REAL TIME IN VIVO DETECTION OF CELLULAR SENESCENCE THROUGH THE CONTROLLED RELEASE OF THE NIR FLUORESCENT DYE NILE BLUE

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Introduction: Cellular senescence is a stable state of cell cycle arrest necessary for maintaining the organism homeostasis. Evidence supports that accumulation of senescent cells is involved in the pathophysiology of many age-related diseases. Senescence state involve change in morphological and physiological cell characteristics. One of the most widely used markers is the overexpression of lysosomal β-galactosidase. However, one general drawback in the use of probe for the senescence detection, even in realistic senescence models, detection is only possible after the animal sacrifice. Consequently, the development of suitable methods for *in vivo* senescence detection remains an unresolved problem. NB chromo-fluorophore has several remarkable features as *in vivo* imaging agent. NB is an organic dye approved by the Food and Drug Administration (FDA) for human use and exhibits near infrared (NIR) emission at 672 nm, which avoids auto-fluorescence background in cells and tissues. Moreover, NB is an aromatic planar fluorophore which is highly quenched at high concentrations or in confined spaces

Objectives: The development of mesoporous silica nanoparticles (MSN) system loaded with the NIR-FDA approved Nile blue (NB) dye and capped with a galactohexasaccharide (**S3**) for the real time *in vivo* detection of senescent cells.

Materials and Methods: Final probe **(S3**) is easily prepared from MSN (**S0**), which are loaded with NB (**S1**), externally functionalized with APTES (**S2**) and finally capped with β(1,4)-hexagalacto-saccharide. The mesoporous structure of **S3** and the starting material (**S0**) was clearly observed by HR-TEM and by powder X-ray diffraction. Surface area, pore volume and pore size of initial **S0** material was reduced in **S3** due to the loading of the pores with NB and grafting of the galacto-saccharide. DLS measurements showed an increase in the hydrodynamic nanoparticle diameter, from 144 nm for **S0** to 282 nm for **S3**. Moreover, the content of NB in **S3** was determined to be 0.45 mmol g^{-1} of solid

Results and Discussion: The prepared **S3** MSN are therefore poorly emissive, yet NB release in senescent cells is expected to result in marked emission enhancement in the NIR zone. In vitro studies demonstrated a remarkable enhanced emission observed in palbociclib-treated SK-Mel-103 and 4T1 senescent cells and incubated with **S3** due to the delivery of the NB dye in comparison with control SK-Mel-103 and 4T1 cells. **S3** was *in vivo* validated in BALB/cByJ female mice orthotopically injected with 4T1 cells to generate breast tumours and treated with palbociclib. In vivo IVIS images showed a remarkable emission enhancement (4.3 fold) in tumours in mice treated with palbociclib and intravenously injected with **S3**, whereas negligible signal was found in control mice. Ex vivo IVIS images showed that fluorescence ascribed to NB was only observed in senescent tumours (ca. 17.6-fold enhancement) but not in control tumours or other organs.

Conclusions: As summary, we describe the use of capped MSN loaded with FDA approved NB and capped with a galacto-oligosaccharide for the real time *in vivo* detection of cellular senescence. The performance in terms of selectivity and sensitivity makes **S3** and efficient OFF-ON probe for the in vivo detection of senescence.

Structural and physicochemical characterization of amphiphilic cyclodextrin derivatives as a nanoparticulate drug delivery platform

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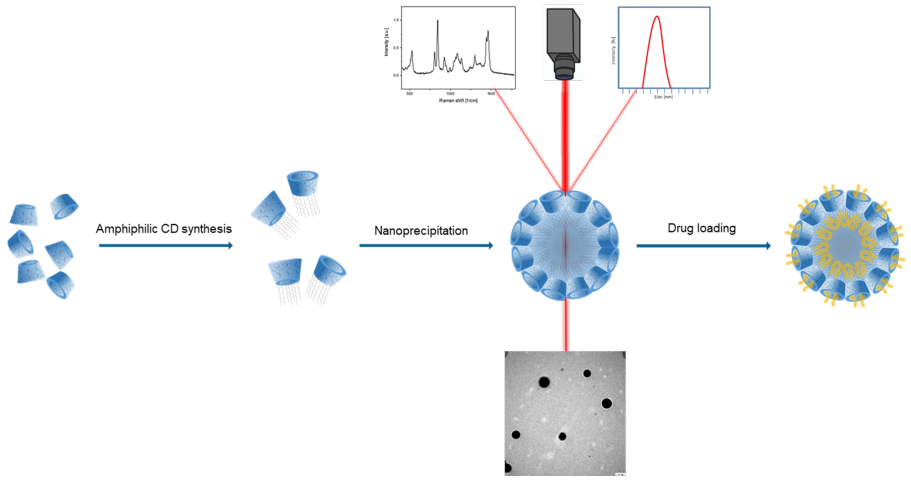
Introduction: Cyclodextrins (CDs) are natural and biocompatible polysaccharides. They are widely used as pharmaceutical excipients to increase drug solubility, stability as well as bioavailability by formation of inclusion complexes. By grafting aliphatic chains onto the CD surface, the amphiphilicity of CDs can be increased. Such amphiphilic CD derivatives self-assemble into nanoscale structures, such as micelles, vesicles, or particles. These nanocarriers are promising platforms for various biomedical applications including drug, gene as well as biomacromolecule delivery, providing high encapsulation efficiency, increased drug stability, sustained drug release, and high drug bioavailability.
Objectives: We aimed at identifying the critical physicochemical properties of CD derivatives that determine their self-assembly and drug encapsulation properties. For this purpose, amphiphilic CD derivatives were synthesized and physicochemically characterized. Further, the self-assembly properties of the derivatives were investigated with respect to their potential as a nanocarrier platform for hydrophobic compounds.
Materials and Methods: Amphiphilic CDs were synthesized in a one-step approach by grafting aliphatic chains of different lengths onto native β-cyclodextrin. Mass spectrometry (MS), nuclear magnet resonance spectroscopy (NMR), Raman spectroscopy, and attenuated total reflection infrared spectroscopy (ATR-IR) were used to characterize the physicochemical properties of the synthesized derivatives. Nanoparticles were prepared by nanoprecipitation and were characterized using dynamic light scattering (DLS), electrophoretic light scattering (ELS), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Particle stability was investigated over time upon storage at 4°C. The amount of drug encapsulated in the nanoparticles was quantified by high-performance liquid chromatography and loading capacity (LC) and encapsulation efficiency (EE) were calculated.
Results and Discussion: The successful synthesis of the amphiphilic CD derivates with a yield of at least 70%, was verified by ^{13}C NMR, ^{1}H-NMR, and MS, as well as ATR-IR- and Raman-spectroscopy. MS data of the derivatives proofed complete derivatization of the seven primary OH-groups. All derivates exhibited spontaneous self-assembly into nanosized structures with a homogenous size distribution (PDI < 0.2), hydrodynamic diameters below 250 nm, and zeta potentials below -20 mV upon nanoprecipitation. SEM and TEM images visualized spherically shaped nanoparticles. A hydrophobic model drug was successfully loaded into the nanocarriers in high EE of at least 70%. Nanoparticles were stable for up to 3 months. The aliphatic chain length was identified as a critical key characteristic of the derivatives determining particle size, surface charge as well as EE.
Conclusions: In the current study, we gained a deep understanding of the influence of physicochemical properties of amphiphilic CD derivatives on self-assembly and drug encapsulation into nanocarriers. The amphiphilic CD nanocarrier platform offers great potential for the encapsulation, protection, and delivery of hydrophobic drug molecules. Further investigations with different drug molecules, *in vitro* and *in vivo* experiments will be carried out to evaluate the whole potential of cyclodextrin-based nanoparticles in drug delivery.



COMPARISON OF OCULAR FAMCICLOVIR FORMULATIONS FOR THE TREATMENT OF HERPES ZOSTER

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Introduction: Herpes Zoster (HZ) is a neuropathic viral disease caused by herpesvirus 3. This virus may stay dormant during years, although many factors are able to reactivate it in the ganglion root. The clinical manifestations usually include severe skin rashes[1]and it is estimated that 50-70% of the patients suffer direct ocular affection[2].

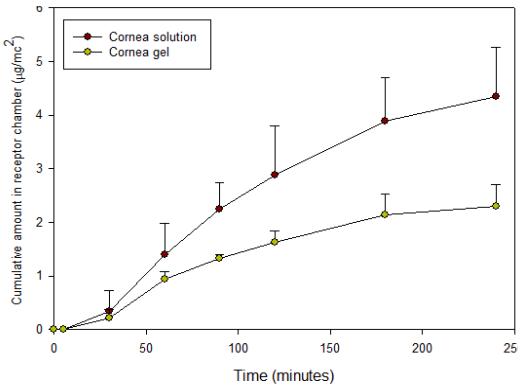
Oral aciclovir is the standard treatment for ocular HZ in spite of its low bioavailability and adverse reactions. Famciclovir (FCV) is a drug that is also used to treat HZ, it has higher bioavailability and can also treat HZ complications like Post-herpetic Neuralgia. Currently FCV is only administered orally[3]. As there are not FCV ocular formulations available, ocular irritation needs to be evaluated.

Objectives: The principal objective of this project is the elaboration of ocular formulations of FCV: a gel and solution. Both formulations will be compared using ocular diffusion studies using rabbit eyes. Additionally, the Hen´s Egg Test on the chorioallantoic membrane (HET-CAM) assay will be used to determine the potential ocular irritancy of the formulations under evaluation.

Materials and Methods: HET-CAM test was performed using fertile chicken eggs. They were incubated for 10 days at 37ºC, and then the formulations were analysed.

Both formulations were compared using diffusion studies using Franz cells with rabbit corneas as membrane. 200µL samples were extracted at different times from the receptor compartment of the cell. Samples were analysed by High Performance Liquid Chromatography, with the objective of knowing which formulation allows a higher permeation of FCV through the eye structure. Corneas previously used in the diffusion test were introduced in an extraction solution, with the aim of analysing the amount of FCV released from ocular tissue.

Results and Discussion: HET-CAM showed a negative ocular irritancy in both formulations. The table below shows the amount of FCV that permeates rabbit's cornea, which is greater when applied in solution than gel. The amount of FCV retained in cornea was again higher when applied as a solution than as a gel.



Conclusions: Irritancy studies show the possibility of incorporating these formulations in the eye. FCV applied both as solution and as a gel penetrates the eye and are retained in the rabbit cornea Penetration through and retention in the cornea are greater when FCV is applied in solution.

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A NEW NANOPROBE FOR THE SENSITIVE DETECTION OF BENZENE METABOLITE T,T-MUCONIC ACID IN URINE

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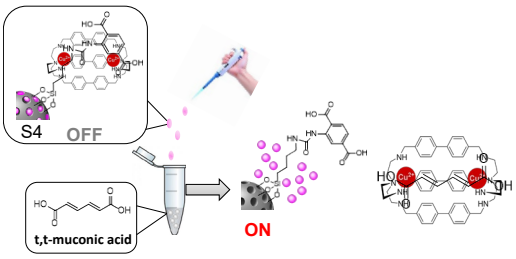
Introduction: Benzene is a highly toxic aromatic hydrocarbon. Inhaling benzene can cause dizziness, vertigo, headaches, aplasia, mutations and, in the most extreme cases, cancer. Trans, trans-muconic acid (t,t-MA) is one of the metabolization products of benzene. Although different analytical methods have been reported for the determination of t,t-MA, these are often expensive, require trained personnel, are not suitable for on-site measurements, and use hazardous organic solvents. For these reasons, the development of reliable, selective and sensitive methods for rapid and in situ detection of t,t-MA are of importance.
Objectives: The development of reliable, selective and sensitive material for the rapid and in situ detection of t,t-MA in urine.
Materials and Methods: MCM-41 nanoparticles (NPs) were prepared following well-known procedures using tetraethylorthosilicate (TEOS) as silica precursor and hexadecyltrimethylammonium bromide (CTAB) as a micellar template. The as-made solid was then calcined at 550^{0}C to obtain the starting NPs with empty pores (**S0**). Surface NPs was functionalized with a derivative of terephthalic acid (**S1**) and subsequently deprotonated (**S2**). Pores were loaded with sulphordamine B (**S3**). The final material (**S4**) was obtained after stirring an acetonitrile suspension of **S3** and Cu_2-bistren complex previously synthesized. Prepared solids were characterized using standard techniques, e.g. powder X-ray diffraction (PXRD), transmission electron microscopy (TEM), porosimetry and elemental and thermogravimetric analyses**.**
Results and Discussion: We describe a new nanoprobe based on gated mesoporous silica NPs for the selective and sensitive t,t-MA detection in buffered aqueous solution and in urine. The sensing mechanism arises from a displacement reaction by the formation of an inclusion complex between t,t-MA and the Cu_2-bistren complex that results in cargo delivery. Pore opening and payload release is selectively induced with t,t-MA, whereas this is not observed for other dicarboxylates. A limit of detection for t,t-MA as low as 0.027 mM in HEPES is determined. Finally, **S4** demonstrate to effectively determine the concentration of t,t-MA in spiked urine samples.



