (DoE) for this type of development. The objective of this study is the re-formulation of a dipyrone oral solution which has stability problems. DoE is used to study the factors affecting the stability. Several works have reported stability problems in liquid formulations containing the same active ingredient, without finding an explanation for the resulting turbidity. Previous studies have identified six potential factors: pH, buffer concentration, antioxidant concentration or the presence of essence, sorbitol or sucrose and paraben. The aim is to optimize those that are relevant. A fractional factorial design derived from a D-optimal model is used to assess all main effects and most of the combined two-factor effects. To achieve a design with a maximum of 16 trials, some unimportant combined effects were strategically removed. By studying simple observations such as appearance, pH and density, all the studied factors are found to have some influence showing complex interactions between them. The 16 formulations are monitored over time by observing the solution colour, aroma and

turbidity. A model explaining the data obtained was elaborated, improving notoriously the knowledge of the developed product. This enables us to propose successful modifications to the tested formulations and to the processes.

Use of statistical methods to optimize the production process of tablets for oral use.

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Keywords: pharmaceutical development, mathematical model, statistical methods

Direct compression method for tablet manufacturing has been a great progress in recent years. A state Pharmaceutical Production Laboratory (Dorrego-ASSE) reforms its solid plant to adapt it to new technologies, with the need to re-formulate their traditional products. For this reason, la Universidad de la República, through Instituto Polo Tecnológico de Pando is working together with this laboratory in order to improve the process. ICH Q8 standard guidelines applied to pharmaceutical development are incorporated.

In the current study, 7 mm diameter tablets obtained by direct compression in a Kilian rotary tablet press using direct compression excipients are used. The tablet press is adjusted to obtain a weight tablet range from 165 to 180 mg, pressure is adjusted properly. For a pool of tablets, height, weight and hardness are measured simultaneously for each one with appropriate instrument. A mathematical model reflecting the incidence between these parameters is sought in order to be used in future product development. Data from tablets produced under different setting conditions are used.

Data are processed to evaluate a mathematical model relating height, weight and hardness. We worked on several simple models both linear and quadratic or even linear with interactions. After an exhaustive data selection and the study of all models, the linear model was found to best fit the experimental data restricted to a suitable range. Measured values do not differ by more than 0.1 mm from the values predicted by the model, being able to say that at least half of the data differs by less than 0.02 mm from the predicted values. The statistical parameters indicate that the model is significant as well as all the calculated coefficients. This model allows us to provide important information for the final design of the production process.

Influence of the feed composition on the production of polylysine-indomethacin microparticles by spray-drying

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Keywords: Indomethacin. Spray drying. Product-process relations.

Indomethacin (IN) reduces pain and inflammation in osteoarthritis, rheumatoid arthritis and tendinitis. Inhaled administration was proposed to overcome gastrointestinal side effects. Spray drying (SD) is an adequate technique to manufacture inhalable powders. The aim of this work is to produce a novel material constituted by IN (model acid drug) and polylysine (PL, cationic polylectrolyte) evaluating relationships process-product quality.

PL was combined with IN to obtain $(PL\text{-}IN)_x$ Y% solutions with different neutralization degrees (25-50-75%, identified with subscripts) and total solid contents (identified as percentages, 1.3-1.6-2.6%w/v). The amount of drug in the solutions, drying performance and residual moisture of the powders were assayed. IN content in the product and glass transition temperature (Tg) of the material were analyzed.

Drug content in the feed was in accordance with the amount of IN used for preparing the solutions, except for the $(PL\text{-}IN)_{75}$ 1.6% solution where partial precipitation of the drug was detected. This behavior was associated to the poor solubility and high hydrophobicity of IN. SD yields were adequate for lab scale. At fixed feed solid content, yields decreased from 54.38-46.23% when the neutralization degree of PL changed from 25-75%. When solid content of (PL-IN)50 was modified from 1.3-1.6%, yield was around 50% but it substantially increased up to 67% when solid content was 2.6%. Residual moisture was low (3.504.84%) indicating that drying was efficient.

Drug content in the product was in good agreement with the amount of IN in the feed. Tg values decreased from 138.50-126.07 °C with the IN content in the product. It is due to the plasticizing effect of the drug on the polymer structure. Stickiness in the chamber could explain the yield behavior of the tested samples.

Feed solid content and neutralization degree were variables that modify the process yield and product quality.

Design pharmaceutical solid forms from S*ilybum marianum* (L.)

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Keywords: *Silybum marianum*, Aeroperl® 300, Cellactose 80®.

Standardized extracts from the fruit seeds of *Silybum marianum* (L.) are used in humans for thetreatment of liver diseases of different etiologies. The therapeutic use of these flavolignans is partly restricted by their insolubility in water. In particular, silybin, the main constituent, is sparingly soluble in water and spontaneouslytends to form non-absorbable microcrystals, resulting inan unfavorable pharmacokinetics.

The oral bioavailability of thisextract is therefore limited and it is strictly dependent on the galenical preparation as shown for varioussilymarin products on the market.

In order to overcome the challenge biopharmaceutical raised, hydroalcoholic extracts from the ground dried fruit of *Silybum marianum*in concentrations 8:2, 7:3, y 6:4 respectively were elaborated. Later Aeroperl® 300 Pharma was added. The dispersion granules (DG) were prepared using a technique which involved the preparation of a hydroalcoholic extract followed by its adsorption into the surface of Aeroperl®, an inert absorbent using the solvent evaporation method. Cellactose 80®, microcrystalline cellulose PH200 and magnesium stearate (DGE) were added. DGE for obtaining herbal tablets were

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processed by direct compression. The rheological properties of DG and DGE were evaluated and DGE showed a remarkable improvement.Process controls: uniformity of weight, friability, hardness and disintegration were evaluated according to codified FA methods. The dissolution test was performed in a Hanson Research SR 8 Plus (900 ml) equipment maintained at 37±1°C for 1 h at 100 rpm, 5 ml were extracted at 5, 10, 15, 45 and 60 minutes. The samples were further analyzed by HPLC.The in-vitro dissolution rate of these forms was significantly better in comparison with commercial forms. Physical characterization enabled us to understand the effects of formulation variables on the sylimarin tablets.

Physical characterization and stability of ranitidine and diclofenac amorphous binary system

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Keywords: diclofenac; ranitidine hydrochloride; binary system; solid-state characterization.

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used in the symptomatic relief of musculoskeletal or joint pain. The drug is poorly water soluble and several salts are employed in commercial formulations, as is the case of sodium, potassium, diethylamine and pyrrolidine ethanol diclofenac salts. NSAIDs are well known to cause side effects including gastrointestinal disturbances. Ranitidine hydrochloride, a H_2-receptor antagonist, is widely used in the treatment of stomach ulcer and to counteract gastric irritation resulting from NSAID treatment. It is therefore reasonable to co-administer these drugs in a single formulation.

The binary system was prepared by co-precipitation of ranitidine hydrochloride and sodium diclofenac in water in a ratio similar to that of their therapeutic daily dose. Fourier-transform infrared spectroscopy evidenced that both active pharmaceutical ingredients (APIs) are present in the spectrum of the obtained solid; the characteristic frequencies of those functional groups, able to form the necessary supramolecular synthons, showed slight shifts. Further confirmation was obtained by tandem mass spectrometry. The solid demonstrated to be amorphous according to its powder X-ray diffraction pattern and remained stable in that condition up to three months during storage at 20 °C. Thermogravimetric analysis indicated the sample starts decomposition at 145 °C. Besides, an endothermic event consistent with a fusion process at 104 °C – well below the corresponding ones for each API – was observed in the differential scanning calorimetry curve, after a weak signal. These results seem promising for exploring the solubility properties of this binary system.

Effect of plasticizer agent on mechanical properties of mucoadhesive films

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RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

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Keywords: mechanical properties, mucoadhesive films, plasticizer.

An ideal mucoadhesive film should have a high tensile strength, which will allow it resist the mechanical stress of buccal cavity. In addition, it should have a high percent elongation at break (ductility) and a low elastic modulus (stiffness), characteristics that will allow it adapted on mucosal surfaces and thus, improving patient's comfort. The objective of this research was to evaluate the effect of a plasticizer agent -glycerol-, on mechanical properties of hydroxypropylmethylcellulose K15M mucoadhesive films. The mucoadhesive films were prepared using *film casting method* adding increasing percentages of glycerol, from 0% to 35%. A Lloyd TA1 texture analyser equipped with eccentric roller grips, 500N load cell and NEXYGEN Plus software was employed to evaluate the mechanical properties. Each formulation was tested in triplicate and according to *International ASTM: International Test Method for Thin Plastic Sheeting* (D882-12). The results of tensile strength, percent elongation at break and elastic modulus were obtained and compared using a one-way analysis of variance and Tukey's test to determine the results that led to statistical differences using *GraphPad Prism* 5.0 *software* ($p < 0,01$). The results showed that is necessary a 10% of glycerol to increase percent elongation at break and decrease stiffness of the films significantly. In the case of tensile strength, glycerol concentrations upon 10% did not produce significant statistically differences on the obtained results. To conclude, glycerol concentrations from 10% would allow improve the mechanical properties of mucoadhesive films made with HPMC due to the glycerol plasticizer effect. Calorimetric studies could be performed to assess the different physicochemical phenomena that explain this behaviour.

Evaluation of metronidazole release from a bilayered mucoadhesive system.

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Keywords: periodontal disease, mucoadhesive film, dissolution profile.

Periodontal diseases are pathologies located on the buccal cavity, which are currently treated with dental hygienic techniques and systemically administered antibiotics. This treatment can trigger the onset of bacterial resistance, adverse effects and pharmacological interactions. Thus, development of a system able to administer in a topical way this kind of drugs would allow avoid these problems. The objective of this research was to evaluate the dissolution profiles of metronidazole from mucoadhesive films, which were prepared using film casting method. The first layer, composed of hydroxypropylmethylcellulose K15M and glycerol, generates a mucoadhesive surface. The second layer composed of Eudragit® NE 30D polymer and metronidazole, it in charge of drug controlled release. To evaluate its effect, mucoadhesive films with different drug:Eudragit® NE 30D proportions were prepared (1:5, 1:7,5, 1:10, 1:20) and evaluated trough dissolution profile studies using an adapted method of USP apparatus 5. The studies were performed for 6 hours and released metronidazole was quantified trough UV spectrophotometry

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

(320 nm). The result showed that the formulation with the lowest drug:Eudragit® NE 30D proportion reach a release percentage of 85.93%. The formulations that included a drug:Eudragit® NE 30D proportion of 1:7,5 and 1:10 showed a more retarded release, achieving release percentages of 73,58% and 53,36% respectively. The formulation that include the highest drug:Eudragit® NE 30D proportion showed a lower drug release, reaching a release percentage of 13,81% after 6 hours. Whereas it the only variation between the different formulations was the Eudragit® NE 30D proportion added, we can conclude that metronidazole retarded release is proportional to quantity of Eudragit® NE 30D which is explained for the insolubility and poor permeability of this polymer in aqueous solutions hindering water diffusion into the system, diminishing drug solubilisation and subsequent drug diffusion.

Natural plant promoters of biofilms and bioemulsifiers increase healthy bacteria resistance as a new promising strategy for pharmaceutical biotechnology

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Keywords: Natural plant promoters, Health beneficial *Lactobacillus*, biofilms and bioemulsifiers´ production.

Lactobacilli, are well known to have a positive effect on the maintenance of human health. These probiotic bacteria constitute natural microbiota, are recognized as potential interfering bacteria by producing various antimicrobial agents such as organic acids, antimicrobial substances, bacteriocins, and adhesion inhibitors, such as biotensioactives, among others. They have antimicrobial activity and capability to interfere with the pathogens adhesion on epithelial cells of urogenital and intestinal tracts, and for their anti-biofilm production on catheter devices and voice prostheses. Some *Lactobacillus* strains have biofilm forming ability, and release thermostable glycoproteins as main resistance strategies regulated by quorum-sensing mechanism.

The aim of this research was to increase biofilms and biotensioactives´ biosynthesis of *L. casei* mediated by plant natural products.

L. casei ssp. *paracasei* C2 isolated from regional cheeses was screened for their ability to produce biofilms, and biotensioactives. A dewaxing chloroform extract from *Flourensia fiebrigii* (Asteraceae), a fraction thereof that containing prenylated flavonoids, 2',4'-dihydroxychalcone (DC) at 50 μg/ml, ascorbic acid (AA), and a mixture of DC with AA (12.5 μg/ml of each one) were analysed as potential promoters. The natural products were incorporated to bacterial culture and incubated for 24 h at 37°C. Then, biofilms formation was measured by crystal violet technique (8 replicates).The strongest increase of biofilm formation was observed when medium was supplemented with DC (295%), and also with AA (256%) with respect to control strain (100%). Biotensioactives´ synthesis was also increased because all supernatants (and replicates) obtained displace the oil and spread in the water more than supernatant control (from 136 to 200%). These results would be promissory in pharmaceutical biotechnology to improve *Lactobacillus* adaptation and the solubilisation of hydrophobic drugs by environment friendly bioemulsifiers.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Development of a highly-concentrated nelfinavir mesylate aqueous micellar formulation for pediatric anti-HIV therapy.

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Keywords: Nelfinavir mesylate; D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS); *In vivo* oral pharmacokinetic studies; nano-sized micelles.

Worldwide an estimated of 3.4 million children are living with Human Immunodeficiency Virus (HIV). Prolonged High Activity Antiretroviral Therapy regimes could present low-patient compliance, especially in children, affecting therapeutic success. Nelfinavir mesylate (NFV) is a non-peptidic HIV-1 protease inhibitor recommended for children over 2 years-old. It exhibits pH-dependant aqueous solubility which results highly restricted at physiological pH value. This represents a clinical limitation due to the reduction on drug absorption and unpredictable drug bioavailability. Moreover a liquid formulation of NFV is not commercially available worldwide. In this framework, the present work reports the development and characterization of a highly concentrated aqueous micellar formulation of NFV employing D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) as micelle-former biomaterial. TPGS critical micellar concentration in distilled water (0.02 %w/v) and phosphate buffer pH 7.4 (0.013 %w/v) was determined by the hydrophobic probe solubilization method at 25 °C. A sharp increase on NFV aqueous solubility in both, distilled water (19.7-fold) and buffer pH 7.4 (17,698-fold) was observed where drug aqueous solubility was increased up to 80.3 mg/mL (TPGS 10 %w/v). Micellar size and size distribution of NFV-loaded TPGS micelles (10 %w/v) was 5.6 nm (PDI: 0.29) as determined by dynamic light scattering. Micelles presented a spherical morphology as it was characterized by transmission electronic microscopy. An almost linear *in vitro* NFV release profile was observed over 6 h for TPGS micellar dispersions (10 %w/v) where total drug cumulative release was 56%. Finally, i*n vivo* data showed a significant ($p<0.01$) increase of Area-Under-the-Curve between 0 and 24 h for NFV encapsulated in micelles in comparison with a NFV control suspension, representing an increment on drug oral relative bioavailability of 1.72-fold.

Overall, this novel micellar formulation represents an excellent and simple nano-technological strategy for the development of liquid pediatric formulations for a more appropriate management of anti-HIV therapy.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Development and evaluation of extended release matrix tablets containing metformin HCl (850 mg)

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Key words: Metformin HCl, extended release matrix tablets, RetaLac, Hypromellose.

Diabetes is one of the major causes of death and disability in the world. Metformin HCl (MHCl) is an oral antidiabetic drug in the biguanide class. It is the first-line drug of choice for the treatment of 2 diabetes type, in particular, in overweight and obese people and those with normal kidney function.
Hydrophilic matrices represent a popular and widely used approach for extended release drug delivery.
The purpose of the study was to formulate MHCl (850 mg) sustained release matrix tablets, to analyzing the influence of hydrophilic polymers in the rheological properties of the granules and sustained release of the active ingredient.
Granules of MHCl were prepared using the wet granulation method. Two granulates different were obtained from 30% w/w of RetaLac (Meggle) or Hypromellose (HPMC K100M), used as matrix former, named MHCl-R and MHCl-H, respectively. Granules were evaluated for loose bulk density, tapped density, compressibility index, hausner ratio and angle of repose. All the granules were lubricated and compressed using 14 mm flat-faced punches. The matrix tablets were evaluated for uniformity of weight, friability, hardness and *in vitro* dissolution. The drug delivery was analyzed according to USP XXX. The amount off drug release was measurer for 24 h at one hour interval and was them determined spectrophotometrically at λ 233 nm (UV- 1603, Shimadzu, Japan).
The evaluation of granules showed in both formulations that the powder mixture had good flow properties.
Physico-chemical evaluation of tablets: Both formulations showed uniformity of weight. MHCl-R showed friability 2.6%, while the MHCl-H was 0.3%. The hardness of tablets from all formulation was between 5 and 6 kg/cm^2. The dissolution release profile of formulation made with HPMC was comparable with the market formulation.
Hence MHCl-H formulation was found to be the best formulation to accomplish the aim of this study.

Optimization of skin permeation/retention studies from nanoemulsions containing extract of *Achyrocline satureioides*

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Keywords: *A. satureioides*, nanoemulsion, skin permeation.

Topical nanoemulsions containing extract of *Achyrocline satureioides* (AS) have been described for the treatment of skin disorders such as herpes simplex. The main pharmacological activities of the AS extract

are related to the flavonoids quercetin, luteolin and 3-O-methylquercetin. The characterization of the permeation/retention of bioactive compounds from formulations through the skin/mucosa is a key consideration to predicttheir applications. However, only few studies describe the optimization of the experimental conditions to perform these studies, especially from complex matrices such as nanoemulsions containing plant extracts. Thus, this work aims to evaluatetheeffect of the amount applied on porcine ear skin on the permeation/retention of the main flavonoids from nanoemulsions containing AS extract. Nanoemulsions composed of medium chain triglycerides, egg lecithin, vitamin E, polysorbate 80, and AS hydroethanolic extract (1% of dry residue) were obtained by spontaneous emulsification technique. Formulations showed mean particle size close to 300 nm, polydispersity index of 0.117±0.03 and zeta potential of -42±3 mV. Permeation studieswere carried out withFranz cells apparatus using porcine ear skin. Increasing amount of nanoemulsion (250, 500 and1000 µL) was added to the donor compartment. After 8h, the epidermis was separated from the dermis and the flavonoids were extracted and determined by a validated HPLC method (Bidone et al. *Die Pharmazie* 69: 5-9, 2014). A progressive increase of the retention of the flavonoids was detected with the addition of nanoemulsions (1.29 to 2.95µg/cm^2 of the sum of quercetin, luteolin and 3-O-methylquercetin). Flavonoids were not detected into fluid receptor from all amounts of evaluated nanoemulsions. The results showed thatthe amount of nanoemulsion used in permeation/retention studies is very important, once interferes directly in the amount of flavonoids retained into skin.

Permeability determination of Acetazolamide in the presence of reference standards

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Key words: Acetazolamide, intestinal permeation, permeability markers, SCB

Introduction: Acetazolamide (ACZ) is a potent inhibitor of carbonic anhydrase that acts by reducing the rate of fluid formation, which makes it useful in the treatment of glaucoma. However, ACZ has low aqueous solubility, low permeability and often causes damage to the intestinal mucosa when administered orally.

Have developed numerous experimental models to determine the potential amount of drug absorbed in the intestinal lumen and its absorption mechanism. The Simple Pass Intestinal Permeation (SPIP) is the *in situ* technique most frequently used because it provides the closest conditions to which a drug is after oral administration. Using specific permeability markers enables a simple screening test for classifying a drug by its permeability.

Objectives: To determine the values of P_{eff} of ACZ using as standards of reference atenolol (ATE), a low permeability marker, propranolol (PRO) and verapamil (VER), a high permeability markers.

Methodology: Studies SPIP developed in Wistar male rats. The rats were anesthetized. Once the animals reached the plane of anesthesia was performed an incision in the abdomen and intestinal segment of approximately 10 cm was isolated and canalized at both ends. The first sample was taken 15 min after the start of infusion of a solution containing the test compound at a rate of 0.2 ml / min. The outlet solutions

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of the intestinal segments, were collected each 7 min into Eppendorf tubes and stored until analysis by HPLC.
Results: Peff values obtained were: 4.4 ± 1.4; 7.7 ± 1.9; 7.6 ± 5.3 and 27.9 ± 1.5 x10^{-5} cm/seg for ATE, PRO, VER and ACZ respectively.
Conclusions: From the results obtained it was concluded that ACZ permeability is higher than the obtained for PRO and VER and also higher than that obtained for ATE. Therefore, these results indicate that ACZ is a high permeability drug according to the SCB.

Encapsulation and subsequent freeze-drying of beneficial lactobacilli for their potential inclusion in pharmabiotic formulations for vaginal applications

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Key words: Vaginal lactobacilli, encapsulation, pharmabiotics

One of the main challenges during the design of pharmabiotic products is that the select microorganisms resist to the processes of biomass production, combination in the final product and storage during the shelf life period. In this work, the resistance of biofilm-forming vaginal *Lactobacillus reuteri* CRL 1324 to encapsulation, freeze-drying and storage were assayed. *L. reuteri* was encapsulated applying the extrusion-ionic gelation technique, using microbial polymers (1% xanthan gum-0.75% gellan gum). Capsule aliquots were freeze-dried with or without lyoprotectors (12% lactose-6% skim milk). Capsules were stored at room and refrigeration conditions for 150 days. The following evaluations were performed: viability of bioactive ingredient (i.e. viable *L. reuteri*) before and after encapsulation, freeze-drying and storage; microorganism release and resistance to the genital tract conditions in a medium simulating the vaginal fluid (pH = 4.2), and maintenance of the beneficial properties (biofilm formation and *Streptococcus agalactiae* NH17 inhibition). High yield of capsules, high encapsulation efficiency and viable entrapped lactobacilli were obtained. The resistance of encapsulated *L. reuteri* to lyophilization was higher with lactose/milk. At 150 days of storage, viable cells from freeze-dried capsules (with or without lyoprotectors) stored at refrigeration temperature were only recovered. From the optimal system (lyophilized capsules with lyoprotectors), *L. reuteri* was released in a culture medium simulating vaginal fluid, maintaining its viability during 24 h at 37°C and the capability to form biofilm and to inhibit *S. agalactiae* NH17 growth. In conclusion, the combination of encapsulation (by extrusion-ionic gelation) and freeze-drying processes in presence of lyoprotectors, and the subsequent storage at refrigeration conditions favored the maintenance of *L. reuteri* CRL 1324 viability and functionality. Encapsulated and freeze-dried beneficial lactobacilli can then be included in a suitable pharmaceutical form for vaginal application, to prevent or treat female urogenital infections.

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Physicochemical conditions and culture media design to optimize the production of pharmacologically active metabolites for chronic wounds.

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Keywords: Chronic wounds, Pharmaceutical biotechnology, *Lactobacillus plantarum*

Lactobacillus plantarum culture supernatants (LAPS) have antimicrobial, pro-healing and anesthetic properties, so our medical team applied it inchronic wounds with very encouraging results. The composition of LAPS was determined and its action mechanism was established. The metabolites responsible for the properties act synergistically, which positions the complex mixture of LAPS as a single active pharmaceutical ingredient (API). However, the need for personalized treatments to suit different types of chronic wounds led to devise and develop this study. Based on this, the aim of this work was to design methods and culture media that increase the therapeutic effectiveness of the supernatants.

Multiple modifications were made in MRS broth composition and *L. plantarum* was cultivated in the resulting media with different physicochemical conditions to obtain modified supernatants ($LAPS_m$). The bacteriostatic, bactericidal and anti-biofilm activity (crystal violet) of $LAPS_m$ was tested on four strains typically isolated from chronic wounds (*Pseudomonas aeruginosa, Staphylococcus aureus, S.epidermidis* and *Serratia marcescens*). The concentration of barbiturates in $LAPS_m$ was quantified by GCMS and its relative anesthetic potency in healthy volunteers was evaluated.

The $LAPS_m$ obtained from media with high concentrations of yeast extract (0.4%) possessed the greatest barbiturates concentration and maximum anesthetic power. Those containing greater amount of meat extract (0.1%), cations(Na, K and Mg salts 0.3%) and surfactants (Tween 80 0.4%) had the highest capacity of biofilm disruption. When the glucose (0.3%) and galactose (0.3%) concentration were increased in media, the $LAPS_m$ had the greatest bacteriostatic and bactericidal power as well as those grown with higher concentrations of CO_2 (10%). Finally, media with boric acid (0.1%) had higher inhibitory capacity of biofilm formation.

The results obtained in this work will allow in the future a $LAPS_m$ manufacture with a greater therapeutic effectiveness and even custom properties for each type of wound.

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Production and characterization of co-spray-dried drug-polymer microparticles for inhalation: Influence of polymer type on the process yield and product properties

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Keywords: inhaled particles spray drying, sodium cromoglicate-polymer microparticles.

Inhalation therapy is used to deliver drugs to the respiratory tract, especially for local diseases. Solid formulations are preferred, being their success dependent on particles characteristics, breathing conditions and mucociliary clearance.

Spray-drying is a process that converts liquids into powders. The feasibility of manipulating its operating conditions and the liquid composition allows the optimization of particle characteristics.

The aim of this work was the production and characterization of novel spray-dried polymeric microparticles for inhalatory administration and sustained delivery of the antiasthmatic drug Sodium cromoglicate (SC).

SC and mucoadhesive polymers, namely carboxymethylcellulose (CMCNa), alginate (AlgNa) and hyaluronate (HLNa) sodium salts, were employed. These polymers may modulate the drug delivery and particles' residence time. At fixed operating conditions, previously selected, SC:polymer (1:0.16) aqueous solutions were spray-dried. The process yield (PY) was gravimetrically measured. Many particles characteristics, such as moisture content (MC), mean volumetric diameter ($D_{4,3}$), size distribution width (span factor), density (D_p), morphology and estimated aerodynamic diameter (D_{aer}), were evaluated. Significant effects were determined using ANOVA.

Table 1 presents the obtained results. Statistically analysis showed that the liquid formulation had a significant effect ($p<0.05$) on PY and some product responses (MC, D_p, span). The PYs were adequate for lab scale and powders MCs were lower than 7.20 wt%, usual values for spray-dried SC. Particles were rounded with wrinkled surface.

Liquid composition did not have significant effect on $D_{4,3}$ and D_{aer} ($p>0.05$). All the D_{aer} were lower than 8.25 µm, appropriate sizes for inhalatory administration. Additionally, all the span factors (< 2) indicated narrow distributions. The SC:HLNa powder showed the best attributes for the desired application (lowest D_{aer} and span).

The selected drying conditions and formulation allowed the production of SC-polymer particles with appropriate D_{aer}, span, MC and acceptable PY for inhalation

Table 1. Process yield, moisture content and particles´ characteristics.

Sample	PY (%)	MC (%)	D_p (g/ml)	Span	$D_{4,3}$ (µm)	D_{aer} (µm)
SC:CMCNa	60.38 ± 1.06	6.15 ± 0.05	2.130 ± 0.027	1.12 ± 0.02	5.65 ± 0.06	8.25 ± 0.08
SC:HLNa	55.50 ± 1.15	6.78 ± 0.20	1,897 ± 0.022	1.03 ± 0.05	5.58 ± 0.34	7.68 ± 0.47
SC:AlgNa	60.66 ± 2.83	7.01 ± 0.12	2,099 ± 0.074	1.13 ± 0.05	5.39 ± 0.04	7.79 ± 0.05
SC	61.22 ± 0,77	7.20 ± 0.09	1.864 ± 0.020	1.21 ± 0.04	5.79 ± 0.33	7.90 ± 0.45

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Dexamethasone-21phosphate loading and release from pH-sensitive copolymers of 2-hydroxyethyl methacrylate and 2-(diisopropylamino)ethyl methacrylate.

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Keywords: pH-sensitive polymers, dexamethasone-21phosphate, 2-hydroxyethyl methacrylate, 2-(diisopropylamino)ethyl methacrylate.

Smart polymers like pH-sensitive systems can improve the pharmacological treatment of diseases. Topical application is perhaps the most typical treatment practice in ophthalmic therapies; however it is inefficient and sometimes produces side effects. A possibility to improve drug residence time and bioavailability is the use of a pH-sensitive hydrogel as a vehicle to control the drug delivery.In this work the behavior of copolymers containing 2-hydroxyethyl methacrylate (HEMA) with different proportions of 2-(diisopropylamino) ethyl methacrylate (DPA) and different amounts of cross-linker agent, ethylene glycol dimethacrylate (EGDMA) are evaluated as drug delivery systems for ophthalmic therapies. Drug load and release studies at different pH values were evaluated using dexamethasone-21 phosphate (DXP) as a model drug. The interaction between the polymer matrix and the drug was studied by IR spectroscopy (FTIR), and drug distribution and film morphology at different pH valueswere studiedby scanning electron microscopy (SEM). The results show thatthe loading of DXP increaseswith DPA content and crosslinking degree. Also the incorporation of DXP into the matrix depends on the medium pH and increase at basic pH. SEM images show important morphological changes when varying the medium pH and it can be seen the presence of the DXP loading in the polymer matrix. FTIR spectra of copolymers show an interaction between the drug and the polymer matrix through the shifting of the carbonyl stretching band. The release of DXP from copolymers of HEMA and DPA is sensitive to small pH variations in the range of 7.00 and 7.80. Kinetics releases shows case-II or anomalous diffusion behavior depending on the HEMA/DPA ratio.At medium ocular pH (7.40) the exponents values (*n*) obtained for the hydrogels containing 10 wt. % of DPA are close to 1.0, suggesting a case-II transport and for hydrogels containing 30 wt. % of DPAthe *n* values are between 0.5 and 1 indicating an anomalous behavior.

Development and *in vitro* dissolution studies of solid dispersions containing thalidomide.

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Key-works: thalidomide, solid dispersion, dissolution, gelucire, TPGS.

Thalidomide (THD) is a drug with promising therapeutic action on injuries associated with erythema nodosum leprosum, aphthous ulcers, in HIV+/AIDS and chronic degenerative diseases, among others.

However, this drug is poorly soluble in water and therefore slowly absorbed from the gastrointestinal tract. The aim of this study was to develop solid dispersions prepared with self-emulsifying carriers and thalidomide in hard capsules and evaluate the improvement of the drug dissolution. Formulations containing thalidomide (THD), Gelucire (GEL), d-alpha-tocopheryl-polyethyleneglycol-1000-succinate (TPGS), polyvinylpyrrolidone (PVP) were developed. The dispersions were named according to the carrier used in the following proportions (w/w): THD+TPGS (1:4), THD+TPGS+PVP (1:3:1), THD+GEL (1:4) and THD+GEL+PVP (1:3:1). For the preparation of formulations, thalidomide was dissolved in acetone and carriers in acetone (GEL) or methanol (TPGS + PVP). Afterwards, the solvents were mixed and evaporated under reduced pressure to form a solid dispersion. The formulations were placed in gelatin capsules and drug content was assayed by HPLC. For comparison, THD+starch (1:4) was also encapsulated and used as a formulation control, since starch widely used as excipient without the self-emulsifying carrier characteristic. The samples were assessed with respect to the *in vitro* drug dissolution profiles at 75 rpm, 37°C, dissolution apparatus 2, with ammonia acetate buffer (pH5.5) as medium. The samples were collected at 15, 30, 60, 90, 120 minutes and thalidomide was quantified on HPLC. At 60 minutes after starting the dissolution test, thalidomide dissolved approximately 80% from formulations containing the carriers while only 47% from the capsule containing only starch. At the end of the assay, the formulations showed the following dissolutions percentages: THD+STARCH (70.36±2.96), THD+TPGS (88.09±4.37), THD+TPGS+PVP (105.05±3.29), THD+GEL (90.68±5.02) and THD+GEL+PVP (89.90±2.89). Similarity factor (f2) for THD+GEL+PVP, THD+GEL, TLHD+TPGS+PVP and TLDH+TPGS formulations were lower than 50%, with different dissolution profile in relation to the control formulation, TDH+STARCH. Therefore, the combination of THD with lipid carriers increased the dissolution of the drug.

Synthesis and characterization of novel stimuli-responsive hybrid polymers with potential applications in controlled drug release

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Keywords: stimuli-sensitive polymers, drug release, polyurethane

Previous works have shown the stimuli- responsive characteristics of some acrylic monomers to different changes in the environment, like pH or temperature, which makes them useful for drug delivery applications. Within that group of monomers, the 2-(diethyl amino) ethyl methacrylate (DEA), the N-isopropylacrylamide (NIPA) and the 2-(diisopropylamino) ethyl methacrylate (DPA) can stand out.
However depending on the conditions, films prepared from the homopolymers are brittle and difficult to handle. A convenient way to improve the physical characteristics of films is to copolymerize with vinyl-terminated polyurethanes (PU) to produce good film-forming hybrid materials.

In this work, hybrids containing PU polymerized with DEA (PU/DEA), NIPA (PU/NIPA) and DPA (PU/DPA) monomers were prepared and characterized using FTIR, swelling and in-vitro drug release at different pHs and temperatures, using Rhodamine 6G (Rh6G) as a model of an active pharmaceutical ingredient (API).

The results show changes in the swelling degree when the temperature or pH was modified. FTIR spectra of hybrid polymers indicate an interaction between the PU and the acrylic monomer.