Scheme 1. In the presence of t,t-MA, the dicopper(II) bistren azacryptand is dethreaded with subsequent pore opening and SRh B release.
Conclusions: The nanodevice used is achieved using mesoporous silica nanoparticles loaded with a dye reporter and capped with a dicopper(II) azacryptand. Pore opening and payload release is induced rapidly (10 min) and selectively with t,t-MA in urine, using a simple fluorimeter without sample pretreatment. This, or similar nanoparticles, could be useful for an effective control of benzene exposure in environments in which this highly toxic pollutant is used.

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TARGETED THERAPIES FOR THE EFFECTIVE TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER BY COMBINING SENESCENCE-INDUCING CHEMOTHERAPY WITH SENOLYTICS

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Introduction: Triple-negative breast cancer (TNBC) represents an aggressive type of breast cancer subtype with a poor prognosis and limited effective therapeutic options. The induction of senescence, a persistent state of cell cycle arrest, has been explored as a barrier against tumour progression and is used as a therapeutic option for breast cancer patients. However, some patients still relapse, perhaps due to the subsequent accumulation of senescent cells in the body that can promote tumor recurrence.

Objectives: In this study, we explored the combination of senescence induction (senogenesis) and the subsequent elimination of senescent cells (senolysis) as an alternative approach to improve outcomes in TNBC patients. To this end, senescence-inducing compounds have been developed, including cyclin-dependent kinase (CDKs) inhibitors such as palbociclib and among senolytic drugs, navitoclax has shown effectiveness in vivo in reducing cancer relapse and delaying the onset of aging diseases.

Materials and Methods: We have performed two targeted strategies to allow the specific release of the drugs in the target cells as well as minimize the associated side effects. On one hand, we developed a galacto-conjugated navitoclax (Nav-Gal) as a senolytic pro-drug that can preferentially be activated by β-galactosidase overexpressed in senescent cells. On the other hand, the effect of the treatment using nanoparticles that communicate by modifying the environment (stigmergy) was evaluated. First, a palbociclib-loaded nanoparticle specifically targeted to MDA-MB-231 breast cancer will induce senescence in the tumor. Once senescence has been established, a second nanoparticle loaded with the senolytic navitoclax and capped with β(1,4)-galacto-oligosaccharides, for specific drug release in senescent cells, will kill the senescent tumor cells.

Results and Discussion: We demonstrate that a combination treatment using palbociclib and navitoclax, selectively eliminates senescent cells, delays tumor growth, and reduces metastases in a mouse model of aggressive human TNBC. Concomitant treatment with palbociclib and Nav-Gal or the two nanoparticles in vivo results in the eradication of senescent human TNBC cells with the subsequent reduction of tumour growth while reducing the cytotoxicity of navitoclax.

Conclusions: Collectively, our results support the effectiveness of combined senescence-inducing therapies with senotherapies for human TNBC as well as the development of targeted approaches as effective and safer therapeutic opportunities.

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SELECTIVE DUALPLEX LATERAL FLOW ASSAY FOR SIMULTANEOUS SCOPOLAMINE AND CANNIBAL DRUG DETECTION BASED ON RECEPTOR-GATED MESOPOROUS NANOPARTICLES

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Introduction: Drug facilitated sexual assaults (chemical submission) consists in the criminal administration of psychoactive substances without the permission of the victim. Among these compounds, scopolamine (SCP), commonly well-known as burundanga, has emerged as the perfect psychotropic substance for aggressors. Physiologically, an appealing feature of SCP is their relevant role as a non-competitive muscarinic acetylcholine receptor antagonist with rapid and robust antidepressant effects in humans and other species.[1] Particularly, SCP is able to block or dampens the M_2-AChR biological response owing to the binding to an allosteric M_2-AChR site that is separate from the active recognition site of acetylcholine, thereby inhibiting the effects of the natural substrate and even of some potent agonists such as bethanechol.[2]
Objectives: Synthesis, characterization, and evaluation of a nanosensor based on MSNs with M_2-AChR for scopolamine *in situ* detection in saliva using a dualplex lateral-flow assay.
Materials and Methods: Fluorescence spectroscopy measurements were taken on a Fluoromax4 from HORIBA Scientific. Photographs were taken with a Samsung Galaxy S7. *Solvents:* All solvents were ACS reagent grade or better quality and were used without any further purification. *Chemicals reagents:* M_2-AChR was purchased from Abcam.
Results and Discussion: The nanosensor consists of MSNs loaded with rhodamine B and functionalised on the external surface with a bethanechol derivative. In addition, the loaded system is finally capped by interaction between the grafted bethanechol derivative and M_2-AChR. The sensing mechanism was confirmed from kinetics release experiments where a remarkable enhancement in the fluorogenic response is only observed in the presence of SCP, which allows releasing entrapped dye molecules from the pores when M_2-AChR is displaced. Moreover, the probe shows a highly sensitivity in buffer solution (LOD of 92 μM) and in a competitive environment such as saliva (LOD of 103 μM). Other drugs (such as cocaine, heroin, morphine, MDMA and MDPV) induce a negligible cargo release. Therefore, the material was incorporated into strips for lateral-flow assays coupled to smartphone readout while guaranteeing fast overall assay times of <15 min. The assay has fast response times, good selectivity, and exceptional sensitivity, reaching a LOD as low as 0.04 μM in aqueous solution and of 18.7 μM in extracted saliva samples. Finally, a dualplex lateral flow assay capable of detecting *in situ* and *at site* SCP and cannibal drug was developed.
Conclusions: A straightforward portable strip in combination with bio-inspired hybrid nanomaterials for SCP drug *in situ* and *at site* detection was developed. The strips allow direct identification of SCP in diluted saliva down to 40 nM in less than 15 min using a smartphone for readout. Additionally, to a single-track strip, the extraordinary modularity of the hybrid biosensor materials in combination with the strip-pattering technologies enabled us to obtain a dual-channel strip for SCP and cannibal drug detection in a simple way, providing comparable analytical performance, while enabling further tailoring to the user's needs.

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GHB SENSING IN STRIPS BY LATERAL FLOW TEST USING A DYE-DISPLACEMENT ASSAY

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Introduction: Gamma-hydroxybutyric acid (GHB) is a natural metabolite, which is currently used as a date rape drug. Particularly, GHB is rapidly eliminated and its detection in realistic environments is only possible within 6–12 h after ingestion. Owing to the increasing use of GHB for criminals purposes, a need has arisen to develop simple and *in situ* efficient assays for its identification in aqueous and alcoholic drinks. Based on the above, we report herein an indicator displacement assay (*IDA*) for GHB detection that consists of a Cu^{2+} complex with a tetradentate ligand and the fluorescent dye, coumarin 343. Likewise, copper complex was incorporated into a coated PEG-glass fibre (PEG-GF) membrane to obtain a highly robust and sensitive lateral flow assay for GHB detection in diluted gin in less than 1 min coupled to smartphone readout.
Objectives: Synthesis and evaluation of a colorimetric sensor for GHB detection in soft and alcoholic beverages using a lateral flow assay.
Materials and Methods: Copper (II) trifluoromethanesulfonate was purchased from Acros Organics. Fluorescence spectroscopy measurements were taken on a JASCO FP-8300 spectrofluorometer. Photographs were taken with a Samsung Galaxy S7.
Results and Discussion: Firstly, the tetradentate ligand was synthesized by reductive amination of a 1,2-cyclohexyl diamine with 2-quinolinecarboxaldehyde. Subsequently, this ligand was reacted with $Cu(CF_3SO_3)_2$ to obtain the corresponding copper complex, which generates the final sensing ensemble by coordinating with coumarin 343. The sensing mechanism relies on a displacement of coumarin 343 from the sensing ensemble as a consequence of the higher binding constant between GHB and complex, showing a high sensitivity in MES buffer (50 mM, pH 6.0) (detection limit of 0.03 μM). System design and optimization led to a straightforward integration into a lateral-flow assay without further treatment or conditioning of the test strips while guaranteeing fast overall assay times of 1 min (Figure 1). Finally, we demonstrated the remarkable robustness of the probe that is able to detect GHB in spiked alcoholic drinks with a detection limit of 0.1 μM.

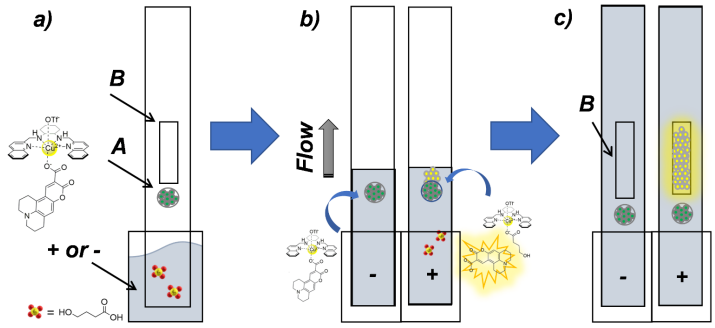

Figure 1. Design of the lateral flow assay on strip with copper complex integrated into a coated PEG-glass fibre membrane.

Conclusions: A rapid and sensitive copper complex integrated to PEG-GF strips was developed for GHB detection in diluted gin in less than 1 min. The lateral flow assay approach using mobile phones for fluorescence measurements offers a promising methodology for the construction of rapid test kits for practical applications such as roadside drug testing and the detection of substances in the workplace or recreational settings.

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Organic Molecular Cages as Drug-Delivery Systems

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Supramolecular chemistry has as objective to replicate biology with designed synthetic systems that are not found in nature and that can intervene in biological functions. In this context, the use of organic molecular cages as drug-delivery systems supposes an innovative application of supramolecular chemistry that has gained an increasing interest in recent years.[1] Molecular cages are defined as supramolecular entities that possess a shape-persistent structure with permanent, accessible cavities that can contain guest molecules like drugs.[2]

The formation of molecular cages is promoted by the molecular recognition and the self-assembly multiple molecules of two building blocks, an aldehyde and an amine derivative, by noncovalent interactions that under thermodynamic control constitute well-defined aggregates.[3,4] Among other molecular cages, imine-based architectures seem to be the most innovative since the lability of imine bonds leads to the most stable assemblies and allows the release of the guest molecules by a wide variety of stimuli.[5]

In this sense, the use of imine-based molecular cages as drug-delivery systems opens the possibility to contain a wide variety of drugs that can be isolated from the harsh physiological conditions and release the drug by a specific stimulus in the desired location.[6] Therefore, this communication will describe the design, synthesis, and potential uses of molecular cages as innovative drug-delivery systems.

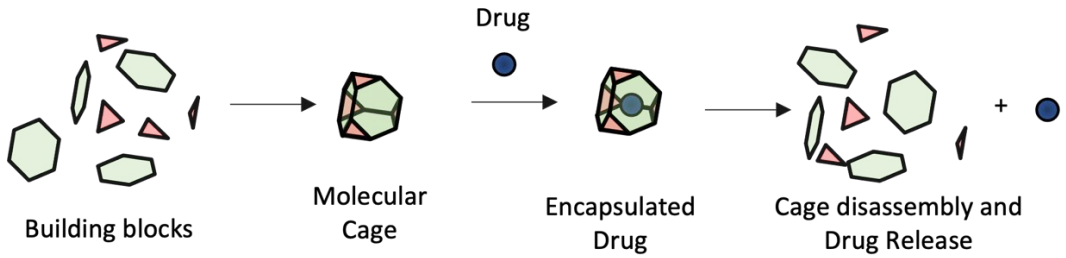

Figure 1. Schematic process of the self-assembly of a molecular cage from their building blocks followed by the encapsulation of a drug and the release by the disassembly of the cage by a specific stimulus.

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TESTING A NEW MULTI-LAYERED DRESSING TO CONTROL *PSEUDOMONAS AERUGINOSA*

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Introduction: Many medical advances have been made possible thanks to *in vitro* and *in vivo* investigations. To study biofilms in wound-like settings these models are required and ideal to collect data for the development of a better wound care formulation. The development of antibiotic resistance by the wound infecting bacteria is a serious issue when using antibiotics. In chronic wounds, *P. aeruginosa* is the most common pathogen capable of forming biofilms, which makes *P. aeruginosa*-infected wounds difficult or impossible to treat with antibiotic therapy [1]. Thus, to overcome antibiotics' ineffectiveness, other antimicrobials are being used, for instance bacteriophages (phages) that have an active killing capability against multidrug-resistant and biofilm-forming bacteria [2]. Additionally, local distribution of drugs in the form of dressings is more convenient than systemic treatment since it delivers a higher concentration of the medicine to the intended location and is less likely to cause bacterial resistance [3].
Objectives: This work aims to find a solution for the control of *P. aeruginosa*-infected wounds by the development of a multi-layered dressing containing phages and poly-L-lysine.
Materials and Methods: The gelatin-alginate (GA) film was prepared by dissolving different amounts of gelatin and alginate, as well as glycerol to improve the film's flexibility. A suspension of phage (1×10^{10} PFU/mL) in SM buffer was added and homogenized to ensure an even distribution of phage on the GA films. The mixture was poured and dried at 40ºC for 24h. After, the film was subjected to $CaCl_2$ crosslink and redried. The gelatin (G) film was prepared by dissolving different concentrations of gelatin and glycerol. Phages and PLL were added. The solution was poured and dried at 40ºC for 24h. All the films were kept in desiccators at 53% relative humidity (RH) and 20ºC. The multi-layered dressing was made by layering the two separate films with the help of 1% carboximetilcellulose. The inhibitory and eradicative effect of the two-layered membrane against *P. aeruginosa* biofilms formed in an *in vitro* biofilm wound model was next studied. In brief, Ø=1 cm membrane samples were placed in wells with artificial dermis immediately after inoculation (inhibition) or in 24h biofilms (eradication), and the wound model was incubated at 37°C for 24h. The complete contents of each well were taken, put in 9 mL physiological water, and serial dilutions were performed and plated for CFU determination.
Results and Discussion: All the phage and PLL loaded membranes demonstrated a significant biofilm inhibitory activity (2.38 log reduction). Moreover, these membranes had a great eradication activity against 24h biofilms with a reduction of 99.7%.
Conclusions: The multi-layered dressing herein described has a great potential for the control of *P. aeruginosa*-infected wounds. Furthermore, this newly developed dressing is cost-effective, and user-friendly which makes it more appealing for patients and pharma industry.

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A REVIEW OF EXTENDED-RELEASE TRANSDERMAL MICRONEEDLE FORMULATIONS

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Introduction: It is estimated that only 50% of patients with chronic diseases adhere to their long-term medication (Sabaté and World Health Organization, 2003). There are several reasons for this, such as complex treatment regimens, prematurely stopping treatment, following dosing instructions incorrectly etc. (Hugtenburg *et al.*, 2013). Extended-release formulations can reduce the dosing frequency, by sustaining the plasma drug concentration over longer durations. This makes the treatment regimen less onerous for the patient, thus promoting medication adherence. In addition they can reduce side effects due to the maintenance of plasma drug concentrations. (Ingersoll and Cohen, 2008)(Aulton, 2018) A transdermal microneedle patch comprises micron-sized projections/needles attached to the patch. Developed to administer drugs across the skin surface into the dermal microcirculation, transdermal microneedle patches can offer many clinical benefits, including the ability to deliver macromolecules that conventionally have required parenteral injections, whilst being easy and painless to administer, unlike parenteral injections. (Donnelly, Raj Singh and Woolfson, 2010)

Objectives: To evaluate the scientific literature on extended-release microneedle formulations with a view to informing the development of a novel, self-administrable microneedle platform for prolonged transdermal drug delivery.

Methods: Peer-reviewed scientific publications were retrieved from online databases, including PubMed and Web of Science. A narrative review was constructed based on the reading and interpretation of these publications.

Results and discussion: The majority of extended-release microneedle formulations are based on polymeric materials. Desired polymer properties include biocompatibility, biodegradability, ready availability, cost effectiveness and tunability. The mechanisms of extended drug release include slow biodegradation and swelling of the polymers to slow drug diffusion from the polymer matrix. A variety of polymers have been explored in formulating extended-release microneedles including poly(lactic-co-glycolic) acid, polylactic acid, polycaprolactone, silk fibroin and chitosan. The drug release profile can be finetuned by adjusting the physicochemical properties of the polymers such as molecular weight and substituent groups. Such polymer-based microneedle patches can achieve extended drug release from days to weeks, with potential applications across various disease states, including in the delivery of contraceptives and diabetic medications. Continued skin adhesion and drug loading capacity are potential challenges for extended-release microneedle patches. (Singh *et al.*, 2019) (Pahal *et al.*, 2021)

Conclusion: To date, research into extended-release microneedle patch formulations has produced promising results. The existing scientific literature in this area provides a realistic benchmark for evaluating the extended-release capabilities of the novel, self-administration microneedle platform being developed in this project.

Microemulsion of progesterone for retinitis pigmentosa: Comparison of ocular diffusion between different animal species

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www.uchceu.es/

Introduction: Retinitis pigmentosa (RP) is the most frequent retinal hereditary degenerative disease, which affects visual photoreceptors and it usually produces blindness. An effective treatment is still in investigation, but some authors have described that progesterone (PG) could be used to slow down the progression of the disease[1]. Microemulsions (ME) are thermodynamically stable systems composed of oil, water and surfactant mixtures (and/or co-surfactant). ME are capable to solubility of PG (a very hydrophobic drug[2]) can be enhanced by means of ME.
Objectives: The main objective was to elaborate ME formulations with PG and to compare ocular diffusion of PG from ME through cornea and sclera of different animal species: sheep and rabbit.
Materials and Methods: For ME preparations the right amounts of mixture surfactant (labrasol) and co-surfactant (transcutol HP) were dissolved in the oily phase (lauroglycol FCC) and then bidistilled water was added. Diffusion studies of ME were performed using Franz cells using cornea and sclera (from rabbit and sheep) as membranes. Propyleneglycol: Water (40: 60% w / w) pH 7.4 was filled in the receptor chamber. During the 240 minutes assay, samples (0.2 mL) were taken manually from the receptor chamber at standard times. PG in the samples was quantified by HPLC-UV. [Column C18 (150 x 4.6mm), Mobile phase Acetonitrile: Water (80:20), flux 1mL/min, volume injection 50 µL, UV detection 240 nm].
Results and Discussion: Accumulated amounts of PG in receptor chamber at 4 hours were 13.49 ± 1.52 and 7.09 ± 0.92 µg/cm^2 in rabbit´s corneas and scleras respectively and 10.09 ± 0.78 and 10.14 ± 1.77 µg/cm^2 in sheep´s in corneas and scleras respectively. Figure 1 show the Kp of PG. Similar results are obtained in cornea and sclera for each species. However, the permeability coefficient was approximately double in rabbit than in sheep. These results coincide with the thickness of both membranes: sheep's cornea and sclera are twice as thick as rabbit´s.

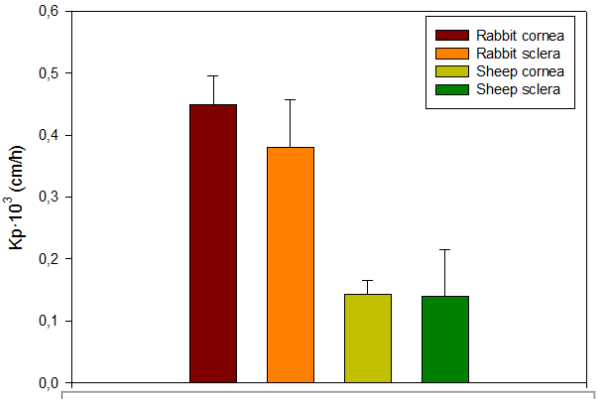

Figure 1. Permeability coefficient (Kp, cm/s) of PG loaded in ME through rabbit and sheep cornea and sclera. (n=6).

Conclusions: PG can be loaded in ME. Statistically significant differences were found in the diffusion of PG. Permeation was twice as high in rabbit than sheep.
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EVALUATION OF RIFAMPICIN RELEASE FROM MICROPARTICLES LOADED IN COATING HYDROGELS FOR ORTHOPEDIC PROSTHESES.

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Introduction: Prosthetic surgeries in orthopedics involve the (partial or total) replacement of a joint with a prosthesis. Some of the most prevalent complications of this type of surgery are the appearance of infections at the surgical site[1], commonly treated with systemic antibiotic therapy.[2] Repeated administration of high doses for long periods of time can lead to the appearance of adverse effects, development of antibiotic resistance or therapeutic failure due to poor patient's compliance with pharmacological therapy.[3] Local delivery systems, that also allow extended release of the antibiotic, would overcome all these problems.

Objectives: The aim of this work was to compare different strategies for local delivery and prolonged release of rifampicin. Specifically, the release of rifampicin from different hydrogels was evaluated, as well as its extended release from biodegradable microparticles.

Materials and Methods: Two hydrogels were prepared; i.e, one with Soluplus® (13% PEG 6000/ 57% vinyl caprolactam/ 30% vinyl acetate) (Basf Pharma), and another one with polyvinyl alcohol (PVA, PM 30,000-70,000 Da, Sigma-Aldrich) in Milli-Q® demineralized water (Millipore) and ethyl alcohol (96º Panreac AppliChem), made with a thermostatized magnetic stirrer.

The release test was carried out using a phosphate buffer solution (PBS) pH 7.4 with 0.3% sodium hyaluronate (Fagron Ibérica) as release medium, which was extracted for analysis and subsequently replaced in each sampling time. Sampling times were 3 and 6 hours, and 1, 3, 6, 10, 14 and 21 days. Before rifampicin quantification, the use of hyaluronidase was required as a breaking agent for the sodium hyaluronate residues present in the transfer medium.

Rifampicin was quantified by HPLC, using acetonitrile: saline buffer (50:50) as mobile phase, at a flow rate of 1.5 mL/min and at a wavelength of 254 nm.

Rifampicin microparticles were prepared by the solvent evaporation technique based on simple O/W emulsions, using the poly(lactic-co-glycolic acid) copolymer Resomer® RG 502 H (Evonik Industries).

Results and Discussion: The in vitro rifampicin release study was performed for 21 days, considering that the maximum drug release was reached when 90% of the incorporated rifampicin was released. To evaluate the release of rifampicin from the hydrogels, the antibiotic powder was incorporated forming a homogeneous suspension in the gels and, prior to the release assay, the prostheses were coated. As a result, the Soluplus® and PVA hydrogels were able to control rifampicin release for 8 and 6 days, respectively, at which time 90% of the incorporated antibiotic had been released. Rifampicin microparticles with a size 14.37 µm and a loading of 163.7 mg of antibiotic per 10mg of microparticles controlled drug release for 15 days, and was characterized by a marked burst effect in the first hours of study. Finally, the microparticles were incorporated into the hydrogels and rifampicin release was evaluated in these systems, determining that 90% of the drug was released after 10 days in the case of the microparticles incorporated into the PVA gel and after 15 days in the case of the Soluplus® gel.

Conclusions: Release studies demonstrate that hydrogels alone can constitute a suitable system for local delivery of rifampicin, also providing an extended release of rifampicin for 6-8 days. However, the use of microparticles and, in addition, the incorporation of the microparticles into hydrogels, specifically Soluplus® hydrogel, would be a more promising strategy, as it not only allows drug release for two weeks, but also avoids the bursting effect present in microparticle release.

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CHITOSAN-DECORATED (CORE/SHELL) NANOSTRUCTURES WITH POTENTIAL APPLICATIONS IN ANTITUMOUR MAGNETIC HYPERTHERMIA

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Introduction: Loading of chemotherapeutics to nanoparticulate systems have reported promising results against cancer. In this line, superparamagnetic iron oxides, e.g. magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), can help in engineering nanoplatforms maximizing drug accumulation into targeted cells, given their magnetic field responsiveness. In addition, they can provide antitumour magnetic hyperthermia functionalities.[1]

Objectives: Develop a reproducible procedure to formulate biocompatible magnetopolymeric nanostructures for combination cancer therapy (hyperthermia plus chemotherapy). These (core/shell)/shell nanoparticles (NPs) will consist of (Fe_3O_4/poly(ε-caprolactone))/chitosan [(Fe_3O_4/PCL)/CS] and (γ-Fe_2O_3/poly(D,L-lactide-*co*-glycolide))/chitosan [(γ-Fe_2O_3/PLGA)/CS]. Size, and electrokinetic and magnetic properties will be characterized. The *in vitro* magnetic hyperthermia effect will also be evaluated.

Materials and Methods: The interfacial polymer disposition and nanoprecipitation solvent evaporation techniques allowed the formulation of the Fe_3O_4/PCL and γ-Fe_2O_3/PLGA NPs, which were then coated with CS by a coacervation procedure (n = 3)[2,3]. Dynamic light scattering and electrophoresis allowed to characterize particle size and zeta potential of the particles, and the hysteresis cycle of the NPs was then evaluated. The capacity of the nanosystems as magnetic fluid hyperthermia agents was analysed by exposing the colloids (10 mg/mL) to an electromagnetic field (250 kHz and 4 kA/m). Then, an MTT assay was used to evaluate the capacity of the NPs (0.4%, p/v) as hyperthermia treatment agents against the T-84 colonic adenocarcinoma cell line (n = 3).