The PU/DEA system releases at pH 4 a large amount of Rh6G compare to pH 8. As it was expected, this behavior is also observed in the PU/DPA system in the same conditions. Additionally, this system shows a thermo-sensitive response which can be observed in the increment of Rh6G released when the temperature is modified, from 37 °C to room temperature (22 °C). For the PU/NIPA system the amount of Rh6G released was higher at 22 °C compare to 37 °C, indicating a thermo-dependence behavior.

In conclusion, the copolymerization between PU and different functional acrylic monomers allows preparing hybrids polymer films with good mechanical properties and stimuli-responsive characteristics suitable for drug delivery applications. Depending on the monomer used, systems with pH or/and thermo-sensitive properties can be obtained.

Serum production against *Tityus serrulatus* scorpion venom using cross-linked chitosan nanoparticles as immunoadjuvant

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Keywords: Scorpion, Chitosan, Nanoparticles, *Tityus serrulatus*, Immunization

Several species of scorpions are known to cause accidents which can lead to death, most of them belonging to the genus *Tityus*. *Tityus serrulatus* is considered the most dangerous scorpion in South America. In Brazil, *T. serrulatus* is responsible for serious accidents, including deaths, which occur mainly with children and elderly people. Anti-scorpion sera are routinely produced by various institutions, and suitable technologies have been investigated for encapsulation and release recombinant or native proteins capable of inducing antibody production. In this context, biocompatible and biodegradable polymers, such as chitosan, have been employed for this purpose. This study aimed to obtain a protein release system for the peptides or proteins from *T. serrulatus*, based on cross-linked chitosan nanoparticles (CN) in order to generate a new model of immunization in animals, and consequently a potentially novel polyclonal serum, namely an anti-*T. serrulatus* venom. CN were successfully obtained by ionic gelation using the polyanion tripolyphosphate (TPP), which demonstrated a suitable particle size of about 200 nm, with maximum encapsulation efficiency (100%) and enhanced antigen-specific antibody titers of 72%. The serum production data revealed that CN were equipotent to

aluminum hydroxide, the traditional adjuvant for immunization. This study demonstrates that chitosan nanoparticles are a promising and safe system for peptide/protein delivery for *T. serrulatus* scorpion.

Study of benzinidazole tablet to evaluate API and excipient distribution using Near Infrared Hyperspectral imaging

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Keywords: Benznidazole, tablets,excipients, NIR, imaging

Benzinidazole (BNZ) tablets were manufactured as solid dispersion, aiming to improve the solubility of the hydrophobic drugs, thereby improving bioavailability. For this purpose, hydrophilic polymers were used (polyvinylpyrrolidone – PVP) to increase solubility, degree of dissolution and inhibit crystallization. Hydroxypropyl methylcellulose (HPMC) was employed to reduce the release time of the API, allowing use of drug as a long duration formulation. In this work, BNZ tablets were manufactured based on two formulations with different amounts of HPMC: LB1 (25% HPMC) and LB2 (35% HPMC). Near Infrared hyperspectral images (HSI-NIR) of 6 tables (triplicates of each formulation) were collected using a SisuCHEMA (Specim) hyperspectral camera in the spectral range 1000-2500 nm. Averaged spectra were obtained from the pure compound images and employed as pure spectra (sp) in MCR-ALS (Multivariate curve resolution using alternating least squares) modeling. MIA (Multivariate Image Analysis) was applied to the concentration distribution maps (CDM's) to analyze the correlation structures of the mixture (internal correlation structure). As a result, internal negative correlation between BNZ and PVP was observed in the third principal component (PC3), i. e., and the presence of one occurring in the absence of the other (Fig 1). Gray Level Co-occurrence Matrices (GLCM) was generated with the API CDM's and then a PCA analysis was made to evaluate similarities in homogeneity. Evaluating the score and loading plots (Fig 2), one of the samples of LB1 formulation (LB1_1) is less homogenous than the other (high energy in PC1). The other 5 tablets presented similar homogeneity concerning the entropy value (PC1). The LB2 samples, however, seem to have a better homogeneity because of the high value of contrast (local differences). HSI-NIR and chemometrics are helpful to understand mixing behavior concerning API and excipient surface distribution, which leads a better development of new formulations.

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Study of the counterionic condensation between the phosphate groups of deoxyribonucleic acid (DNA) and model drugs possessing basic groups.

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Both DNA and Ribonucleic acid (RNA) have phosphate groups in each monomer unit. Taking DNA salmon sperm as a model, the ionic interaction of their phosphate groups ($(-O)_2$-PO_2^-) with drugs (B) currently used in therapy that posses basic groups was performed. Such acid-base interaction generates a certain degree of counterionic condensation (CC) [$(-O)_2$-PO_2^- BH^+] according to equations 1 and 2:

$$(-O)_2\text{-}PO_2H + B \rightleftharpoons (-O)_2\text{-}PO_2^- + BH^+ \rightleftharpoons [(-O)_2\text{-}PO_2^-\ BH^+] \quad (1)$$

or

$$(-O)_2\text{-}PO_2^-Na^+ + BH^+ + Cl^- \rightleftharpoons [(-O)_2\text{-}PO_2^-\ BH^+] + Na^+ + Cl^- \quad (2)$$

The aim of this study is the generation of a detailed knowledge of the affinity between DNA and B to form ion pairs according to 1 and 2 under different conditions. Such information would have projections towards basic and applied areas in the field of pharmacotherapy. The selected B were Atenolol, Propranolol, Lidocaine and Metoclopramide, that were subjected to interact with DNA at different molar ratios (B /DNA) yielding stable aqueous dispersions. The distribution of species at equilibrium was determined through dialysis methodology using spectrophotometric analysis. The results revealed a high degree of CC [$(-O)_2$-$PO_2^-BH^+$] with affinity constants (log Kcc) above 5. Besides, the resultant dispersions of the complexes DNA-B were characterized by light scattering, circular dichroism, and potentiometric determinations. They exhibited a negative electrokinetic potential that are lowered from nearly -30 mv to – 15 mv as the ratio B /DNA was raised. In line with the high degree of CC, they exhibit a slow release rate of B towards water or simulated biological fluids placed in the receptor compartment of two-compartment diffusion cells. Such results confirm the reversibility of the ionic interaction already described.

The set of results let to conclude that the ionic equilibrium described here are the main interactions between DNA and the model drugs studied. However, due to the complex structure of DNA other kind of interactions would also be present.

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Sodium Hyaluronate Minitablets for Ocular Therapy

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Keywords: minitablets – hyaluronan – ocular – glaucoma.

Introduction: Topical administration is preferred for ocular drug delivery to the structures of the front part of the eye, such as the cornea and the conjunctiva. Traditional ocular topical dosage forms have limitations in solving many eye diseases. The main reason could be attributed to the rapid and extensive drainage loss of the formulation in the precorneal area as a result of blinking and tear replacement. A promising alternative to increasing the residence time of formulations in the area of application is the use of bioadhesive systems capable of releasing controlled amounts of the desired drug.
In this work we propose the design of minitablets (MTs) for topical ophthalmic application; using hyaluronic acid, a well-known biomaterial widely used in ophthalmology, and hydroxypropyl methylcellulose, an excipient widely used in pharmaceutical industry for oral, ophthalmic and skin formulations.
MTs loaded with timolol maleate (TM) were prepared by direct compression method and characterized in terms ofthe rheology of powders, crushing strength, friability and drug delivery.
Results: Studies showed limitedflowproperties in powder mixture. Angle of Repose values are slightly higher than 34º, Compressibility Index higher than 21 and Hausner Ratio superior to 1,25 (higher acceptable values for each index). On the other handstrenght, friability and release assays showed good performance. Crushing strength values were 26,6 ± 1,25 N and the friability 0,403%. Finally drug release showed a slow release ratio, reaching the 80% of TM loaded in 4 h.
Conclusions: New MTs designed showed slow release ratio for TM, low strenght but good friability. However the formulation must be optimized in order to reach better flow properties of poder mixture.

Topotecan biocompatible hydrogels with potential implications in metronomic treatment of retinoblastoma

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Keywords: retinoblastoma, injectable gel, controlled release, topotecan.

Retinoblastoma is the most common primary cancer to affect the eyes of children. Despite the survival rate is over 95% in Argentina, the main objective is to increase the eye salvage rate while not affecting the overall survival or the local and systemic toxicity. In contrast to other oncological diseases, retinoblastoma treatment lacks of a maintenance phase after the initial reduction of the tumor mass. In this sense metronomic treatment (low doses administered over prolonged intervals) has proven to stabilize the

disease in some pediatric tumors. An approach to the metronomic treatment without repeated intravitreal injections would be the use of a controlled released system for topotecan, a well-known agent with activity against retinoblastoma. The aim of the present work was to synthesize an extended release formulation of a biocompatible and biodegradable injectable gel of a triblock copolymer loaded with topotecan. Triblock copolymers of polyethylene glycol (PEG) and epsilon-caprolactone (PCL-PEG-PCL) were synthesized by the ring opening polymerization of CL by PEG in presence of tin(II) 2-ethylhexanoate (SnOct). Three different PEGs were used (1000, 4000 and 6000 g/mol) with the same ratio of 1.1 of CL/EG (characterized by ^{1}H RMN). PCL-PEG-PCL 35% w/w hydrogels were loaded with 0.05, 0.1 and 0.2 mg of topotecan/g of gel. The *in vitro* topotecan released was performed placing the gel in microdyalisis membranes immersed in phosphate buffer pH 7.4 at 37°C. The release profiles showed a burst effect followed by a sustained released for 7 days. The cumulative release of topotecan for the three gels with PEG4000 was around 65% after 7 days. The other two copolymers showed the same behavior. Considering these results, these new formulations open a potential new dose and schedule of treatment for retinoblastoma and will be supported by *in vivo* data in an animal model in further studies.

Novel Soluplus® nano-sized micelles for encapsulation of antineoplastic paclitaxel: *In vitro* characterization.

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Keywords: Antineoplastic drugs; Paclitaxel; Polymeric micelles; Poly(vinyl caprolactam)-poly(vinyl acetate)-poly(ethyleneglycol) graft copolymer (Soluplus®).

One of the most effective antineoplastic drugs used for the treatment of various solid tumors including ovarian cancer and metastatic breast cancers is paclitaxel (PTX), which is commercialized as a mixture of Cremophor EL® and dehydrated alcohol (1:1, v/v) (Taxol®) due to PTX hydrophobic nature and its low-aqueous solubility (0.3-0.5 µg/ml). However, seriously hypersensitivity reactions have been reported associated with the use of Cremophor EL®. Thereafter, the development of novel PTX formulations with enhanced drug aqueous solubility represents a technological challenge. In this framework, nanotechnology explores different strategies to improve apparent solubility and stability of poorly-soluble drugs. The present study investigates PTX encapsulation within nano-sized polymeric micelles employing the commercially available poly(vinyl caprolactam)-poly(vinyl acetate)-poly(ethylene glycol) graft copolymer (Soluplus®) as micelle-former biomaterial. The aggregation behavior of the copolymer was characterized by the hydrophobic probe solubilization method where the critical micellar concentration was 0.55 x 10^{-4}% w/v at 25 °C. Micelle hydrodynamic diameter (Soluplus® 10% w/v) was 91.6 ± 0.6 nm (PDI: 0.22) as determined by dynamic light scattering (DLS). Also, the nanocarrier exhibited a spherical morphology as characterized by transmission electronic microscopy (TEM). *In vitro* solubility assays demonstrated that PTX was efficiently encapsulated within Soluplus® polymeric micelles where the drug apparent aqueous solubility was increased up to 11.3 mg/mL (copolymer 10% w/v). PTX encapsulation

was also confirmed by nuclear magnetic resonance of hydrogen (NRMH). Moreover, the increment on PTX aqueous solubility (~60,000-fold) is being reported for the first time for this nano-sized polymeric carrier.

Finally, this novel PTX micellar formulation represents an excellent nanotechnological platform to improve anticancer therapy. Further studies will be focused on PTX *in vitro* cytotoxicity and *in vivo* oral bioavailability for drug-loaded micelles compared with commercially available Taxol®.

Development and characterization of nanostructured formulations containing saquinavir planned for oral administration

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Keywords: Saquinavir, nanotechnology, oral administration.

More than 35 million people worldwide are infected with the human immunodeficiency virus, and more than 600 thousand are children. Saquinavir is a commercial drug for this virus treatment, with low bioavailability and presented in pharmaceutical forms that are difficult to administer to children. A promising alternative to improve their biopharmaceutical characteristics is nanotechnology. The objective of this work is to develop nanostructured liquid formulations with saquinavir. Formulations were developed by combining poly-ε-caprolactone (PCL) and poly-ε-caprolactone triol (PCL-T) through the interfacial deposition of preformed polymers. The average diameter and particle size distribution were determined by laser diffraction, while the encapsulation rate and the drug content by high performance liquid chromatography. The pH was determined by potentiometry and zeta potential by electrophoretic mobility. The stability was evaluated at 0, 15, 30 and 60 days, and the release profile with dialysis membrane. The drug content ranged from 870 ± 50 to 946 ± 119 µg/mL. All formulations presented nanometric size between 173 ± 21 and 250 ± 44 nm and a good size distribution. The drug content associated with the nanocapsules resulted in more than 99% of encapsulation rate and the zeta potential was negative between -10 ± 1.5 and -13 ± 3 mV. The evaluated stability parameters remained constant over time. The *in vitro* release assay showed a controlled release profile of the drug in the formulations when compared to the free drug. The increase in PCL content resulted in higher delayed drug release. The results demonstrated that the developed formulations had appropriate nanotechnological features and that the association of polymers is effective for saquinavir nanoencapsulation. The study of *in vivo* pharmacokinetics is included as a perspective of this study.

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Experimental and theoretical studies over the stability in co-amorphous samples

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Keywords: Famotidine, Ibuprofen, stability amorphous systems, DFT, molecular simulations

The solubility, bioavailability and processability of crystalline drugs can be improved converting them into an amorphous state. However, a particular issue related to amorphous solids is that they are high-energy states and therefore metastable. In this work, the stability of the amorphous binary system formed by the H_2 receptor antagonist (famotidine, FMT) and a NSAID (ibuprofen, IBU) in 1:1 molar ratio was studied. The sample was prepared by cryo-milling process; its amorphous nature was confirmed by the lack of the diffraction peaks and the observation of the Tg (transition glass) signal. This sample was physically stable when stored for 60 days at different temperatures. The high stability, confirmed by FTIR analysis, is due to a solid state interaction between the guanidine group of FMT and carboxylic acid of IBU forming a heterodimer trough the heterosynthon N-H···O. Two conformers were proposed for the heterodimer and the lowest energy conformer was analyzed to determine the presence of bond critical point in order to deeper understand this interaction. Besides, molecular dynamics simulations were conducted. Physical consequences of these results are discussed for the co-amorphous phase in terms of thermal properties (Tg), hydrogen bonding distributions and molecular diffusion, since these properties are important to justify the stability of this metastable material. The estimated Tg is in good agreement with the experimental value. In addition, the radial distribution functions suggest that the heterosynthon N-H···O is found in this amorphous model. Taking all together, our results suggest that, the high stability observed in this amorphous sample is due to a strong interaction (N-H···O) between both drugs as was confirmed by FTIR and DFT calculations. Moreover, molecular simulations could be employed to predict physical properties and justify the stability of this amorphous phase.

Impact of Crystal Habit on *in vitro* Biopharmaceutical Performance of Nitazoxanide

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Keywords: microcrystals/ micronized sample/ poorly-water soluble drug / powder dissolution rate

Poor biopharmaceutical performance of Biopharmaceutical Classification System (BCS) class II active pharmaceutical ingredients (APIs) is a major hurdle in the design and development of pharmaceutical formulations. Polymorphism and the external appearance of crystals (habit) of such APIs can have considerable influence on physicochemical properties and, therefore, on product performance.
In the present investigation, a molecule centered approach is presented toward crystal habit modification of nitazoxanide (NTZ), a poorly-water soluble antiparasitic and antiviral API, and its effect on dissolution behavior.

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Three samples of NTZ with different habits [acicular (NTZ-a), plate-shaped (NTZ-p) and microcrystals (NTZ-m)] were prepared and evaluated for internal structure (X-ray powder diffractometry, XRPD), surface characteristics (confocal microscopy), wetting behavior (contact angle measurements), particle size distribution (PSD, laser diffraction) and powder dissolution rate [Apparatus 2, 500 mL of Tween 80/triethanolamine (0.25:0.25 % w/w), 75 rpm and 37 ºC] in comparison with a micronized raw-powder (NTZ-rp). The dissolution parameters evaluated were initial dissolution rate (iDR), dissolution efficiency (DE) and dissolution kinetics. The cumulative amount of NTZ dissolved was determined by high performance liquid chromatography assay.

According to PDRX, the four samples were isomorphic but their PSD, wettability and dissolution behavior were different. The microcrystals (NTZ-m) exhibited higher iDR and DE than NTZ-rp, NTZ-a and NTZ-p. The kinetics models that fitted better were Probit function for NTZ-rp and Weibull model for NTZ-m, NTZ-a and NTZ-p.

This study establishes the potentially significant contribution of the crystal habit on the dissolution performance of NTZ. The preparation of microcrystals by a precipitation technique is suitable for the enhancement of the dissolution of NTZ.

Preparation and physicochemical characterization of nitazoxanide- and tixozanide-polyvinylpyrrolidone K30 films

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Keywords: co-evaporated samples; DSC; PDRX; solid dispersions.

The improvement of the solubility and dissolution rate of poorly water-soluble active pharmaceutical ingredients (APIs) represents an important challenge. Such APIs may benefit from formulation approaches that overcome a poor aqueous solubility and/or a dissolution rate-limited bioavailability and, for this purpose, solid dispersions (SD) are frequently proposed.

Nitazoxanide (NTZ) and Tixozanide (TIX) are two antiparasitic and antiviral compounds practically insoluble in water, and polyvinylpyrrolidone (PVP) a soluble hydrophilic polymer able to form a SD. The goal of this work was to associate NTX and TIX with PVP K30 in a SD to improve their solubility.

To obtain the SDs, each API and PVP K30 were dissolved in ethanol and leading to a homogeneous system after the evaporation of the solvent. The weight ratios of the NTZ-PVP and TIX-PVP mixtures were kept constant to 1:1. The NTZ-PVP and TIX-PVP dispersions were analyzed by Raman, Attenuated Total Reflection and Diffused reflectance Infrared Fourier Transform Spectroscopy (ATR-IR and DRIFT), Differential Scanning Calorimetry (DSC), Thermogravimetry (TG), Hot-Stage Microscopy (HSM), Powder X-ray diffractometry (PDRX) in comparison with the APIs and the respective physical mixtures (PMs). Contact angle and solubility (water and buffer phosphate pH 6.8, 37 ºC) measurements were also performed.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

After evaporation of the solvent, two yellow transparent films were thus obtained, suggesting that NTZ and TIX were dispersed in PVP K30. The diffractograms of the NTZ-PVP and TIX-PVP dispersions exhibited no reflection lines of TIX and only a few low intense peaks of NTZ. DRIFT and DSC corroborated the dispersion of NTZ and TIX in the carrier. The solubility measurements indicated that both dispersions exhibited better solubility than the PMs and the APIs.
The solubility enhancement can be attributed to improved wettability, as indicated by contact angle measurements, as well as particle size reduction and disruption of the TIX and NTZ crystallinity.

Vesicles as a carrier for administration of rPsaA protein of *Streptococcus pneumoniae*.

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Keywords: vesicles, *Streptococcus pneumoniae*, vaccine

Mucosal vaccines have the advantage of stimulate systemic and mucosal immunity. Despite of the potential immunological, practical and logistical benefits compared to the parenteral route, mucosal administration of typical subunit vaccines does not strongly stimulate immune response and protection. Therefore, the development of antigen delivery systems for enhancing the immunogenicity of poorly immunogenic recombinant antigens is necessary.
Streptococcus pneumoniae is a serious public health problem in developing countries due its high rates of mortality and morbidity. Among the vaccines against this bacterium, none of them is able to protect against about 90 different serotypes. For this reason the search of common protein antigens for all serotypes, which solves the problem of coverage and low immune response in young children is an interesting scientific challenge. One of the proteins studied to date is the pneumococcal surface adhesin A (PsaA), lipoprotein of 37 kDa, located in the membrane of the bacterium.
The objectives of this study were the expression and purification of recombinant PsaA protein, the evaluation of the immunogenicity of the protein in a nasal and oral administration, and the encapsulation and characterization of the protein in vesicles.
The psaA gene was expressed in the E. coli BL21 (DE3) strain with IPTG induction of the plasmid pET15b. The pellets samples of expression cells were subjected to lysozyme treatment and disrupted by sonication to obtain the total protein extract for SDS–PAGE analysis. The total protein extract was harvested by centrifugation to obtain the soluble fraction, which was cleaned-up through a weak anion exchange column. Bound protein was eluted and quantified by BCA (Bicinchoninic Acid Kit for Protein Determination) using BSA as a standard protein. Vesicles were prepared using the thin layer evaporation method and size was adjusted with ultrasonic homogenizer. Vesicles were morphologically characterized using TEM, size and zeta potential were determined by DLS, using a nanoZ-sizer.
It was possible to express the protein in E. coli BL21DE3 and purify on a column of affinity for histidine. Serum antibodies from immunized rabbits with recombinant PsaA, recognized the native protein of serotypes 7 and 14 of *Streptococcus pneumoniae*. Preliminary results indicate that it is possible to encapsulate the protein in vesicles with an efficiency of 40%.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Meloxicam-Poloxamer solid dispersions by spray drying

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Key words: Meloxicam, Poloxamer, Solid Dispersion, Spray Drying

Meloxicam (MX), a non-steroidal anti-inflammatory drug used in various chronic diseases, has low solubility. To improve it, solid dispersions (SDs) were successfully prepared by the fusion method using Poloxamer (PX) as carrier. Considering that spray drying is a relatively flexible, low cost, continuous and easy scaling process, it was explored as an alternative technique to obtain MX:PX DSs, facing the challenge of processing an aqueous suspension containing a hydrophilic low melting point polymer (58 °C). In this context, the aim of the present work was to determine a set of operating conditions with acceptable process yield.

MX dispersions were prepared in PX solutions, according to a MX: PX=1:1 ratio. The samples were sonicated for 10 min (to favor the dispersion of MX in the PX solution) and spray dried in a Mini Spray Dryer Büchi B-290, varying the drying air inlet temperature (Tin: 45-85 °C) and flowrate (Qd: 35-38 m^3/h), the liquid feed flowrate (Qf: 2-3 mL/min) and solids concentration (Cin: 1-8 wt%) and the atomizing air flowrate (Qa: 414-601 L/h).

The highest yield (19.92%) was obtained for Cin = 1%, Tin = 50 °C, Qd = 38 m^3/h Qf= 2 mL/min and Qa = 473 L/h, maintaining the liquid feed between 37 and 40 °C under agitation.

MX:PX=1:1 DSs microparticles were obtained by spray drying an aqueous suspension of the polymer (avoiding the use of organic solvents) with acceptable performance in agreement with the PX hydrophilicity and low melting point and the lab scale of drying unit.

Achyrocline satureioides extract-loaded nanoemulsions: mucosa retention/permeation and antiherpetic activity

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Keywords: plant extract, nanoemulsion, antiviral

Achyrocline satureioides (AS) - Asteraceae is a medicinal plant widely studied in South America. The activity of AS extracts against Herpes Simplex Virus has been recently demonstrated and was attributed especially to presence of flavonoids quercetin (Q), luteolin (L) and 3-*O*-methylquercetin (MQ). The local treatment of the herpetic infections includes the application of medicines on the skin and/or the oral mucosa and can be performed before and after herpetic lesions appear. Recently, our research group described the feasibility of obtaining AS extract-loaded nanoemulsions. Such nanoemulsions have been

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considered for local treatment of herpetic infections. The present work aimed to evaluate the Q, L and MQ retention in intact and impaired mucosa from AS-loaded nanoemulsions and to determine the antiherpetic activity of the formulations. The nanoemulsions were obtained by spontaneous emulsification. The mean droplet size and ζ-potential were respectively close to 300 nm and -40 mV. The retention/permeation assay was carried out using Franz type diffusion cells and porcine esophageal mucosa cuts as membranes. After 8 hours, the Q, L and MQ were determined into mucosa, as well as in receptor fluid. The impaired mucosa was obtained by removing of superficial epithelium layer. The flavonoid retention in intact porcine esophageal mucosa was of 0.93±0.20, 0.78±0.16 and 2.98±0.5 µg/cm^2 of Q, L and MQ, respectively. After 8 hours only MQ was detected in receptor fluid (0.68±0.14 µg/cm^2). The removal of the superficial stratum from mucosa conduced to an increase of flavonoid retention (being 2.25±0.47, 1.47±0.34 and 7.47±1.39 µg/cm^2 for Q, L and MQ, respectively). These results were confirmed by confocal microscopy. The permeation also increased from impaired mucosa, being Q, L and MQ detected in concentrations of 1.69±0.5, 2.71±0.53 and 3.22±1.26 µg/cm^2, respectively. Finally, the antiherpetic evaluation showed an IC_{50} for AS extract of 12.8 µg/mL. The incorporation of this extract in nanoemulsions decreased 5-fold the IC_{50} of the viral plaque formation and increased more than 8-fold the selectivity index. Thus, we concluded with this work that AS extract loaded-nanoemulsions are promising in herpes treatment.

Characterization of bioadhesive gels for skin application

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Keywords: Bioadhesive gels, sensory characterization, *in vitro* release.

The main advantage of bioadhesive gels lies in their potential to increase contact intimacy and residence time at the delivery site, improving active substance bioavailability and reducing administration frequency. However, bioadhesive excipients could increase stickiness of formulations. Considering that patient acceptance plays a major role in treatment compliance, the development of bioadhesive gels should take into account their sensory characteristics.

In this context, the aim of this work was to characterize bioadhesive gels in order to select formulations suitable for skin application.

Eight gels were prepared by using carbomer homopolymer type C (Cb) or potassium salt of kappa carrageenan (Cg), in binary combination with either carbomer copolymer type B (C), guar (G) or xanthan gum (X), together with propylene glycol, methylparaben, sorbic acid, deionized water and caffeine (model active substance). The pH of the formulations was adjusted to 5.5.

Twenty one untrained assessors used a check-all-that-apply (CATA) question to evaluate the sensory characteristics of the formulations. Data were analyzed using Multiple Factor Analysis (MFA) and Cluster Analysis.

Physicochemical characterization was performed using spreadability tests (between plates), adhesion measurements (texture analyzer) and in vitro caffeine release studies (Franz cells). Data were analyzed using analysis of variance.
MFA provided a sensory map of the samples, which identified their main characteristics. Cluster analysis grouped samples in three clusters. One of them was composed by all gels with positive sensory characteristics. These formulations were selected for spreadability, adhesion and caffeine release testing. Gels containing Cb with C or X presented good adhesion results and gradual caffeine release, which reached approximately 70% in less than 6 hours.
Sensory and physicochemical studies enabled the identification of bioadhesive gel formulations with positive characteristics for skin application.

Molecular modelling investigations of chloramphenicol Supramolecular systems

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Keywords: molecular modelling, chloramphenicol, multicomponent complexes.

Molecular modeling (MM) combining theoretical methods and computing techniques are currently used to aid the study of molecular interactions involved in supramolecular systems of pharmaceutical interest. In this work, MM was applied to investigate the interaction between chloramphenicol (CP) and β-cyclodextrin (βCD) in presence of several aminoacids (AA): [glycine (GLY), L-cysteine (CYS) or N-acetyl-L-cysteine (NAC)].
CP structure was subjected to conformational analyses, after which complexes with βCD were predicted by molecular docking using the software designed by Open Eye Inc. The complex was afterwards subjected to molecular dynamics (MD) simulations, confirming the inclusion of CP within the βCD hydrophobic cavity, which is stabilized by electrostatic (-30.6 kcal/mol) and van der Waals (-23 kcal/mol) interactions. The electrostatic stabilization is originated in hydrogen bond interactions between hydroxyls of CP and hydroxyls located in the wide rim of βCD. The binary complex conformation was afterwards used to dock GLY, CYS and NAC (in ternary complexes). When the three dimensional structure of the ternary complexes was analyzed, it was observed that the three AA are bound on the wide rim of βCD, as intercalators between CP and Bcd and establishing hydrogen bond interactions with CP and βCD. A competition by native hydrogen bonds between CP and βCD was observed in the ternary complexes, which may originate the experimentally observed lowered affinity of CP to βCD in the ternary systems if compared to the binary counterpart. To further confirm the inclusion behavior, MM studies were also supported by 1D 1H and 2D ROESY NMR analyses, with results being in agreement.
Based on the presented results, the three dimensional structures of the CP:βCD:AA complexes were elucidated and analyzed, with good agreement between MM studies and experimental observations. MM studies arise as a powerful technique to further select the suitable AA for CP:βCD complexes.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Preparation and preformulation studies of albendazole solid dispersions using poloxamer 407 as carrier polymer

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Keywords: carrier, drug delivery, solubility

The permeability and solubility of some drugs can be limiting conditions for oral absorption with the consequent decrease of bioavailability. Solids dispersions (SDs) have been proposed as alternative for improvement of dissolution rate of different drugs using carriers like poloxamer. The aim of this work was to increase the dissolution rate of Albendazole (ABZ) through its incorporation in SDs using Poloxamer 407 (P407) as carrier, by means of the study of the pharmacotechnical properties of the prepared formulations.

In all prepared formulations (5, 10, 25 and 50 % w/w) it is shown an improvement of dissolution rate while the concentration of P407 decreases. In SDs with lower P407 percentage (SD3 and SD4) reaches nearly 60% of dissolved ABZ during the initial stage while SD1 and SD2 hardly reach 20%. This effect can be explained because when SDs takes contact with the dissolution medium at 37°C, P407 would form a gel-layer in the particles surface that can be able to modulate the release of ABZ.

Physical mixtures (PMs) were prepared from the studied formulations, showing a different behavior to the SDs.This is possible because they are mechanical mixtures, the ABZ particles can be distributed in a non uniform way in the dissolution medium. Although P407 contributes to increase the dissolution rate of ABZ it is not done in function of the proportion that it is in the system.

ABZ (pure drug) and PMs have very poor flow properties. However, this property is improved when the drug is incorporated in the SDs. For example the angle of repose of the MF4 was 57±1 while for DS4 was 34± 2.

The addition of P407 as carrier in SDs containing ABZ markedly improves its dissolution properties. SDs seems to be advantageous over PMs for manufacturing of acceptable quality solid dosage forms.

Evaluation of albendazole release from composite thermosensitive polymeric matrices poloxamer - chitosan

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Keywords: injectable depot, *in situ* formation, veterinary use

A polymer solution capable of gelling *in situ* at a temperature close to the physiological one might be attractive for the development of drug delivery systems for veterinary use. The scope of the present contribution was to explore the potential of novel thermosensitive drug delivery platforms designed by combination of two different poloxamers (P407 and P188) with chitosan (CH).

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The release experiments were performed using the membraneless model since this procedure allows direct contact between gel and release medium, and gel erosion can also be considered. In membraneless model, two phenomena are involved: the fickian diffusion of the drug and the dissolution of poloxamer.
To assess the influence of the concentration of poloxamer and chitosan in the drug release, gels of P407, P188 and CH in different proportions (formulations 28/12, 28/15, 28/12/0.5, 28/12/1, 28/15/0.5 and 28/15/1) % w/w were studied. The viscosity of all formulations was suitable for an injectable administration. The addition of chitosan decreased the release of albendazole from the gels containing 12% of P188, but this effect was less in the gels with 15% of P188. Release data were then processed using the power model. The *n* values obtained were between 0.5 and 1 for all formulations, suggesting that, besides the diffusion mechanism, other mechanisms are involved in the kinetic control of drug release. Therefore, dissolution and relaxation polymer processes could be involved in drug release mechanism. The poloxamer system is one of the swelling-controlled systems, which functions by a process of continuous swelling of the polymer carrier that is associated with simultaneous or later dissolution of the polymer.
Depending on the composition of the poloxamer blend, chitosan decreased albendazole release rate. This study demonstrates that the addition of chitosan into poloxamers blends can be considered a useful tool to design thermosensitive injectable depot systems, if added in suitable amounts.

Evaluation of the variables with three different disintegrants in diclofenac sodium tablets by desings of experiment.

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Keywords: DOE, disintegrant, dissolution

We used the design of experiment techniques to define three variables over three types of disintegrants to reach a dissolution test Q = 75% + 5 % in 45 minutes for sodium diclofenac tablet.
One of the variables is the type of the disintegrant: sodium croscarmellose, crospovidone and sodium starch glycolate. The other variables are percentage on the formula and the intragranular proportion.
The formula used was 41.7% diclofenac Na, microcrystalline cellulose PH 101 (FMC) as diluent and the final weight adjustment of formula and magnesium stearate (Mallinckrodt) 0.8% as the lubricant, the binder was povidone K30 (ASHLAND) to 5.9% in alcohol for the granulation. Disintegrants are used from 0.4 to 2.0%.
The process was wet granulation, drying, milling, lubrication, and compressing at the same weight.
The experimental model was a full factorial type 2FI and 27 treatments were performed jointly to avoid bias and blocks. The Design Expert Ease Stat v8.0 software was used.
The depend variables were: time of disintegration and the value of Q in the dissolution test according to USP technique XXXVI.
Croscarmellose reached the target at a concentration of 1.2% and was slightly influenced its presence inside or outside the granules.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Crospovidone also behaved as croscarmellose but was strongly influenced his intragranular presence between 0% and 50%, outside of granules did not reach the level of Q.
Sodium starch glycolate required high concentrations to achieve the target and its presence inside or outside the granules showed little influence.
This use of DoE allowed to reach the equation that relates all parameters for each disintegrating and can display design space that facilitates determining minimum levels for each parameter Q dissolution test achieves 75% +5%.