Results and Discussion: Particle size was 308 ± 3 and 326 ± 8 nm for the (Fe_3O_4/PCL)/CS and (γ-Fe_2O_3/PLGA)/CS nanostructures, respectively. The electrokinetic analysis confirmed the complete coverage of the core/shell particles by CS. The initial susceptibility and saturation magnetization values were ≈ 0.156 × 10^{-3} m^3/Kg and ≈ 11.34 Am^2/Kg for the (Fe_3O_4/PCL)/CS particles, and ≈ 0.071 × 10^{-3} m^3/Kg and ≈ 4.98 Am^2/Kg for the (γ-Fe_2O_3/PLGA)/CS particles. It was characterized how the (Fe_3O_4/PCL)/CS and the (γ-Fe_2O_3/PLGA)/CS colloids generated the minimum antitumour hyperthermia temperature (≈ 41ºC) in ≈ 30 and ≈ 32 min, respectively. This promising quality crystalized in an efficient antitumour activity against T-84 cells: compared to control groups, cell viability was reduced to 36 ± 4% and 39 ± 5% when exposed to the (Fe_3O_4/PCL)/CS and (γ-Fe_2O_3/PLGA)/CS hyperthermia treatment nanosystems, respectively.

Conclusions: Reproducible methodologies have been established to obtain these (core/shell)/shell NPs. They had demonstrated potential capabilities as antitumor magnetic hyperthermia agents. Research is in progress to define their use in cancer theranosis.

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INFLUENCE OF DIFFERENT HYDROGELS IN SKIN: A BRIEF HISTORY OF INCUBATION TIME, STRUCTURE AND EFFECTS

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Introduction: A rational design in nanotechnology focuses not only on the molecule used in a specific treatment, but also on the vehicle, and which properties might be determinant in the therapy success and patient adherence, particularly for topical administration. Hydrogels have been reported as interesting cutaneously applied formulations due to their high-water content that can regulate dry skin conditions, their structure versatility, ability to carry and give purpose to all kinds of drugs, capacity to be modulated and responsive to stimuli, making them excellent candidates for drug delivery vehicles in skin research. In this work, natural origin k-carrageenan (CRG) polysaccharide was combined with synthetic polyvinyl alcohol (PVA) or polyvinylpyrrolidone (PVP), originating two different blends of polymeric hydrogels. CRG is a marine origin sulphated polysaccharide, used for drug delivery and tissue engineering purposes due to its viscoelastic and gelling properties. CRG is non-toxic, hydrophilic, and biodegradable, and it is widely used in cosmetic applications in hydrogel form, with moisturizing, conditioning, and water-retaining properties for skin. Synthetic PVA and PVP are neutral, water-soluble, biocompatible, non-toxic, and have interesting mechanical properties to be used in industrial, biomedical, and pharmaceutical applications. These hybrid hydrogels combine the biocompatibility and safety of natural polymers with the mechanical properties of synthetic ones.
Objectives: Preparation of two hybrid hydrogels, CRG-PVA and CRG-PVP, proceed to their characterization, investigate their interplay with skin layers regarding different incubation times and address their effect on skin permeation profile using Rhodamine B as a model compound.
Materials and Methods: The designed hydrogels were characterized in terms of rheological properties. Their interplay with different skin layers was evaluated performing extensive infrared micro-spectroscopic analysis through synchrotron-based Fourier Transform Infrared Microspectroscopy (SR-FTIRM) by determination of lipids organization and protein structure, after 3h and 24h of incubation time. To complement characterization and spectroscopic data, *ex vivo* porcine skin permeation assays were conducted to evaluate the permeation of both hydrogels at the same defined intervals using Franz diffusion cells. The distribution of both hydrogels in the different skin layers was also addressed through the confocal microscopy analysis of histological sections of permeated skin at 3h and 24h.
Results and Discussion: CRG-PVA and CRG-PVP present similar rheological properties, presenting a pseudoplastic behavior and good resistance to deformation, positive characteristics for skin application. The *ex vivo* skin permeation assay showed that hydrogels permeate at different extents through the skin. Rhodamine B allowed the fluorescent analysis of permeation extent through histological sections of skin, particularly in the 3h timepoint, where CRG-PVP shows more accumulation in the *stratum corneum* region. The SR-FTIRM information confirmed changes in terms of epidermal lipid organization for CRG-PVP at 3h, shifting from orthorhombic to hexagonal (less ordered, fluidized) packing when compared to untreated skin.
Conclusions: The polymers' choice and their combination in hydrogels can be of great importance when designing cutaneous formulations, particularly aiming for topical - limited penetration through upper skin layers - or transdermal delivery - deeper layers of the skin, reaching the bloodstream.

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STIMULUS-RESPONSIVE NANOSYSTEM FOR EFFECTIVE PEPTIDE DELIVERY FOR THE TREATMENT OF IBD

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Introduction: IBD encompass series of chronic inflammatory conditions affecting the gastrointestinal tract (GIT). The prevalence of IBD is increasing worldwide, emerging as a public health challenge. Current treatment options are often impaired by serious adverse effects and limited therapeutic efficacy.
Objectives: This innovative project aims to develop ROS-sensitive PEGylated nanoparticles (NPs) for the effective oral delivery of peptides with high potential in IBD treatment. The PEGylation allows to trigger an efficient transposition of intestinal mucus, while the cleavable behaviour, provided by the presence of a thioketal (TK) linker, in response to environmental oxidative features of diseased tissues (ROS), will help to maximize the interaction of NPs with target cells and tissues.
Materials and Methods: Peptide-loaded NPs were produced by the double emulsion technique and characterized for hydrodynamic diameter and polydispersity index (PdI) by DLS and zeta potential (ZP) by LDE. The peptide association efficiency (AE%) was calculated directly by HPLC-FL analysis. After, the ROS-responsiveness profile of the TK linker was analysed. Briefly, both the *co*-polymer mPEG-TK-PLGA and the NPs were incubated in water containing H_2O_2 and $CuCl_2$. After 24 h, all mixtures were centrifuged, washed and freeze dried for further ^{1}H NMR analysis.
.
Results and Discussion: The final NPs formulation is composed of 10mPEG-TK-PLGA:90PLGA. NPs present sizes around 200 nm, being advisable for the intestinal peptide transport through the GIT [2] and PdI values lower than 0.3, suggesting a homogenous distribution of the particle size. The almost neutral ZP reveals the effective surface PEGylation of NPs. The AE of the peptide in the NPs was around 55%. Regarding to the ability of TK to be cleaved under ROS-simulated conditions, the results clearly demonstrate the absence of the characteristic peaks of the TK (~2.59 and 2.81ppm) after the exposure to ROS. The decrease of the PEG peak is also evident. Mucus-diffusivity tests are being performed to confirm the diffusive profile of the nanosystem.
Conclusions: The nanosystem presents good characteristics for intestinal drug delivery and the ability of the TK linker to cleave in the presence of ROS, will allows the internalization of the NPs in the cells.

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RECOVERY OF POLYPHENOLS FROM SPENT COFFEE BY PULSED ELECTRIC FIELDS AND SUPERCRITICAL FLUID EXTRACTION

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Introduction: There is a great demand for the recovery of bioactive compounds from food by-products due to their potential use in food, pharmaceutical and cosmetic industries. Within these components the polyphenols, are very interesting molecules that have one or several phenolic rings in their structure and are characterized by their antioxidant capacity[1]. Coffee, one of the most consumed non-alcoholic beverages worldwide, has a large amount of antioxidants such as chlorogenic acid and caffeic acid[2]. For the extraction of these biological compounds, different solid-liquid extraction methodologies have been proposed[3]. Nowadays, more sustainable extraction methods like pulsed electric fields (PEF) are being evaluated[4]. Besides, the use of supercritical fluids extraction (SFE) have also been proposed as an interesting technology[5].

Objectives: The aim of this study was to extract polyphenolic compounds from spent coffee solids by applying PEF and SFE and quantify the total phenolic compounds and total antioxidant capacity.

Materials and Methods: Four extraction methodologies were compared: **a)** PEF pre-treatment (2 and 3 kV/cm, Total energy intake 100kJ/kg); **b)** SFE (10 MPa, 40ºC, 90:10 CO_2:EtOH, 1 h); **c)** Microwave (T = 80ºC, t = 10 min) and **d)** Water (T = 20ºC, t = 30 min). The extracts were collected after centrifugation at 3500 rpm for 10 min. The ABTS protocol was used to determine the total antioxidant capacity (TEAC) and by the Folin-Ciocalteu methodology the total phenolic content was analyzed (TPC).

Results and Discussion: The recovery of phenolic compounds was higher in the extracts obtained with microwave heating and SFE. In the first case, the short heating time and the high temperature could be responsible of the higher amount of extracted compounds[6]. In the case of SFE, the pressure could influence the recovery of the phenolic compounds. However, from the point of view of ABTS results, the higher antioxidant capacity corresponds to the microwave extract and the SFE had the lower. This could be related to the selectivity of extraction phase of SFE.

Conclusions: The different extraction methodologies allowed to provide extracts with high antioxidant activity and rich in polyphenols, that could potentially find several applications as dietary supplement, ingredient for cosmetic formulations or as additives in food. Further process optimization and analysis are necessary to support the results and find the best extraction methodology from a sustainable point of view.

Figure 1. Extraction methodology

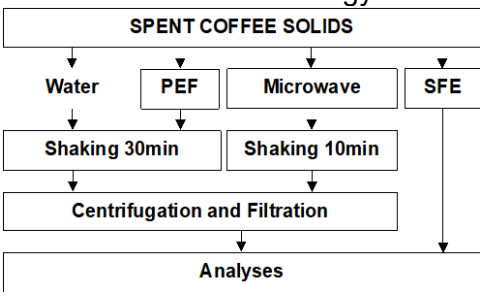

Table 1. TPC and TEAC of extracts

	Polyphenols (mg/g)	TEAC (μmol/g)
Water	1.10 ± 0.04^{ab}	21.68 ± 2.27^{b}
Microwave	1.87 ± 0.28^{c}	33.21 ± 1.37^{c}
2 kV/cm	1.04 ± 0.21^{ab}	19.14 ± 1.36^{b}
3 kV/cm	0.79 ± 0.19^{a}	20.77 ± 1.05^{b}
SFE	1.64 ± 0.85^{bc}	7.17 ± 0.57^{a}

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DEFORMABLE LIPID VESICLES AS TOPICAL DRUG DELIVERY SYSTEMS FOR TREATMENT OF CUTANEOUS LEISHMANIASIS

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Introduction: Cutaneous leishmaniasis (CL) is a neglected tropical skin disease with increasing incidence worldwide, characterized by disfiguring and deforming skin lesions. Current available treatments options suffer from limitations related to toxicity, efficacy, high cost and invasive administration route.[1] Topical treatment represents a convenient and safe option since the drug goes directly to the CL skin lesion without the use of needles which improves patient compliance facilitating treatments.[2] Deformable Lipid-based carriers have great potential for dermal and transdermal drug delivery.[3]

Objectives: To explore Deformable Lipid Vesicles (DLV) as topical delivery systems for two active drugs in order to enhance their percutaneous penetration and anti-leishmanial activity and reduce the drug's adverse effects.

Materials and Methods: Amphotericin B (AmB) and a hemisynthetic dinitroaniline analogue (TFL-A13) were incorporated in Deformable Lipid Vesicles (DLV) composed of a mixture of phospholipids and surfactants.[4] Physicochemical characterization of the developed DLV formulations included drug to lipid ratio, incorporation efficacy, vesicle size and surface charge assessments. The DLV skin permeation through newborn pig using Franz diffusion cells and lipid-bilayer deformability through a permeability barrier assays were performed to understand the behavior of the prepared vesicles when in contact with the skin. In vitro cytotoxicity against the keratinocyte cell line HaCaT and the macrophage-like cell line THP-1 was assessed. The antileishmanial activity of the drug loaded DLV formulations was also evaluated *in vitro* against both the promastigote and amastigote forms of *L. major* parasites.

Results and Discussion: All the DLV formulations prepared demonstrated homogeneity, a translucent fluid gel-like aspect and a yellow color. Their average mean size was between 125 and 135 nm (PdI < 0.1) with zeta potential values around zero (mV) and incorporation efficiencies higher than 96 %. Permeation and penetration assays suggest that these loaded DLV formulations are suitable to be incorporated in a topical formulation since both drugs were detected in the epidermal and dermal skin layers. Cell viability results in THP-1 macrophage-like cells indicate that the drug's associated cytotoxicity is reduced when loaded in DLV. No reduction in HaCaT cell viability was observed for the tested concentrations (< 10 µg/mL). Treatment of infected THP-1 macrophage-like with *Leishmania* amastigotes showed that drug loaded DLV inhibited the growth of parasites proportional to the drug concentration.

Conclusions: Deformable Lipid Vesicles could be a promising vehicle for dermal delivery of AmB and TFL-A13 overcoming skin permeation issues and a new low-cost and safe therapeutic option in CL treatment.

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ALBUMIN NANOPARTICLES AS CBD CARRIERS: DESIGN AND OPTIMIZATION OF ELABORATION PROCESS

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Introduction: Cannabinoids have demonstrated a potential therapeutic utility in cancer disease not only as palliative agents but also as antitumor drugs per se[1]. Cannabidiol (CBD) is one of the most promising cannabinoids due to its lack of psychotropic effects and its ability to inhibit angiogenesis, growth, and metastases of several carcinomas such as glioblastoma, breast cancer, prostate cancer and ovarian cancer among others and to its ability to potentiate the effect of conventional antineoplastics such as paclitaxel and doxorubicin[2]. Nevertheless, CBD shows a high lipophilicity that hampers its parenteral administration. In this context, the use of albumin nanoparticles as CBD carriers would resolve this challenge and would allow the parenteral administration of this cannabinoid without using organic solvents. Moreover, albumin nanoparticles tend to accumulate at certain types of tumors such as breast carcinomas, which would allow a more selective location of CBD at the tumor[3]. Therefore, a higher anticancer effect could be achieved.
Objectives: The objective of this work was to design, develop, and characterize optimized albumin nanoparticles as CBD carriers.
Materials and Methods: Albumin nanoparticles were prepared by desolvation technique, using bovine serum albumin (BSA) and glutaraldehyde at 4% as crosslinker. Initially, a a Plackett-Burman experimental design was carried out with unloaded nanoparticles to evaluate the effect of several elaboration process variables (albumin concentration, solvent, pH of albumin solution, volume of glutaraldehyde and stirring rate) on particle size and polydispersity index (PDI). Then, nanoparticles loaded with CBD (1.5, 3, 6 and 9 mg) were prepared and characterized by determining their morphology, particle size and PDI. Drug loading was also evaluated by HPLC.
Results and Discussion: Unloaded nanoparticles obtained in Plackett-Burman DoE showed a particle size of 165-528nm and a PDI of 0.03-0.25. Changes on precipitation solvent, pH, concentration of albumin solution, and stirring rate showed a statistically significant influence (p value<0.05) on nanoparticle size, being solvent the most influent parameter. However, their effect on PDI was not significant (p value>0.05). Changes on the volume of crosslinker (glutaraldehyde at 4%) did not show a statistically significant (p value>0.05) influence on either particle size or PDI. Unloaded nanoparticles prepared with acetone as precipitating solvent, a stirring rate of 500 rpm, 75µL of glutaraldehyde and an albumin solution of 60 mg/ml and a pH value of 7, showed a small particle around 170 nm and a low PDI (0.11). These were selected as the optimal parameters for the elaboration of CBD loaded nanoparticles. While CBD nanoparticles prepared with a CBD:albumin ratio of 1.5:180 showed a similar particle size than unloaded nanoparticles (around 170 nm), nanoparticles prepared with a CBD:albumin ratio of 3:180 showed a particle size around 200 nm and those elaborated with ratio of 6:180 and 9:180 showed an even higher particle size of around 270 nm due to the higher amount of entrapped CBD. In all cases, spherical nanoparticles with a suitable PDI value (<0.2) were obtained. Finally, the higher initial drug loading, the higher entrapment efficiency (EE), as although EE values higher than 45% were obtained in all CBD loaded formulations, CBD nanoparticles prepared with the highest CBD:albumin ratios (6:180 and 9:180) showed EE above than 90%.
Conclusions: Albumin nanoparticles that were prepared with acetone as precipitating solvent, a stirring rate of 500 rpm, a volume of glutaraldehyde at 4% of 75µL, an albumin solution of 60 mg/ml and a pH value of 7, and a CBD initial loading of 9 mg and that showed a particle size around 270 nm, a low polydispersion (<0.2) and drug loading of 4.44 mg of CBD/100 mg of nanoparticles could be a suitable nanocarrier for the delivery of this cannabinoid to tumors.

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A MICRONEEDLE-MICROPLATE PLATFORM TO DETECT BIOMARKERS IN THE SKIN

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Introduction: Recent global health crises such as the COVID-19 and Ebola outbreaks have underscored the need for sensitive and rapid point-of-care (PoC) diagnostic tests. Microneedle arrays comprising small (~1 mm in length), solid projections can be inserted into the skin painlessly[1–3] to selectively capture specific biomarkers[4–6]. This technology could vastly accelerate and improve disease diagnosis as a PoC system. However, it has previously lacked a facile detection technique for large-scale deployment.
Objectives: To integrate a microneedle immunocapture platform with off-the-shelf microplate spectrophotometry to demonstrate skin biomarker detection and facile signal quantification.
Materials and Methods: A miniature sandwich enzyme-linked immunosorbent assay (ELISA) platform for porcine IgG (as a model biomarker) was prepared by covalently immobilising the capture antibody on to polylactic acid (PLA) microneedle arrays. Following blocking with 5% w/v bovine serum albumin (BSA), the microneedle arrays were pressed into porcine skin to capture IgG from the dermal interstitial fluid. Experimental controls included IgG immunocapture arrays incubated in (a) 50 ng/ml IgG solution (positive control) or (b) 1% w/v BSA solution (negative control). The microneedle arrays were then incubated with a horseradish peroxidase (HRP)-conjugated detection antibody, washed and dried, before being placed on a 384-well microplate. Designed to align with the wells, the microneedles were inserted into an o-phenylenediamine (OPD) solution in the wells. The arrays were removed from the microplate after a 45-minute incubation then the plate was read at 450 nm.
Results and Discussion: Using the integrated microneedle-microplate platform, native IgG was detected in the excised porcine skin with a good signal-to-noise ratio (Figure 1). This was corroborated by the visible colour change in the OPD solution in the microplate. The higher absorbance in the test microneedle array compared to the positive control suggests that the IgG concentration in the skin sample was >50 ng/mL, although hydrodynamic differences in the skin matrix and the control solution may affect IgG capture and complicate this interpretation. Further assay optimisation may be required to further enhance the absorbance values, and thus the sensitivity of the assay.
Conclusions: The microneedle-microplate platform successfully detected native IgG in excised porcine skin. The results demonstrate the potential of immunocapture microneedles as a simple and rapid PoC diagnostic platform by integrating with existing high-throughput analytical systems.

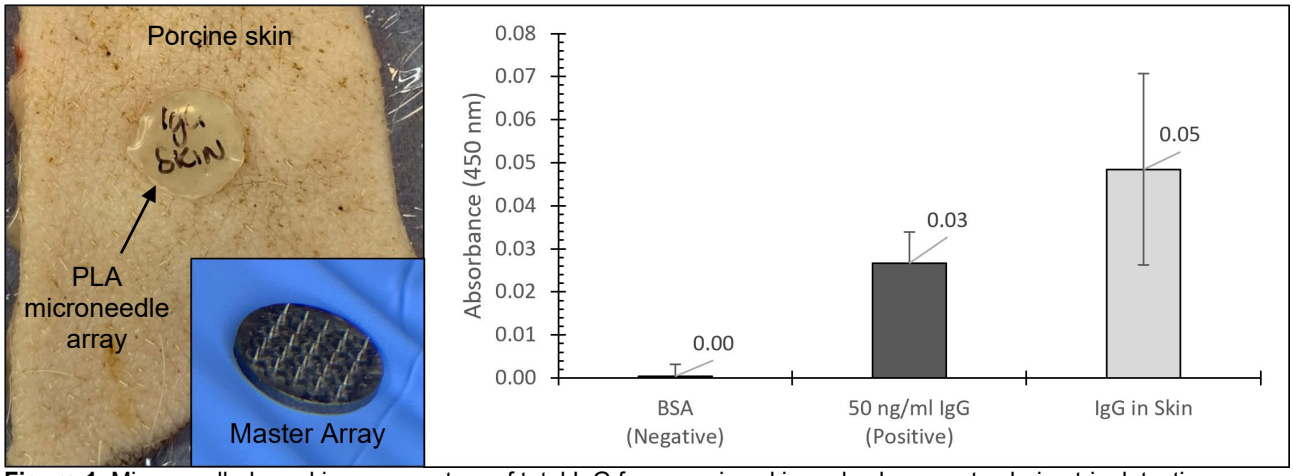

Figure 1. Microneedle-based immunocapture of total IgG from porcine skin and subsequent colorimetric detection.

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THE FORMULATION VARIABLE OIL/SURFACTANT RATIO DRIVES THE PREPARATION OF LIPID NANOCAPSULES BY PHASE INVERSION METHODS

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Introduction: Nanoemulsions are nonequilibrium systems, so energy input is required for their formation. To this end, high- or low-energy emulsification methods can be used. High energy methods are mostly influenced by preparation variables whereas low energy methods are influenced by formulation variables. Low-energy emulsification that exploit the chemical energy of the system, such as that associated with the change in the spontaneous curvature of polyethoxylated surfactants during the emulsification process, are known as phase inversion methods. These changes to obtain O/W nanoemulsions can be achieved through two different routes[1]: (i) maintaining a constant composition while varying the temperature (phase inversion temperature (PIT) method), and (ii) maintaining a constant temperature while varying the composition (phase inversion composition (PIC) method). In a previous study[2], we demonstrated that a linear univariate model allows size-tailored lipid nanocapsules (LNCs) to be obtained by PIT as a function of the oil:surfactant ratio.
Objective: The aim of this study was to compare both phase inversion methods (i.e., PIT and PIC) to prepare LNCs and demonstrate if a linear univariate model as a function of the formulation variable oil: surfactant ratio can be defined for both methods for different excipient combinations.
Materials and methods: Deionized MiliQ® water and NaCl were use as aqueous phase; Labrafac® lipophile WL 1349 or Labrafil® M 1944 CS were used as oily phase; and Lipoid® S75, Kolliphor® HS 15 or Kolliphor® ELP were use as surfactants. Three combinations of surfactant and oil phase (i.e., a) Kolliphor® HS15 - Labrafac® lipophile WL1349; b) Kolliphor® ELP - Labrafac® lipophile WL1349; c) Kolliphor® HS15 - Labrafil® M1944 CS) were prepared by each method. For each combination, four different formulations in terms of oil:surfactant ratio (PIT: F1 – F4, PIC: F5 – F8) were prepared in triplicate. The average volume diameter and polydispersity index of LNCs were determined by dynamic light scattering using Microtrac®-Zetatrac analyzer.
Results and discussions: On the one hand, the linear univariate model (R^2>0.98) was observed for prediction of particle size of LNCs prepared from the three distinct excipients combination by both PIC and PIT; i.e., LNCs size increased as a function of the oil:surfactant ratio. The reduction in particle size with increasing ratios of surfactant can be accounted for by the decrease in interfacial tension.
On the other hand, the results indicated that highly monodisperse LNCs were obtained in all cases. There were not statistically significant differences among polydispersity indexes obtained for the three oily phase-surfactant combinations tested by any of the phase inversion methods ($p>0.05$).
Conclusions: Preliminary characterization studies of LNCs let conclude that the mechanism whereby nanoemulsions are formed through low-energy phase inversion methods is analogue for both PIT and PIC methods. Accordingly, we have proved that LNCs size can be predicted for both methods with a linear univariate mathematical model as a function of the oily phase/surfactant ratio for distinct oily phase-surfactant combinations. Importantly, this size-tailoring can be achieved maintaining monodisperse size distributions in all cases as the polydispersity index did not vary significantly between the different oily phase-surfactants combinations.

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BIOCOMPATIBLE NANOMOTOR TOWARDS TUMOR ELIMINATION

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Introduction: Controlled and localized drug delivery for tumor elimination is an area of particular interest, due to its great advantages over standard treatments. One of the strategies that has been followed to achieve this is the encapsulation of anticancer drugs in nanoparticles, which are retained to tumors due to the EPR effect. Nevertheless, their arrival to target areas is passive, resulting in poor tumor penetration, poor cytotoxic results, and toxic side effects. For this reason, in recent years have emerged the employment of self-propelled nanoparticles (nanomotors), that show active movement. This strategy results in better tumor reach and tumor penetration. However, most of the nanomotors reported until date use high concentrations of toxic and exogenous fuels, mainly H_2O_2.

Objectives: Synthesis and validation in several tumor-like models of a multifunctional nanomotor type Janus Pt-mesoporous silica nanoparticle (MSN) powered by a biocompatible fuel, glucose, capable of penetrate tumors and deliver an anticancer drug in a controlled way.

Materials and Methods: Janus Pt-MSN nanomotors were synthetized by the Pickering method. Next, the MSN face was loaded with Doxorubicin (Doxo) and the pores were blocked with the highly active enzyme glucose oxidase (GOx) joined by amide bonds. GOx acts as both gatekeeper and first propellant element of the nanosystem. The construction of the nanomotors was analyzed using the standard nanomaterials characterization techniques: powder X-ray diffraction (PXRD), N_2 adsorption-desorption, thermogravimetric analysis (TGA), dynamic light scattering (DLS), transmission electron microscopy (TEM) and scanning transmission electron microscopy coupled with electronic energy dispersive x-ray spectroscopy (STEM-EDX). The motion of the nanomotors was evaluated with the NTA software in a Nanosight NS300, as well as in a tumor-like microenvironment made up of collagen. Finally, the antitumor performance of the nanomotors was evaluated in HeLa cell culture, HeLa tumor spheroids, and in HeLa xenograft Balb/c mice by viability assays, confocal laser scanning microscopy (CLSM) and TEM.