Liquid crystalline emulsion as controlled liberation system compared to a conventional emulsion. Study of physical stability and rheological behavior.

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Keywords: liquid crystals, controlled release, stability, rheology.

The lyotropic liquid crystals[1,2,3] proved to be suitable systems for the controlled release of hydrosoluble and liposoluble drugs[4]. We work on biphasic systems with a vegetal oil-soluble active rich in essential polyunsaturated fatty acids. In this work we have studied the stability[5] and physical characteristics of a liquid crystal compared with a conventional emulsion both with addition of *Rosa mosqueta* oil. Two emulsions were essayed, one was prepared by conventional technics (1) and the other one was prepared by a method of formation of liquid-crystals (2)[6]. The oil phase of the essayed emulsions contained stearic acid (15.00 %), mineral oil (20.00%), *Rosa Mosqueta* oil (1%) and propylparaben (0.03%), while the aqueous phase was formulated with triethanolamine (4.14%), water (59.76%) and methylparaben (0.07%). The stability of the emulsions was evaluated by centrifugation for 30 minutes at 3000 rpm and by storage during 12 months at 40ºC. The formation of liquid-crystalline structures was verified using a polarizing microscope Carl Zeiss with a digital camera Olympus. The reological profile was done with a viscosimeter Brookfield of coaxials cylinders; a rotor Nº 29 was used (25ºC). The pH was determined with pH-meter model Metrohm. All determinations were done in triplicate. Both systems turned out to be stable against centrifugation and to storage during 12 months at 40ºC. Only the emulsion (2) provided liquid crystalline structures and secondary droplets. Rheologically they proved to be plastics bodies with thixotrophy; values corresponding to sample (1) are superior to those of sample (2). The pH was 7.2. The proposed methodology allows obtaining emulsions with liquid crystalline structures (2). We suppose that the decrease of the viscosity is related with the incorporation of the Rosa mosqueta oil into the lamellar multilayer structure surrounding the emulsions drops, besides being part of the internal phase. The presence of liquid crystals and secondary droplets increase the physical stability of biphasic systems. The pH is suitable for topical application.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Liposome composition and preparation method affect the insulin entrapment capacity into modified liposomes.

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Key words: Insuline, Pulmonary delivery, Modified liposomes

Nowadays, diabetes treatment is uncomfortable for the patient because of the injections as well as the frequency of administration. Consequently, other administration routes are being investigated.
The objective of our research was to develop and characterize new formulations containing insulin-loaded liposomes, due to a high affinity of these vesicles through the pulmonary surfactant. Specifically, the effect of phospholipid, as main component of liposomes, and the preparation method (thin layer evaporation (TLE), detergent dialysis and extrusion), have been evaluated.
Firstly, we studied the most suitable experimental conditions for the insulin testing and its behaviour in such conditions. Following this study, we studied the influence of some operational factors on drug stability, by using the design of experiments tool. Liposomes were elaborated by TLE and detergent removal method by using three different phospholipids: phosphatidylcholine (PC), distearoyl phosphocholine (DSPC) and dipalmitoyl phosphatidylcholine (DPPC). TLE samples were also extruded. Afterwards, some parameters were determined to characterize the different batches: the percentage of drug entrapment by HPLC, vesicle size, polidispersión index and zeta potential.
From the study, high percentages of drug entrapped were obtained in DPPC liposomes using the dialysis of detergent method, being 53.76%. This result offers an interesting possibility for pulmonary delivery of insulin because the presence of the palmitoyl group in DPPC opens the space between pulmonary cells. Moreover, the smaller sizes (between 200-300 nm) and lower polydispersion indexes (<0.3) obtained, also would suppose an advantage for this route. Finally, the zeta potential (-14 mV) contributes to maintain the colloidal stability.

Regression analysis as statistical tool for optimizing formulations of timolol-loaded transfersomes

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Keywords: regression analysis, transfersome, design of experiment, optimization

Transfersomes are ultradeformable hydrophilic lipid vesicles that cross the membrane under the influence of a transepidermal water activity gradient. In this study, we planned to optimize the effect of formulation variables on the timolol entrapment and physicochemical stability of transfersomes. For this, the statistical tool of design of experiments was used and, specifically, regression analysis.

In the screening phase, five factors: amount of cholesterol (F1), amount of edge activator (F2), place of addition of drug (F3), stearylamine (F4) and type of edge activator (F5) were selected as the causal factors. According to the number of factors and levels required, an orthogonal array L16 Taguchi was selected.

The vesicle size (VS), polidispersion index, zeta potential (ZP) and percentage of drug entrapment, were the responses (dependent variables) evaluated for each formulation. All these responses were evaluated before and after the extrusion process.

ANOVA test was performed to determine which test factors were statistically significant for each response.

Optimization process was carried out by regression analysis, which is a statistical approach to estimate the relationship between a dependent variable and one or more independent variables. Furthermore, for each of the responses a confirmatory experiment was performed, based on which we calculated the prediction error. The adjustment of each response was justified by the adjusted R-squared coefficient.

Once the statistical significance of the factors was obtained from ANOVA, low prediction errors may be observed in the optimization process, which was expected based on the R^2_{adj} values obtained. The largest errors are found for VS (before) and ZP (after).

The optimized conditions by regression analysis demonstrated that seven of the eight studied responses achieved an adequate estimation. From the study, we can conclude that regression analysis is an interesting tool for prediction and forecasting in further studies.

Development of curcumin-loaded liposomes: solubility and stability studies

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Keywords: Curcumin, liposomes, solubility, stability

Curcumin is a compound isolated from the turmeric plant that has a variety of pharmacological actions, such as anti-inflammatory, anti-carcinogenic and antiviral properties, as well as promising clinical applications due to its low toxicity. It has extremely low aqueous solubility and presents a fast intestinal and hepatic metabolism, resulting in poor oral systemic bioavailability. Therefore, curcumin-loaded liposomes were designed in order to enhance the water-solubility properties. Also, the optimal formulation of curcumin liposomes to obtain the best encapsulation efficiency was investigated, and finally, a comparative study of the stability between free and encapsulated curcumin was developed.

Firstly, a solubility study was carried out, by using different mixtures of cosolvents in order to determine the composition that enhances the drug solubility. The solvent mixture that allowed the best solubility properties was selected to prepare liposomes. They were obtained by TLE method, comprising a lipid phase (phosphatidylcholine, cholesterol, stearylamine and curcumin) that were dissolved in chloroform/methanol 2:1 and a lipid film was made with a rotary evaporator. Afterwards, the appropriate aqueous phase was added and vesicles were formed after vortexing the samples. Finally, liposomes were characterized in terms of percentage of drug entrapment (PDE), size and surface charge.

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From the solubility studies, the cosolvency mixture propylene glycol (PG), PEG 400, Tween 80 and Hepes buffer pH 7.4 was selected for further formulations. The lipid film was hydrated with the solvent mixture where curcumin was dissolved. Once fixed the liposome composition, we proceed to quantify the PDE over time. From the stability study, a decrease in both the curcumin standard and curcumin in sample was obtained, being free curcumin less stable than drug entrapped into liposomes.
In conclusion, the strategy of including curcumin into lipid vesicles, such as liposomes, could represent an alternative in administering this antioxidant assuring the maintenance of activity.

Physicochemical properties of carrier affect morphological, dimensional and release behaviour of acetazolamide-loaded proniosomes

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Keywords: Proniosomes, carrier, chitosan, acetazolamide.

Nowadays, lipid vesicle systems such as liposomes, niosomes or transfersomes represent an interesting therapeutic alternative due to diverse advantages such as biodegradability, biocompatibility and non-immunogenicity. However, the most important disadvantages are their physical and chemical instability. For these reasons, technological alternatives are the proniosomes which incorporate the drug in a suitable carrier together with nonionic surfactants and other lipids, which can be converted into niosomes when they are reconstituted.
The aim of this study was to analyze how the carriers (maltodextrins, sorbitol, trehalose, maltose, alginate and chitosan) can affect the characteristics of solid proniosomes containing Acetazolamide (AZM), a carbonic anhydrase inhibitor (CAI) commonly used orally for the reduction of intraocular pressure (IOP) in patients suffering from glaucoma. The study was completed with a stability analysis.
Proniosomes were made from the slurry method, previously described in the literature. Samples were morphologically characterized by scanning electron microscopy. Then, reconstituted niosomes were characterized in terms of size, by dynamic light scattering, and zeta potential by photon correlation spectroscopy; the percentage of encapsulation (PDE) was determined by centrifugation (9000 rpm, 4 ºC and 60 minutes) and drug content was quantified by HPLC. Stability studies were developed along one month, and PDE was monitored.
Generally, results showed homogeneous reconstituted samples with a negative zeta potential and PDE from 25% to 80%. Dimensional parameter ranged from 1100 to 1900 nm, except for alginate, which had a higher size. Stability testing demonstrated a high stability of acetazolamide into niosomes, because a very low percentage of drug was lost.
With respect to the carrier, studies indicated a great influence of AZM on the dimensions of proniosomes. In this sense, samples made with maltodextrins and sorbitol had smaller sizes and relative homogeneity, making them especially useful. However, alginate was rejected from the study because proniosomes were

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high in size and instability of reconstituted niosomes was appreciated. Regarding the chitosan and trehalose, interesting results were found in terms of characterization parameter.

Different binary soluble inclusion complexes of triamcnolone with β-cyclodextrins

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Keywords: Triamcinolone**;** Cyclodextrins; Inclusion Complex.

Introduction: Triamcinolone (TRI) like many corticosteroids has its application limited by poor water solubility. Several strategies have been used to improve water solubility of drugs and consequently to enhance the bioavailability. One of the most efficient alternatives for this purpose is the cyclodextrins inclusion complex formation.
Objective: The aim of this work was to obtain different spray dried inclusion complexes of TRI with three types of cyclodextrins (beta-cyclodextrin (βCD), hydroxypropil-beta-cyclodextrin (HPβCD) and randomized-methilated-beta-cyclodextrin (RMβCD).
Methods: Equimolar drug:cyclodextrins solutions were spray dried under suitable conditions in order to produce solid complexes. The physico-chemical aspects studied included X-ray diffraction, SEM images, and finally drug loading and drug dissolution profile.
Results: The spray dried drug (TRISD) and βCD systems showed crystalline patterns, while isolated RMβCD, HPβCD and the three inclusion complexes showed an amorphous aspect. SEM images demonstrated the obtainment of TRISD spherical particles. All complexes exhibited like cenospheres with toroid aspect. The drug release profile and dissolution efficiency values (DE%) for different samples demonstrated that spray drying procedure increase considerably drug dissolution rate. Among binary complexes, the best results were observed for TRI:RMβCD > TRI:HPβCD> TRI:βCD.
Conclusions: Amorphous complexes were successfully produced by using spray drying. Spray dried TRI exhibited a faster dissolution rate than non-spray dried drug, TRI:HPβCD and TRI:βCD complexes. However, the best results were obtained with TRI:RMβCD, demonstrating the potential of this new raw material.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Genipin-crosslinked chitosan nanoparticles for colonic delivery of triamcinolone

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Keywords: Nanoparticles, Chitosan, Genipin, Triamcinolone

Colon drug delivery has gained increased importance due to therapeutic benefits obtained for treatment of chronic inflammations. Polymeric nanoparticles are potential systems for colonic delivery due to ability to across gastrointestinal tract and biologic barriers. Chitosan is a natural polysaccharide considered suitable for the delivery to specific sites of the intestine. Genipin, a cross-linker of natural origin, has been used to change stability, permeability and release properties of chitosan systems. Triamcinolone is an anti-inflammatory steroid of the current choice to treat chronic inflammations. The aim of this study was to develop and characterize nanoparticles based on natural polymer chitosan crosslinked with genipin containing triamcinolone as a novel colon-delivery system. The nanoparticles (NP) were prepared by coacervation/ionotropic gelation technique using sodium sulphate as precipitant and genipin as chemical crosslinking at different times of reaction (3, 6, 12 and 24 h). The average particle size, polydispersity index (PDI) and zeta potential were measured. The degree of crosslinking with genipin of nanoparticles was determined by ninhydrin assay. The nanoparticles were obtained using drug polymer ratio of volume 1:1, 0.1% sodium sulphate and 0.1 mM genipin. The results of particle size, PDI and zeta potential obtained ranged from 235.1 to 334.4 nm, 0.321 to 0.392 and 26.92 to 32.07 mV, respectively. The degree of crosslinking of systems (20 to 50%) was dependent of the reaction time. The optimum system of 235.0 ± 13.9 nm, PDI 0.392 ± 0.009 and zeta potential 32.07 ± 4.2 mV was obtained with 6 h of reaction. Thus, the proposed technique using sodium sulfate and genipin was effective to obtain chitosan nanoparticles containing triamcinolone as a potential innovate alternative for the treatment of chronic inflammatory diseases.

In vitro evaluation of mucoadhesive buccal films loaded with miconazole nitrate

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Keywords: miconazole nitrate, polymeric films, full characterization

Buccal mucoadhesive films, releasing drugs in the oral cavity provide alternatives to the traditional dosage forms for treatment of oral candidiasis. The aim of this work was the development and full characterization of polymeric films loaded with miconazole nitrate (MN) as model drug. Three different formulations containing MN, polyethylene glycol 400 (PEG 400), as plasticizer, and chitosan (CH) and pectin (PC) as film-forming polymers, were prepared by the casting method. Films were characterized in

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terms of thickness, mechanical strengths, and swelling capacity. Adhesion of films was assayed using pig buccal mucosa. Interactions between polymers were analyzed by Fourier-transform infrared spectroscopy, thermal gravimetric analysis and differential scanning calorimetry. Morphology of the matrices was investigated by scanning electron microscopy. MN release and *in vitro* microbiological effectiveness against *Candida parapsilosis* were evaluated. CH was solubilized in aqueous lactic acid solution (2% v/v) and stirred overnight. PC solutions were prepared by dissolution in water. CH solutions were dripped to the PC solutions, then MN solubilized in PEG 400 was added and the mixture was stirred at 200 rpm during 2 h. The solutions were cast on petri dishes and dried in an oven at 35 °C. The compositions of matrixes were combinations between CH and PC, all loaded with MN. Thickness the films ranged from 0.6818 to 0.7389 mm and its weights from 406.9 to 653.7 mg. Adhesion (0.20 to 0.30 N) and mechanical properties (tensile strength 0.5 to 0.95 N/mm and elongation 3.39 to 10.88 mm) were in adequate range. The swelling percent of the films after 6 h ranged from 68.8% to 197.5%. The antifungal *in vitro* activity was confirmed by the generation of inhibition halos (31 to 52 mm) in candida culture dishes. Thus, CH-PC buccal films containing MN could be a convenient approach for the treatment of oral candidiasis.

In Vitro Assessment of Platelet Lysate-Loaded Porous Silicon Microparticles onto Fibroblast Cell Proliferation

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Key-words: Porous Silicon; Platelet Lysate; *In Vitro*; Wound Healing, Microparticles

Introduction. Porous silicon (PSi) is a mesoporous material prepared from Si-wafers via a top-down electrochemical etching process, with excellent properties being non-toxic, biodegradable, biocompatible and even bioactive depending on the pore size. In the literature topical applications of mesoporous materials with good results in drug delivery are reported. Therefore, this makes PSi a very interesting material to be studied also for wound healing applications.

Objectives. Given these premises, the aim of this work was to load PSi microparticles with platelet lysate (PL) and to assess the ability of the developed formulation to enhance fibroblasts proliferation *in vitro* in order to further develop the PSi-based formulation for future wound healing applications. PL indicates the solution of bioactive molecules obtained by platelet destruction by freeze-thawing starting from a platelet rich plasma sample in presence of an anticoagulant agent.

Results. PSi particles with different surface chemistries were tested for *in vitro* cytotoxicity in order to select the particles with the best behavior toward fibroblasts; at the same time, PSi particles with different sizes were also tested to find the least cytotoxic particle size. No statistically significant differences between the PSi particles with different sizes were observed. Amongst the PSi particles with different surface chemistry tested, the PSi particles with the lowest (thermally hydrocarbonized, THCPSi) and the highest (thermally carbonized, TCPSi) cytotoxicity were chosen to be assessed in further tests. PSi

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particles loaded with PL were tested in a proliferation assay that showed an increase in cell proliferation in comparison to the control (medium without serum).

Conclusions. The results clearly showed that PSi particles loaded with PL induced fibroblast proliferation at levels comparable with those of fresh PL control and superior to the particles without PL. Overall, the results demonstrate that the PL-loaded THCPSi particles are not cytotoxic toward fibroblasts and enhance fibroblast proliferation.

Preparation and comparison of Human Serum Albumin (HSA) Nanoparticles Stabilized by Gantrez ES 425 and Glutaraldehyde for the treatment of Glaucoma

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Keywords: nanoparticles, ophthalmic delivery, Gantrez ES-425®, glaucoma, timolol

Topical ocular application is a common route for drug administration. However, the protective mechanisms of the eye dramatically decrease the bioavailability of drug.

Nanoparticles (Nps) have emerged as a suitable vehicle for drugs administration. There are a large variety of materials for the preparation of NPs. Among them, natural biopolymers present more advantages than synthetic materials. In this context, human serum albumin (HSA) offers a number of advantages including its biodegradability and acceptation by Regulatory agencies.

The aim of this work was to prepare and evaluate HSA nanoparticles coated with Gantrez ES-425® for ocular delivery of timolol (TM).

Nanoparticles were prepared by a desolvation method, adding absolute ethanol to a solution of HSA, and stabilized with either glutaraldehyde (1.5 ug/mg HSA) or Gantrez ES-425® (0.5 mg/mg HSA). Then, the nanoparticles were purified by centrifugation and freeze-dried. The physico-chemical characteristics and the *in-vitro* release profiles of the different systems were studied.

The results indicate that the interaction between the COO^- groups of Gantrez ES-425® and $NH3^+$ of the protein was adequate to stabilize the surface of the nanoparticles. In addition, these nanoparticles displayed a similar stability than NPs cross-linked with glutaraldehyde. Regarding the use of these NPs as carriers for timolol, the nanoparticles stabilized with glutaraldehyde displayed a size of about 160 nm and zeta potential of -35.42 mV with a TM loading of 30%. On the other hand, nanoparticles coated with Gantrez showed a mean size of 210 nm, a zeta potential of -38.92 mV and a drug loading of 40%.

In both cases, the *in-vitro* released profiles showed that after 24 hours, 75% of the TM encapsulated was released.

In summary, Gantrez–coated HSA nanoparticles displayed adequate physico-chemical characteristics for ophthalmic delivery and appropriated controlled release properties for the ocular delivery of timolol maleate.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Physicochemical characterization of novel albendazole suspensions for pediatric treatment of helminth infections

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Keywords: suspensions, microparticles, zeta potential, rheological properties.

Helminth diseases may be the commonest cause of chronic infection in children, in many low income countries. The recommended drug is albendazole (ABZ), a benzimidazole carbamate with a broad-spectrum activity. ABZ low water solubility, which may cause a variable bioavailability after oral administration, as already reported. Several attempts to increase the aqueous solubility/dissolution rate of ABZ by using solid dispersions, liposomes, microparticles and cyclodextrins have been reported.
The aim of the present research work was to prepare and full characterize novel ABZ-microparticles suspensions to improve the drug release.
Chitosan (CH) microparticles were prepared by spray drying technique and further characterized by means of X-ray powder diffractometry, scanning electron microscopy, and dissolution profiles. Then the microparticles-suspensions were formulated employing hydrophilic polymers and electrolytes (NaCl and sodium citrate) to regulate the zeta potential, viscosity and ionic strength in order to determine the interactions among the microparticles and the rheological properties.
The microparticle-suspensions increased significantly the ABZ dissolution rate values in comparison to the pure drug. ABZ is poorly soluble in water (1 µg/mL) and it is well demonstrated in the extent of drug dissolved after 60 min (7%). ABZ-CH microparticles released 80% after 60 minutes, while after 180 minutes it was observed nearly the 100% of drug released. The diffractograms of ABZ-CH microparticles displayed amorphous patterns, indicating that the drug underwent a transition from a crystalline to an amorphous state.
The presence of NaCl up to 1.5% (w/v) did not increase the viscosity of the suspensions significantly ($p > 0.05$). The rheological behaviour of the formulations showed a non-Newtonian behavior, due to the reorientation of solids in the flow field and solid-solid interactions.
Therefore, the ABZ-CH microparticles may be employed as a promising alternative for the delivery of ABZ from oral suspensions.

Physicochemical characterization of carboxylic β-cyclodextrin derivatives and albendazole inclusion complexes.

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Keywords: cyclodextrin derivatives, dissolution rate, nuclear magnetic resonance.

Albendazole (ABZ), a benzimidazole carbamate, is an anthelmintic compound widely used in the treatment of systemic nematode infections. Nevertheless, its effectiveness is limited by its poor water solubility (1 µg/mL at 25 °C) and the consequent poor bioavailability, producing an unpredictable

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therapeutic response. Several strategies may be employed to increase solubility, dissolution rate and oral bioavailability of poorly water soluble drugs, including the formulation of cyclodextrin (CD) inclusion complexes. CD derivatives presenting acidic groups in their structure may interact strongly with basic drugs modifying the inclusion complexes stability. Therefore, the aim of this work was focused in the synthesis of a succinyl-β-CD (S-β-CD) derivative to design oral delivery systems and to improve the solubility and dissolution rate of ABZ.

Initially, the syntheses of S-β-CD derivative was carried out according to similar methodology previously reported by Garcia et al. The characterization of β-CD derivative was performed by carboxylic titration, mass spectrometry, infrared spectroscopy and nuclear magnetic resonance. The interaction between ABZ and S-β-CD was evaluated by phase solubility analysis, nuclear magnetic resonance and mass spectrometry. Additionally, solid systems were studied comparing the techniques: physical mixture and spray dried. The results obtained from the phase solubility analysis and mass spectrometry, verified that the complexes were formed in a 1:1 molar ratio. The characterization studies confirmed the drug inclusion complex in the solid state. The proposed systems allowed to increase significantly the ABZ solubility and the dissolution rate values in comparison to the pure drug and the complex prepared by ABZ:β-CD.

Solid-state NMR Studies on Albendazole and Cyclodextrin Albendazole Complexes

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Keywords: Albendazole, cyclodextrin, solid-state NMR.

Albendazole (ABZ), methyl [5-(propylthio)-1-H-benzimidazol-2-yl] carbamic acid methyl ester is one of the most effective broad-spectrum anthelmintic agents, albeit its poor solubility in water. This fact has been of major concern regarding the preparation of ABZ formulations. ABZ:β-cyclodextrin complexes are among the several approaches that have been used to improve the physicochemical properties of ABZ. However, solubility is strongly dependent on solid-state properties, particularly when polymorphism occurs, which solid-state NMR enables probing under non invasive and non destructive modes. ^{13}C cross polarization/ magic angle spinning (CP/MAS) and ^{15}N CP/MAS NMR spectra were obtained from ABZ and from ABZ:β-cyclodextrin (CD), ABZ:methyl β-CD, ABZ:hydroxypropyl β-CD and ABZ: citrate β-CD systems, which were obtained by solvent evaporation. Solid-state ^{13}C and ^{15}N NMR spectra suggest two different kinds of chemical interaction between ABZ and the CDs used in the present study. For CDs with higher hydrophobic cavity (methyl β-CD and hydroxypropyl β-CD), were detected three ABZ molecules with different imidazole ring electronic environments, most probably corresponding to free ABZ and to two ABZ tautomers. On the other hand, the spectra from complexes of ABZ with β-CD and citrate β-CD are consistent with ABZ carbamate NH groups being involved in hydrogen bonding, and this effect is more pronounced for ABZ: citrate β-CD. Solid-state NMR data has contributed therefore to explain the differences in ABZ solubility enhancement found with the different CDs.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

A recombinant smart elastin-like tetrablock-copolymer for the controlled release of acetazolamide

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Keywords: glaucoma, drug delivery system, release medium, ophthalmic formulations

Acetazolamide (AZM) is used for the treatment of glaucoma. Topical administration is the most common route for ophthalmic medications, but it presents some limitations, such as the short precorneal residence time and the poor bioavailability of most eye-drop solutions. Several approaches to increase the ocular bioavailability of drugs are subject of research.
The aim of this work was to evaluate the ability of a recombinant smart elastin-like tetrablock-copolymer $(E50I60)_2$ to release AZM for potential use as a drug delivery system platform. The influence of the medium (NaCl 0.9 or Dextrose 5%) on the release profile of AZM from the tetrablock hydrogel was investigated at physiological temperatures over a period of 6 days, and the release kinetics were determined using the Korsmeyer kinetic model.
The results showed that AZM release was sustained over the period of time studied, with a visible absence of burst release at the initial time-points. The release kinetics of AZM from tetrablock hydrogels were markedly influenced by the release medium, with rate constants, K, of 5.19 and 1.03 for NaCl and dextrose respectively. Although similar quantities of released AZM were detected after 6 days (cumulative release of 79% in NaCl, and 70% for dextrose), the shape of the released profile was totally different, pointing out the differences in the drug delivery mechanism.
The data for the release profile of AZM from the hydrogel on dextrose solution fitted an anomalous (non-Fickian) mode of diffusion (n= 0.84), thus suggesting a combination of pure AZM diffusion and polymer relaxation/swelling mechanisms.On the other hand, when the medium was NaCl the release profile followed a Fickian diffusion model(n=0.58), evidencing a pure diffusion mechanism.
In conclusion, the dispersing medium influences the released profile of AZM and tetrablock hydrogel has great potential for use as a component of ophthalmic pharmaceutical formulations.

Preliminary studies in the development of triamcionolone acetonide lipid nanocapsules.

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Keywords: lipid nanocapsules, triamcinolone acetonide, retinal diseases.

Triamcinolone acetonide (TAA) is a synthetic corticosteroid used to treat a broad spectrum of retinal diseases. The development of TAA dosage forms is limited due to its poor solubility in water and/or

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physiologically acceptable solvents[1]. The purpose of this work was to develop and characterize a novel lipid nanocapsule (LNC) formulation as drug delivery system for TAA.
LNCs were prepared using an optimized phase inversion-based method[2]. Due to the poor solubility of TAA in the oily phase of the original formulation, two co-surfactants (captex® 500p -Glyceryl triacetate- and oleic acid) in three proportions: 20, 30 and 50% were tested. The average particle size, polydispersity index (PI) and zeta potential were measured.
Acceptable results were obtained only with a 20% of both co-surfactants. LNCs with captex® 500p leads to about (42.3±0.2) nm size nanoparticles with a narrow size distribution (PI<0.2) and a negative z potential (-4±1) mV while LNCs with oleic acid showed an average particle size of (37.0±0.5) nm and a PI below 0.1 with a negative z potential (-11 ±5) mV.
The obtained formulations allow the encapsulation of TAA and it could be a potential strategy to treat several ocular diseases of the posterior segment by intravitreal route.
Further studies will be carried out to test the encapsulation efficiency and the long-term stability of these formulations.

Development of chloramphenicol supramolecular systems to improve dissolution rates

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Keywords: chloramphenicol, aminoacids, supramolecular systems, solid state characterization.

Chloramphenicol (CP), a lipophilic antibiotic, has poor solubility and dissolution rate in aqueous solution, which affects its application in clinical therapy.
In this contribution, CP supramolecular systems with aminoacids (AA) were prepared by different methods: freeze-dried (M I) and drop assisted grinding (M II). In solid state, the systems were characterized by X-ray diffraction (XRD), Fourier transform infrared (IR) spectroscopy and confocal laser scanning microscope (CLSM). In addition, the dissolution rate studies of CP (alone and from CP/AA systems) were conducted in a dissolution apparatus using the paddle method, in 900 ml simulated gastric fluid without enzymes, at 37.0 ± 0.5 ºC and stirring at 50 r/min.
When CP was processed with leucine or arginine maintained its crystalline structure, but displayed different crystal habit. The CLSM images showed that crystal shape was changed and the particle size was reduced, compared to control untreated drug. The crystals obtained from M I produced rods shaped crystals and those obtained from M II exhibited irregular surfaces with a tendency to form agglomerates. The IR spectra of CP/AA systems showed the same characteristic vibrations for CP observed in the raw materials and no molecular interactions between the drug and AA could be detected. Lastly, the dissolution rate of the newly developed solid forms was found to be greater than that of the pure drug.
It was concluded that the supramolecular systems with both AA showed improved dissolution rate compared to the pure CP. These increases in dissolution appear to be derived from a combination of changes to crystal habit and particle size.

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Preparation and characterization of coamorphous glibenclamide system

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Keywords: glibenclamide, coamorphous systems, dissolution.

Glibenclamide (GLB) is an oral hypoglycemic agent used in the treatment of type II diabetes. GLB belongs to class II compound according to the biopharmaceutics classification system due to its low aqueous solubility. Coamorphous drug arrangements were recently introduced as potential drug delivery systems for poorly water soluble drugs. In this approach, an active pharmaceutical ingredient and small molecular weight excipients are chosen and combined in order to prepare coamorphous mixtures with improved dissolution properties. Based on these above considerations, the present investigation was aimed to develop and characterize GLB coamorphous mixtures with arginine (ARG) to improve the drug dissolution characteristics.

X-ray powder diffractometry, infrared spectrometry (IR) and confocal laser scanning microscopy (CLSM) were used to investigate the physicochemical characteristics of the solids. The comparative dissolution behavior of the newly developed solid form and that of the untreated GLB were also studied, for which the prepared sample and the raw drug were added to 500 ml of pH 7.4 phosphate buffer, at a temperature of 37.0 ± 0.5 °C and paddle-stirred at a rotation speed of 50 rpm.

The diffractogram of GLB/ARG system was distinguishable from GLB and ARG raw materials, and showed the absence of sharp diffraction peaks, indicating the formation of a coamorphous solid. The CLSM analysis confirmed the differences in the particles morphology between GLB/ARG system and the raw materials. IR spectra showed molecular differences between the coamorphous system and the untreated drug. Furthermore, it could be shown that the dissolution rate of GLB/ARG in pH 7.4 phosphate buffer was faster than that of the crystalline pure drug.

Therefore, it can be concluded that the amorphous form of GLB and ARG produced in this study has shown promising results *in vitro*, and is a candidate for further studies aimed at improving the bioavailability of GLB.

Antiparasitic microparticles: a promising strategy for the treatment of trichinellosis.

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Keywords: albendazole, microparticles, controlled precipitation.

Bulk microstructured materials have been the subject of intense investigations over the last decades due to the unusual properties and their promising applications.

Microsuspensions are microsized dispersion systems stabilized by surfactants and/or polymers. A suspension platform is an efficient drug delivery system for water-insoluble drugs because of the increase of the saturation solubility and the surface area available for dissolution.

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Albendazole (ABZ) is a benzimidazole carbamate widely used for the treatment of helminthic diseases. ABZ has an unfavorable bioavailability after oral administration, leading to a variable oral absorption.
The main objective of this study was to design and prepare ABZ microparticles. This can be achieved by controlled precipitation or crystallization and evaporation.
The antipasasitic drug was dissolved in acetic acid:ethanol solution, and then precipitated in a polymeric solution (cellulose derivatives and non ionic surfactants). Finally, the microsuspensions were sprayed using a Büchi Mini Spray Dryer B-290.
Different techniques were used to thoroughly investigate the structural characteristics and morphology of the particles. The scanning electronic microscopy images confirmed the uniform size (5-20μm) and spherical shape. Powder X-ray diffraction patterns for ABZ exhibited several reflection peaks revealing its crystalline nature. The diffractograms of the microparticles displayed amorphous patterns**,** indicating that the drug underwent a transition from a crystalline to an amorphous state.
The best results obtained by the dissolution profiles of ABZ loaded in the microparticles exhibited the 100% drug release after 30 minutes of the running assay while ABZ (pure drug) exhibited a 2.8%.
Additionally, the microparticles were *in vitro* evaluated employed infective larvae of *Trichinella spiralis.* Two different concentration of ABZ (0.5 and 1 μm/mL) were employed during 72 h. Cellulose derivatives microparticles were the most effective systems reducing the percentage of infective larvae. These results remarkably demonstrate the improvement of the dissolution rate of ABZ, loaded in the microparticles.

Development and Optimization of ivermectin-lipid nanocapsules intended for oral administration

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Keywords: ivermectine- lipid nanocapsules- P-glycoprotein

Introduction. P-glycoprotein (P-gp), an ATP-dependent drug efflux pump, plays a major role in the transport of various drugs. Lipid nanocapsules (LNC) emerged as promissory alternative as drug delivery system.The main advantages of LNC lie in their ability i) to increase the solubility of hydrophobic compounds, (ii) to increase intracellular internalization, (iii) to improve *in vitro* and *in vivo* stability.
IVM is a broad-spectrum antihelmintic agent reported as a P-gp substrate. Taking into account these, the goal of this work has been to develop LNC suitable for the encapsulation of IVM.
Materials & Methods. The LNC were prepared by the phase inversion process and optimized using a DoE approach to obtain nanoparticules within the size range of 50 nm. This related domain was named the 'feasibility domain'. Once the feasibility zone was identified, IVM was loaded into the LNC. Size, polydispersity and zeta potential were analyzed by photon correlation spectroscopy. IVM encapsulation efficiency was determined by HPLC technique. CaCo-2 cell viability was estimated by the MTS assay and the stability in simulated gastrointestinal media was also evaluated.
Results & Discussion. Following the optimization process, both blank and IVM-loaded LNC formed monodispersed populations with a mean size around 50 nm and a slightly negative charge surface. Due to

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its hydrophobic character, IVM was efficiently encapsulated into the optimized LNC with an encapsulation efficiency of about 90%.
The cytotoxicity studies indicated that both formulations were not toxic in the evaluated concentrations. Finally, the stability in gastric and intestinal media confirmed that this formulation could be suitable for oral administration.
Conclusions. We report evidence about the feasibility of a lipid-core nanoformulation optimized for IVM encapsulation. The high entrapment of IVM in LNCs supplies a new pharmacological tool to treat endo and ecto parasites. Additional studies are underway to determine the potential therapeutic activity of IVM-LNC after an oral delivery in rats.

Biocompatible and bioadhesive films based on polyelectrolyte-drug complexes using novel dendronized chitosan loaded with Ciprofloxacino.

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Keywords: topical controlled-release, ionic exchange, Draize test

The use of polymeric films as topical controlled drug delivery systems is an interesting alternative to conventional treatments. New dendronized chitosan derivatives (WChi) were synthesized as biomaterials with improved mechanical properties (1). The aims of this work were the preparation of film based on Chi-Ciprofloxacino ionic complexes (Chi-Cip) and the *in vitro* evaluation of relevant biopharmaceutical behaviours.
An appropriate Chi and Weisocyanate-Chi (WChi) were partially (50%) neutralized with Cip, in aqueous dispersions. Polyvinyl-pyrrolidone (PVP) 1% as plasticizer was added. Films based in Chi-Cip complexes were obtained by solvent casting at 40ºC for 24h using a Petri dish. Dried hydrogel films were appropriate cutted into 5-mm diameter disks. The Cip content and uniformity was assayed. *In vitro* release studies towards buffer solution pH 5.8 and 7.4, using Franz cell's were performed and bioadhesion studies on mucin gel layer (30%). Release profiles were analyzed using Zero order, Higuchi and Peppas equations.
The Cip content and uniformity was demonstrated, (2.2±0.2) and (1.9±0.2) $\mu g.cm^{-2}$ of Cip into Chi and WChi films, respectively. Release profiles showed a slow and controlled Cip release rate (Q_{24h}>70% and Q_{24h}<50% from (Chi-Cip) complexes at pH 5.8 and 7.4, respectively). Opposite results showed (WChi-Cip) complexes and a favorable change in kinetic control was observed (Anomalous release in (Chi-Cip) and Zero order in (WChi-Cip)).This results could be attributed to a structurally ordered network and hydrophobicity of dendritic polymers in which the swelling rate, complexes relaxation and ionic exchange are the predominant release mechanisms from the films. Appropriate mucoadhesion was observed and non-irritant skin behaviour was evaluated in *in vivo* Draize test.
Novel biocompatible and bioadhesive films based on ionic complexes between dendronized Chitosan derivatives and Ciprofloxacino were prepared with potential use in topical controlled release.

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Solubility of Ketoconazole in polymeric micelles: Fluorescence studies

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Key words: Ketoconazole, poloxamer, fluorescence

Introduction. Ketoconazole is a poorly soluble drug, traditionally used in the treatment of local and systemic fungal infections. Since poor aqueous solubility reduces drug bioavailability, several strategies have been developed in order to improve it. Poloxamers are water soluble, non ionic amphyphylic triblock copolymers (PEO-PPO-PEO); they can form micelles in aqueous medium and incorporate variable amounts of hydrophobic or/and poor soluble drugs. Ketoconazole fluorescence emission was used to obtain information about the solubility process and its location in the poloxamer micelles.

Materials and Methods. Ketoconazole (KTO) and Poloxamers 188, 237, 338 and 407 were purchased from Sigma Chem. Fluorescence measurements were performed in a Aminco Bowman S2 spectrofluorometer.

Results and Discussion. Fluorescence emission spectra of KTO in different poloxamer concentrations were recorded. In the absence of the polymer the intensity was low, while it was enhanced and no shifts were observed in the maximum emission wavelength upon increasing poloxamer concentration. The increase in fluorescence emission indicates an interaction between KTO and the poloxamers tested, even at lower concentration of the polymers.

The plots of fluorescente intensity versus poloxamer concentration showed an hiperbolic pattern; the analysis of the non-linear regression allowed us to obtain the minimal amount of polymer to dissolve one mg of KTO.

Quenching fluorescence studies were carried out using acrylamide as quencher. The results were expressed as Stern Volmer plots which yield a straight line, where its slope is the quenching constant. The quenching constants for KTO in poloxamer solution were lower than the value observed for KTO, suggesting a moderate protective effect to the quencher and therefore its localization in the outside of the micellar coronna.

Conclusions. The poloxamers assayed are effective solubilizing agents for KTO, showing a protective effect on the KTO molecule due to its inclusion in the poloxamer micelles.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Polymer coating procedure for avoiding phenolic compounds degradation in *Melissa officinalis* based pellets

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Key words: pellets – stability – coating

The official drug *Melissa officinalis* L. (Lamiaceae) 'melisa' is widely cultivated throughout the world because of its medicinal properties. *M. officinalis* is rich in terpenoids, flavonoids and phenolic acid derivatives, with rosmarinic acid (RA) as the main component, generally used as analytical marker in the quality control assays. However, it is well known that phenolic compounds are sensitive to many different factors such as high temperature, UV radiation, air oxygen and metal ions. For this reason, strategies to avoid the phenolic components degradation are necessary. In the present work, 'melisa' based dried extracts were obtained and used for pellets preparation. Also, a procedure for protecting unstable components was applied to the pellets.

Infusions of *M. officinalis* were prepared according to FNA VI ed. (10% w/v) and dried in a Mini-spray BÜCHI Dryer 290, inlet temperature ranging between 90 and 140 °C, with Aerosil ® as carrier. A rheological study of the dried extracts was performed, the obtained solids showed good compressibility and fluidity.

By modifying different parameters in the granulation process and using sequentially the procedures of extrusion and spheronization, the dried extract material was used for pellets preparation. The nature and quantity of formulation components, was also evaluated.

A polymer film coating was applied to the pellets with the aim of protecting the phenolic compounds against humidity and temperature effects. Suspensions with different percentages of polymer were evaluated for applying an optimal coating film to the pellets. The polymer selected for the coating was Opadry® AMB in a concentration of 20% (w/v). The coated pellets were used for gelatin capsules filling. The content of RA in the coated pellets was analyzed by, showing LC-MS/MS, that the degradation of phenolic compounds was avoided with the described procedure.

Polyelectrolyte-Drug ionic complex: Species distribution in the dispersions using dialysis tube techniques.

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Keywords: Ionic complex; Drug Carrier; Polyelectrolites; Counterionic Condensation.

One of the main objectives in the design of new drug delivery systems (DDS) is to generate platforms with the ability to carry high amounts of drugs (D) and release them steadily over time or in specific sites. Despite there are many techniques used in the determination of the loading capacity of DDS, many of

them require a lot of steps and the use of organic solvents. Here we present a simple technique for the determination of species present at equilibrium using a water dispersion/water system and a dialysis tube. Sodium Hyaluronate (HiNa) and Hyaluronic acid (HiH) were used as polyelectrolyte carriers while Doxorubicin (Dx) was selected as a counterionic model D.

The proportions of the species *Dx*, DxH^+, and the complexed one $[R\text{-}COO^-DxH^+]$ were determined by dialysis using a tube of cellulose membrane (12.000 Da). Ten mL of $HiNa\text{-}Dx_{25}$ and $HiH\text{-}Dx_{25}$ aqueous dispersions at a 0.25% w/v were put into the dialysis tube (donor compartment) which was introduced into a flask containing 500 mL of water (receptor compartment). After that, the pH of donor and receptor phases was recorded and Dx concentration in the receptor compartment was spectrophotometrically assayed at 480 nm to get the apparent Dialysis Ratio (DRapp). The affinity constant for the counterionic condensation (Kcc) was also calculated.

A high proportion of Dx was ionically condensed (higher than 78%) with both complexes $HiNa\text{-}Dx_{25}$ and $HiH\text{-}Dx_{25}$, yielding counterionic condensation constants (Kcc) of the order of 1×10^8.

We developed a simple technique in the determination of the complexation capacity of a polymer, it is a one-step procedure, it does not require the use of organic solvents and yields comparable results with other techniques.

Nanoclays Aluminum-Magnesium: Influence on the thermal stability of sodium diclofenac

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Keywords: nanoclay, sodium diclofenac, thermal stabilization.

Layered Double Hydroxides or anionic clays are biocompatible compounds with application in the pharmaceutical fields. Particularly, much attention has been focused on the use of layered double hydroxides as support for controlled release systems of drugs, vitamins, biomolecules, etc. Layered double hydroxides or anionic clays are composed by positively charged hydroxides sheets, of few nanometers and interlayer exchangeable anions. The anions insertion in the interlayer zone is carried out by two routes, coprecipitation (direct method) and anion exchange (indirect method). With indirect route was prepared first host laminar solid by coprecipitation and then the desired anion introduced by the contact of a given time with stirring and the temperature. Sodium diclofenac is a non-steroidal antiimflamatory drug used for the relief of symptoms of osteoarthritis and rheumatoid arthritis. In the present work studied sodium diclofenac intercalated into nanoclays layered double hydroxides as previously mentioned. The host laminar solid and direct method was prepared by coprecipitation of Aluminum-Magnesium salts at pH 10±0.2; on N_2 atmosphere. By X-ray diffraction was observed the drug incorporation into nanoclay. The basal spacing obtained suggests that the drugs molecules are arranged in partially interdigitated bilayers. The amount of intercalated sodium diclofenac was determined by UV-visible spectroscopy. By the direct method greater incorporation was obtained. Differential scanning calorimetry thermogram of sodium diclofenac shows two endothermic peaks at 270°C and 350°C due to the melting point and the oxidative of degradation. Differential scanning calorimetry of sodium diclofenac incorporated into layered double hydroxides present an exothermic peak

at 255°C probably corresponding to oxidative degradation of the superficial drug. At higher temperatures (410°C) the decomposition of the drug and destruction of the layers of the layered double hydroxides is observed. This behavior indicated a thermal stabilization of the drug.

Risedronate sustained release system reduces the formation of calcium insoluble complexes

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Keywords: *Risedronate - Calcium interaction – Eudragit E100*

Risedronate´s (Ris) high chelating ability leads to the formation of calcium (Ca) insoluble complexes, which result in severe gastrointestinal irritation and reduce its oral bioavailability. The new sustained-release system of Ris obtained by complexation with Eudragit E100 (EuE100-Ris) showed a reduced irritation potential and a significant increase in Ris oral bioavailability in fed conditions. The goal of this work was to identify those mechanisms involved in these events, specifically the role of EuE100-Ris complexation in the interaction with Ca.

Ris solubility in the presence of Ca was comparatively evaluated for EuE100-Ris_{50} and RisNa at a concentration equivalent to 70 mg dose/250 ml in simulated intestinal fluid. The samples (in triplicate) were stirred and maintained at 37°C, then added with $CaCl_2$ to achieve Ris/Ca molar ratios of 1.7, 1 ,0.5 and 0.2. Samples taken at time 0, 0.75, 2 and 24 hs were centrifuged and the supernatant assayed by UV spectrophotometry. The results were compared using one-way ANOVA. The precipitated solid was separated by filtration, dried and evaluated by FTIR to identify the presence of Ris (pyridine group) and EuE100 (ester carbonyl groups).

In the presence of Ca, Ris precipitation leads to a supernatant concentration decrease, which progressed with time. The precipitation was significantly slower in EuE100-Ris_{50} compared to RisNa ($p$value$\leq$0.05). At any time, Ris supernatant concentration from EuE100-Ris_{50} was 20-50% higher than RisNa except in Ris/Ca ratio of 0.2 where differences were less evident.

The precipitated solids showed FTIR bands corresponding to the protonated Ris pyridine groups ($C=N^+H$ 2304 cm^{-1}) in both samples. The C=OOR band at 1732 cm^{-1} were also observed in EuE100-Ris_{50} samples, indicating EuE100 coprecipitation.

These results suggest that EuE100 prevents and slow Ris precipitation in the intestinal lumen due to its interaction with Ca. This behavior could be a reason for gastric damage reduction and the increased bioavailability in the presence of food observed previously with EuE100-Ris_{50}.

The EuE100-Ris_{50} complexes are potentially safer and efficient alternatives for oral bisphosphonates treatment.

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In vitro release evaluation of two model NSAIDs from interpolyelectrolyte-drug complexes in solid state.

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Keywords: interpolyelectrolyte-drug complexes, drug release, dissolution

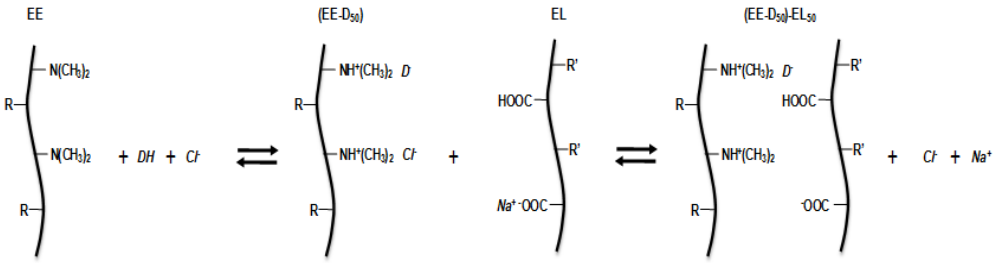
Interpolyelectrolyte complexes with ionizable drugs of opposite charge (IPEC) are an attractive strategy, which has been exploited in order to obtain new carrier and modified drug release systems (1,2). The IPEC can be obtained by spontaneous electrostatic association or acid-base reaction, in aqueous dispersion or hydroalcoholic mixtures. The objectives of this work were to obtain IPEC in solid state, based on two polymethacrylates of opposite charge (Eudragit-EPO and Eudragit-L100) neutralized with Naproxen and Ketoprofen and to evaluated the release behavior according to the composition of the system, contained of NaCl and pH of medium.

The IPEC were obtained by two methods: 1) complex coacervation in aqueous medium (Scheme-1) and subsequent obtention of the solid state complex by lyophilization, 2) Wet blending of the solids materials and evaporation of solvent, using hydroalcoholic mixture as reaction medium (not contain NaCl). The solids were sieved (30/40-mesh). Dissolution of IPEC, contained in hard gelatin capsules (200-mg) were carried out in 900-ml of buffer solutions of pH 1.2 and 6.8 (Apparatus-1, 100-r.p.m., 37°C, by triplicate). Drug released determination was performed by UV-spectrophotometry.

The IPEC obtained by lyophilization showed: at pH 1.2, controlled release of ketoprofen and release rate changes with the composition of IPEC (Q_{4h} between 80 and 20%) were observed. While naproxen was released more slowly, showing a minor dependence with IPEC composition (Q_{4h} between 45 and 25%). At pH 6.8 a significant changes and opposite release profiles were observed. The release profiles from IPEC without NaCl showed decreased in release rate and changes in kinetic control, which can be attributed to the greater influence of ion exchange and wettability of the solids on release performance.

These complexes behave as a reservoir that slowly released of NSAIDs, thus the IPEC systems exhibited interesting properties to design multiparticulate drug delivery systems for oral route.

Scheme 1: Representation of the acid-base equilibria between polyelectrolytes and oppositely charged anionic drug in IPEC



Formulation of glibenclamide nanocrystals by a solvent change process. Influence of polymers on the dissolution rate.

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Key words: Glibenclamide, nanoprecipitation, poloxamers freeze drying

Glibenclamide (GB), originally described as a K+-ATP transporter blocker that interacts with sulfonylurea receptors, is a second generation sulphonylureas oral hypoglycemic agent used for the management of diabetes mellitus. Glibenclamide belong to BCS class II. Thus, its bioavailability is erratic and incomplete. Reducing the particle size of a drug to a nano-scale leads to an increased surface area-to-volume ratio, increased dissolution velocity and adhesiveness, and improved *in vivo* performance of poorly soluble drugs. The aim of this work was to formulate GB nanocrystals via nanoprecipitation. The influence of preparation parameters on particle size, stabilizer type and concentration, and type of drying were evaluated. For a basic formulation, GB and Eudragit RLPO were dissolved in acetone/etanol (2:1) at 25°C to form uniform organic solution. The prepared organic solution was then injected slowly dropwise with the help of a syringe into an aqueous phase, with or without PEG6000, containing a defined amount of a poloxamer derivative (P-188 or P-407) kept under high-speed agitation to get desired dispersion. The resulting dispersion was then stirred magnetically at 500 rpm at room temperature for 12 h to evaporate organic solvent. Solids were collected after filtration, washed with deionized water and dried at 40 ^{o}C or freeze drying. All samples were prepared in triplicate. The results showed that the GB crystals size and stability was dependent on the type of polymer used. Micron and sub-micron size particles were obtained by modifying the drying process. The results also showed that the dissolution of GB crystals was affected by changing the drug: polymer ratio and also the type of poloxamer. In conclusion, GB nanocrystals, consisting of pure drug and a minimum of surface active agents required for stabilization is a convenient approach to modify the drug dissolution rate.

Benznidazole and cyclodextrin benznidazole complexes studied by ^{13}C solid-state NMR

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Keywords: Benznidazole, cyclodextrin, inclusion complex, solid-state NMR.

Benznidazole, N-benzyl-2-(2-nitroimidazol-1-yl) acetamide (BZL) is the drug of choice for treating Chagas' disease but its absorption in gastrointestinal fluids is limited due to its poor aqueous solubility. The formation of water-soluble inclusion complexes of BZL with cyclodextrins (CDs), cyclic carbohydrates with a hydrophobic cavity and hydrophilic exterior, is expected to increase the drug solubility, which is strongly dependent on solid-state properties, like polymorphism. The aim of this study was to evaluate the formation of such complexes by ^{13}C solid-state NMR. This technique enables the analysis of these systems by probing local changes on electronic environment and/or mobility of carbon

atoms, under non invasive and non destructive modes. The BZL-CDs complexes were prepared by the solvent evaporation method at 1:1 and 1:2 (mol:mol) drug:CD ratio. ^{13}C cross polarization/ magic angle spinning (CP/MAS) NMR spectra were obtained from BZL and from the prepared BZL:□□cyclodextrin (CD), BZL:methyl □-CD and BZL:hydroxypropyl □-CD complexes. The results of this solid-state ^{13}C NMR study allowed us to postulate the absence of chemical interaction between BZL and CD, because BZL carbon signals recorded from the pure drug and from BZL:CD can be superimposed. However, when BZL:CDs were prepared with methyl □-CD, hence with higher CD hydrophobic cavity, BZL spectral changes occur, particularly in the benzene frequency region (namely, a new signal is identified at 139.4 ppm). Such effect, which appears not depend on the drug:CD ratio, clearly indicates the inclusion of BZL in methyl □-CD through its benzene ring. A similar species is assigned in BZL:hydroxypropyl □-CD spectra, which corresponds to a complex with lower concentration, owing to the presence of lower intense signals. In conclusion, solid-state NMR was a valuable approach to elucidate the different oral bioavailability of these systems.

In vitro characterization of superoxide dismutase (SOD)-loaded microparticles on fibroblasts

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Keywords: SOD, microparticles, fibroblasts, antioxidant activity.

Superoxide dismutase is an enzyme counteracting Oxygen Reactive Species (ROS) in cells, thus preventing damage to tissues. Topical delivery of SOD to treat skin and mucosal deseases/injuries could benefit from the embedding of SOD into polymeric microparticles with the aim of improving stability and controlling drug release. The aim of this work was the evaluation of biocompatibility and antioxidant activity of Manganese-SOD chitosan-based microparticles onto a fibroblast cell line. Two protocols were developed to formulate SOD into non-crosslinked (sample 1) and crosslinked chitosan microparticles (sample 2). In the case of sample 1, SOD was dispersed into an acidic chitosan solution and the mixture was spray dried under specific conditions. Sample 2 was prepared by adding the SOD into a sodium sulphate solution. Then, this mixture was poured into the acidic chitosan solution while stirring. The microspheres were separated by centrifugation. The morphology and surface of the microparticles was study by SEM and the yield and encapsulation efficacy was also evaluated. SOD-microparticles were tested onto a bNHDF cell line and cell viability was assessed by MTT test after 24 h of incubation. A preventive oxidative test was then conducted on sample 1 onto bNHDF fibroblast to evaluate the protective effect (microparticles left in contact for 24h before insult) against the oxidative damages produced by hydrogen peroxide (incubation with 2 mM H_2O_2 for 24 h) chosen as model oxidant

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substance. The cytotoxicity results clearly show that SOD microparticles have the same viability as the complete medium, although dependent on the concentration used, and no significant differences are shown between the two different samples. The antioxidant activity test performed on sample 1, and evaluated at different dilutions (1:5; 1:10; 1:20; 1:40) in 10% FBS DMEM (standard medium), demonstrated that the 1/40 dilution was able to produce a significant antioxidant activity against H_2O_2.

Formulation of praziquantel binary and ternary solid dispersions into hard-gelatin capsules. Effects of the carriers on the drug dissolution profile.

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Keywords: praziquantel, solid dispersions, dissolution rate, hard-gelatin capsules.

Solid dispersions (SDs) are one of the most promising tools to improve the solubility, dissolution rate and oral bioavailability of drugs with low aqueous solubility. Even though this technique has been widely applied in the last decades, there is still limited knowledge about the successful formulation of binary and ternary SDs into final solid dosage forms. The aim of the present study was to evaluate the dissolution of praziquantel (PZQ), as model drug, through the formation of binary and ternary SDs, and to compare the dissolution profiles of the SDs with those of formulated SDs in hard-gelatin capsules. SDs systems were prepared by the solvent evaporation method using as polymeric carrier polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG) and poloxamer 237 as surfactant agent. Dissolution studies, differential scanning calorimeter (DSC), X-ray diffractometry and infrared spectroscopy were used to characterize the SDs of PZQ. The results showed that PZQ release rates from the binary SDs were evidently higher than the dissolution rate of drug alone. Corresponding physical mixtures also demonstrated higher dissolution profiles than PZQ alone. However, it was observed a similar dissolution profiles between the SDs and the PMs. The dissolution rate of PZQ from the ternary SDs was very similar to the binary systems showing that the influence of the surfactant on the drug dissolution was negligible. On the other hand, minor differences were found in the PZQ dissolution rate from capsules in the binary and ternary systems indicating that further processing of the solid dispersions did not greatly modify the drug dissolution rate. By DSC, it was observed a remarkable difference between the thermograms of PZQ alone and the SDs. In conclusion, PZQ binary and ternary SDs formulated into hard-gelatin capsules were confirmed as a valid approach to the improvement of drug dissolution.

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PHARMACEUTICAL EDUCATION

Assessment based on survey results about contents learnt in the subject Pharmaceutical Technology to improve the skills of future professionals.

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Keywords: survey, professional skills, PharmaceuticalTechnology I.

The aim of this work was to assess, via a survey, the correlation between the degree of knowledge provided by the subject Pharmaceutical Technology I at the UNS and the development of professional activity which includes both the development of professional practice during the career and the professional activity performed after graduation.
The survey was carried out at random by e-mail and the selected four questions were:

1. Year in which the career has been started.
2. Year in which the course has been attended.
3. Rating from 1 to 10: How useful the degree of knowledge provided by the subject Pharmaceutical Technology I to their professional development or to their compulsory period of professional practice was.
4. Some suggestions.

It is important to remark that the suggestions and the recommendations were suggested by students or professionals and grouped into seven categories. The number of surveys answered was 86 and the average was 7.71.
From the suggestions, it has been shown that there are topics considered to be very important for the professional lives, which are not deeply dealt with in this subject. However, the study of the GMP has already been included in the past years; and, also, the number of students per commission in the laboratory practices has been reduced. On the other hand, there are tasks which are not currently develop in the subject and considering the survey results, it is clear that the implementation of the survey suggestions must be done in the near future.

First experience in problem-based learning in general chemistry of the pharmacy career

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Key words: general chemistry, pharmacy education, problem-based learning

Problem-based learning (PBL) is an educational method focused on self-directed learning, small groups discussion with facilitators and working through problems to acquire knowledge. This

method can be an important tool in healthcare career education, in which students learn by working with real-life cases. The PBL curriculum seems to improve the academic performance of pharmacy students when compared to the traditional method of instruction (1).

This first approach was performed under the hypothesis that this teaching strategy gradually applied throughout a career would improve the quality of learning.

Methodology PBL was applied in General Chemistry course (Stoichiometry problems), in April of 2014. The experience involved 87 students, 2 research fellows and 5 teachers.

It was performed the following: characterization of student population, design of problems, developing support materials and problems guide, application of the methodology, group and individual evaluation and anonymous survey of student opinions.

Qualitative results indicated that:

- The purposes and way working were properly explained, the teachers guidance and the supporting materials were satisfactory (80 % to 86 %).
- The methodology helped them to learn to work together, it was facilitated theory and practice integration, it was useful for taking decisions and they could develop skills in oral and written communication (68 % to 79 %).
- This strategy allowed them to understand theoretical contents, it favored self-learning and the time spent at work was enough (60 % to 64 %).

According to students the evaluation complexity was similar to those it was developed (73 %). Quantitative results showed that group evaluation was approved by 72 % of students. However, the individual evaluation was approved by only 34 %.

The overall assessment of the experience is encouraging (even with some difficulties) so that commits us to optimize the work to continue applying it.

Teaching Good Documentation Practices using problem-based learning. Implementation of a practical class in the chair of Industrial Pharmacy at Universidad Nacionaldel Sur

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Keywords: Documentation, Good Manufacturing Practice, Industrial Pharmacy, Problem-based learning.

Documentation is an essential requirement of Good Manufacturing Practices (GMP). It defines procedures and specifications of all products and methods of manufacturing and control that ensure the quality of medicines. It is considered that about 20-30% of the deviations detected during inspections to the pharmaceutical industry are related to documentation. For this reason it was identified as an important topic to be addressed through a practical class during the implementation of Industrial Pharmacy (a new optative chair in the career of Pharmacy at UNS). The aim of activity designed was to enhance student's

skills in the identification, preparation and correction of documents under international standards using problems as a tool for the acquisition and integration of concepts.

Through fictitiousproblematic situations, documents required for establishing quality policy of an industry and ensuring product quality were identified. Functionality, purpose and requirements of each identified document were determined. Besides, strategies of document redaction were considered.

The practical activity was divided in two sections and took around 90 min. First, the type of documents required in pharmaceutical industry, as well as their structure and contents were introduced by the tutor using a brief presentation. Thus, it was possible to work in the resolution of problematic situations following the documentation requirements demanded by GMP during the second part of the activity. Students were able to develop a production order for capsules and appropriately rewrite a standard operating procedure for cleaning lab-countertops in production areas. The sharing at the end of the class allowed the group enrichment of knowledge acquired. The final test demonstrated the objective achievement.

The implementation of the practical class about documentation was didactic and simple as well as effective to improve the abilities of students of Pharmacy at UNS to face their future challenges.

Development of a new theoretical and practical activity about pharmaceutical aerosols for the Pharmacy students of the Universidad Nacional del Sur

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Key words: teaching methodology, theoretical-practical seminar, inhalatory route, pharmacy students

The inhalatory route is employed for the treatment of respiratory diseases. In such cases, local therapy has shown to be more effective than systemic administration. An adequate access to this via requires the use of medications (i.e. formulations and complex devices) and their appropriate administration technique. Lack of efficacy and/or safety in this therapy is generally related to the inappropriate use of the devices involved. Therefore, pharmacists should be prepared to intervene and advise patients on this subject.

In this context, it was considered relevant to improve the students' knowledge of inhalation therapy. The goal of this work was to develop a theoretical-practical activity in order to allow students to make a connection between the technological concepts of formulations to their correct use. In this sense, this activity will be useful for students' professional performance.

To this end, a theoretical-practical seminar was designed after a search in books and scientific articles and the study of different therapeutic devices and educational videos. The activity's estimated time of completion is 3 hours. It comprises exercises aimed to understand the performance and characteristics of different types of pharmaceutical aerosols (metered-dose inhalers, dry-powder inhalers and nebulizers). For this purpose, diagrams, tables, videos and devices were employed. The activity was designed to be executed by groups and assisted by the teachers.

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In conclusion, this seminar is expected to improve the understanding of this topic and raise students' awareness of the importance of an adequate use of pharmaceutical aerosols. The activity will be carried out in the subject Farmacotecnia II of the career of Pharmacy at the Universidad Nacional del Sur.

Is acetylsalicylic acid stable? An undergraduate laboratory experiment.

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Keywords: Kinetics, acetylsalicylic acid, surfactants.

Aspirin (ASA) is the main salicylate belonging to the AINEs family drugs. It has been widely used due to its analgesic, anti-inflammatory and antipyretic properties. The decomposition reaction of ASA generates salicylic (AS) and acetic acids, which takes place inalkaline medium.

Among others, surfactants have the capacity to modify chemical reaction rates and equilibrium,and play an important role in pharmaceutical formulations.

The purposeof this work wasto investigate,in three days, the ASA hydrolysisin presence and absence of surfactant's micelles.

A kinetic study of the alkaline hydrolysis of ASA at 37°C in presences of cationic (hexadecyltrimethylammonium bromide, CTAB) and anionic (sodium dodecyl-sulfate, SDS) surfactants (0-0.02 M)were evaluated. HannaE25 conductivity-meter and Cary50-UV-Visible spectrophotometer,wereused.

Day 1: Critical micelle concentration (*cmc*) determination.The obtained *cmc*values were7.82×10^{-3}M (SDS)and 1.07×10^{-3}M(CTAB).

Day 2:UV-spectrophotometric study of ASA hydrolysis (without surfactants).The reaction rates were studied at 296 nm (λ_{max}AS). The kinetic reactions were carried out at four pHs and analyzed using the expressions for pseudo first-order kinetics,

$$ln(A_\infty - A_t) = -k_{obs}\, t + ln\, A_\infty$$

$$k_{obs} = k\,[OH]$$

A_t and A_∞:absorbance at different times and t=∞, respectively.Rate constants,k_{obs}, increase linearly with an increase in [NaOH].

Day 3: Micellar media effect.Higher and lower concentrations of surfactant than *cmc*, were used. Solutions with low [CTAB] were shown a catalytic effect. However, this reaction was inhibited at higher surfactant concentrations. In the case of anionic micelle,a very low catalytic effectoccurs.Both surfactant behaviors were more evidentat pH 12.5.

The ASA hydrolysis was determined (k = $9.52\times10^{-2}M^{-1}min^{-1}$).The presence of both surfactant showed a catalytic effect on ASA decomposition reaction.

The proposed technique is useful in chemical kinetics teaching.Theseexperiences allowpharmacystudents a comprehensive study of the drugs stability.

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Knowledge and acceptability of generic drugs in the city of Rosario.

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Key words: generic drugs, law 25649, descriptive observational study.

In order to evaluate the use of generic drugs and the knowledge, acceptance and applicability of the law 25649, we carried out an observational descriptive study in the Central district in the city of Rosario. 341 prescription drug buyers and 67 dispensing drugs were surveyed.
From the analysis of the surveys to dispensing, we note that the main difficultythat it is perceived in the implementation of the law is the distrust from the buyer towards the quality of medicines which are not the best known brands.
While the pharmacist is able to provide information at the time of the dispensation and expressconfidence about the quality of medicines with the same international common denomination, the respondents claimed not to give alternatives to that contained in the recipe at first instance. In general, they provide different options depending on the type of buyer or wait for theirs' request.
On the other hand, the analysis of buyers' surveys reflects the confidence that exists from the majority of them towards the different alternatives, what is contradicted by what is perceived by the dispensers.
In turn, we note that only in 20% of the cases they reach the selection instance among similar drugs, 80% of them have opted to replace them.
These results show the importance of the pharmacist advice during dispense, and the responsibility for them to incorporate in their daily practice the habit to offer different alternatives.
Also it makes evident that generic's policy isa tool that enables pharmacists to reassert their profession before the society, supported by the buyer's trust.

Team-based learning applied to pharmaceutical technology teaching.

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Keywords: team-based learning, active learning, critical-thinking abilities.