Results and Discussion: *In vitro* tests carried out prove that the nanomotors can release Doxo specifically in response to cell proteases, move in response to increasing concentrations of glucose, and cross a tumor-matrix like environment composed of collagen. *In vivo* tests demonstrate that nanomotors effectively reach deep into tumor, deliver Doxo, and induce cell apoptosis, killing cancer cells. Results pointing in the same direction were obtained in cell culture and tumor spheroid assays.

Conclusions: Here we present the successful design, fabrication, and validation of nanomotors propelled with a bioavailable and non-toxic fuel, that shows high diffusion values in comparison with other previously reported nanomotors. This results in deep penetration into tumors and faster cell uptake and drug release, resulting in a significant tumor size reduction. In summary, this strategy could be a milestone in the approach to the treatment of cancer, a major public health problem nowadays.

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ENZYME-ACTUATED DNA NANODEVICES USING HYBRID NANOPARTICLES FOR SENSING, CONTROLLED RELEASE AND COMMUNICATION

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Introduction: DNA structural properties enable this molecule to be treated as a modular system susceptible to modify its own conformation upon medium chemical changes in solution.[1] Recently, several pH-responsive DNA nanodevices capable of triple-duplex transitions or loading and release of cargo have been reported. In this regard, the integration of DNA nanodevices on nanoparticles holds great potential to design smart nanosystems for diverse applications.[2] Here, we developed enzyme-directed pH-modulated DNA nanodevices by using Janus gold–mesoporous-silica nanoparticles (Au–MSNPs).[3]
Objectives: (1) To monitor structural changes in responsive DNA nanostructures, able to undergo triplex-duplex transitions and ligand loading and release. (2) To prepare Janus Au–MSNPs functionalized with DNA and enzymes. (3) To employ the resulting nanodevices for sensing, controlled release of DNA and communication between nanoparticles.
Materials and Methods: DNA nanostructures are labelled with fluorophores and characterized by fluorescence spectrophotometry to confirm structural changes. In addition, a urea nanosensor based on the release of Cy3-labeled oligonucleotide from enzyme-functionalized Janus Au–MSNPs (S1) was developed. The Janus particles were functionalized on the silica face with amino groups by APTES to which the labelled oligonucleotides were attached by electrostatic interactions, whereas the gold face was used for grafting urease enzymes by EDC/NHS method.
Results and Discussion: Fluorescence measurements confirmed conformational changes of DNA nanostructures. In addition, the Janus Au–MSNPs functionalized with DNA and enzymes were able to release the fluorescent oligonucleotide through the enzyme-mediated hydrolysis of urea to ammonia and the subsequent deprotonation of amino groups on the sílica face. Other hybrid nanosystems with enzyme-actuated DNA nanodevices under development
Conclusions: Our results demonstrate the potential of using DNA nanodevices and hybrid Janus nanoparticles to develop advanced nanosystems. This is a general new paradigm that will be applied to the design of optical probes for the detection of target analytes, controlled release of DNA strands and communication between nanoparticles.

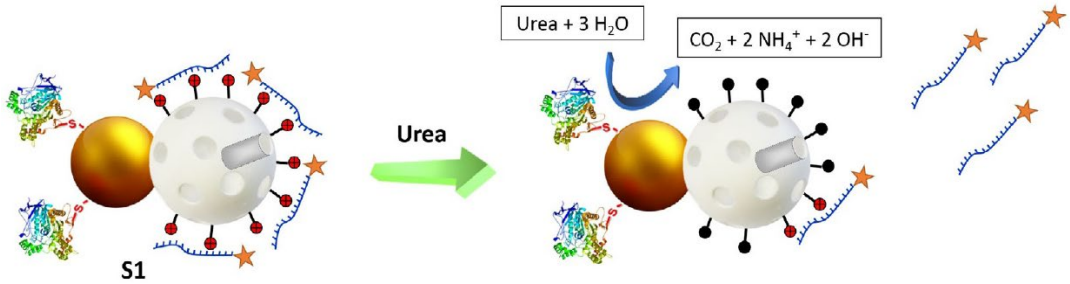

Figure 1. Representation of the design and sensing performance of the nanosensor S1.

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USE OF INNOVATIVE TECHNOLOGIES TO REDUCE MYCOTOXINS IN FOOD

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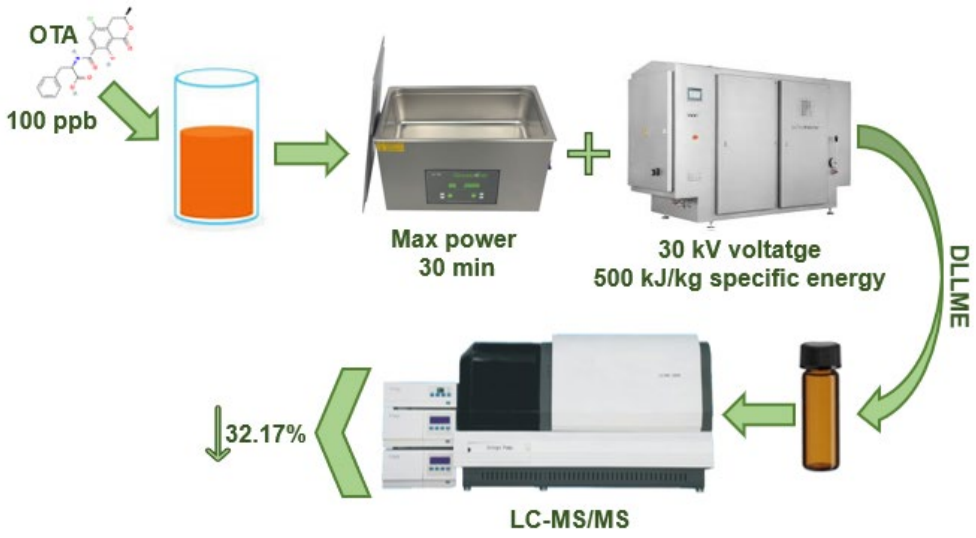
Introduction: Pulsed electric fields technology (PEF) and ultrasounds (US) technologies link with green chemistry and eco-friendly characteristics [1]. The food industry is looking for these innovative technologies of food processing that can reduce toxic compounds from food, such as mycotoxins, without producing toxic by-products and without affecting the organoleptic characteristics and nutritive value of food [2]. PEF involves the application of short pulses (μs to ms) of electric fields in the order of 100-300 V/cm to 20-80 kV/cm, to a product placed between two electrodes. US is a technology that produces sound waves, comprised between 20 kHz and 100 MHz, that are propagated through a liquid medium. Nowadays, the mycotoxins represent a global public health problem due to their incidence in food and feed and their harmful effects linked with cancer induction, mutagenicity and estrogenic effects. Ochratoxin A (OTA) is a widely studied mycotoxin produced by *Aspergillus* and *Penicillium* species. It is classified as 2B (possibly carcinogenic) by IARC and it is related with nephrotoxic effects [3]. It mainly contaminates cereals and wine, but its presence has also been detected in other products such as juices [4].
Objectives: The aim of the present study is to investigate the application of US and PEF for OTA reduction in orange juice with milk.
Materials and Methods: Orange juice with milk samples were prepared in the laboratory and spiked with OTA at concentration of 100 μg/L, after that samples were treated by PEF + US or by US + PEF. US conditions were maximum power for 30 min and PEF conditions were voltage of 30 kV and specific energy of 500 kJ/kg. Then, OTA was extracted using dispersive liquid-liquid microextraction method (DLLME) and determined by liquid chromatography coupled to tandem mass spectrometry with triple quadrupole (LC-MS/MS-QTRAP). The results were analyzed by analysis of variance (one-way ANOVA). A probability value of $p < 0.05$ was considered to be significant.
Results and Discussion: The OTA reduction percentages obtained were 41.32% in PEF + US and 32.17% in US + PEF in the juice. The results obtained were not statistically different. Therefore, the order of the treatments does not affect the reduction percentage.
Conclusions: The combination of US and PEF technologies could be a useful tool in the reduction of OTA in fruit juices**.**



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Solidification of self-emulsifying drug delivery systems (SEDDS): Impact on storage stability of a therapeutic protein

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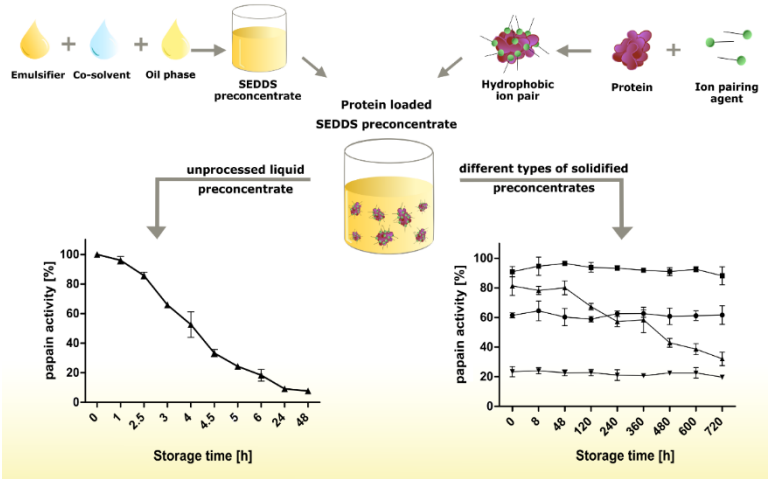
Introduction: By combining hydrophobic ion pairing (HIP) technique to increase protein lipophilicity with self-emulsifying drug delivery systems (SEDDS) for the oral delivery of protein drugs bioavailabilities ranging from 5% to 25% could be achieved in recent studies. Despite these encouraging results SEDDS, as liquid formulations, are still facing shortcomings. During processing or storage especially, macromolecular drugs tend to precipitate or denature. Moreover, liquid SEDDS in general face multiple shortcomings such as phase separation tendencies, oxidative degradation as well as dosage form leakage. Up to now, these crucial criteria have limited the industrial relevance of SEDDS.

Objectives: Development of suitable solidification methods for protein loaded, liquid SEDDS to improve drug and formulation stability during storage.

Materials and Methods: Papain was loaded in SEDDS via hydrophobic ion pairing (HIP). Liquid SEDDS (l-SEDDS) were solidified by adsorption to solid excipients such a magnesium-aluminometasilicate via wet granulation (ssilica-SEDDS), carbohydrates via lyophilisation (scarbo-SEDDS). These conventionally solidified SEDDS relying on high amounts adsorption material were compared to novel sSEDDS solidified using high-melting SEDDS components such as PEG-surfactants (sPEG-SEDDS) and triglycerides (soil-SEDDS). L- and s-SEDDS were compared regarding intrinsic emulsion properties, solid-state form of papain, macro and microscopic appearance as well as their enzyme stability and activity during storage.

Results and Discussion: HIP with deoxycholate showed a precipitation efficiency of 82% and papain maintained 90% of its initial activity after HIP. Papain HIP incorporated in SEDDS was present in an amorphous state, confirming a molecular dispersion in all preconcentrates. In comparison to l-SEDDS each solidification method investigated improved the storage stability of incorporated papain. Neither precipitation nor phase separation was observed for s-SEDDS. sPEG-SEDDS demonstrated with 87.8% the highest enzymatic activity and displayed according to the following rank order: sPEG-SEDDS > soil-SEDDS > ssilica-SEDDS > scarbo-SEDDS > l-SEDDS the highest remaining papain activity after 30 days of storage. Moreover, the absence of pharmacologically non-relevant adsorption materials and the simplicity in design of novel s-SEDDS outperformed adsorptively solidified SEDDS.



Conclusions: By suitable and innovative solidification methods SEDDS storage stability for therapeutic proteins can be significantly improved. In particular, solidification methods without non-relevant adsorption materials represent a promising approach easing industrial production. By eliminating SEDDS storage stability issues, we are getting another step closer to bridge the gap between academic research and industrial manufacturing.