Teachers wanting to engage students in the classroom seek methods to augment the delivery of factual information and help student move from being passive recipients to active participants in their own learning.
Team-based learning (TBL) is a new teaching method in the field of pharmacy education that has a widespread history of proven success in medical and business schools. This method encourages students to be prepared before class and has students working in teams in the classroom. Key benefits to this pedagogy are student's engagement, improved communication skills, and enhanced critical-thinking abilities. The aim of this work is to evaluate the applicability of this methodology to the teaching of pharmaceutical technology and the student´s response to this new educational strategy.
TBL has 4 phases: class preparation, application of an evaluation at the beginning of the class that will be done individually and then in groups, teacher feedback and finally, conducting a group work, after which

students evaluate their peers and themselves as well. This methodology was applied to 104 students, additionally; a survey to measure the satisfaction of the students on this learning strategy was applied.
It was observed that the individual average grade was 4.6 (scale 1-7), meanwhile the group average grade was 6.8. Regarding to the survey, 71% of students surveyed said that this learning strategy has more advantages than the traditional approach and 63% believe that this way of working promotes long-term learning.
These results indicate that team-based learning allows the achievement of an appropriate academic performance and student satisfaction with the learning methodology.

Promotion of pharmaceutical compounding: an approach related to pharmaceuticals, legal and social aspects.

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Keywords: professional service, compounding pharmacy, semi structured interview

Introduction. The health promotion is one of the major concerns of pharmacist and one of its essential functions in our society.
Pharmacist manufacturers, working in their own community pharmacies play a central role in the sanitary system. This effort of each professional is strengthened by the decision to work grouped in Commissions on CFC. Using this strategy we set work goals to analyze and solve common situations.
It is very important to remark that these commissions have been currently made in forming organs and consultation for different social problems. A clear example of this is the preparation and application of a new natural compounding medicine for the control of lice in school-age children from an area of the city of Rio Cuarto.
From the commission on "preparaciones magistrales y homeopatia" (CPHM), was raised to know more about this professional service.
The contribution of pharmaceutical processors was crucial to obtain conclusions.
Objectives. Analysis and dissemination of this Pharmaceutical Professional Service from the legal, pharmaceutical and social point of view.
Diagnose the current situation of the compounding pharmacies in Córdoba.
Methodology. The analysis of this professional service is conducted through a survey (semi structured self-interview) directed to compounding pharmacist. It was proposed and carried out by a sub commission formed from the CPHM.
Results. The following results were obtained: 79% of pharmacist considered that professional achievement is the main motive for initiating the compounding service. 100% a very expensive service to implement. On the other hand 79% are motivated to continue offering it. We note that 100% of respondents make emphasis a positive impact on a professional level and 86% also considered to be profitable.
Conclusion. The service of officinal preparation promotes professional development of pharmaceutical, increasing the profitability of the pharmacy and provides solutions to specific needs of the society. This type of survey allows design strategies and policies to improve the pharmaceutical practice.

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Evaluation of teaching activities in Pharmacognosy

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Keywords: Pharmacognosy, survey, teaching.

This study collects data on the opinion of 66 students during the course of Pharmacognosy in the Pharmacy career at Universidad Nacional del Sur. The selected research design was the descriptive type (anonymous survey). A questionnaire was designed and grouped according the opinion about I) Practical activities developed in the Pharmacognosy course (laboratory, oral presentations, monographs, etc.), II) Theoretical aspects related to the classroom activities (clinical cases, relationship with other subjects, preferences about contents to be impaired, etc.), III) Learning process of the course, IV) Aspects related to teachers´ performance during the learning process, V) Aspects to be improved, modificated or incorporated.

The students indicated that the oral exposure was their preference to develop an assigned topic (52%) and this may contribute to a better development in their professional future (95%). It was observed that the analysis of clinical cases related to the use of medicinal plants was their preference (77%) and they found a relationship between the contents seen in Pharmacognosy and the pharmaceutical products that may find in their laboral environment.

It was also mentioned that the teachers helped the students to enhance their interest for the subject (100%) and that teachers promoted and encouraged the participation of students in the development of the activities (100%). Students considered to have had sufficient core knowledge (from previous subjects) to address the learning of Pharmacognosy (90%).

It was observed that the most important future improvement could be the possibility of incorporating a study trip (58%).

The present experience helped us to identify the strengths and weaknesses of the subject during the learning process of the students. Their identification can be helpful for organizing strategies that motivate and stimulate the learning process in the classes and aquire a better understanding in the concepts related to Pharmacognosy.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

PHARMACEUTICAL MICROBIOLOGY

Antibiofilm and Phytochemical Evaluation of *Capsicum baccatum var.pendulum* (Solanaceae)

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keywords: pepper, capsicum, biofilm, antimicrobial.

Bacterial resistance against antibiotic and host immunologic deficient are the first cause of death into intensive-care units worldwide. Bacterial adhesion and colonization are required to infection and pathogenesis, thus biofilm has been an important virulence factor, present in 80% of human infections as endocarditis, osteomyelitis, periodontitis, characterizing it as a severe public health problem. Hence biofilm is a complex matrix wrapping microorganisms/communities irreversible adhered to a biotic/abiotic surface that can avoid the antibiotic accesses.Thus, rather than focusing on therapies that target bacterial growth, such as conventional antibiotics, an alternative approach is to search for new mechanisms of action, targeting virulence factors, including biofilm formation. *Capsicum baccatum var. pendulum*, a red pepper cultivated on many tropical and temperate regions worldwide, shows a broad variety of therapeutic applications, being underexplored pharmacologically. Accordingly, we aimed to identify antibiofilm compounds from *C.baccatum* seeds and fruits,extracting them separately with different polarity solvents. Antimicrobial and antibiofilm activities from each extract were evaluated monitoring bacterial growth on 600 nm and via violet crystal method.The aqueous seeds extract (AqSE) was the most promising extract, it was able to inhibit in 80% and 60% *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* biofilm, respectively. Scanning electron microscopy images showed no biofilm in treated samples. Interestingly, AqSE did not inhibit the growth of these bacteria, indicating that the inhibition of biofilm is independent of bacterial cell death. By phytochemical evaluation of AqSE we detected polyphenols and quantify it in 70.29mg/g with Folin-Ciocalteu reaction. Saponins were detected with foam test, haemolysis assay, Liebermann-Burchard and Salkoski reactions. Tannins were detected with $FeCl_3$ and gelatin solution precipitationand with KIO_3 reaction (15 mg/g of tannic acid equivalents). After acid hydrolysis the chromatography indicated gallic acid and anthocianidins characteristics bands. Our results expected that *C. baccatum*is source of innovative bioactive compounds.

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Optimizacion of biosynthesis and characterization of silver nanoparticles using *Pseudomonas aeruginosa.*

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Keywords: silver nanoparticles, *Pseudomonas aeruginosa*, biosynthesis process

The worldwide escalation of microbial resistance to conventional antibiotics represents a challenge for the scientific community to develop new bioactive compounds and novel approaches for coping with this problem. Particularly, the silver-based nanomaterials attract enormous attention for potential prevention of microbial infections due to their documented antimicrobial and disinfectant properties, being the use of microorganisms for the eco-friendly synthesis of these materials in the limelight of current nanotechnology. The objectives of this study were production of silver nanoparticles using *Pseudomonas aeruginosa* extract and optimization of the biosynthesis process. The effects of quantity of extract, $AgNO_3$ concentration, temperature and influence of the synthesis medium on the formation of silver nanoparticles were studied. The biosynthesized nanoparticles were characterized using techniques such as UV-vis spectroscopy, Dynamic Ligth Scattering (DLS), and Transmission Electron Microscopy (TEM). The formation of silver nanoparticles at the end of the reaction was confirmed with the obtained UV-vis spectroscopy results. The more efficient synthesis was obtained from a silver nitrate concentration of 10 mM with a percentage of bacterial supernatant from 30% v/v and a temperature of 37 °C for 18 hours. The use of tryptic soy broth or Luria Bertani broth favored the biosynthesis process. The analysis of the TEM images and the results of the DLS technique confirmed that silver nanoparticles were spheroidal and posses a relatively uniform size distribution. We reported a simple and green chemistry approach for the biological synthesis of silver nanoparticles using the culture supernatant of a *P. aeruginosa* reference strain at 37 °C and without any harmful reducing agents.

Effectiveness of various processes for cleaning and disinfection of nebulizers used by patients with cystic fibrosis

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Keywords: cystic fibrosis, nebulizers, disinfection, *Pseudomonas aeruginosa*.

Appropriate cleaning and sterilization/disinfection of reusable nebulizers are biosecurity measures to prevent infections of Cystic Fibrosis (CF) patients associated with respiratory therapy equipment. According to a survey about home disinfection carried out to CF patients, was found that the main microorganism isolated was *Pseudomonas aeruginosa*, the type nebulizers used was jet, the maximum storage time one day, and that there is no single pattern of cleaning and disinfection. Moreover, it is

known that N-acetylcysteine (NAC) acts as an antimicrobial agent and destroys biofilms formed by *P. aeruginosa.*

According to survey data, the aim of this study was to evaluate the effectiveness of NAC and a number of different disinfectants and cleaning/disinfection processes used by patients against *P. aeruginosa*. To complete this objective was studied the effectiveness by microbiological control of the different disinfectants, disinfection and washing conditions in nebulizer pipettes contaminated with an inoculum standardized of *P. aeruginosa* by counting the colony forming unities (CFU/ml) in trypticase soy agar before and after cleaning/disinfection. The results obtained indicated that the use of NAC 0.5% by immersion 20 min decreased 7 log the initial inoculum. While ethanol 70° dip 20 and 5 min, the boiling water 5 min, and washing drag by cold running water, liquid soap and brushing were effective eliminating inoculums of 10^5 CFU/ml. The evaporative drying, with hair dryer or paper towel, did not alter the effectiveness of alcohol 70°. Disinfection of articles with alcohol 70 ° with subsequent drying and wrapping them with paper napkin allowed keep it disinfected for 24 h. Spray with alcohol 70 ° and subsequent evaporation was not an effective approach.These results indicate that home care hygiene and proper disinfection of equipment are useful tools for CF patient to reduce the risk of infection in the lungs and that NAC may be included as an appropriate disinfectant.

A silver-plated gold heart. A promising alternative against resistant bacteria

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Keywords: nanoparticles, antibiotic resistant strains, visible light

Increase of bacterial resistance to available drugs is a matter of concern for pharmaceutics all around the world. In this work, we compare gold nanoparticles (AuNPs) and a novel kind of coreshell nanoparticles to be used as photosensitizers in Photodynamic Antibacterial Chemotherapy (PACT), an efficient alternative therapy against bacterial strains not sensitive to clinically used antibiotics.

When a metal nanoparticle interacts with light, having a wavelength longer than the dimensions of the particle itself, Surface Plasmon Resonance (SPR) takes place. In case of gold, SPR absorptions occur in the visible range; this allows us to use cheap and harmless LEDs (525nm) as radiation source.

Gold nanoparticles were synthesized according to Turkevich method. The new nanomaterial reported here consists of a gold core, encapsulated in a silver shell and stabilized with a dipeptide, specifically aspartame (Asp). The gold core is designed for efficient heat delivery through established plasmonic mechanisms. The silver shell retains the antibacterial properties that are now well characterized in the case of AgNP. The surface protection with aspartame (Asp) leads to excellent aqueous stabilization with

long shelf life. Further, aspartame is non-toxic, remarkably inexpensive and easy to replace by another molecule of interest.

Time kill curves show that bactericidal activity was only achieved in samples treated with Asp@Ag@AuNPs and irradiated of three clinical *Pseudomonas aeruginosa* strains and for a clinical strain ofExtended-spectrum β-lactamases (ESBL)*Escherichia coli*.Nine hours of irradiation were necessary when bacterial suspensions were treated with AuNPs and just 6 hours were needed when Asp@Ag@AuNPs were used. Moreover, Transmission Electronic Microscopy images revealed membrane structural damage of the bacillus.

These results would suggest that bactericidal activity was achieved earlier in samples irradiated and treated with Asp@Ag@AuNPs rather than with AuNPs. This could be attributed to the synergism between the silver shell and the plasmon excitation of gold core.

Antibacterial activity of N-benzenesulfonyl derivates against *Staphylococcus aureus*

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Keywords: *Staphylococcus aureus*, reactive oxygen species, N-benzenesulfonyl derivatives.

The indiscriminate clinical use of many very efficient antibiotics has led to an unfortunate emergence of bacteria able to resist them. The call for greater effort to develop new classes of antibiotics is fully justified. A lead discovery project, a library of N-benzenesulfonyl derivatives of bioactive heterocyclic compounds was design and prepared. The approach was based on the combination of two groups that are known to be active, benzenesulfonyl (BS) and 1, 2, 3, 4-tetrahydroquinoline heterocycle (THQ). The objective of this study was to evaluate the antibacterial activity of BS-THQ and to investigate the production of reactive oxygen species.

Minimum inhibitory concentration (MIC) of BS-THQ was determined in *Staphylococcus aureus* ATCC 29213 and a methicillin-resistant *S.aureus* ATCC (MRSA) 43300 by using the standard tube dilution method according to Clinical Laboratory Standard Institute (CLSI). The reactive oxygen species were evaluated by Nitro Tetrazolium Blue (NBT) assay and by Fluorescent Microscopy (FM). Transmission electronic Microscopy (TEM) provides useful insight into the mechanism of action of antibacterial agent.

The MIC of BS-THQ was200 µg/mL for both isolates tested. The generation of ROS in *S.aureus* ATCC 29213 was particularly higher than the MRSA, showing 15.6 and 2.8% of ROS, respectively, at sub-MIC concentrations of BS-THQ. Similar results were obtained by the qualitative method FM.

BS-THQ (sub-MIC concentrations) affects the morphology of *S.aureus* ATCC 29213. Clear disorganization of the cytoplasmic membrane was revealed by TEM. Hydrophobic properties of BS-THQ could explain the interaction with the bacterial membrane. This interaction would facilitate the generation of ROS, altering the bacterial physiology and the oxidative stress balance.

As a result, the disruption of the membrane and the generation of reactive oxygen species are suggested as factors that compromise the viability of *S. aureus* strains exposed to BS-THQ.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Could oxidative stress be involved in cellular damage generated by Linezolid?

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Keywords: Reactive oxygen species, reactive nitrogen species, *Staphylococcus aureus*,

Antibiotics disturb the physiological homeostasis of bacterial cells by interfering with essential cellular functions or structures. This physiological response is specifically tailored to overcome the inflicted damage and, thus, closely linked to the antibiotic target and mechanism of action. Linezolid is an Oxazolidinone, its binds to the 50S subunit of the prokaryotic ribosome inhibiting protein synthesis. It has activity against Gram positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) as one of the last therapeutic option.

The objective was to evaluate the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in *S.aureus* ATCC 29213 and *S.aureus* ATCC MRSA 43300 treated with LZD at different concentrations, as a tool to know if reactive species are involved in the mechanism of action of this antibiotic.

ROS generation was evaluated by two different methods, the reduction of the Nitro Tetrazolium Blue (NBT) and by Fluorescent Microscopy using 2,7- dichlorodihydrofluorescein diacetate. On the other hand, RNS was measured by the Griess assay. Indeed, the Ferrying reducing antioxidative power (FRAP) was measured to investigate bacterial response to stress.

The generation of ROS and RNS in the *S.aureus* ATCC 29213 was particularly higher than in the *S.aureus* ATCC 43300; ROS measurement was 23% and 17% respectively. Similar result was obtained by fluorescent microscopy. *S.aureus* ATCC 43300 was less affected by stress as demonstrated by FRAP assay.

The results show that LZD produces oxidative stress in *S.aureus* strains as well as a methicillin resistant strain. This reactive species could be involved in the mechanism of action of this antibiotic which causes cellular damage.

Effect of drugs on virulence factors of *Candida albicans* isolated from oral lesions

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Key words: *Candida albicans*, aspirin, chlorhexidine, nystatin

Candida albicans is an opportunistic fungus widely found in oral cavity, causing stomatological lesions. Chlorhexidine (CLX) and nystatin (NYS) are frequently employed in clinical dentistry. There are evidences of the inhibitory role of aspirin (AAS) on virulence factors for fungal adhesion and infection as lipases (LIP) and biofilm formation (BF).

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

The objective of this study was to evaluate the effect of AAS, CLX and NYS on BF and LIP activity of *C. albicans* isolated from oral lesions.

The strains were isolated from patients with different lesions (chronic candidiasis CC n=6, lichen planus LP n=5, and oral cancer OC n=5) and were identified in chromogenic medium and biochemical tests. A reference strain was employed. BF by XTT method, LIP activity, by rhodamine assay, and inhibition of LIP activity with AAS were assessed. Sub-inhibitory concentrations of CLX, NYS and AAS were used. Data were analyzed by Wilcoxon test ($p \leq 0.05$).

All isolates showed LIP activity and BF (LIP=1.16±0.07; BP=305.4± 188.7). LIP did not showed significant differences between oral lesions. BF values were higher in OC and CC ($p=0.05$ y $p=0.04$, respectively). LIP activity showed lower values with AAS and CLX treatments ($p < 0.0001$). In OC isolates, the highest values of LIP inhibition were observed (1.88 mM, $p<0.0001$). BF diminished significantly with CLX ($p=0.03$). When the oral lesion was considered, AAS treatment showed a diminution of BF in CC ($p=0.004$) and CLX, in OC ($p=0.015$).

The most important inhibition of virulence factors studied was observed with AAS and CLX. Although that CC and OC strains showed the highest values of LIP and BF, these were the most sensible to the treatment with these drugs.

Considering the role of virulence factors in fungal pathogenicity, the present study may mean a contribution in the search of a better therapeutic option.

Inhibition of *Staphylococcus aureus* biofilm formation by fungal metabolites of *Fusarium sp.* 3300

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Keywords: *Fusarium sp.*, Biofilm, *Staphylococcus aureus.*

Entomopathogenic fungi (EF) attack insects and consume them as a nutrient source. However, on the cuticle of insects and in their intestine, there are numerous bacterial species that also try to consume it, particularly when the insect dies. One of the most common organisms present on epithelia, including human skin, is *Staphylococcus aureus*. The objective of the present work was to determine the MIC_{50} and antibiofilm activity against *Staphylococcus aureus* ATCC 6538 of the extracts of *Fusarium sp*. 3300 [NRRL 25102] ARSEF cultures produced in presence of insect fragments. The EF was grown up in potato dextrose broth (3%) in presence and absence of 1% p/v *Spodoptera frugiperda* traces. In addition, an uninoculated control with insect remains (1% p/v) was used. After the incubation, the mycelium and insoluble material were separated by vacuum filtration and then extracted with ethyl acetate (EtOAc) and methanol (MeOH). In addition, an EtOAc extract of the filtered supernatant was performed. Chemical profiles of the nine obtained extracts were analyzed by thin-layer chromatography (TLC). The effect of (50, 100, 200 and 400) ug/ml of the EtOAc and MeOH extracts against *S. aureus* growth in Müller-Hinton (MH) media was in microplate reader at 560 nm. The effects on the biofilm production were evaluated by the crystal violet micro-technique.

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None of the methanolic mycelium extracts was able to inhibit bacterial *S. aureus* growth but all of them were able to inhibit biofilm formation (between 30 – 60%). Among the EtOAc extracts of filtered supernatant, the most potent was the one obtained in presence of insect traces (MIC_{50} 66.7 ug/ml) and inhibiting biofilm formation by 68%.

These results indicate that EF are able to produce antimicrobial compounds, probably to detect and control the accompanying flora in insects. These metabolites from eukaryote origin should be further investigated in their antibiotic potential.

Mandarin fruit essential oil inhibit *P. aeruginosa* virulence factors

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Keywords: Mandarin essential oil and terpenes, *Pseudomonas aeruginosa*, Quorum sensing.

Antipathogenic compounds don´t kill bacteria or stop their growth. They rather control bacterial virulence factors like biofilm formation or elastase activity, and prevent the development of resistant strains. *P. aeruginosa* employs *Quorum sensing* (QS) to coordinate the communal behavior. The bacterial virulence factors have been shown to specifically involve the recognition and response to self-generated secreted small molecules called autoinducers. Gram negative bacteria produce *N*-acylhomoserine lactones (AHL) as quorum signal molecules.

The interruption of QS by antipathogenic natural products from mandarin fruits was study. Mandarin oil is extracted from *Citrus reticulata* of the Rutaceae family.

Essential oil and terpenes were obtained from regional industry while, bacterial strains came from ATCC collection, and from clinical isolates. The techniques used were: Crystal violet to measure biofilm formation, MTT assay to determine metabolic activity, Congo Red Test to measure elastolytic activity and Miller Test to quantify the AHL production.

The higher biofilm inhibition was displayed by 4 mg/ml of terpenes against both strains *P. aeruginosa*: ATCC 27853 (88%), and clinical isolated (92%), in concordances with the higher viability inhibition (21% and 61%, respectively). The essential oil of mandarin, produce lower inhibitions against both strains (2-19%).

Both mandarin natural products inhibit elastase activity (higher than 80%) even at 0.1 mg/ml, and this action is correlated with the lower AHL formation (inhibition between 30 to 50%).

The use of local production of essential oil to inhibit *P. aeruginosa* virulence is a key strategy to employ this product to prevent food contamination by pathogenic bacteria and foodborne diseases.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Antibacterial interaction between extracts of *Fridericia caudigera* and *Cuspidaria convolute*

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Keywords: synergistic effect, Bignoniaceae, fractional inhibitory concentration, multi-resistant bacteria.

An interesting approach to the treatment of infectious diseases and prevention of the development of resistant microorganisms is the combination of antimicrobial agents. The aim of this work was to evaluate the antibacterial effect of the association between the extracts of *Fridericia caudigera* and *Cuspidaria convoluta* (Bignoniaceae) on several human pathogenic bacteria. The antimicrobial interaction between these antibacterial extracts was evaluated by the checkerboard method. The microorganisms used were Gram-positive bacteria: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, four clinical isolates of methicillin-sensitive *Staphylococcus aureus* and two methicillin-resistant clinical isolates of *S. aureus*; also Gram negative bacteria: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and antibiotic-resistant clinical isolates of *Enterobacter cloacae* (two strains), *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The fractional inhibitory concentration indices for all combinations were determined. *Klebsiella pneumoniae* and *P. aeruginosa* strains were resistant at used combinations. Nevertheless, synergism was demonstrated against the other Gram-negative bacteria. Furthermore, no antagonic effect was detected against Gram-positive bacteria, while synergism was found against four strains: *E. faecalis*, two ampicillin and methicillin resistant strains of *S. aureus* and ampicillin resistant *S. aureus*. The combinations had additive effect against the other three *S. aureus* strains and indifferent effect against *S. aureus* ATCC 29213. The results revealed that most of the combinations selected could efficiently inhibit the growth of tested bacterial strains at lower concentrations than those required for the individual extract. Considering the scarcity of plant extracts with good antibacterial activity against Gram-negative bacteria, these combinations offer good and promising prospects for the treatment of diseases caused by these bacteria in traditional medicine.

Influence of nutritional conditions on antibacterial activity of *Bacillus* sp SL-6

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Keywords: Carbon source, antibacterial activity, *Bacillus* sp

Introduction: In the last years, antibiotic development continues stagnate, so that the isolation of antibiotic-producing microorganisms from different environments became relevant in pharmaceutical, agronomic and food industries. *Bacillus* species are considered as "cell factories" producing a large number of bioactive compounds against bacteria, fungi, protozoa and viruses.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

This report studies the effect of different culture media and several carbon sources of a chemically defined broth in the estimation of antibacterial activity of *Bacillus* sp SL-6.

Materials and Methods*: Bacillus* sp. SL-6 was grown in Synthetic Mineral Broth (SMB) with orbital shaking at 200 rpm for 24 h at 30ºC. Glucose was replaced by glycerol, lactose, sucrose, maltose and starch in SMB at a final concentration of 10g/l. Samples were centrifuged and filtered to obtained cell-free supernatants (CFS). Screening of antibacterial activity of CFS on Mueller-Hinton, Brain Heart Infusion, Tryptone Soy Agar, Plate Count Agar and Glucose Peptone Agar (GPA) was tested against *Staphylococcus aureus* ATCC 29213 and *Yersinia enterocolitica* W1024 by agar well diffusion method.

Results: The detection of anti-*Yersinia* activity was clearly manifested in all solid media used. In contrast, the anti-*Sthaphylococcus* activity showed a haze of growth inside inhibition zone, with exception of observed activity on GPA.

CFS of cultures in SMB supplemented with glucose and glycerol gave maximum anti-*Staphylococcus* activity, while that those added of maltose and lactose showed activity only against *Y. enterocolitica*. No significant variation ($p<0.05$) was observed between all carbon sources for the anti Gram-negative activity.

Conclusions: These results indicate that the antagonist activity of *Bacillus* sp SL-6 against *S. aureus* was dependent of solid media used. Further, the secretion of these metabolites was modified by carbon source used in the growth of producer strain, giving glucose and glycerol the best values. The activity against *Y. enterocolitica* was evident in all conditions assayed.

Biofilm-forming vaginal lactobacilli inhibit *Escherichia coli* isolated from women with urinary infections

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Key words: Vaginal lactobacilli, uropathogen *Escherichia coli*, organic acid, probiotics

Lactobacilli are the dominant microorganisms of the healthy human vagina and participate in the maintenance of the ecological balance of the urogenital tract. They can inhibit the growth of urogenital pathogens by different mechanisms. The intravaginal administration of beneficial lactobacilli in pharmaceutical products has been proposed to restore the vaginal microbiome. The biofilm formation of lactobacilli is a beneficial characteristic to favor their mucosal colonization and/or permanence. In this study, the virulence factors of clinical *Escherichia coli* isolates and the inhibitory ability of biofilm-forming vaginal lactobacilli against this uropathogen were evaluated. The resistance of *E. coli* 36 and 36a (isolated from a patient with pyelonephritis) and *E. coli* 275 (isolated from a patient with recurrent cystitis) to different antibiotics (determined by disc diffusion method, according to Clinical and Laboratory Clinical Institute recommendations) and the presence of virulence genes (by multiplex PCR) were assayed. Antimicrobial activity of vaginal *Lactobacillus rhamnosus* CRL1332, *Lactobacillus reuteri* CRL1324 and *Lactobacillus gasseri* CRL 1263 was assayed using the agar plate diffusion technique. *Lactobacillus* supernatant aliquots were assayed, untreated, neutralized with NaOH or neutralized and

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

treated with catalase. *E. coli* 36 and 36a were susceptible to all the antibiotics tested, while *E. coli* 275 resulted an ESBL (extended-spectrum-beta-lactamase)-producing strain. In *E. coli* 36 and 36a, *fimH, papAH* (adhesins), *fyuA* (siderophore), *traT* (serum resistance) and *kpsMTII* (capsule) genes were detected. In *E. coli* 275, *fimH, fyuA, traT* and *agn43* (biofilm formation) genes were evidenced. *L. reuteri* CRL1324 and *L. rhamnosus* CRL1332 were able to inhibit the growth of the three *E. coli* strains. The inhibitory activity against *E. coli* disappeared after the supernatant neutralization, indicating that organic acids are responsible of the antagonism. The *Lactobacillus* strains evaluated are promising probiotic candidates to prevent or treat urinary infections caused by *E. coli*.

Effects of antibiotics on Shiga toxin-producing *Escherichia coli* biofilms

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Keywords: Biofilms; *Escherichia coli* O157:H7; antibiotic; Shiga toxin

The use of antibiotics in the treatment of enterohemorrhagic *Escherichia coli* (EHEC) infections is controversial since they are capable of triggering hemolytic uremic syndrome (HUS) by increasing Shiga toxin (Stx) production. In the present study, three antibiotics (ciprofloxacin, fosfomycin and rifaximin) at different concentrations were evaluated on Shiga toxin-producing *Escherichia coli* (STEC) biofilms to determine the relationships among biofilms, cellular stress and ***release*** of ***Stx***. With this purpose, reference and clinical strains associated with HUS were used, and biofilm formation was determined using crystal violet stain. In these biofilms, the reactive oxygen species (ROS) and the reactive nitrogen intermediates (RNI) were detected by the reduction of nitro blue tetrazolium and the Griess assays, respectively. In addition, the activities of the two antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were studied. The effect of an exogenous antioxidant (tiron) was also studied, and the ***Stx release*** of STEC biofilms was evaluated by cytotoxic assays using Vero cells. It was found that ciprofloxacin significantly altered the prooxidant-antioxidant balance, with an increase of antioxidant enzyme activity (SOD and CAT) and a high-level of Stx production. However, this effect was reverted by the addition of tiron. In contrast, fosfomycin and rifaximin produced less alteration to the oxidative cellular balance (antioxidant enzymes/oxidative metabolites) than controls, with minimal cytotoxic effects in Vero cells being observed.

The disturbance in the prooxidant-antioxidant balance induced by different antibiotics provoked oxidative stress in biofilms, and concomitantly had an effect on the production and *release of Stx. Consequently this effect* might play an important role in the pathogenesis of infections caused by STEC. In future, an improved understanding of the mechanisms involved in the release of toxins during biofilm formation would contribute to a better understanding of the pathogenesis of HUS.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Characterization of the bioactive diffusible pigment of an actinomycete isolated from patagonian soil

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Keywords: actinomycete, antibacterial, bioprospecting, streptomyces

Actinomycetes comprise an enormous group of bacteria famous by its ability to produce antibiotics such as erithromycin, kanamycin, gentamicin among a huge amount of clinically successful antibiotics. It is known that the vast genomic potential of these bacteria has been underestimated. The key to unveil such metabolic productivity has not been cleared yet. However, some researchers support that changing the isolation and screening methods, the media and other production factors would lead to new metabolic diversity, and thus, new drug candidates for antibiotic therapy. Up to date, the potential for antibiotic production of actinomycetes growing in Argentinean soils has not been explored. Here, we study the chemical composition of the antibacterial supernatant of *Streptomyces* SUE01, an active strain isolated from patagonian soils.

The strain identified by molecular methods as Streptomyces spp., was selected during a screening of antimicrobial producing strains as the most promising candidate from 60 isolates. *Streptomyces* SUE01 produced a diffusible purple pigment and high antibacterial activity when grown in a medium supporting the production of potent antitumor antibiotics. After lyophilizing the active supernatant, a dark powder was obtained which was subjected to different extraction methods. The agar diffusion assay against *Bacillus subtilis* was used for monitoring the isolation steps and for assessing the potency of the lyophilized supernatant (156 ug/mL). The lyophilized powder was extracted with methanol yielding an antibacterial purple extract. Two dimensional NMR experiments in D20 revealed the presence of oxygenated carbons and double bonds and the absence of alkyl chains in this sample. These studies showed that the selected producing medium activates the production of several active metabolites of highly hydrophilic nature. LC-MS analyses will be needed for further characterization and identification of the actives.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Synthesis and antimicrobial activity of hydrothiazine, hydrooxazine, hydroquinazolinone and hydropyrimidine derivatives

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Keywords: hexahydroquinazolinones, antimicobacterial; inhibitory concentration.

We present here the synthesis, purification and structural determination of ninehexahydro compounds with the aim of evaluate their antimicrobial activity. Their syntheses were performed in solution resulting in all cases very good yields. Furthermore, the antimicrobial activity was evaluated using the following bacterial strains: ATCC *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiellapneumoniae*, *Salmonella entérica*, *Serratiamarcescens*, *Shigella flexneri* and *Staphylococcus aureus*. These bacterial strains were obtained from the "Instituto Nacional de Controle de Qualidade em Saúde" (INCQS/FIOCRUZ, Brasil).

The measurement of the antibacterial effect was performed according to the Clinical Laboratory Standards. All compounds were tested in the concentration range 500-3.91 µg/mL. The same methodology was employed for the positive control (gentamicin -1600 µg/mL). The parameters analyzed were the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

The results were summarized in the table shown below. The nine hexahydro compounds present positive inhibitory activity and six of them demonstrated bactericidal activity. WP2331 presents the lowest inhibitory and bactericidal concentration, 3.91 and 31.25 µg/mL respectively.

Hexahydro compounds	Denomination	MIC (µg/mL)	MCB (µg/mL)
Hexahydroquinazolines	WP2	15,62	125
	WP6	500	-
Hexahydropyrimidines	WP33	500	-
	WP78	250	500
Hexahydrooxazines	WP45	125	500
	WP50	500	-
	WP2331	3,91	31,25
	WP2332	31,25	62,5
Hexahydrothiazine	WP55	31,25	62,5

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Phototoxicity of cationic porphyrin against multi-drug resistant *Pseudomonas aeruginosa*

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Keywords: Photoinactivation, Porphyrin, $TPMyP^{4+}$, Photodynamic Antimicrobial Chemotherapy

Pseudomonas aeruginosa is the cause of various infections. Amongst others, eye and ear infections or those in skin tissue and soft in burn patients represent a major therapeutic challenge. In recent years, photodynamic inactivation (PDI) has been proposed as an alternative treatment for localized bacterial infections in response to the problem of antibiotic resistance. The principle of PDI is to use a non-toxic dye or photosensitizer (PS) to produce reactive oxygen species (ROS) to cause damages on target bacteria while the PS is activated by a harmless visible light. The main purpose of this study was to explore the PDI effect of 5,10,15,20-tetra(4-N-methylpyridyl)porphyrin ($TPMyP^{4+}$) on *P.aeruginosa* ATCC 27853 and a clinical isolate of MDR *P.aeruginosa* since reports of their effects against multi-drug resistant (MDR) bacteria are limited. The porphyrin was evaluated for different doses (2.5, 5, 10, 20 µM), 30 min of irradiation with visible light and cellular suspensions of ~ $1x10^6$ CFU/mL. The PS was not toxic in absence of light for any doses studied. PDI treatment performed against both *P.aeruginosa* strains show a similar performance for all concentrations tested. Cellular suspension treated with 10 µM of $TMPyP^{4+}$ reached an inactivation greater than 99.99% for both strains. Moreover the assay was carried out incubating cells 30 min with porphyrin before irradiation and without this step. As previously, the inactivation was greater than 99.99%. Therefore, the sensitizer is quickly bound to the bacterial cell, thus reducing the treatment time. Additional experiments will be needed to confirm this hypothesis. $TMPyP^{4+}$proved equally effective for photodynamic inactivation of *P. aeruginosa* both sensitive and resistant to antimicrobial agents currently used in therapeutics. This reinforces the potential utility of Photodynamic Antimicrobial Chemotherapy as an alternative to the global problem of antimicrobial resistance.

A prenylated flavonoid enhances the effect of fluconazole on viability of resistant *Candida albicans*

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Keywords: prenylflavonoid- combination- azole antifungal

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The prenylated flavanone 2',4'-dihydroxy-5'-(1''',1'''-dimethylallyl)-8-prenylpinocembrin (**1**) from *Dalea elegans* Gillies ex Hook et Arn. showed antimicrobial properties and inhibition of fluconazole transport in resistant *Candida albicans* (R*Ca*) overexpressing Cdr1p, Cdr2p and Mdr1p pumps. Moreover this compound restores fluconazole efficacy.

In this work we study the effect of **1** and fluconazole on viability of R*Ca*.

Compound 1 was isolated from *D. elegans* roots.

Microorganism: An azole-resistant *C. albicans* isolated from the oral cavity and overexpressing transporter genes was used.

Cell viability of *C. albicans* was evaluated as previously described. A 10^5 CFU/ml starting inoculum was cultured in a microtiter 96-well plate at 37°C. Variable concentrations of **1**, fluconazole, or their combinations were used. Absorbance was measured at 540nm. Each sample was further serially diluted and cultured on Sabouraud agar plates. CFU/ml were counted after 24 h of incubation at 37°C.

At 125µM compound **1** and its combination with equal concentration of fluconazole significantly reduced cell viability (CFU/ml). Survival values (‰ of control) were: 629±71; 979±124 and 285±108 for **1**, fluconazole and the combination, respectively.

By combining fluconazole with 125µM of **1,** survival was reduced in a concentration dependent way. With 1000µM fluconazole, viability was reduced in 3-log (3.3±1.6) with respect to control (1000 ‰), reaching values close to a fungicide effect.

In fungal growth evaluation, starting from a 10^5 CFU/ml inoculum, the combination of fluconazole and **1** was more effective inhibiting the growth of R*Ca*. In fact, inhibitory concentration 50 (IC_{50}) of compounds alone were 1750 and 250µM of fluconazole and **1**, respectively while the same inhibition was achieved by combining fractions of them (1/2 fluconazole IC_{50} with 1/7 compound **1** IC_{50}).

The two compounds combined are more potent in inhibiting the growth of R*Ca* that each compound separately. The combination of both fungistatic compounds at their MIC produced values of survival close to fungicidal effect.

Antifungal activity of a silver-albendazole complex

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Keywords: albendazole, silver complex, antifungal.

Benzimidazole and its derivatives possess a wide variety of useful biological properties (antiviral, fungicides, insecticides, etc.). Among them, albendazole (Fig. 1) is an effective anthelmintics.

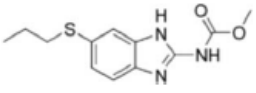

Fig. 1 HAbz

Many active medicinal drugs possess enhanced potential administrated as metal-based compounds. Silver is effective against a broad range of bacteria, fungi, and yeast. As HAbz is poorly soluble in many solvents, which makes it difficult the reaction, the complex Ag–HAbz was synthesized as a white solid in glacial acetic acid (HAbz 0.1327 g -0.5 mmol- plus 0.5 mmol of aqueous $AgNO_3$). ^1H NMR (300.1 MHz) and ^{13}C NMR (50.3 MHz) spectra of the Ag–HAbz complex suggest the

coordination of the Ag(I) with the non-protonated N atom of the imidazole ring. Minimal Inhibitory Concentration (MIC) and minimal fungicidal concentration (MFC) of Ag-Habz and $AgNO_3$ (Table 1) were determined by using broth microdilution techniques according with the Clinical and Laboratory Standards Institute. Both MIC and MFC were confirmed by two replicates.

Table 1: MIC/MFC values in µg/mL of Ag-HAbz complex and AgNO3 acting against human opportunistic pathogenic fungi. In bold: remarkable results

Fungi Sample	Ca	Ct	Cn	Afl	Afu	Ani	Mg	Tr	Tm
Ag-HAbz	31.25/ 62.50	31.25/ 62.50	7.80/ 7.80	**3.40/ 7.80**	**3.40/ 7.80**	**3.40/ 7.80**	62.50/ 62.50	62.50/ 62.50	62.50/ 62.50
$AgNO_3$	6.36/ 12.72	12.72/ 25.44	3.18/ 12.72	12.72/ 12.72	25.44/ 25.44	25.44/ 25.44	12.72/12. 72	6.36/ 6.36	12.72/ 12.72

Ca: *Candida albicans* ATCC 10231; Ct: *Candida tropicalis* C 131; Cn: *Cryptococcus neoformans* ATCC 32264; Afl: *Aspergillus flavus* ATCC 9170; Afu: *Aspergillus fumigatus* ATCC 26934; Ani: *Aspergillus niger* ATCC 9029; Mg: *Microsporum gypseum* C 115; Tr: *Trichophyton rubrum* C 113; Tm: *Trichophyton mentagrophytes* ATCC 9972. ATCC: American Type Culture Collection (Rockville, MD, USA); C: CEREMIC, Centro de Referencia Micológica, FCByF, Rosario, Argentina. Positive controls: Amphotericin B, Ketoconazole,Terbinafine

Microbiological control of bronchofibroscope after high-level disinfection

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Keywords: Bronchoscopy, Glutaraldehyde 2%, High level disinfection

Bronchoscopy is an invasive medical practice to visualize the airway with diagnostic or therapeutic purpose. Spaulding classified the medical devices according to the risk of infection associated with their use, and established the level of disinfection or sterilization required. Critical elements are in contact with sterile cavity or vascular system; semicritical are those in contact with mucosa intact or non-intact skin and non-critical items are used on intact skin. The bronchoscope, necessary equipment to make such practice requires sterilization or at least a high level disinfection after each scan. The process must be effective to avoid further complications.

This work evaluates the process of sterilization of bronchofibroscopes by immersion in glutaraldehyde 2%. Positive control to check the media and the proper concentration of glutaraldehyde used were made. Inoculum *Staphylococcus aureus* ATCC 25923 and 2% glutaraldehyde in equal parts, and *Pseudomonas aeruginosa* ATCC 27853 and 2% glutaraldehyde in equal parts mixed, were picked in blood ram agar and CLDE. On plates which contained glutaraldehyde inoculums no development was observed after 18 h. Then 8 samples after used by patients taken once a week for 2 months were processed, performing external swabbing the bronchoscope and injecting thioglycolate broth in lumens maintaining different contact times. For recovery of aerobic, anaerobic bacteria and fungi the samples were cultivated in different culture media. The results were negative indicating that the method is effective.

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Efficacy of a cationic polymer as an adjunct to antibiotics with different target site against *Pseudomonas aeruginosa*

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Keywords: Eudragit E100®, time-kill assay, ciprofloxacin, vancomycin

Emerging drug resistance, along with the few antibiotic discovery programs in the pharmaceutical industry has forced the scientific community to seek new alternatives.

Numerous reports describe the antimicrobial properties of cationic polymers for pharmaceutical use. However, Eudragit E100® (Eu) has not been characterized in this sense. Recently, we described that Eu causes alterations in the bacterial envelopes of *P.aeruginosa*, but the polymer itself is not bactericidal.

In this study we evaluate the effects, if any, of Eu on the antibacterial action of antibiotics with different site and spectrum of action: Ciprofloxacin (CIP) and Vancomycin (VAN) against *P.aeruginosa*. It is known that *P.aeruginosa* is outside of spectrum of action of VAN. In contrast, CIP is an effective antimicrobial against *P.aeruginosa* but growing resistance in clinical isolates has limited its use.

Bactericidal activity of drug-containing Eu dispersions (Eu-CIP and Eu-VAN) was determined by time-kill assay against a fluoroquinolone-resistant *P.aeruginosa* clinical isolate (PaFQ-R1; MIC_{CIP}: 64µg/mL). Several concentrations and treatment times were tested. Simultaneously, drug solutions (CIP and VAN) and Eu dispersions were evaluated.

Eu-ClP tended to kill Pa FQ-R1 rapidly, exhibiting bactericidal effect ($3log_{10}$ reduction) after 1 h at 8µg/mL CIP, whereas 8-fold higher concentration was required from CIP. Initial inoculums eradication was observed after 6 h with 16µg/mL of drug in Eu-CIP, requiring 128µg/mL of CIP to achieve this effect.

Eu-VAN also exhibited time- and concentration-dependent bactericidal activity against PaFQ-R1, achieving $\geq 3log_{10}$ reduction at low concentrations and short exposure times, and eradicating after 24 h in the whole range of VAN concentrations tested (128-1024 ug/ml). Furthermore, as expected, no effect was shown by VAN in any of the conditions assayed.

Drug-free Eu exhibited bacteriostatic or slightly bactericidal action with regrowth seen at longer exposure times.

The improved bactericidal effect observed using this cationic polymer in combination with two antibiotics against *P.aeruginosa* provides a useful tool to broaden the spectrum of antibiotics whose clinical use is limited by intrinsic resistance and/or have an alternative to prolong the usefulness of conventional antibiotics against multidrug resistant bacteria.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

PHARMACOBOTANY- PHARMACOGNOSY

Antibacterial activity of extracts obtained from plants of central Argentina.

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Keywords: Native plants, natural antibacterials, resistant *Staphylococcus aureus.*

Bacterial infections are dramatically increasing every day for diverse reasons, mainly due to the development of resistance to conventional antibiotics.

In this situation, and given the lack of new highly effective drugs, numerous extracts obtained from plants are being studied for chemical and anti-microbial characterization.

Although many plant families are being investigated as sources of new antibiotics, the plant world is far from being totally explored and this also applies to the native flora from Argentina.

Hence, extracts obtained from 69 native, introduced, adventive and naturalized plants from central Argentina were evaluated for their *in vitro* inhibitory activity on pathogenic bacteria with the aim of selecting the most active ones as new sources of effective antibiotics.

The susceptibility of reference and clinical strains of *Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa,* methicillin-susceptible *Staphylococcus aureus* (MSSA) and gentamicin- and methicillin-resistant *S.aureus* (MRSA) was determined. Among these last, endemic strains of Hospital and Community Acquired *S. aureus* (HA-MRSA, CA-MRSA, respectively) obtained from patients from Córdoba hospitals, were included.

Extracts from *Aloysia citriodora* Palau and *Lepechinia meyenii* (Walp.) Epling were the most active against all Gram positive and negative bacteria tested, being the last one the most potent (MICs and MBCs 0.062-1 and 0.250-2 mg/ml, respectively). Among the assayed extracts, those from *Gaillardia megapotamica* (Spreng.) Baker, *Baccharis salicifolia* (Ruiz et Pav.) Pers., *Wedelia glauca* (Ortega) Hicken, *Viguiera tucumanensis* (Hook. et Arn.) Griseb., *Flourensia campestris* Griseb, *Elaphoglosum lorentzii* (Hieron.) H. Christ and *Angelphytum aspilioides* (Griseb.) H. Rob. showed high inhibition against HA-MRSA and CA-MRSA.

The antibacterial activity shown by the plant extracts suggests that they could become as source of novel antibacterial drugs.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Cytotoxic activity of bioactive compounds isolated from *Flourensia oolepis* S. F. Blake

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Keywords: Natural products, Native plants, Antiproliferative effect.

Previously, five antibacterial flavonoids identified as 2´,4´-dihydroxychalcone (**1**), isoliquiritigenin (**2**), pinocembrin (**3**), 7-hydroxyflavanone (**4**) and 7,4´-dihydroxy-3´-methoxyflavanone (**5**) were isolated by bio-guided fractionation from the ethanol extract of *Flourensia oolepis* S. F. Blake. This time, the cytotoxicity of these compounds against two leukemic cell lines and their respective multidrug resistant counterparts was evaluated.

Compound **1** showed the strongest cytotoxic effect against CCRF-CEM and its MDR variant CEM/ADR5000 (IC_{50} = 1.6 and 2.4 µg/ml, respectively). It also showed a moderate effect against K562 and the MDR line Lucena 1 (IC_{50} = 6.6 and 7.4 µg/ml, respectively). A lack of cross resistance toward the MDR cells was evidenced in opposite to that found in treatments with the chemotherapeutic agent doxorubicin.

In addition, **1** induced a dose and time dependent growth inhibition. 72 h exposure of CCRF-CEM to 1.25 µg/mL and 2.5 µg/mL resulted in 46% and 85% of growth inhibition while in K562 this last grade of inhibition was achieved at 10 µg/mL. Analysis of DNA content by flow cytometry indicated that treatments of CCRF-CEM with **1** resulted in an accumulation of cells in G2/M phase. After 72 h of exposure, the percentage of cells in this phase was 47% compared to 11% in control cells. On the other hand, K562 cells showed a significant increase in the proportion of cells in S and G2/M phases. Annexin V staining showed that **1** induced CCRF-CEM and K562 apoptosis reaching 26 and 27%, respectively, after 72 h of treatment.

As far as we know this is the first time that the anti-proliferative activity of **1** over these leukemic cell lines is reported. These results suggest that **1**-induced suppression on cell growth would be possibly mediated by slowing down cellular progress through G2/M phase or even blocking cells in this phase accompanied by apoptosis on both leukemic lines.

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Foliar micrography and chromatographic profile of flavonoids present in Fuchsia

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Keywords: aljaba, epidermis, flavonoides

F.magellanica "aljaba" is a Patagonian bush whose aerial parts are popularly used as emmenagogue and diuretic by the Mapuche people of southern Chile and Argentina.

Due to the ethnobotanical importance, in the present study the leaf epidermis was observed and a thin layer chromatography (TLC) of polyphenolic compounds was conducted in order to contribute to its knowledge.

Leaves and flowers were collected in the Botanical Garden Spegazzini of La Plata, where has been naturalized.

Epidermal studies were performed using diaphanization technique (Dizeo de Strittmatter, 1973). The leaves were stained with alcoholic safranin 80% and mounted in glycerine jelly. Because of its use as a diuretic and emmenagogue this study was focused on the presence of polyphenols. Hexane, Ethyl acetate and Aqueous extracts were assayed using Shinoda and Rosenheim reagents, ethanol solutions of Cl3Al, Cl3Fe, boric acid and NaOH in order to confirm the presence of polyphenols. TLC on silica gel chromatoplates 0.25mm F254 Merck was done subsequently by appropriate mobile phases for polyphenols (Wagner & Bladt, 1995). Solvent systems used were SI EtOAc/AcOH/ForH/H2O (100:11:11:26) and SII Reference compounds: Methanolic solution (1%) of vitexin, isoquercetin apigenin and caffeic acid. Detection: natural products reagent observed under UV366nm.

F. magellanica has anfistomatic leaves, epidermal cells with sinuous anticlinal walls (upper and lower surface), stomata anomocytic, and simple eglandular trichomes. A lot of raphides are also observed in the mesophyll. Characterization reactions verified the presence of flavonoids in the aqueous and ethyl acetate fractions of leaf and flower extracts. TLC analysis shows the presence of vitexin and isoquercetin and the absence of apigenin and caffeic acid.

The presence of glycosylated phenolic compounds, which have shown various biological and pharmacological properties such as anti-hypertensive, anti-inflammatory, antispasmodic, antimicrobial and/or antioxidant, make of *F.magellanica* a promising specie for medicinal use.

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Preliminary results of *Caladium bicolor* (Aiton) Vent. (Araceae) anatomical and phytochemical studies, species of the ecuadorian traditional medicine.

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Keywords: *Caladium bicolor*, foliar anatomy, flavonoids.

C. bicolor is used in traditional medicine as anti-inflammatory, analgesic, sedative, skin diseases and bites reliever. Thus, the aim of this work is to study the leaves anatomy in order to establish micrographic foliar features, and to characterize the presence of polyphenols.

Fresh leaves were collected at 88 m.a.s.l. during the rainy season at "Valencia" city in "Los Rios" province of Ecuador. Samples were dried in a dark chamber at room temperature. Anatomical studies were performed using the diaphanization technique of Dizeo de Strittmatter (1973). Observations and images were made with an optical microscope equipped with a Gemalux colour CCD PAL camera and Hyper Media Center software. Hexanic, ethyl acetate, methanolic and acqueous extracts were obtained from powdered samples. Each extract was assayed using Shinoda and Rosenheim reagents, ethanol solutions of Cl_3Al, Cl_3Fe, boric acid and NaOH in order to determine the presence of polyphenols. The extracts were assayed by TLC on silica gel 60 F254 using as eluents ethylacetate:formicacid:aceticacid:H_2O (100:11:11:27) and ethylacetate:methanol:H_2O (100:13:10) along with reference compounds. Observations under UV 254 and 366 nm using Natural Products reagent were performed (Wagner & Bladt, 1995).

Leaf in surface view shows no trichomes, epidermic cells with polygonal anticlinal walls and paracitic stomata on both faces. The presence of mucilaginous cells, abundant druses and microcrystals were observed in the mesophyll. Phytochemical assays established the presence of flavonoids in ethyl acetate, methanolic and acqueous extracts. TLC results had shown the presence of rutin and vitexin.

Caladium bicolor leaves can be microscopically identified by their anatomical features. These micrographic diagnostic characters constitute the basis for a future quality control along with phytochemical and chromatographic assays. *Caladium bicolor* traditional use on inflammatory and other diseases involving free radicals and as sedative could be related at least in part to the presence of rutin and vitexin, compounds with antioxidative, antiedematogenic and antinociceptive, and sedative activities previously reported.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Effects of central administration of *Coriandrum sativum* essential oil on anxiety-like behavior in chicks

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Keywords: Coriandrum essential oil, Linalool, Anxiety-like behavior

Coriandrum sativum ("cilantro") is an herb with culinary use. Coriandrum essential oil (CEO) is an aromatic complex mixture of volatile terpenes with antibacterial, antifungal, antioxidant activity and when it is systemically administered it has anxiolytic and antidepressant effects. Linalool is a major constituent of CEO and its inhalation has anxiolytic and sedative effects. Several studies have attempted to elucidate the action of CEO on the central nervous system, however few studies shown the effect of its central administration on the behavior. We evaluated the effect of CEO and linalool centrally administered on anxiety-like behavior in 4-7 day-old chicks. CEO was obtained from seeds of cilantro by hydrodistillation (characterized by GC-MS spectroscopy: linalool major constituent) and pure linalool was purchased from Sigma-Aldrich. Both were intracerebroventricularly injected at doses of 0.01, 0.1 and 1 µl/chick at a volume of 10 µl. Each animal was exposed to open-field test for 10 min and latency to ambulate, number of ambulations and latency to defecate were registered. CEO (0.1 and 1 µl/chick) significantly increased the ambulation latency and decreased the number of ambulations in open-field test. However, no significant increase for defecation latency was observed. On the other hand, linalool significantly decreased the ambulation latency at doses of 0.1 and 1 µl /chick. The number of ambulations and defecation latency significantly decreased only at dose of 0.1µl/chick. Therefore, CEO exerted an anxiogenic-like action when it was centrally administered in chicks exposed to an open-field test. This effect may have been produced by other compounds present in minor proportion in CEO but not by linalool. Respect to the decrease in the number of ambulation observed, a synergic effect between linalool and others terpenes of the essential oil should not be discarded.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Reversal of P-glycoprotein mediated multidrug resistance by pinoresinol in chronic myeloid leukemia cells.

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Keywords: Multidrug resistance, P-glycoprotein, Pinoresinol.

The overexpression of P-glycoprotein (P-gp) in tumor cells is one of the major mechanisms of multidrug resistance (MDR) leading to cancer chemotherapy failures. This MDR pump extrudes out of cells a wide range of anticancer drugs by an ATP-dependent mechanism, resulting in a decreased intracellular concentration of these agents. This fact has encouraged the development of effective MDR reversal agents. Little has been reported about the use of compounds isolated from plants of Argentina as MDR inhibitors.

The aim of this study was to evaluate the ability of 15 compounds, isolated from native and naturalized plants of central Argentina, to reverse P-gp mediated MDR using doxorubicin (DOX) as a P-gp substrate. For this purpose, the chronic myeloid leukemia cell line K562 and its MDR variant due to P-gp overexpression, Lucena 1, were used. The effect of the compounds on DOX cytotoxicity in Lucena 1 was evaluated by MTT. Pinoresinol (**1**), isolated from the ethanolic extract of *Melia azedarach*, showed a high effectiveness, causing a decrease of 9.4 times in the IC_{50} of DOX in Lucena 1 at 40 µg/mL, showing the same level of activity as that of the commercial modulator verapamil. This result was confirmed by flow cytometry as a significant increase in the intracellular DOX accumulation in MDR cells treated with **1**.

Docking studies revealed that **1** binds to aromatic residues of P-gp involved in a "v" vortex formed by the trans-membrane α-helices 4, 5 and 6. Also, P-gp ATPase activity was proved to be inhibited by **1** with an IC_{50} of 7.5 µg/mL. No effect was found on P-gp expression.

In addition, **1** did not affect cell viability of fresh human lymphocytes up to 160 µg/mL.

The results suggest that compound **1** has great potential to be further developed as a P-gp inhibitor.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Rubiadin 1-methyl ether: A natural photosensitizing anthraquinone with antifungal activity *in vitro*

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Keywords: anthraquinones, antifungal activity, *Candida*

Extracts rich in anthraquinones (AQs) of *Heterophyllaea pustulata* Hook. f. (Rubiaceae), have shown *in vitro* antifungal effect against planktonic microorganisms. We have established that their purified AQs exhibit photosensitizing activity type I and/or II. Since the most important group of opportunistic fungal pathogens are **Candida** species, in this study we evaluate the antifungal activity of Rubiadin 1-methyl ehter (RubME), one of the majority AQs from these extracts, against different *Candida* strains, analyzing whether this effect could be increased by irradiation.

RubME was isolated from the benzenic extract of roots by several chromatographic methods; and identified by their spectral data (UV-V and RMN). Minimum Inhibitory Concentration (MIC) of RubME was determined by following the protocols of the Clinical and Laboratory Standards Institute, testing twelve concentrations (0.24 to 500 µg/mL) against two *C. tropicalis* strains (NCPF3111 and clinical) and a *C albicans* strain (NCPF3153). The assay was performed simultaneously under darkness and irradiation with an Actinic Phillips lamp.

RubME decreased meaningfully the growth of all strains in darkness. Thus, the MICs were 250 µg/mL for clinical *C. tropicalis*, 31.3 µg/mL for *C. tropicalis* NCPF3111 and 15.6 µg/mL for *C. albicans* NCPF3153. Under irradiation, this inhibition percentage was achieved at lower concentrations: being 31.3 µg/mL for the clinical strain, 15.6 µg/mL for *C. tropicalis* NCPF3111 and 7.81 µg/mL for *C. albicans* NCPF3153.

RubME exhibits a very good antifungal activity *in vitro*, which can be enhanced by light action, against several Candida species, mainly on *C. albicans*. Therefore, RubME could have application in photodynamic therapy for Candida infections.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Anticandidal and antioxidant activities from leaf extracts of climbers of selected species of Bignoniaceae family

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Keywords: scavenging activity, climbers, *Fridericia*, Candidas.

Bignoniaceae is a family of shrubs, trees and climbers. It comprises tropical species and many are used as traditional medicines. The scavenging activity against DPPH (1,1-diphenil-2-picrylhydrazyl) and ABTS (2,2′-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) radicals and the anticandidal effect of hydroetanolic extracts of leaves of seven climbers were investigated. The selected species were: *Adenocalymma marginatum* (Cham.) DC., *Amphilophium vauthieri* DC, *Cuspidaria convoluta* (Vell.) A. H. Gentry, *Dolichandra dentata* (K. Schum.) L. G. Lohmann, *Fridericia caudigera* (S. Moore) L.G. Lohmann, *F. chica* (Bonpl.) L. G. Lohmann and *Tanaecium selloi* (Spreng.) L. G. Lohmann. The anticandidal activity was determined using agar disc diffusion and microdilution in broth method. The antifungal activity of each extract was tested against: *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 and clinical isolates of *C. albicans*, *C. glabrata*, *C. kruzei*, *C. parapsilosis* y *C. tropicalis*. Zones of inhibition of extracts were compared with that of fluconazole. The results showed that the most active extracts were *A. vauthieri*, *C. convoluta*, *F. caudigera* y *T. selloi.* Their minimal inhibitory concentration ranged from 125 to 1000 µg of phenolic compounds/mL. The antioxidant activity was evaluated by the DPPH and ABTS methods. The scavenging capacity was confirmed in all of cases. The antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC). The TEAC results revealed that the ability to scavenge DPPH (*A. marginatum* ~ *F. chica* ~ *F. caudigera* > *C. convolute* > *T. selloi* > *A. vauthieri* > *D. dentata*), differed from the ability to scavenge $ABTS^{+}$ (*C. convolute* ~ *F. chica* ~ *D. dentata* > *A. marginatum* > *F. caudigera* ~ *A. vauthieri* > *T. selloi*). Overall, *C. convoluta* and *F. caudigera* were the most promising species. Hence, these plants can be used to discover bioactive natural products that may serve in the development of new pharmaceuticals.

Searching for flavonoids with tyrosinase inhibitory activity from extracts of *Dalea pazensis*.

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Keywords: *Dalea pazensis*, Inhibition, Diphenolase, Tyrosinase.

Tyrosinase enzyme (**Tyr**) participates in the biosynthesis of melanin, responsible for the color formation in the skin of mammals, catalyzing two reactions: hydroxylation of L-Tyrosine (monophenolase activity) and L-Dopa´s oxidation to o-dopaquinone (diphenolase activity). The inhibition kinetics for these

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reactions are different because **Tyr** presents two active sites. **Tyr** inhibitory compounds are important in the treatment with abnormal pigmentation diseases and as whitening agents in cosmetics, but they showed toxicity, so research for new inhibitors of **Tyr** becomes primary. In the plant kingdom, flavonoids are the polyphenols's group more studied. Some of them were reported as **Tyr** inhibitors.

The chemical and pharmacological study of the genus *Dalea* in Argentina has been performed by our group informing new prenylated flavonoids with important activity as **Tyr** inhibitors, antioxidant and antimicrobial. The promising results motivate us to study other species in the genus. Previously, we inform that different extracts of *Dalea pazensis*, Bolivian specie, showed significant inhibition of monophenolase activity. In the present study, the inhibitory activity of diphenolase by hexane, benzene, ethyl acetate and ethanol extracts are presented. Also, the isolation, identification and diphenolase inhibitory activity of 5,7,2 ', 4'-tretrahydroxy-5'-(1''', 1'' '-dimethylallyl)-8-prenylflavanone (**8PP**), isolated from the benzene extract and informed in other species of the genus, are reported. The results show a benzene extract as the most active (82.6 ± 1.0) % at 1µg/mL. **8PP** showed an inhibition with an IC_{50} (80.7 ± 0.4) mM, compared with reference inhibitor Kojic Acid (IC_{50} =129.6 ± 0.3) mM, showing two times more active than the reference inhibitor. Previously, **8PP** had shown a significant inhibition on monophenolase activity.

Thus, **8PP** could be a new candidate with potential applications in the pharmaceutical and cosmetic industries, motivating us to search other components that could contribute to the significant activity observed for the benzene extract as **Tyr** inhibitors.

Acute toxicity of *Lomatia hirsuta* in mice

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Keywords: *L.hirsuta* - acute toxicity

Lomatia hirsuta (Lam.) (Proteaceae) leaves ("radal"), used in Patagonian traditional medicine for bronchial troubles, asthma, ulcers, hypertension, constipation, inflammation and as expectorant, were evaluated. Previous experiments in our lab have shown that aqueous extracts of radal exhibit a potential sedative activity but further studies are needed to know more about the mechanisms of this action. Due to the widespread usage of this plant, it is essential to investigate the effects of the crude extract on liver and kidney so as to detect any potential risk to human health. There is no available information on the pharmacological or toxicological properties of radal so the present work has investigated the effects of its aqueous extracts in mice. *L.hirsuta* was collected in February 2012 from El Bolsón (Río Negro), the leaves were dried and crushed and used to prepare infusions according to the traditional method, filtered and administered orally to male adult mice according OECD Guideline for the testing of chemicals 423 with minor modifications. Fourteen days after the single dose, mice were anesthetized, killed by cardiac puncture to obtain blood samples and afterwards the liver and kidneys were removed. The plasma obtained was aliquoted for biochemical analysis using A25 BioSystems Autoanalyzer. The macroscopic appearance of the organs was noted, their weights recorded and they were stored in 10% formaldehyde

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

for routine histology. The crude extract did not produce toxic symptoms in mice in doses up to 2000 mg/kg. Based on biochemical analyses of renal and hepatic functions, such as the level of urea, creatinine, transaminases and alkaline phosphatase, we determined that the extract is generally tolerated by mice. This was also confirmed by histopathological exam. Our results showed that acute administration of *L.hirsuta* is not toxic in male mice, suggesting a safety use by humans.

Quality control of commercial samples of white, green and red tea marketed in Bahía Blanca city (Prov. Bs. As., Arg.)

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Key words: tea, quality control, commercial samples.

A great amount of teas are commercialized around the world, but only young leaves and stems of *Camellia sinensis* are used to manufacturing tea *sensu stricto*, which is the favorite infusion for many people around the world. The aim of this study was to analyze commercial samples of white, green and red tea sells in Bahia Blanca city, determining its quality in direct relation to the amount of trichomes presented in these samples. One commercial sample of white tea, three of red one and four of green tea, corresponding to five trademarks were analyzed. From each package of 20-25 teabags, were analyzed four randomly chosen. The material was dissociated and drained subsamples of 0.1 g were mounted and studied under light microscopy following a greek guard design. All samples correspond to *Camellia sinensis*, although most of them are contaminated with tissues of other plants and animal species. The only studied brand of white tea showed about 300 hairs per subsample. For green tea, three brands had near 200 trichomes per subsample while in the remaining more than 700 were counted. A brand of red tea averaged 100 hairs per subsample, another one 350 and two had highest values, reaching to 1100 trichomes. The quality of raw material with which this type of mass-market teas are produced is notoriously uneven, which contrasts with their high prices due to the current popularity of the antioxidant properties that they own.

Micrographic analysis of ruminal content, its importance in intoxications detection

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Key words: micrography, ruminal content, Wedelia glauca.

The toxic weeds constitute a threat when the animals feed on natural and implanted pastures or on any type of baled plants. Though the majority of the toxic plants are not consumed voluntarily

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

by their low palatability, there exist numerous reasons for which this type of poisonings occurs. Toxic plants can cause slight to serious hurts in different organs and even death according to the toxic principle and the quantity of vegetable material ingested. The aim of this work was to use micrographical tools to study the ruminal content of Tragelaphus strepsiceros, an antelope Great Kudu belonging to the Foundation Temaikén, dead of a sudden form without demonstrating previous symptoms of disease. Six subsamples of 0,2 grs each were taken from the dry ruminal content being processed and mounted using traditional techniques for micrographical studies. They were studied under optical microscope. The results showed: 1-tricellular trichomes of rough surface with triangular apical cell and basal rosette belonging to Wedelia glauca; 2 - unicellular long and warty trichomes, and calciphytoliths (double truncated pyramid type) of Medicago sativa; 3 - eragrostoid type epidermal cells probably belonging to Cynodon dactylon; 4 - typical stellate hairs of some Malvaceae species, and multicellular uniseriate hairs belonging to some sort of eudicotyledon. We conclude that the most abundant diagnostic elements are trichomes of W. glauca and M. sativa, the latter species and C. dactylon are prescribed part of its diet, but not the first species. W. glauca has a hepatotoxic terpenoid, inhibitor of mitochondrial respiration and ATP synthesis, which is a serious threat when accidentally consumed by animals (as part of the pasture or bundles).

Searching for a methodology for assess *in vitro* antiviral activity photosensitized

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Keywords: Neutral Red Uptake, Reduction PFU, photosensitizing rich-AQs extract.

Our research group studies the activity of photosensitizing anthraquinones(AQs), purified from a phototoxic plant, against several viruses.Previously, we have demonstratedthat these AQs exhibit virucidal effect against HSV-1 and JUNV (by inactivating viral particles before they enter into the host cell), and this inhibition is increased by light (photostimulation).To study the antiviral activity of these AQs(on the infected cells, at some stage of viral replication),which can in turn be photostimulated, an appropriate methodology is needed. Therefore, the aim of this work was to assesstwo techniques, Neutral Red uptake assay (NR) and reduction of plaque forming units test (PFU), and establishing the conditions necessary to quantify the *in vitro* antiviral effect photosensitized.