INVESTIGATING MUCUS SURROAGTES ON THE FORMATION OF *PSEUDOMONAS AERUGINOSA* BIOFILMS AND THE EFFECT ON ANTIBIOTIC EFFICACY

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Introduction: Lung infections with *Pseudomonas aeruginosa* can be life-threatening for patients suffering from cystic fibrosis (CF). In this context, the formation of mucosal biofilms affects the diffusion as well as the efficacy of antibiotics but also the presence of pulmonary mucus plays a crucial role in this scenario and both should be considered in *in vitro* studies. Human pulmonary mucus obtained from endotracheal tubes can, therefore, be used as bacterial growth medium in such studies.[1] However, the limited availability of native biological samples demands for suitable surrogates, one of those being the so-called artificial sputum medium (ASM). This liquid medium shows a similar composition to pulmonary mucus, while a modification (ASM_{mod}) also provides it with rheological properties, which are comparable to human tracheal mucus.[2]
Objectives: This study aims to set up ASM_{mod} as a medium to grow *P. aeruginosa* biofilms in a mucus-like environment. The ability of this surrogate to test drug permeation and efficiency against PAO1 biofilms in different media was investigated.
Materials and Methods: PAO1(-GFP) biofilms were grown for 72 h in different media: LB, ASM, ASM_{mod} and human tracheal mucus to investigate growth (CFU) and morphology (CLSM) of bacterial biofilms. Transport studies with mucus and ASM_{mod} were performed with three model drugs – Tobramycin, Colistin and Ciprofloxacin – to test antibiotic permeation. Furthermore, 72 h PAO1 biofilms in LB, ASM and ASM_{mod} were treated for 24 h with different concentrations of the same drugs. MBC-B assays using Presto Blue® as well as CFU analysis provided data on the antibiotic efficacy.
Results and Discussion: Using ASM_{mod} as bacterial growth medium, bacterial survival and morphology of PAO1(-GFP) biofilms comparable to human mucus were observed. Transport studies demonstrated comparable drug permeation through native mucus and ASM_{mod} in a time window of four hours indicating that the diffusion through mucus is limited by the turtosity of this hydrogel as well as by chemical interactions with its glycoproteins. Furthermore, MBC-B assays, as well as CFU analysis, showed that differences can occur according to medium composition (LB vs. ASM) as well as rheology (ASM vs. ASM_{mod}), which depends on the characteristics of the drug. When PAO1 biofilms were treated with Ciprofloxacin, which showed best mucus permeation, the bacterial survival after treatment was comparable for any of the tested media. After 24 h of Tobramycin treatment, PAO1 biofilms in standard LB medium could be completely eradicated, while biofilms grown in ASM only showed some reduction in CFU. The bacterial survival did, however, not change dramatically with high Tobramycin concentrations for biofilms cultured in ASM_{mod}. The efficacy of Colistin was comparable against PAO1 biofilms grown in LB or ASM but also highly limited against those cultured in ASM_{mod}.
Conclusions: This study confirms that the presence of mucus affects the permeation as well as the efficacy of antibiotics. ASM_{mod} simulates the most important barrier features of this hydrogel and we conclude that it can be used for the cultivation of *P. aeruginosa* biofilms as well as for transport studies and drug efficiency testings.

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THE ROLE OF CBD IN GLIOBLASTOMA: IN-VITRO ANTITUMORAL STUDIES AND DEVELOPMENT OF CBD-LOADED LIPID NANOCAPSULES

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Introduction: Cannabidiol (CBD) is a non-psychoactive phytocannabinoid that, apart from its activity in palliating symptoms related to chemotherapy, has shown a powerful antitumor role on glioblastoma, inhibiting the appearance, migration and invasion of tumor cells and altering tumor angiogenesis (1). In addition, it has been shown that CBD potentiates the effect of conventional antineoplastics which allows to reduce their dose administered and consequently its adverse effects (2). However, due to its low aqueous solubility and its stability problems, formulation of CBD often poses dosing problems that can be overcome through its formulation in lipid nanocapsules (LNCs) (3) .

Objectives: the aim of this study is to develop CBD loaded LNCs and to evaluate which one of the four conventional chemotherapeutic agent (paclitaxel (PTX), doxorubicin (DOX), temozolomide (TMZ) and carmustine (BCNU)) is the most suitable to co-encapsulate with the CBD against two glioblastoma cell lines (U-87-MG and U-373-MG).

Materials and Methods: Labrafac® lipophile WL 1349 was supplied by Gattefossé S.A. Kolliphor® HS15 was a gift from BASF. Lipoid® S75 was supplied by Lipoid-Gmbh. NaCl was purchased from Panreac and de-ionized water was obtained from MiliQ® Purification System. CBD was provided by THC-Pharma. PTX, DOX, BCNU and TMZ were purchased from *Fisher Scientific.* 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2Htetrazolium bromide (MTT) and dimethyl-sulfoxide were purchased from Sigma-Aldrich. The U87-MG and U-373-MG cell lines were supplied by ATCC and ECACC, respectively.

Blank LNCs and LNCs loaded with CBD were prepared by the phase inversion temperature method (4). The antitumoral activity of the monotherapy and combination studies was evaluated by the MTT method. Data were processed with the Compusyn software to calculate IC_{50} values and combination indexes (CI).

Results and Discussion: Monodisperse 50 nm LNCs were obtained: for CBD-loaded LNCs there was a significant increase in particle size compared with blank LNCs ($p < 0.05$). However, the CBD loading did not significantly alter the polydispersity index ($p > 0.05$) in comparison with blank LNCs. These nanoparticles will help with formulation problems due to the high lipophilicity of CBD and conventional chemotherapeutics. On the other hand, in monotherapy *in vitro* studies, CBD showed an inhibitory effect on both glioblastoma cells showing and IC_{50} value of 36,68 ± 7,46 μM in U-87-MG line and 31,87 ± 7,17 μM in U-373-MG after 24h of treatment. Concerning combination studies with CBD, a powerful synergistic effect was achieved with PTX (CI < 0,7 in both cell lines). An additive effect between DOX and CBD was observed in U-373-MG cell line (CI 0,7-1,2), whereas an additive/light antagonistic effect was observed in U-87-MG (CI: 0.8-1.7). Finally, TMZ and BCNU combinations showed a light or moderate antagonistic effect in both lines (CI: in the range of 1.5).

Conclusions: These preliminary studies will serve as a basis for the rational selection of PTX and CBD for co-encapsulation within LNCs, thereby overcoming their traditional formulation problems.

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SYSTEMATIC EVALUATION OF INTESTINAL *IN VITRO* MODELS FOR DRUG DELIVERY VIA NANOPARTICLES

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Introduction: Although a consensus is reached within the scientifc community about the relevance of using *in vitro* models for the assessment of oral drug delivery via nanoparticles (NPs), the complexity and culture conditions of *in vitro* models vary immensely between laboratories. For high throughput experiments, simple monocultures of enterocytes are often used, neglecting the influence of mucus on NP permeation. On the other end of the spectrum, complex co-cultures with multiple cell types or primary material are used in microfluidic chips to produce highly physiological systems under flow conditions at the cost of labour-intensive, low throughput experiments. With respect to the physiology of the human intestinal mucosa and the design of NP formulations, some key aspects regarding barrier integrity and mucus secretion should be considered when selecting the right *in vitro* model following the “as simple as possible, as complex as necessary” mindset. Although evaluations of simple *in vitro* models have been performed for different model drugs[1], the interaction of nanoparticles with different models has not been thoroughly assessed so far.
Objectives: In a systematic approach, we evaluated NP interaction with different *in vitro* models of the human intestinal mucosa depending on model complexity and cultivation parameters. Using two NP model formulations, we assess how the selection of cell types and culture conditions can impact transport dynamics into and through the intestinal tissue.
Materials and Methods: *In vitro* models of the human intestinal mucosa were cultured in Transwell® inserts with varying ratios of enterocytes (CaCo-2) and goblet cells (HT29-MTX) under static or dynamic conditions for 21 days. Characterisation of the models was performed via histology, immunehistochemistry and measurement of transepithelial electrical resistance (TEER). Two NP model formulations based on poly(lactic-co-glycolic acid) were used either without or with additional polyethylene glycol coating and loaded with DiI as hydrophobic model drug. NPs were characterised regarding size, zeta-potential and morphology as well as uptake into and transport across epithelial cells, via fluorescence microscopy and semi-quantitative analysis, respectively.
Results and Discussion: Histological analyses of the *in vitro* models allowed for the differentiation between cell types and revealed significant variations in morphology of the models depending on culture conditions. While static cultivation resulted in the formation of monolayers, dynamic conditions induced the formation of multilayered cellular structures and villi-like protrusions. Here, we observed that the threedimensional structure of dynamic *in vitro* models increased the uptake of NPs into the tissue. Models exhibited a decrease in TEER values and a simulateous increase in secreted mucus by increasing the proportion of goblet cells compared to monoculutres. While the thickness of the cell layer was found to influence permeation of both NP formulations, the mucus layer predominently influenced the permeation of uncoated NPs.
Conclusions: The here presented study consititues a systematic evaluation of *in vitro* models of the human intestinal mucosa and changes in cultivation parameters that critically influence the uptake of nanoparticulate formulations. We found that NP uptake and transport was strongly influenced by barrier integrity, mucus production and the morphology of the respective *in vitro* models. By implementing simple alterations in the rational design of the tissue models, a higher throughput approach of more physiologically relevant models of the human intestine can be realised in most laboratories, ultimately resulting in a more efficient formulation of nanoparticulate drug delivery systems.

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PLA IN SITU FORMING IMPLANTS AS NEW STRATEGY FOR CBD ADMINISTRATION

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Introduction: In situ forming implants (IFIs), composed of a biocompatible polymer, a water-miscible solvent, and a drug, have shown several advantages in drug delivery. As we know, IFIs have the advantages of a simple manufacture, an easy injection, and a good stability. Moreover, this drug delivery strategy allows to control drug release, as a sustained release over periods of days to months can be achieved[1]. In fact, several IFIs containing doxycycline or leuprorelin are currently approved by FDA and/or EMA[2,3]. Cannabidiol (CBD), the main non-psychotropic cannabinoid, has emerged as a potential therapeutic tool in oncology, but it shows high lipophilicity and instability that complicates its handling and dosing and restricts its use by a parenteral route.[4] The use of IFIs should be a great strategy to develop parenteral drug delivery CBD formulations.

Objectives: This work aimed on developing poly-D, L-lactide (PLA) IFIs for the subcutaneous administration of CBD, comparing the effect of different water-miscible solvents and several PLA resomers (PLA-202 and PLA-203) on CBD drug release.

Materials and Methods: Several resomers of PLA (PLA-202 and PLA-203) were used as polymer, due to the biocompatibility, biodegradability, and safety of this polymer. N-Methyl-2-pyrrolidone (NMP) and Dimethyl sulfoxide (DMSO) were used as water-miscible solvents. Formulations loaded with a CBD drug: polymer ratio of 2.5:100 and 5:100 were elaborated using the direct injection technique. Firstly, injectability studies (using 23G and 25G needles) were carried out to select the most suitable and manageable polymer, and the optimal polymer:solvent ratio. Optimized CBD loaded implants, were characterized by scanning electron microscopy (SEM) and drug release was determined.

Results and Discussion: Injection tests using DMSO as a solvent, showed that both PLA-202 and PLA-203 solutions exhibited good injectability properties, with an easy and continuous injection using both 23G and 25G needles. However, when NMP was used as water-miscible solvent, PLA-203 showed a bad injectability when a 25G needle was used, probably due to its higher molecular weight of this polymer. In all cases, the optimal polymer: solvent ratio was 100 mg: 400 µL. Therefore, IFIs of PLA-202 and PLA-203 using DMSO as water-miscible solvent and IFIs of PLA-202 with NMP were prepared All the selected solutions led to the easy formation of CBD-loaded ISFIs when injected in phosphate buffer solution. A controlled CBD release was obtained in all formulations. ISFIs prepared with PLA-202 and NMP showed a faster drug release, with around 25% of CBD release within one hour, and around 50% within 2 days. However, when DMSO was used as solvent, a significantly slower drug release in both PLA-202 and PLA-203 formulations, with a significantly lower burst effect (around 7% of the CBD was released within one hour). Around 60% of CBD was released after 28 days. These differences could be attributed to the diffusion of the solvent during IFI formation.

Conclusions: The implants prepared using DMSO and PLA-202 or PLA-203 at a polymer: solvent ratio of 100mg:400µL showed good injectability properties to be administered subcutaneously, and a controlled CBD release for more than one month after a single administration, which would allow to obtain a long-acting effect.