An extract enriched in AQs (benzene extract) was assayedin order to ensure a photostimulated effect against HSV-1. Seven different concentrations were tested ($\leq CC_{50}$, which was estimatedfrom the curve of cellular viability vs. concentrations of extracton Vero cells, by RN assay). The antiviral effect was performed by both techniques in Vero cells,under two simultaneous conditions: darkness and irradiation (actinic lamp 380-480 nm, Philips TL/03). The following variables were evaluated: culture medium

during irradiation, irradiation time on virus-cells-extract, culture medium post-irradiation and incubation time.

The optimal conditions for both methodologies were: PBS 1% DMSO (culture medium during irradiation), 15 minutes (irradiation time on virus-cells-extract), MEM supplemented with 2% FBS (culture medium post-irradiation), 36 h incubation. The RN assay was chosen because it allowed determining the inhibition percentage, which could not be estimated by the PFU test, since it only showed the qualitative toxicity of the photostimulated extract. Besides, during the NR assay, the cytopathic effect (microscopic observation of morphological alterations in cells) could be simultaneously assessed. This is very important because it alloweddiscriminating the agent responsible for the cytotoxic effect.

Total phenolic content from traditional culture and *in vitro* culture of *Physalis peruviana*

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Keywords: Total phenolic content, *in vitro* culture, *Physalis peruviana*

Interesting and various pharmacological properties has described for *Physalis peruviana*, such as anticancer or antioxidants, due to its content of phenolic compounds. As for all medicinal species destined to the elaboration of drugs, the production of stable and high quality material, in relation to content of active substances is essential, it is proposed to *in vitro* culture of the species as an alternative to traditional culture to increase biomass and the production of interesting secondary metabolites. Therefore, the aim of this study was to compare the total phenolic content from plants obtained by traditional culture and from *in vitro* culture.

For this investigation, a wild type of *Physalis peruviana* was cultured by traditional way, beside to *in vitro* culture, in semisolid medium, from different explants of the species, such as seeds, shoots and leaves, using aseptic and germination protocols, as well as direct callogenesis by providing Indole Acetic acid and Benzyl Amino Purine. Once defined and applied the technique of *in vitro* culture, were quantified by the Folin Ciocalteu method, using gallic acid as a reference standard, the total phenolic content in the different cultures, extracting the compounds by maceration with ethanol 95%.

The comparative results for different plants organs obtained from traditional culture and from *in vitro* culture, expressed as mg per litre of gallic acid equivalents (GAE), indicated that for leaves was 192 and 394, for shoots 131 and 157, respectively, and for the callus 150 mg GAE/L, noting that the last component correspond to a clusters of undifferentiated cells without specialization.

It is concluded that the application of exogenous hormones to propagate plant organs and stimulate the generation of secondary metabolites is a valid alternative to provide a stable plant material with high concentrations of active ingredients as compared to that obtained in traditional culture.

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Ipomoea asarifolia neutralizes inflammation induced by *Tityus serrulatus* scorpion venom

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Keywords: Inflammation, *Ipomoea asarifolia*, Therapy, *Tityus serrulatus*.

Envenoming caused by scorpion sting is a serious public health problem. In Brazil, 13,038 accidents caused by venomous animals have been reported. Of this total, 53% of the cases and 14 deaths were caused by scorpions. Furthermore, *Tityus serrulatus* (Buthidae) is the most dangerous scorpion due to the high toxicity of its venom. The treatment is the common supportive therapy and the serum therapy, but some people do not have access to both therapies and seek healing through the use of medical plants. This study evaluated the ability of the crude extract and fractions from the leaves of *Ipomoea asarifolia* in neutralizing the main biological effects caused by *Tityus serrulatus* envenoming in mice. BALB/c mice were pretreated (i.v.) with 100 µL of aqueous extracts and fractions dichloromethane, ethyl acetate, and *n*-butanol (CH_2Cl_2, EtOAc, and *n*-BuOH, respectively) of *Ipomoea asarifolia*, rutin or saline. Then, the animals received 100 µL (i.p.) of venom of *Tityus serrulatus* (0.8 mg/kg). After six hours, the peritoneal lavage was performed with PBS and the number cells were determined using a *Neubauer* chamber. The supernatants were collected for determination of cytokines, such as IL-6, IL-12, and IL-1β.The aqueous extract, fractions and rutin, at all doses, significantly reduced cell migration, which was endorsed by the reduction of the levels of certain cytokines. This is the first study that demonstrated the potential effect of *Ipomoea asarifolia* against inflammation caused by *Tityus serrulatus* venom, suggesting that these extracts and/or their bioactive molecules, especially the flavonoid rutin, have potential use in the therapy of this envenomation.

Membrane interaction of natural ciclic ketones with inhibitory activity on the $GABA_A$ receptor

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Keywords: Gabaergic Ketones, Membrane Interaction, Cytotoxicity

The $GABA_A$ receptor (GABA-R) is the main inhibitory receptor of the Central Nervous System. It possesses binding sites for drugs other than the neurotransmitter GABA, including benzodiazepines, barbiturates, and the convulsant picrotoxine which behave as allosteric modulators or channel blockers.

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The study of this last site is especially relevant since it constitutes the action site of widely used neurotoxic organochlorine pesticides. Our group has studied some cyclic ketones, structurally similar to the convulsant product thujone, demonstrating their ability to inhibit the GABA-R activity. Many compounds that regulate GABA-R function are noticeably lipophilic, which may change the physical properties of the lipid bilayer. Taking into account that all the ketones studied are highly lipophilic, in the present work we studied the effect of six cyclic ketones (including the reference compound thujone) on the microviscosity and stability of model membranes (liposomes) by using fluorescence anisotropy and DLS. These studies were completed with the analysis of the cytotoxic activity of these compounds by mean of a test that evaluates the membrane integrity (LDH test). The results showed that all the ketones included in this research were able to modify the membrane microviscosity incrementing the probes mobility. However, the LDH studies indicated that only one compound showed cytotoxic effect at higher concentrations and longer exposure times. Concluding, all compounds are able to interact with the membrane modifying its properties, although in biological systems the cell would respond to these changes to maintain the membrane integrity.

Identification and quantification of anthraquinones by HPLC in bioactive extracts of *Heterophyllaea lycioides*

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Keywords: HPLC, anthraquinones, *Heterophyllaea lycioides.*

Recently, we have reported that some extracts, obtained from aerial parts of *Heterophyllaea lycioides* (Rusby) Sandwith (Rubiaceae), showed *in vitro*antibacterial activityagainst Gram-positive and Gram-negative bacteria.This plantbelongs to a genus characterized by the presence of anthraquinones (AQs). With the aim to find a relationship between antibacterial activity and chemical composition, a HPLC method was developed for identification and quantificationof AQs in the extracts.

The four extracts previously tested for antibacterial activity, hexane (Hx), benzene (Ben), ethyl acetate (AcOEt) and ethanol (EtOH), were analyzed by HPLC. A C18 columnwas used, with a gradient program of two mobile phases: a) MiliQ water and b) methanol (MeOH) (both with 0,612 % V/V of formic acid) at 0.4 mL/min, and detection at 269 nm.Identification of AQs was performed by comparison of the retention time with the corresponding standard (AQs purified and unequivocally identified by their spectroscopic data). The percentage of each AQ in dried extract was expressed as Soranjidiol (Sor) by means of the external calibration method.

HPLC analysis revealed the presence of Sor, Bisoranjidiol (Bis), Pustuline (Pus), Heterophylline (Het), Chloro-soranjidiol (Cl-sor), 2-OH-3-methyl-anthraquinone (2-OH-3-Me-AQ), and two AQs that are in elucidation process (AQ-1 and AQ-2).Thus, several known AQs that had not been isolated yet from this vegetal species were identified. Sor, Bis and Pus are the most important AQs because they were detected in all extracts, in spite ofAQ-1 was the mostabundant compound but it was only found in AcOEt and

EtOH. Het, Cl-sor, 2-OH-3-Me-AQ and AQ-2 were present in small amounts and only in some extracts. Since the bioactive extracts ,AcOEt and Ben, possess a wide variety of AQs in high amount, we would conclude that exist a strong relationship between the antibacterial activity and the composition of AQs in the extracts.

Identification of antioxidant compounds in seeds of *Cucurbita spp*

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Keywords: antioxidant, gastroprotective, Cucurbita spp

The objective of this study was to determine the antioxidant activity of extracts obtained from seeds of *Cucurbita spp*: Tetsukabuto (hybrid between *C. moschata* y *C. maxima* Duchesne ex Lam.), *C. mixta* Pangalo; *C. moschata* (Duchesne ex Lam.) Duchesne ex Poir. and *C. maxima* Duchesne, in relation to their content of total phenols and flavonoids. Extracts of decreasing polarity were obtained using the following solvents: acidified water, methanol, acetone and ethyl acetate. The total phenols and flavonoids were quantified by the Folin-Ciocalteu and complexation with 5% $AlCl_3$, respectively. The bleaching technique free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was used to determine the antioxidant activity of extracts. The highest values of total phenols was observed in the aqueous fractions of the four varieties of *Cucurbita spp*, with values ranging from 62.79 ± 3.34 to 95.96 ± 3.25 µmol GA/g extract (µmol of Gallic acid per gram of extract). In fractions of ethyl acetate, the concentrations found in all varieties were relevant, with values from 41.29 ± 0.21 to 80.91 ± 1.89 µmol GA/g extract. In contrast, lower values were observed in the fractions with acetone. The four varieties of *Cucurbita spp* showed higher concentration of total flavonoids in the ethyl acetate fractions with values ranging from 49.27 ± 0.18 to 54.74 ± 0.46 (mg quercetin/g of extract); and the lowest values were found in the aqueous and methanolic fractions. The aqueous and ethyl acetate fractions showed higher scavenging capacity of DPPH free radical, which could be related to the content of total phenols and flavonoids in these fractions.

From the results obtained, a relationship between the content of total phenols and flavonoids with the antioxidant activity is observed, so that the seeds of Cucurbita spp may be considered a source of natural antioxidants to produce a beneficial effect on health.

Identification and characterization of fatty acids on extract of pumpkin seeds *(Cucurbita spp*) by FT-IR

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Keywords: *Cucurbita spp*, fatty acids, FT-IR.

Cucurbita spp oil contains 39 to 54% of unsaturated fatty acids, of which the highest concentration corresponds to oleic and linoleic acid, essential and beneficial for humans. The aim of the work was to identify and characterize by infrared spectroscopy extracts from four varieties of *cucurbita spp* seeds*:* Tetsukabuto (hybrid between C. *moschata* y *C. maxima Duchesne ex Lam*.), *C. mixta Pangalo, C. moschata (Duchesne ex Lam.) Duchesne ex Poir*. and *C. maxima Duchesne*. The extraction was performed with a room temperature and a refluxing concentrated hexane. Infrared spectra were analyzed on a FT-IR spectrometer Spectrum 2000 Mod. Characteristics oleic and linoleic fatty acids bands are observed wherein a signal corresponding to the tension band of C = CH 3007 cm^{-1} typical of the unsaturated fatty acids. Two bands at 2925 and 2854 cm^{-1} are associated, respectively, to the CH stretching vibration of symmetrical and asymmetrical CH of CH_2 and the band at 1747 cm^{-1} is associated with the movement of extension of the C = O typical of triglycerides esters. In the region between 1458 and 1376 cm^{-1} a wide band with many peaks associated with the presence of CH bending vibrations of CH_2 and CH_3 is observed. The band at 1164 cm^{-1}, is characteristic of the CO stretching vibration. Finally 722 cm^{-1} are clearly seen, the bending vibrations in the plane (*rocking*) corresponding to $(CH_2)_n$ with n> 4 typical of fatty acids of long chain linear. Compounds flavonoids may appear blurred between 1700-1550 cm^{-1}, so it would be advisable to perform a previous purification. The spectra obtained correspond to IR standard spectra provided by the Spectral Database for Organic Compounds (SDBS) from Japan. It is observed that the spectra obtained are identical in terms of frequency of absorption peaks, so that the extraction method does not affect the oil composition.

Evaluation of antioxidant activity in leaves extracts from *Jodina rhombifolia*

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Keywords: *Jodina rhombifolia*, ROS, RNS, antioxidant activity

Generation of reactive oxygen (ROS) and nitrogen (RNS) species in biological media are responsible for oxidative damage to biomolecules such as DNA, lipids and proteins, producing alterations in its structure and promoting tumoral, ulcer, and inflammatory processes and various degenerative diseases. Scientific evidence indicates that ROS and RNS are implicated in inflammatory etiopathology events.
In this sense, our research group has focused the study of the biological activity of medicinal plants reputed as analgesic and anti-inflammatory and its relationship to the inhibition of ROS and RNS by extracts obtained from them.

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Jodina rhombifolia (Hook. et Arn.) Reissek commonly known as "sombra de toro" is one of the species studied in the group. Barks and leaves of this medicinal plant are used as anti-inflammatory airways (among other uses). Different extracts obtained from this species were studied as antiproliferative, antibacterial, antifungal and anti-inflammatory activity (assessed by producing carrageenan paw edema). Previous studies of our group, informed the presence of alkaloids and other metabolites already reported for this species (phenolic compounds, tannins, flavonoids, steroids.); and evaluated the inhibitory activity on nitric oxide production, from macrophage cell line. In the present work we evaluated the antioxidant properties of chloroformic and ethanolic extract obtained from leaves of *J. rhombifolia* by different in vitro tests: 1) Total antioxidant potential: phosphomolybdenum method (PM) and 2) Radical scavenging capacity by TEAC methodology (Trolox Equivalent Antioxidant Capacity). Results showed an inhibition in both methodologies by the chloroform extract, in a dose-dependent manner. Besides, the extract showed an important TEAC of 98% at 100 ug/mL, being lower in the PM. These results could indicate that the alkaloid-enriched extract is the most active as free radical scavenger. We could infer, at least in part, that the presence of antioxidant compounds could correlate with the anti-inflammatory activity evaluated. However, more chemical studies are necessary in order to indicate which is the metabolite responsible for the scavenger activity.

Effect of usnic acid on oxidative stress of resistant *Candida albicans* biofilms

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Key words: usnic acid, antifungal activity, *Candida albicans*, biofilms

The continued emergence of infections with antifungal resistant *Candida*strains requires constant searching of new antifungal drugs. Thus, native flora is an important source of new chemical structures. Usnic acid (UA) [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione] is a well-known compound obtained from lichen species of *Usnea* genus. No previous studies related to biofilms oxidative stress in UA presence have been reported.

The aim of this work was to characterize the effects of UA on *C.albicans* biofilms by evaluating oxidative stress.

Materials and methods

Compound: UA was purified from benzene extract of the lichen. Spectroscopic data was coincident with those previously reported.

Microorganisms: two strains of *C.albicans* isolated from the oral cavity were used, a sensitive strain (**S*Ca***) and an azole-resistant strain (**R*Ca***), which over expresses transporter genes.

Biofilm formation: was measured by adhesion to 96-well plate and crystal violet stain.

Reactive oxygen species (ROS): was assayedby the reduction of the nitro-blue tetrazolium (NBT).

Total superoxide dismutase (**SOD) activity:** was assayed based on the inhibition of NBT reduction.

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Total antioxidant capacity of biofilms: was assayed by FRAP method.

Results

UA at 4 µg/mL reduced both strains biofilms (86.50 and 82.58% of inhibition with respect to the control for R*Ca* and S*Ca*, respectively). In both biofilms treated with 4 µg/mL ofUA, ROS measurements increased four-fold.SOD levels were higher (seven-fold) than basal ones. The basal levels of the total antioxidant capacity of biofilm were similar in both strains. However, S*Ca* evidenced a considerable increase in FRAP in presence of UA.

Conclusions

C.albicans biofilms were reduced by UA, even in R*Ca*. As expected for a sensitive strain, the more significant inhibition of biofilms was observed in S*Ca*. There was an increase of ROS and antioxidant system in response to stress.These preliminary results encourage the study of mechanisms of action and combination assays with other antifungals.

Phytoextract effects on lipids of murine telencephalon and their redox implications

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Keywords: central nervous system, essential fatty acid, Ilex paraguariensis, oxidation.

Extracts from native plants contain phenolic compounds, which reach the central nervous system. These phytochemicals might affect redox state and composition of CNS tissues. Thus, our aim was to assess superoxide anion (SO, 600 nm) and γ-glutamyltranspeptidase (GGT, 450 nm) by spectrophotometry, and fatty acids (FA by gas chromatography) in telencephalon of Balb/c mice treated orally for a month with 100 mg/Kg/d of *Ilex paraguariensis* A. St.-Hil. (IP), *Aspidosperma quebracho-blanco* Schltdl. (AQB) or *Lantana grisebachii* Stuck. (LG) (Aqueous extracts from plant infusion at 95°C). Statistics were ANOVA and Pearson correlation (PC) ($p<0.05$). First, FA were correlated to SO (sat=-0.3, ω3=-0.2, ω6=0.2, ω9=-0.6) and GGT (sat=0.1, ω3=0.6, ω6=-0.4, ω9=0.4) under the different treatments, depending on their family. Then, concerning extracts, saturated FA were decreased by IP and slightly increased by LG (C=35.1±1.6%, LG=39.9±2.9%, AQB=34.1±1.6%, IP=29.8±2.3%), with essential ones being conserved. IP-related changes were due to 18:0 decrease and ω3 elevation ($p<0.06$). AQB slightly increased the ω3 family. The ω6/ω3 index was not affected. Summing up, *I. paraguariensis* extract improved lipid profile and consequently their oxidative response.

Marine Pharmacognosy: brown seaweeds from Golfo San Jorge (Argentina) with potential application in health

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Keywords: *Undaria*, *Lessonia*, alginate, bioactivity, marine pharmacognosy.

Products from seaweeds are important in the treatment of many diseases and serve as compounds of interest both in their natural form as well as templates for synthetic modification. Coasts of the Golfo San Jorge present an important biological diversity with native and invasive brown seaweeds; *Lessonia vadosa* Searles (Lessoniaceae) is a native species, *Undaria pinnatifida* (Harvey) Suringar (Alariaceae) is an invasive algae, the latter of unknown potential. In this work we are presenting advances in the study of these species collected at the Comodoro Rivadavia coasts.

Metabolites were extracted from the dried powder of the fronds from seaweeds. The principal products were analyzed by chemical characterization and bioactivity; alginate was applied in the preparation of the microparticles.

Alginate yields from *L. vadosa* and *U. pinnatifida* collected in summer were 30 and 62 %, respectively. Alginate from *L. vadosa* showed no proteins or phenols; its physical properties were: viscosity 10 cp, Mw 89 kDa, m.p. 212, characteristic crystals. We have obtained microparticles by the gelation ionic technique using paracetamol as active ingredient; these showed 100 μm of size and irregular form; the yield was 95 % and encapsulation efficiency of 46 %.

U. pinnatifida showed complex chemical composition. In summer, their ethanolic extraction yield were 22 and 50 %, at room temperature and 70 °C, respectively. Carbohydrates, phenols (include flavonoids e.g. quercetin), lipids (phosphatidylcholine and sulfolipids), peptides, pigments, steroids, cardenolides were the principal constituents.

Alginate from both seaweeds showed antiproliferative activity *in vitro*; products the *L. vadosa* showed activity on the colon cancer Caco-2 cells.

These results illustrate the potential of these algae for their use in health and the importance of integral studies to generate a rational use of natural resources and, in case of invasive species, a control of their expansion.

Weight and redox effects of infusive phytoextracts on overweight female mice treated for fifteen days

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Keywords: bioavailability, brain, oxidative stress, phenolic, spleen.

Extracts from native plants contain phenolic compounds and can affect animal organism and tissue redox response. There are many attempts to discover plant-based drugs which will be useful for the treatment of chronic metabolic diseases. Thus, our aim was to evaluate body weight and redox markers in tissues of overweight female mice. Animals were treated orally for 15 days with 100 mg/Kg/d of *Ilex paraguariensis* A. St.-Hil. (IP), *Aspidosperma quebracho-blanco* Schltdl. (AQB) or *Lantana grisebachii* Stuck. (LG) (aqueous extracts from plant infusion at 95°C), and respect to controls (C). Then, phenolic bioavailability was assessed in brain and spleen using a fast blue bb-based technique. Statistics included ANOVA and Spearman correlation (SC). For the three extracts, body weight gain was inversely correlated to phenolic concentration in brain (telencephalon SC=-0.70; diencephalon SC=-0.71, $p<0.05$), without relation with splenic content of phenolics (SC=-0.05, $p<0.9$). AQB increased phenolics 2.26 times in telencephalon, 3.76 times in diencephalon, and 1.14 times in spleen. Thus, mice treated with AQB controlled weight gain respect to C ($p<0.02$), whereas IP and LG did not show significant effects. Nonetheless, weight gain was mildly associated with lower splenic lipoperoxidation (SC=-0.60, $p<0.1$) and strongly with superoxide levels (SC=-0.75, $p<0.03$), whereas nitrites showed a SC=0.37 ($p<0.4$). Summing up, the *A. quebracho-blanco* extract prevent body weight gain depending on its phenolic content in brain, although splenic oxidative stress could be a collateral effect, which encourage further studies.

Preliminary phytochemical analysis and antioxidant capacity of *Calycera crassifolia* (Calyceraceae)

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Keywords: *Calycera crassifolia*, phytochemical screening, scavenging activity.

Calycera crassifolia Hicken (Calyceraceae) is a native species popularly known as "cardo de las dunas". As several authors have pointed out in different parts of the world, many dune ecosystems support high plant richness and diversity values. In order to improve the knowledge of argentine medicinal flora, particularly that belonging to the coastal dune area from southern Buenos Aires, the chemical composition of the leaves of the plant as well as the scavenging activity were investigated as potential utility in medicinal field.

Plant material was extracted with ethanol under reflux (2 h, 1:10 w/v) to get fraction A (FA). Half of the volume of FA was evaporated to dryness and the residue suspended in HCl 1% and filtered (acid suspension). The remaining residue was extracted with the same volume of chloroform (3X) to obtain fraction B (FB). The acid suspension was alkalinized and partitioned with the same volume of chloroform (3X) to reach chloroformic fraction (FC) and aqueous residual fraction (FD). Antioxidant activity of FA was determined based on the reduction of DPPH absorbance at 517 nm.

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The main compounds found were carbohydrates (FA), flavonoids (FA and FD), phenolic compounds (FA and FD), lipids (FA), steroids (FB), triterpenes (FC) and proteins (direct reaction). The presence of flavonoids as well as other phenolic compounds is well known as scavengers and useful in the prevention of diseases related to oxidative processes; their effects on human nutrition and health are considerable and may explain partially the scavenging activity found in this case (40.2 %).
The results of this work are very useful as a first approach to the knowledge of this species found in the coastal area of Buenos Aires since dune ecosystems support a wide variety of species with promising economic value.

Phytochemical analysis and antioxidant capacity of the aerial parts of *Senecio subulatus* (Asteraceae)

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Keywords: aerial parts, antioxidant activity, phytochemical screening, *Senecio subulatus*.

Senecio subulatus D. Don ex Hook. & Arn. (Asteraceae) is a native species used in traditional medicine as expectorant, antiasthmatic, anticephalalgic and to treat bronchitis, dyspnea, chronic colds and earache.
In order to improve the knowledge of argentine medicinal flora from the coastal dune area from southern Buenos Aires, the chemical composition of the aerial parts of *S. subulatus* as well as the scavenging activity were investigated considering the biological effects of this species in phytotherapy.
Plant material was dried at 40ºC and extracted with ethanol under reflux (2 h, 1:10 w/v) to get fraction A (FA). Half of the volume of FA was evaporated to dryness and the residue suspended in HCl 1% and filtered (acid suspension). The remaining residue was extracted with the same volume of chloroform (3X) to obtain fraction B (FB). The acid suspension was alkalinized and partitioned with the same volume of chloroform (3X) to reach chloroformic fraction (FC) and aqueous residual fraction (FD). Antioxidant activity of FA was determined based on the reduction of DPPH absorbance at 517 nm.
The main compounds found were carbohydrates (FA), flavonoids (FA and FD), phenolic compounds (FA and FD), lipids (FA), steroids (FB) and proteins (direct reaction). The presence of flavonoids and carbohydrates in the aerial parts of the plant was remarkable. Flavonoids and other phenolic compounds are well known for their effects on human nutrition and health and may explain the higher scavenging activity found in the plant (92 %).
The results of this work are very useful for the knowledge of this species found in coastal dunes systems and have important biological and agronomic benefits, including the rational use of native resources and the national economic development.

Flavonoid content and scavenging capacity of *Solidago chilensis* (Asteraceae)

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Keywords: flavonoids, inflorescences, scavenging capacity, *Solidago chilensis*.

Flavonoids are commonly found in medicinal plants and they have been reported to exert several biological effects, including antioxidant activity. Many species containing these compounds are recognized to possess medicinal properties and beneficial impact on health. *Solidago chilensis Meyen* (Asteraceae) is a widespread species used in the traditional medicine of South America and has been reported to possess anti-inflammatory, diuretic, antimicrobial and gastroprotective properties.

The objective of this work was to determine the total content of flavonoids and the scavenging capacity of the decoction of the plant and its derivative product. For this, a decoction (D) of the inflorescences was prepared and dialysed for 24 h in water using a cut-off membrane of 6000 - 8000 Da to get a dialysed solution (discarded) and a non dialised solution (NDi). Total flavonoid content was measured at 360 nm and expressed as rutin. The stable DPPH radical was used for determination of free radical-scavenging activity of the samples at 517 nm; butylhydroxytoluene (BHT) was used as a comparative standard.

The contents of flavonoids observed were 16,5% (D) and 9.8% (NDi). The scavenging activities detected were 90.5 (D) and 83 % (NDi).

It has been recognized that flavonoids exhibit antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of these compounds are through scavenging or chelating process.

The higher scavenging activity of the decoction (D) could be explained by the higher content of flavonoids as compared to NDi. This is very interesting since free radicals are involved in many disorders like gastric ulcers, inflammation, neurodegenerative diseases and cancer. These facts suggest that some biological activities previously informed for *S. chilensis* could be related to presence of these phenolic compounds in the plant.

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PHARMACOLOGY

Jodina rhombifolia repeated oral administration in adolescent and adult rats: differential effects in ethanol consumption

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Keywords: antialcoholic, *Jodina rhombifolia*, adolescent rats, adult rats

Worldwide, alcohol (ethanol) causes about 3.3 million deaths/year (5.9% of all deaths), and 5.1% of global burden of disease is attributable to alcohol consumption. Abuse and alcohol dependence play an important role in public health due to both the clinical consequences as economical cost. Empirical data from traditional medicines manifest the use of plants for treatment of alcoholism and alcohol abuse. Leaves of *Jodina rhombifolia* (Hook. & Arn.) Reissek (SANTALACEAE) are used in Argentine folk medicine as anti-alcoholic. In present study we analyzed the effect of leaves aqueous extract (0, 125 and 250 mg/kg) on voluntary ethanol intake in adolescent [postnatal day (PD) 35-40] and adult (PD 70-75) male Wistar rats. Infusion to 10% was prepared according VIII Ed Argentine National Pharmacopoeia; plant material was separated by filtration and aqueous extract was concentrated and lyophilized to preserve it. The extract was administrated twice daily (1 ml/200 g; *p.o.*). Animals (n=6) were individually housed in standard plastic cages with wood chip bedding. Throughout the duration of experience, ethanol was offered in home-cage, two-bottle free-choice regimen between an ethanolic solution (20% in tap water, v/v) and tap water, with unlimited access for 24 h/day for 10 consecutive days. Rats used in present study had never experienced alcohol before the start of experiment. Ethanol presentation was initiated at start of day 1. Results are expressed as mean±S.E.M. of 10-days experimentation. Ethanol consumption for 0, 125 and 250 mg/kg, respectively: **adolescent rats**: 7.89±0.40, 4.43±0.89 (p<0.001) and 2.49±0.26 g/Kg (p<0.001); **adult rats**: 6.32±0.14, 2.62±0.17 (p<0.001) and 2.38±0.15 g/Kg (p<0.001). A significant decrease in ethanol consumption was observed throughout the treatment period, without significant changes in food or water intake. The results obtained in the present preliminary study show that repeated administration of extract, markedly reduces ethanol voluntary intake in adolescent and adult male Wistar rats.

Novel Gemini Vitamin D Analogue: Antitumoral Effects on Cancer Cell Lines and Lack of Calcemic Activity in Mice.

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Keywords: analogue, antitumor agents, calcitriol, cancer, cell lines

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1α,25-dihydroxyvitamin D_3 (calcitriol) shows potent growth-inhibitory properties on different cancer cell lines although its hypercalcemic effects have severely hampered its therapeutic application. In collaboration with the laboratory of Organic Chemistry of the University of Vigo we synthesized a novel Gemini analogue of calcitriol, called UVB1, in order to maintain or increase the antitumor effects and decrease the calcemic activity. The aim of this study was to evaluate the antitumor action of UVB1 on different tumor cell lines, comparing its effects with those elicited by calcitriol, and studying its calcemic activity and its toxicity in mice. The analog exerts a significant decrease in cell count after treatment with UVB1 in HCT116 (human colorectal cancer), U251 (human glioblastoma), HN13 and HN12 (human head and neck squamous cell carcinoma) and T47D (human breast adenocarcinoma) cell lines. Also, UVB1 reduces cell migration of T98G (human glioblastoma multiforme), HN12 and LM3 (murine mammary adenocarcinoma) cell lines, while cell motility of HC11 (normal murine mammary epithelial cell line) is not affected. Since calcitriol has been shown to generate ROS which is involved in its antiproliferative activity, ROS production was determined in HN13 cell line after treatment with UVB1. An increase in the levels of ROS was observed. Cell cycle analysis by flow cytometry on the same cell line shows that UVB1 induces arrest in the G_1/G_0 phase and this result is accompanied by a decrease in cyclin D1 levels. The novel analogue, in contrast to calcitriol, did not cause hypercalcemic effects in BALB/c and nude mice. Additionally, histological examination of livers and kidneys showed no pathological changes. Furthermore, animals did not experiment changes in behavior, weight loss or haematocrit alterations. In conclusion, these results suggest that this Gemini analogue may have therapeutic potential as an antitumor drug.

Could variations in nNOS levels drive expression of cocaine sensitization?

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Key words: cocaine, sensitization, nitric oxide, hippocampus

Behavioral sensitization is known as the increased sensitivity to locomotor stimulating effect after repeated psychostimulants administration, and it is believed to be relevant to drug addiction and craving in humans. Repeated cocaine induces behavioral sensitization and modulates synaptic plasticity in the hippocampus, an important brain region for the associative learning processes occurring during addiction. Nitric oxide (NO) is a neurotransmitter involved in several effects in the central nervous system including synaptic plasticity and complex behavioral responses. We have previously demonstrated a key role of neuronal NO synthase (**nNOS**)/**NO**/soluble guanylate cyclase (**sGC**)/cyclic guanosine monophosphate (**cGMP**) signaling pathway in the development of cocaine sensitization and in the associated enhancement of hippocampal synaptic plasticity. In the present work, we attempted to determine constitutive differences in nNOS protein levels between sensitized and non-sensitized groups by western blot, and whether nNOS inhibition after sensitization reverses the behavioral effect of cocaine and the associated hippocampal synaptic plasticity. We administered five daily cocaine injections (15 mg/kg, i.p) to 35 days old Wistar rats, followed by five daily 7-nitroindazole (nNOS inhibitor, 50 mg/kg, i.p) or

vehicle injections. We tested development of cocaine sensitization, by measuring locomotor activity, and the threshold for long-term potentiation in hippocampus, using electrophysiological multiunitary extracellular recordings, as indicator of synaptic plasticity. We observed that only sensitized rats showed significant increases in nNOS protein levels compared to non-sensitized group. Furthermore, nNOS inhibition reversed behavioral sensitization and the highest level of hippocampal plasticity. In conclusion, we can speculate that repeated cocaine exposure has differential impact within subjects, increasing nNOS protein levels only in sensitized animals. Then, nNOS inhibition can restore NO function in hippocampus and reverse cocaine sensitization. Moreover, changes in NO function and nNOS activity in other brain areas related to the reward system that contribute to cocaine sensitization must be considered.

Analysis of adverse drug reactions reports concerning the ATC classification group in the Chaco province during the 2006-2013 period

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Keywords: ADR, Nervous System, Pharmacovigilance, Chaco.

The aim of this study was to analyze and classify Adverse Drug Reactions (ADR) concerning the Nervous System Group of the ATC classification. Reports from 2006 to 2013 were made to Chaco's Peripheral Reporter for the National Pharmacovigilance System, part of the National Administration of Drugs, Food and Medical Technology (ANMAT). Chaco's Peripheral Reporter operates at the National University of the Chaco Austral. A retrospective descriptive study was conducted to analyze the reports of suspected ADR, using data collected from ANMAT's Yellow Cards. The World Health Organization Adverse Reaction Terminology (WHO-ART) Dictionary and the Anatomical Therapeutic Chemical (ATC) Classification System were used for their evaluation. Causality was assessed using the Naranjo Algorithm. Regarding severity, three categories were established (WHO): mild, moderate and severe. From a total of 664 reports received during 2006-2013, 101 corresponded to the ATC Classification N Group.According to the ATC code, subgroups involved were distributed as follows: Analgesics (N02, 34%), Antiepileptics (N03, 24%), Psycholeptics (N05, 23%), Psychoanaleptics (N06, 12%), Anesthetics (N01, 5%), Anti-parkinson (N04, 1%) and other nervous system drugs (N07, 1%). Regarding the causality of ADR they were probable (68%), possible (25%), certain (6%) and unlikely (1%). Relative to the severity were accounted moderate (56%), severe (24%) and mild (20%). Regarding the gender distribution the female sex prevailed over males by 60%. The most reported age group was 45-59 years old (21%).Among the most reported drugs were included dipyrone (21%), haloperidol (9%), paracetamol (7%), fluoxetine (6%), carbamazepine (5%) and pregabalin (5%).The most reported ADR were related to Disorders of Skin and Appendages (25%), Central and Peripheral Nervous System Disorders (18%), Body as a Whole-General Disorders (11%) and Psychiatric Disorders (7%). A profile of ADR concerning the ATC classification N group in the Chaco Province during the 2006-2013 period was obtained.

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Blockade of brain ethanol metabolism by centrally-administered cyanamide: effects on ethanol intake, locomotor activity and participating enzymes in a perinatal lead exposure model

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Keywords: lead exposure, ethanol, acetaldehyde, catalase, ALDH

In pursuing the putative mechanism for the increased vulnerability to consume ethanol reported in developmentally-lead (Pb)-exposed rats, we have demonstrated that catalase activity (CAT, the main enzyme involved in brain ethanol metabolism) is elevated in the blood and brain of these animals. Moreover, we have also reported that the ethanol ingested in the free-choice test is sufficient for the Pb-exposed animals to evidence hyperactivity. We thus postulate that brain ethanol-derived acetaldehyde could be in part responsible of the increased motivational and stimulant properties of ethanol in Pb-exposed rats. To this end, cyanamide, an ALDH2 blocker (being ALDH2 the enzyme responsive for acetaldehyde degradation) was administered in the lateral ventricle to interfere with acetaldehyde metabolism in the brain. Male thirty-five day-old animals were subjected to a 2-h free-choice test (2 bottles filled with water and 2 bottles filled with ethanol solutions at 2-10%). Once 10% ethanol intake was stabilized and Pb animals evidenced elevated ethanol intake in comparison to controls, they were microinfused with vehicle, 0.1, 0.2 or 0.3 µg cyanamide immediately before the free-choice session; and their ethanol-induced hyperlocomotion assessed immediately thereafter. At the end of the one-hour locomotor activity session they were sacrificed to determine brain CAT and ALDH2 activity. The results demonstrate that cyanamide increase ethanol intake and locomotor activity in control animals at all the three doses evaluated whereas, only the two higher doses were able to increase the same behaviors in the Pb-exposed group. In relation to brain CAT activity, a reduction was observed only in the control group at the higher dose evaluated. However, we failed to evidence a significant reduction in brain ALDH2 activity. These results support the importance of brain ethanol-derived acetaldehyde as a putative metabolite implicated in the motivational and stimulant effects of ethanol.

Effects of *Aristolochia argentina* on gastric lesion induced by ulcerogenic agents in rats

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Keywords: *Aristolochia argentina,* NSAIDs-induced gastric lesions, histamine, reserpine

Aristolochia argentina (family Aristolochiaceae) (*A.a.*) is popularly know as "charrúa". The roots of this plant are used in folk medicine. In the present study we investigate the effect of *A. argentina* on experimentally induced lesions in rats. Phytochemical assays were performed. Non-steroidal anti-inflammatory drugs (NSAIDs)-induced gastric lesions were produced using aspirin (200 mg/kg, *p.o.*),

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indomethacin (30 mg/kg, *p.o.*) and phenylbutazone (200 mg/kg, i.p.) in rats. Histamine (80 mg/kg, s.c.) and reserpine (10 mg/kg, i.p.) were administered as ulcerogenic agents. Phytochemical screening indicated the presence of flavonoids, alkaloids, saponins, tannins among others compounds. NSAIDs used resulted in the production of gastric lesions, mainly in the glandular segment of stomachs. Pretreatment with *A. argentina* root infusion (500 mg/kg, *p.o.*) produced a decrease in the gastric lesion induced by indomethacin (14.17 ± 3.37 mm^2 vs. *A.a.*: 4.62 ± 0.39 mm^2, $p<0.05$; at 6 h) and aspirin (Ulcer index: 3.58±0.20 vs. *A.a.*: 1.16±0.10, $p<0.0001$; at 4 h), whereas in the phenylbutazone-treated group (at 6 h) did not inhibit gastric lesion. Indomethacin is a potent inhibitor of prostaglandin biosynthesis, aspirin is a gastric mucosal barrier breaker and phenylbutazone inhibits mucopolysaccaride synthesis by gastric mucosal cells. Histamine is a powerful gastric secretagogue and evokes a copious secretion of acid from parietal cells. No difference was seen in lesion between the *A. argentina* treated rats vs. the histamine (at 4 h) and reserpine (at 24 h) treated rats. The protective effect against (NSAIDs)-induced gastric lesions could be due, at least in part, to the presence of flavonoids in this plant. Flavonoids are responsible for protective effect in others vegetables. The suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration. Present findings suggest the possible involvement of prostaglandins in the antiulcer effect of *A. argentina.*

Mechanism of action involved in the gastroprotective activity of *Lithraea molleoides*

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Key words: *Lithraea molleoides*, gastroprotective activity, mechanism

Lithraea molleoides (Vell.) Engl. (Anacardiaceae) is a medicinal plant traditionally used in South America to treat various ailments, including diseases of the digestive system. Previously, we have demonstrated that *L. molleoides* prevents the formation of gastric lesions and protects the gastric mucosa against injuries caused by several necrotizing agents in rats. In this study the mechanism of the gastroprotective effect of *L. molleoides* was investigated with regard to the roles of prostaglandins (PG), nitric oxide (NO) and gastric endogenous sulfhydryls groups (SH).

The gastroprotective activity of the infusion of *L. molleoides* (500 mg/kg) was assessed in according to the method of Robert *et al.* (1979) in Wistar rats. Absolute ethanol administered orally was employed as necrotizing agent. The degree of erosion was assessed from a scoring system. The involvement of PG, NO and SH was investigated through the pretreatment with specific inhibitors: indomethacin (10 mg/kg, s.c.), a non-selective cyclooxygenase inhibitor, N^G -nitro-L-arginine (L-NNA, 40 mg/kg, i.v.), an inhibitor of nitric oxide synthesis and N-ethylmaleimide (NEM, 10 mg/kg, s.c.), a sulfhydryl-blocker. Indomethacin, L-NNA and NEM antagonized gastroprotective activity of *L. molleoides* (***$p<0.001$ vs. gastroprotection groups).

PG exerts a gastroprotective action through maintenance of gastric mucus synthesis and secretion. NO acts by increasing mucosal blood flow, regulating the secretion of mucus and bicarbonate, and inhibiting the secretion of gastric juice. SH provide protective effects through binding free radicals formed by ethanol treatment and by controlling the production of mucus.

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These findings suggest that gastroprotective mechanism of *L. molleoides* against ethanol induced gastric mucosal damage depends on PG, NO and SH.

Cyclosporin A induced renal fibrosis was prevented by trimetazidine

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Keywords: Trimetazidine, Cyclosporin A, Renal fibrosis

Cyclosporin A (CyA) is an immunosuppressor used in transplanted patients. Adverse effects like nephrotoxicity and fibrosis, were described by our group. Our previous papers demostrated that Trimetazidine (TMZ) is able to reverse the CyA-induced toxicity. This work describes the mechanism by which TMZ prevents renal fibrosis induced by CyA. The experimental design was carried out for 120 days. Included four groups (n = 8) of male Wistar rats treated with: A control; B treated with CyA 25 mg/kg/day; C treated with TMZ 20 mg/kg/day; D pretreated with TMZ 20 mg/kg/day for 20 days, then for 120 days with TMZ 20 mg/kg/day and CyA 25 mg/kg/day. Biochemical markers were studied. Structural studies were investigated with hematoxylin-eosin and Mallory staining to evidence the renal fibrosis. Labeling was quantified using Image Pro Plus software (NIH). Renal ultrastructure was analyzed by transmission electron microscope (TEM). Expression Collagen I, Monocyte Chemoattractant Protein-1 (MCP-1) and Transforming Growth Factor beta-1(TGF β1) were investigated by immunohistochemistry using specific antibodies. The renal parenchyma from animals of group B showed mononuclear cell infiltration and increments of 20 % in renal fibrosis. Ultrastructure analysis showed edematous mitochondria with loss of its internal structure and positive immunoreaction for Collagen I, TGF β1 and MCP-1. Pretreatment with TMZ in group D showed preserved structural and ultrastructural studies and negative immunoreactions for Collagen I, TGF β1 and MCP-1.
TMZ prevents the incorporation of CyA to cell membranes and thereby preserves mitochondrial inner membranes and the structure and function of the Mitochondrial Respiratory Chain. TMZ reverses the pro-inflammatory process and expression of the molecules involved in the renal fibrosis.

Saliva: a new challenge for antiretrovirals detection? Insights from mass spectrometry

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Keywords: Saliva, Antiretroviral, UFLC-MS/MS, Bioequivalence.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Saliva contains only the non-protein-bound fraction of total drug and allows an adequate correlation between drug concentration and its pharmacological effect. The antiretroviral Efavirenz (EFV) needs constant monitoring and comparative relative bioavailability (RBA) studies of EFV in an alternative matrix required advanced technology.

The main objectives were: (i) to develop and to validate a simple method by UFLC-MS/MS useful for a RBA study with human saliva and (ii) to find a correlation between saliva and plasma results.

A C_{18} column (100 mm x 2.1 mm, i.d, 3 µm) was used. Elution was performed in an isocratic mode using: methanol–water (10:90 (v/v)), and 5mM ammonium formate in 97% MeOH as mobile phase. Sample preparation involved a simple protein precipitation anddilution.

An RBA study of 600 mg EFV tablets was performed in a single-dose, randomized-sequence, open-label, two-way crossover study, in 16healthy men. Saliva and plasma samples were taken at the same time. Pharmacokinetic parameters were calculated byWinNonlin®.

The method showed linearity (r>0.999) over the working range (1.00–99.85 ng/mL). Accuracy (85-115%) and precision (%CV<15) were according to the international bioequivalence criteria. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$for test and reference formulations were:(24.19 and 29.41) ng/mL,(539.82 and 795.24) ng/mL/h,(702.45 and 951.85) ng/mL/h, respectively. These results were comparable to those thrown by the study in plasma,which was performed by HPLC-UV-vis (λ=250 nm). The EFV concentration could be obtained in saliva by the relation: [Saliva EFV] = 0.009 [Plasma EFV].There was a goodlinear correlation (r =0.84) between the EFV concentrations in both fluids.

A rapid and accurate UFLC-MS/MS method was developed. Salivademonstrated to be a suitable surrogate to evaluate EFV tablet pharmacokinetics and it has been fairly comparable to plasma with excellent biosafety advantages for RBA and therapeutic drug monitoring studies.

Development and validation of a LC-MS/MS method for quantitative determination of Tenofovir, Emtricitabine and Lamivudine in Human Plasma, and its Application to a Bioequivalence Study of Tenofovir

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Keywords: Tenofovir, Lamivudine, Emtricitabine, Bioequivalence, LC-MS/MS.

A SPE-LC-MS/MS method has been developed and validated for simultaneous analysis in human plasma of one nucleotide Tenofovir disoproxil fumarate (TDF) and two nucleosides Emtricitabine (FTC) and Lamivudine (3TC) reverse transcriptase inhibitors. Plasma samples were prepared by solid-phase extraction (SPE) using tenofovir-d8 as internal standard, 0.7 mL plasma sample and Waters Oasis® MCX

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

cartridges. Chromatography was performed on a C-18 analytical column, the injection volume was 30 µL and the run time was 4 min.

The proposed method was specific, intra- and inter-day precisions were <10.8% with an accuracy within 87-108%. A linear dynamic range of 13.2–396.8 ng/mL, 10.8–5434.3 ng/mL and 10.9–5452.2 ng/mL was established for TDF, FTC and 3TC, respectively. The limits of quantitation were consistent with trough plasma concentrations, allowing its application for therapeutic drug monitoring and clinical pharmacokinetic study.

The validated method was applied to a bioequivalence study between a new pharmaceutical equivalent tablet formulation containing 300 mg of TDF and the innovator product. A randomized, single-center, open-label, single-dose, two-way crossover bioequivalence study in 24 healthy adult subjects was conducted. Dosing was separated by a wash-out period of 7 days. All subjects signed an informed consent form. In each study period, 17 blood samples were collected in Vacutainers™ containing EDTA over 48 h. Rate and extents of absorption were similar between products. The 90% confidence interval (CI) of the ratio of the geometric means for log-transformed C(max), AUC (last) and AUC (inf) values were used to assess bioequivalence between the two formulations using the equivalence interval of 80 and 125%. In healthy subjects, the point estimate and 90% CI of the ratios of Ln (Cmax), Ln (AUC last) and Ln (AUC inf) values for TDF were 90.9% (85.0-97.2%), 92.30% (87.2-97.7%) and 91.6% (85.5-98.1%), respectively. It was concluded that the new pharmaceutical formulation was bioequivalent to the innovator.

Antimutagenic and promutagenic activity of*Limonium brasiliense*(Boiss) Kuntze extract

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Keywords: *Limonium brasiliense*, antimutagenic, antipromutagenic activity

Limonium brasiliense Kuntze (Plumbaginaceae) is a medicinal plant, known as “guaycuru” from southern Argentina. Infusion from the roots is popularly used in the treatment of hemorrhage, menstrual disorders, genito-urinary infections and cardiac diseases.

The increasing research on the isolated active components from this plant reveals a large number of components in the roots, which have been the subject of many studies that show biological activity. To these active compounds are attributed antioxidant, and antiinflammatory properties. We have also found antimutagenic properties *in vitro* and *ex vivo*, being the main objective in the present study. The mutagenicity of the extract and the isolated components of *L.brasiliense* were evaluated using the Ames assay with strains *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of exogenous metabolic activation (S9 fraction from rat liver), at concentrations between 50 - 0.05 mg/mL.

In the tests without metabolic activation, the mutagens utilized were sodium azide (NaN_3), 2,4,7-trinitro-9-fluorenone (TNF), 4-nitro-0-phenylenediamine (NPD), 4-nitroquinoline *N*-oxide (4NQO),

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

methylmethanesulfonate (MMS) and mitomycin C, while in thetests with metabolic activation, the mutagen 2 amine acridine (2AA) was used. The entire assay was performed in triplicate.

The aqueous extract and the compounds isolated from the roots of *L.brasiliense* (myricitrin and epigallocatechingallate dimer (EGCG)), acted as strong antimutagens against the mutagens previously mentioned. In the absence of metabolic activation their inhibition resulted in a range from 61.76% to 86.02 %. While the inhibition of mutagenic effect with metabolic activation, resulted in a range from 68.93%-95.01%, calculated in the same concentration.

These results demonstrate that EGCG dimer, myricitrin and the aqueous extract from the roots of *L. brasiliense* are not mutagenic. They are able to reduce or inhibit the lesions in the DNA or mutations that induce cancer cells and diseases caused by genotoxic agents.

Cytokine gene expression and nitric oxide regulation of *Atriplex undulata* ethanolic extract in RAW 264.7 macrophage cells

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Keywords: *Atriplex undulata*, Cytokine, gene expression

Atriplex undulata (Moq) D. Dietr. (Chenopodiaceae) commonly known as "zampa crespa" or "cachiyuyo", is an endemic species that is found in Patagonia and the South of Buenos Aires province.

The anti-inflammatory activity and the chemical composition of the aerial parts of *A. undulata* have been evaluated. Ethanolic extract and different successive extracts showed significant anti-inflammatory activity. Moreover, ethanolic and ethyl acetate extracts exhibited better anti-inflammatory activity against interleukin-1β than the other extracts.

The ethyl acetate extract was fractionated by column chromatography over silica gel and HPLC, to isolate and purify the active compounds. These compounds were identified by RMN and evaluated for their inhibitory activity of nitric oxide (NO) production in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophage cells.

The free and glycosidated flavonoids of ethyl acetate extract have been isolated, such as kaempferol, quercetin, rutin, isorhamnetin and isorhamnetin-diglucopyranoside.

The flavonoid fractions and isolated compounds have been evaluated measuring release of interleukins (IL-1β), tumor necrosis factor (TNF-α), expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) by reverse transcriptase-polymerase chain reaction (RT-PCR).

In conclusion, this study demonstrated that the isolated components from the ethyl acetate extracts of *A. undulata* inhibit the production of NO, TNF-α, IL-1β in LPS-stimulated macrophages, in a dose dependent manner. The anti-inflammatory effect was also found to be achieved by the suppression of the production of iNOS and COX-2 inflammatory mediators. Interestingly, the flavonoid glycosides fractions elicited greater anti-inflammatory effect than the isolated compounds. This could be explained based on a

synergistic effect between the components of this extract. Therefore, this crude extract is a valuable source of anti-inflammatory compounds that has not yet been explored.

Safety and pharmacological evaluation of *Ziziphus mistol* arrope: anti-tussive and expectorant activities

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Keywords: *Pharmacology* evaluation, toxicity assays, ethnopharmacology

Ziziphus mistol (mistol) derivative product (arrope) has been traditionally used as food and folk medicine for the treatment of a wide variety of diseases including bronchopulmonary disorder.
The objective of this study is to evaluate the antitussive and expectorant effects and safety of arrope of mistol in male Wistar rats (200-250 g). The antitussive activity was evaluated using a model of ammonia induced cough in rats (positive control: codeine). The expectorant activity was evaluated by the volume of phenol red in rat's tracheas (positive control: bromhexine). The tested doses of arrope were of 500 and 1000 mg/kg, p.o. For the safety evaluation of arrope, a chronic toxicity test in rats (1 and 2 g/Kg, p.o.) was conducted during 90 days. The results show that the arrope of mistol at dose of 1000 mg / kg administered for three days, causes an increase in the latency period of the cough of 225.7% and a reduction in cough frequency of 79.68%. The antitussive effect would be due to the opioid properties of arrope. These results were previously determined when the arrope antinociceptive activity was tested in rats, using morphine and naloxone as a positive control and opioid antagonist respectively. The results show that mistol at doses of 1000 mg/kg significantly increased expectoration of bronchial secretions in the tracheal lumen in 85.5% compared to the negative control, while the bromhexine increased them by 89.1%. This study has shown that the arrope possess significant antitussive and expectorant effects and the toxicity assays suggested that arrope did not induce or cause any damage to the liver and kidney of rats and can be considered non toxic for these animals at tested doses. These findings seem to justify use of the plant in traditional medicine.

Structural interaction of dehydroleucodine with cyclooxigenase COX_1 and COX_2 in antiinflammatory mechanism

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Key words: dehydroleucodine, docking, COX, arthritis induced by Freund's adjuvant carrageenan

Previously, we have demonstrated that dehydroleucodine (DhL), a sesquiterpene lactone isolated from *Artemisia douglasiana* Besser (Asteraceae), prevented the inflammation in arthritis induced by Freund's adjuvant carrageenan (J. Ethnopharmacol. 2003; 88:195-8).

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Here we study the DhL effect to probe the structural basis of possible interaction with cyclooxigenase (COX_1 and COX_2).

Wistar rats were treated according Mizushima (1972) with doses of DhL (2.5 to 40.0 mg/kg). Pedal edema was determined by measuring the paw volume at 3, 5, 24, 48, 72 and 96 h after carrageenan administration using a plethysmograph and was expressed as the difference between both paw volumes. Inhibition percentage was calculated against the control (100% inflammation). Results obtained for the higher doses in acute phase were 22 and 34% (3 and 5 h), while in chronic phase were observed 53 and 41% (72, 48 h) of inflammation inhibition. DhL inhibited both chronic and acute adjuvant carrageenan-induced inflammation phases.

DhL dockings into the crystallographic structure of COX_1 and COX_2 were done using AUTODOCK4.

DhL docking with COX_1 showed that binding site is located at the mouth of active site (region C1-C7) acting like a cap in hydrophobic channel but not penetrate it, interacting hydrophobically with Leu357, Leu93, Tyr355 and Met113. Contrary DhL coupling with COX_2 showed that it binds to protein active site, a long and deep hydrophobic channel, occupying the same region as other NSAIDs occupy in the crystal structure of these complexes with COX_2. The different binding mode DhL with COX_1 and COX_2 inhibition would suggest that serious stronger in the case of COX_2.

Endothelial effects of magnetic nanocarriers composed by magnetite, oleic acid and chitosan intended to drug delivery devices

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Keywords: Magnetic nanoparticles, targeted drug direction, endothelial cells, viability.

Introduction. Magnetic nanoparticles (MNPs) are promising systems intended for target and delivery of several drugs due to their ability to orient the therapeutic agent to a desired part of the body by the application an external magnetic field. Two magnetic nanodevices were evaluated in endothelial cells (ECs) cultures to study cell viability. Formulations consist in a magnetic core composed by magnetite functionalized with oleic acid and recovered with crosslinked (N1) and non-crosslinked chitosan (N2).

Materials and Methods. Primary cultures of ECs were obtained from aortic strips of Wistar rats. Cell viability (measured by MTT assay) and nitric oxide (NO) production (Griess reaction) were evaluated in ECs exposed for 48 hs to aqueous dispersions of both MNPs in concentrations of 1, 10 and 100 μg MNPs/ml; respective controls (vehicle alone) were also processed.

Results

MNP(ug/mL medium)	NO production (nmol NO/μg protein)*
Control	0,021±0,005
N1(1)	0,023±0,002
N1(10)	0,024±0,003
N1(100)	0,020±0,005
N2(1)	0,018±0,004
N2(10)	0,022±0,002
N2(100)	0,021±0,002

*Results are expressed as media ± S.D.

Table 1 shows the results obtained for NO production and for MTT assay. The results obtained by MTT assay indicate no significant differences between viability of control cells and of those exposed to the MNPs.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

No statistical differences were found in NO production between control and both MNPs formulations. After treatment with MNPs, ECs response to the NO production agonist, Acetylcholine (AC) was assayed. The treatment (30 minutes) with 10μM AC induced NO production in the ECs treated with both MNPs (0,027±0,003; 0,065±0,008; 0,062±0,011; 0,055±0,006; Cont; 10μMAC, N1(100)+AC, N2(100)+AC nmol NO/mgProtein) $p<0,05$.

Conclusion. The study of cell viability on ECs exposed to both formulations of MNPs indicated that treatment with the studied concentrations do not affect EC viability and bioactivity, demonstrating that these MNPs do not result toxic for this cell line.

Amphetamine neuroadaptations involve neurocognitive alterations: angiotensin II AT_1 receptors role.

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Key words: Candesartan, Cold stress, Long term memory, c-fos.

The Renin-Angiotensin System (RAS) was first described as a peripheral hormonal system. Over the years several local RAS have been described in different organs. In the brain, the RAS is a well-documented neuromodulator of multiple brain circuits. AT_1 receptor (AT_1-R) activation is involved in learning and memory processes, in the stress response and in catecholamine neurotransmission.

Exposure to amphetamine (AMPH) induces neuroadaptations that modify behavioral responses to future pharmacological or environmental challenges. Alterations in working, recognition and long term memory have been reported in AMPH users.

Our aim was to evaluate the involvement of brain RAS, through its AT_1-R, on long term amphetamine-induced modifications in learning processes.

Wistar male rats (250-300g) treated with AT1-R blocker (candesartan 3 mg/kg p.o., days 1-5), followed by AMPH (2,5 mg/kg, ip, days 6-10), were evaluated 1 week later on the passive avoidance test after receiving a pharmacological (AMPH 0.5 mg/kg) or physiological (cold stress: 4ºC for 4 h) challenge. Following the test session, the animals were sacrificed and the brains were processed for c-fos immunoreactivity (fos-IR) as a marker of neuronal activation. The results were analyzed with Kruskal Wallis test (step trough latencies) and ANOVA (fos- IR).

The results indicated that history of repeated AMPH administration does not allow the expression of the deleterious effect in long term memory induced by acute exposure to AMPH or cold stress. In both situations the AT_1-R blockade prevented this modified response. C-fos expression in the hippocampus, indicates a diminished neuronal activation in animals receiving an AMPH or cold stress challenge in all studied groups.

In agreement with our previous findings, we conclude that AT1-R play an active role in AMPH-induced neuroadaptations altering learning and memory processes. It should be taken in consideration the potential use of AT1 receptor antagonist in the therapy of drug of abuse disorders.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Improvement of the anxiolytic-like effect of candesartan by its superoxide dismutase mimetic copper (II) complex

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Key words: Candesartan, AT1 receptors, anxiety

There is a large body of evidence, supporting a key role for Ang II in the stress response acting through its AT_1 receptors. It has been already found that candesartan cilexetil (AT_1 receptor blocker) administered orally has an anxiolytic effect when tested in the plus maze. Most of the sartans are composed of an appropriately substituted heterocyclic nucleus coupled to an acidic group (carboxylic or tetrazole) bearing biphenyl system through a methylene linker. Candesartan has the benzimidazole moiety substituted with carboxyl functional group at the 7-position. Modifications of the structure of candesartan by the generation of pharmacodynamic hybrids with antioxidants or NO releasing drugs showed to improve the biological effect of the sartans. Based on this evidence, it was undertaken the design of another class of hybrids by synthesizing coordination compounds with the biometal copper(II) and candesartan. The aim of the present work was to compare the anxiolytic-like effects of candesartan cilexetil (Cand) and the new candesartan complex $[Cu(Cand)(H_2O)_4]$(Cu-Cand). This compound was tested in vivo for the first time. For this purpose, Wistar male rats (250-320 g) were administered orally with Cand or Cu-Cand (3mg/kg), vehicle ($NaHCO_3$ 0.1N) or Cu during 5 days. On day 6 the animals were tested for 5 min in the plus maze to analyze the time spent in the open arms and extreme arrivals as anxiety index and total entries and closed arm entries as locomotor activity index. The data were analyzed using Anova. The results showed an increase in the anxiolytic parameters in the Cu-Cand respect Cand group. The group Cu was not significant from vehicle. Neither of the compounds affected the locomotor activity. Cu-Cand showed to be more effective as anxiolytic than Cand. This study provides a new insight into the development of copper (II) complexes as potential drugs.

Methotrexate increases the urinary excretion of the Organic Anion Transporter 5 in rats

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Key words: Methotrexate, Acute Kidney Injury, Organic Anion Transporter 5

Methotrexate (MTX) is used in the management of certain types of cancer as well as several auto-immune diseases. However the efficacy of this agent often is limited by severe side effects and toxic conditions. The development of acute kidney injury (AKI) following MTX administration remains a significant management challenge. Prompt recognition and treatment of MTX-induced renal dysfunction are essential to prevent potentially life-threatening MTX-associated toxicities, especially myelosuppression, mucositis, and dermatitis.

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Organic Anion Transporter 5 (Oat5) is expressed exclusively in the kidneys where it is found in the apical membrane of proximal tubule cells. Our group was pioneering in detecting Oat5 in urine. Preclinical animal results obtained in our laboratory proposed that Oat5 urinary excretion (Oat5u) might potentially serve as a non-invasive early biomarker of mercury, ischemic and cisplatin-induced AKI. Oat5u was elevated when no modifications of traditional markers of renal injury were still observed.

The aim of this study was to evaluate Oat5u in rats treated with a nephrotoxic dose of MTX. Adult male Wistar Rats were treated with a single intraperitoneal dose of MTX (360 mg/kg b.w.) 48 h before the experiments (MTX, n=3). A control group of animals was processed (C, n=8). Biochemical determinations were performed in plasma and urine samples. Oat5u was evaluated by immunoblotting. Statistical analysis: Student's t-test, (*) $p < 0.05$.

Results (media ± standard error):

	C	MTX
Kidney/Body weight ratio ($x10^{-3}$)	7,04±0,07	7,83±0,23*
Urine volume (ul/min/100g)	3,57±0,29	5,10±0,65*
Plasma urea (g/L)	0,27±0,01	1,19±0,12*
Plasma creatinine (mg/L)	5,79±0,20	8,17±0,88*
Creatinine clearance (mL/24h/100g b.w.)	868±34	560±49*
Urine alkaline phosphatase activity (UI/g creat)	117±5	742±143*
Urine Proteins (g/g creat.)	1,07±0,05	1,57±0,12*
Oat5u (%)	100±5	845±169*

Our results show a significant increase in Oat5u at a nephrotoxic dose of MTX. Further studies are required to evaluate if Oat5u could be proposed as a n early biomarker of MTX-induced AKI.

Urinary excretion and renal expression of caveolin-2 in rats with cisplatin induced acute kidney injury

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Keywords: Caveolin, Cisplatin, Acute Kidney Injury

Caveolins belongs to a family of integral membrane proteins that are components of caveolar-type lipid raft domains. Caveolins transport macromolecules, compartmentalize signaling molecules, and are involved in various repair processes. Dysregulation of caveolae raft microdomain expression is involved in the induction and maintenance phases of ischemic and toxic forms of experimental AKI. Cisplatin (cispt) is a widely used chemotherapeutic agent but nephrotoxicity limits its clinical application. The aim of this study was to evaluate Cav2 renal expression and urinary excretion in a preclinical model of cispt induced AKI. Adult male Wistar rats were treated with a single injection (i.p.) of cispt at 5 mg/kg body weight (T, n=6) and controls rats (C, n=16) received the cispt vehicle. The studies were performed 4 days after treatment. 24h urines were collected to determine urine output and to obtain urinary exosomes by ultracentrifugation. Plasma samples and renal tissue were also collected. To corroborate cispt AKI model, creatinine clearance (Clcr) was estimated by conventional formulae and creatinine levels in plasma and urine were determinate spectrophotometrically. Cav2 in urinary exosomes (Cav2ue), and Cav2 in renal homogenate samples (Cav2H) and in apical membranes (Cav2Mb) were evaluated by immunoblotting.

RICiFa 3era Reunión Internacional de Ciencias Farmacéuticas

Statistical analysis: Student´s t-test, (*) p< 0.05. Results: ClCr(mL/24h/100g): C=656±45, T=25±13*; Cav2H(%): C=100±5, T=125±6*; Cav2Mb(%): C=100±3, T=24±1*; Cav2ue(%): C=100±11, T=270±33*.

Cav2 was found significantly decreased in apical membranes and increased in renal homogenates. Modifications in Cav2 expression could be involved in renal damage observed in cisplatin treated animals. To our knowledge, this is the first time that Cav2 is found in urinary exosomes. The significant increase observed in Cav2ue after cispt treatment could lets us to propose it as a novel non-invasive biomarker of cispt induced AKI.

Impact of systemic cyanamide administration in developmentally low-level lead-exposed rats: ethanol motivational and stimulant effects and enzymatic biomarkers

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Keywords: Lead exposure – Alcohol – Acetaldehyde - Cyanamide

We have demonstrated that the developmental neurotoxicant lead (Pb) increases ethanol intake in a free-choice paradigm, which is associated with hyperlocomotion assessed immediately thereafter. Peripheral ethanol oxidation to acetaldehyde is exerted by ADH (alcohol dehydrogenase) -with catalase (CAT) playing a minor role- while ALDH2 (aldehyde dehydrogenase 2) is involved in acetaldehyde oxidation to acetate. Cyanamide (CYAN), an ALDH2 inhibitor (which also produces slight CAT inhibition) is prescribed to treat alcoholism in several countries due to peripheral toxic acetaldehyde accumulation. Thus, in the present study we sought to determine whether systemically-administered CYAN blunt the elevated ethanol intake and associated hyperlocomotion evidenced in Pb-exposed animals. Male thirty-five day-old rats exposed to Pb (220 ppm) or tap water (control group: C) through gestation and lactation were offered four bottles, two containing water and the other two increasing ethanol concentrations (2% to 10%) in a 2-h free-choice test. On test day 25, once 10% ethanol intake was stabilized and a higher ethanol intake was evident in Pb-exposed animals, they were injected with saline (SAL) or CYAN (25 mg/kg i.p.) thirty minutes before the last four ethanol intake sessions. This was followed by a locomotor activity test and decapitation thereafter to collect blood, brain and liver tissue to determine CAT and ALDH activities by spectrophotometric analysis. CAT activity was measured by the H_2O_2 disappearance at 240 nm, while ALDH2 activity was determined by NADH formation at 340 nm. The behavioral data revealed that ethanol intake (mg/kg) was significantly reduced in the Pb-exposed animals as a consequence of CYAN administration: C-SAL: 0.70$\pm$0.09; C-CYAN: 0.70$\pm$0.14; Pb-SAL: 2.03$\pm$0.18; Pb-CYAN: 0.89$\pm$0.08. In the same line, ethanol-induced locomotor activity was considerably abrogated in Pb-exposed rats. Preliminary results indicate the absence of differences in brain and blood CAT activity; ALDH in brain seemed to be reduced only in the cerebellum in both, control and Pb-exposed animals. We postulate that peripheral acetaldehyde accumulation produces aversive symptoms that can account for the reduction in the excessive ethanol intake observed in developmental Pb-exposed rats, a finding that provides predictive validity to our model.

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