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PREPARATION OF CHITOSAN-DECORATED POLY(ε-CAPROLACTONE) NANOPARTICLES WITH POTENTIAL APPLICATIONS IN DRUG DELIVERY

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Introduction: Poly(*ε*-caprolactone) (PCL) is an aliphatic polyester offering interesting properties for the delivery of chemoterapeutics, i.e. adequate loading capacity and sustained (biphasic) release profiles.[1,2] Chitosan (CS) is a polysaccharide that could be considered as a promising alternative to polyethylene glycol (PEG) chains in the formulation of long-circulating nanoparticles (NPs), while additionally providing tumour pH-triggered drug release capabilities.[3,4]
Objectives: Reproducible formulation of a core/shell nanostructure comprising PCL particles embedded into a CS nanomatrix. Size and electrokinetics will be characterized.
Materials and Methods: It was prepared an aqueous media containing acetic acid (2% v/v), Kolliphor® 0.5% (w/v), PCL NPs (mean size ≈ 200 nm, obtained by an emulsion/evaporation method), and CS. Then, sodium sulphate (20% w/v) was added drop-wise under ultrasonic agitation (10 min, sonicator input 4). Mechanical stirring at 1,200 rpm was continued for 50 min. Cleaning of the NPs was done by centrifugation (5000 rpm, 30 min). Particle size and the width of the size distribution were determined by photon correlation spectroscopy. The electrophoretic properties of the NPs were characterized as a function of both pH and ionic strength.
Results and Discussion: PCL/CS (core/shell) particles of suitable and moderately monodisperse size were obtained (546.5 ± 20.4 nm). The efficacy of the CS coating onto the PCL NPs was demonstrated by comparing the zeta potential (*ζ*) values of the PCL/CS particles with those of pure CS and pure PCL NPs. The *ζ*-pH trends of the core/shell NPs were almost identical to those of the pure CS. The CS shell efficiently hides PCL, rendering the surface of the PCL/CS NPs indistinguishable from that of the CS particles. To confirm these results, it was also determined the *ζ* as a function of KNO_3 concentration. Again the similarities between the electrokinetics of CS and PCL/CS NPs, and their differences with PCL were clearly observed.
Conclusions: A reproducible methodology has been optimised to obtain PCL/CS (core/shell) particles with promising applications as a stealth and pH-sensitive drug nanocarrier. Work is in progress to characterize the drug vehiculization properties.

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ENHANCING TRANSMUCOSAL DRUG DELIVERY USING MULTIRESPONSIVE NANOGELS

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Introduction: The treatment of local diseases in mucus-covered tissues, such as the gastrointestinal tract, lung, eye, and bladder exhibit as main challenge overcoming the mucus gel layer that protects the underlying epithelium. Thiol-bearing materials recently gained increased interest due to their ability to deliver drugs to submucosal layers in sufficient concentrations. In an aqueous environment, disulfides and thiols are in a dynamic equilibrium resulting in the occasional presence of free thiols for disulfide-containing materials as well. We thus hypothesize that disulfide containing materials will perform in a similar manner to thiol bearing particles and are exciting candidates to overcome the mucosal barrier and deliver therapeutic actives to submucosal cells.
Three-dimensional hydrogel nanoparticles, also called nanogels (NGs), are shaping up as a potential strategy to deliver small molecule drugs as well as therapeutic biomacromolecules through different biological barriers. Their crosslinked networks offer abundant reservoirs for cargo encapsulation and their properties can be tuned by incorporating certain functionalities in the NG scaffolds. In order to overcome the mucus barrier, the chemistry of NGs can be manipulated to promote mucoadhesion and mucopenetration, thus, prolonging drug-residence time and deep penetration into the mucus gel layer. In this context, we hereby present the development of redox-sensitive NGs for overcoming restrictive barriers in mucosal drug delivery of therapeutics.
Objectives: development of redox-sensitive NGs. *In vitro* evaluation of the NG interaction with mucus for their application in transmucosal drug delivery.
Materials and Methods: N-Isopropylacrylamide (NIPAM)-based nanogels were prepared by precipitation polymerization using the disulfide-bearing crosslinker N,N'-bis(acryloyl)cystamine (BAC). Also, N,N'-Methylenebisacrylamide (BIS) was used as a non-degradable crosslinker. Different crosslinker and monomer feed ratio were evaluated in order to obtained degradable NGs under reductive conditions. The interaction of NGs and with mucose were studied by dynamic light scattering (DLS) and gel permeation chromatography (GPC). *Ex vivo* experiments using Franz diffusion cells were performed to study the penetration of a model cargo into bovine small intestine.
Results and Discussion: A comprehensive screening of monomers and crosslinkers was performed to control the number and location of disulfide linkers within the NGs' structure. Synthesized NGs showed diameters in the 50-200 nm range that yield low polydispersity and intact disulfide-bonds. Aspects related to the crosslinker functionalization, reaction temperature, monomer/crosslinker ratios, and nature of the monomers showed to play a pivotal role. The use of BAC as a crosslinker yielded fully degradable NGs, while using BIS yielded NGs that did not degrade when they were incubated in reductive environments. NG interactions with the mucus and potential mucopenetrating properties were tested with commercial mucin and with mucus of excised bovine small intestine. The results demonstrated that the incorporation of disulfide bonds provides the NGs not only with degradable points that later enable the cargo release but also with a programmable capacity for mucoadhesion or mucopenetration. Moreover, the Franz diffusion cell experiment revealed that NGs allowed the transport of a model protein through the small intestine. Next experiments with *ex vivo* small intestine will evaluate the penetration of NGs and cargo by fluorescence microscope. Further applications against cystic fibrocys, bovine mastitis and Chagas disease are currently being explored.
Conclusions: Degradable NGs upon reductive conditions were successfully synthesized. The incorporation of disulfide bonds provided degradable points that later enabled the cargo release and conferred mucoadhesive or mucopenetrated properties. Different *in vitro* experiments were set up to study the NG interaction with commercial mucin and actual mucus, which demonstrated their ability to overcome the mucus barrier.

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STUDY OF THE SURVIVAL TO LYOPHILIZATION OF AN ADENOVIRAL VECTOR WITH 5 CRYOPROTECTANTS

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Introduction: Stability of recombinant viral vectors during storage and transport of formulations containing has lately resurfaced due to the pandemic situation(1). The use of freeze-drying technology has shown great perspectives for stabilization of adenovirus(2).
Objectives: Comparison and selection of the best cryoprotectant for the lyophilization of adenoviral vector type 5.
Materials and Methods: Materials: Type 5 adenovirus with E1 and E3 gene deleted; lactose; mannitol; trehalose; sucrose; PVP; tris buffer pH=7.5; Hewlett Packard 8453 spectrophotometer; TG-SDTA 851e de Mettler-Toledo; Syrius luminometer; Virtis Genesis 12 EL freeze dryer; Zetasizer Nanoseries Malvern Instrument. Methods: Differential Scanning Calorimetry; lyophilization; bioluminescence viral titration; spectrophotometry; thermal-gravimetric analysis.
Results and Discussion: The next parameters of the crude adenoviral vector pre-lyophilization were characterized: Tertiary structure (with the second derivative of UV spectrum), quaternary structure and activity of the vector (through luciferase gene expression). After lyophilization of 5 formulations, each with a different cryoprotectant [Lactose (9,75% w/v), Sucrose (9,25% w/v), Trehalose (5% w/v), Polyvinylpyrrolidone (5% w/v) and Mannitol (5,57% w/v)], the glass transition temperature, and the residual humidity of each formula was found out; the ratio of survival of the vector and its tertiary and quaternary structures were assessed, as well. Analytical results obtained with samples are the following: Survival ratio (%): Lactose≈100; Mannitol≈0; PVP≈80; Sucrose≈100; Trehalose≈60. Residual Humidity (% p/v): Lactose=1.11; Mannitol=0.12; PVP=1.56; Sucrose=0.75; Trehalose=0.80. Glass transition temperature (ºC): Lactose=-39.31; Mannitol=ND; PVP=-44.12; Sucrose=-43.29; Trehalose=-46,75. Apart from the formulation with mannitol, the second derivative of UV spectrum showed than tertiary structure remains unchanged after lyophilization, being the best results from the sucrose formulation. The adenoviral vector quaternary structure remains stable in every lyophilizate, except the one with PVP; this last one showed a 10% of aggregates with a size over 5500nm.
Conclusions: The acceptance threshold for survival ratio was predefined over 90%, hence, only lactose and sucrose showed to be effective enough cryoprotectants to accomplish with stablished requirements. Further experimentation will be developed to assess how the different cryoprotectants assayed affect the stability of the lyophilizates over time.

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DEVELOPMENT AND EVALUATION OF PHYSICAL AND BIOPHARMACEUTICAL PROPERTIES OF QUERCETIN EMULSION GELS FOR TOPICAL DRUG DELIVERY

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Introduction: Natural flavonoid quercetin is widely present in fruits and vegetables. Quercetin is a lipophilic compound that exerts various pharmacological effects such as anticancer, antiinflammatory, antioxidation, antianemic, antiplatelet, hypolipidemic and cardioprotective action.[1] Emulgels are semisolid dosage forms which consist of a mixture of emulsion and gel, used for dermal delivery of lipophilic substances such as quercetin. Nowadays emulsion gel formulations are commonly used because it is easy to use and enhances patient compliance. Physical characteristics of emulgels depend on proper selection of oil phase, emulsifier and gelling agent.[2]
Objectives: The objective was to formulate a quercetin emulsion gel for topical application with satisfactory physical properties and to examine the influence of gelling agent, oil phase and emulsifier on the emulsion gel characteristics.
Materials and Methods: Eight emulsion gel formulations containing 0.5% quercetin were prepared with concentrations of carbomer 940 as a gelling agent of 0.25% and 0.75%, concentrations of castor oil as an oil phase of 10% and 20%, and concentrations of Tween® 20/Span® 20 mixture (1:2) as an emulsifier of 1,5% and 3%. Emulgels were evaluated for their physical appearance, pH, spreadability, occlusive properties, quercetin content and its release from formulations.
Results and Discussion: The prepared emulgels had soft creamy consistency, homogenous texture, shiny appearance and favorable tactile properties. Quercetin emulsion gels were different in homogeneity and consistency, but all having adequate pH values (6.3-6.5) and quercetin contents (0.50-0.52%). The proportion of gelling agent was the predominant factor in determining the spreadability and the release of quercetin. Emulgels exerted mild to moderate occlusive effect and the concentration of oil phase had the greatest impact on occlusivity, although the other components also contributed.
Conclusions: The formulation composition significantly affects the physical characteristics of quercetin emulsion gels. Emulgel with 0.75% gelling agent, 10% oil phase and 1.5% emulsifier can be considered as the optimal quercetin formulation.

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ENHANCEMENT OF CLINDAMYCIN HYDROCHLORIDE DISSOLUTION RATE USING BILE ACIDS IN HYDROGEL PHARMACEUTICAL FORMULATIONS

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Introduction: Clindamycin hydrochloride is a widely used antibiotic in therapy of mild to moderate cases of acne and skin infections. Although widely used in dermatology, the major issue of conventional clindamycin topical formulations for effective treatment is the hydrophilic nature of clindamycin, not suitable for skin penetration and accumulation in the pilosebaceous structures. Therefore, new approaches in the development of clindamycin topical formulations are of great importance and several vesicular and particulate nanodelivery systems of clindamycin have been developed.[1] The use of bile acids as penetration enhancers in both conventional dosage forms and novel drug delivery systems has been increasingly investigated due to their biocompatibility and specific physicochemical properties.[2] To the best of our knowledge, topical formulations of clindamycin with bile acids have not been previously investigated.
Objectives: The aim of this study was to formulate 1% clindamycin hydrogel formulations for topical application with good physical properties and adequate release of the active substance, as well as to investigate the effects of the type of gelling agent, the type and concentration of bile acids as penetration enhancers, on the clindamycin dissolution rate using in vitro model.
Materials and Methods: Eight formulations of 1% clindamycin hydrochloride gel were prepared with two different gelling agents, two different bile acids as permeation enhancers in two different concentrations, adapting a 23 full factorial design. Carbomer 940 and carmellose sodium were used as gelling agents. The release and permeation enhancers in the hydrogel formulations were deoxycholic acid and cholic acid in concentrations of 0.1% and 0.5%. The hydrogels were evaluated for physical appearance, pH, drug content, drug release, and permeability parameters. An in vitro release test of clindamycin hydrochloride from hydrogel formulations was performed in an apparatus, resembling a Franz diffusion cell, containing a cellulose dialysis membrane. The amount of the drug released and permeated per unit surface area (μg/cm2) was plotted versus time (hours). The values of clindamycin permeability parameters in different formulations were calculated from dissolution curves. The interactions of clindamycin hydrochloride and bile acids were investigated also by molecular mechanics calculations (MM2).
Results and Discussion: Although formulations with carbomer as the gelling agent exerted optimal sensory properties, carmellose sodium hydrogels had significantly higher release rates and permeation of clindamycin hydrochloride. The permeability of clindamycin increased with the addition of bile acids, particularly after the addition of cholic acid in a higher concentration. Similarly, the permeability of clindamycin in carmellose sodium gels increased with the addition of bile acids. It was more pronounced after the addition of cholic acid than deoxycholic acid, and slightly more after the addition of cholic acid at a higher concentration in comparison to a lower concentration. Following the molecular mechanics calculations, it was shown that the total energies of both clindamycin/bile acid complexes were lower than the sum of the potential energies of the two single components optimized by molecular mechanics calculations, indicating that the formation of the complexes induced a stabilization of the system. Clindamycin/cholic acid complex was demonstrated to be more stable in comparison to the clindamycin/deoxycholic acid complex.
Conclusions: The type and concentration of bile acid affect the dissolution rate and permeation of clindamycin. In summary, the hydrogel containing carmellose sodium as a gelling agent and 0.1% cholic acid as a penetration enhancer can be considered as the formulation of choice.

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GALENUS INTERNATIONAL WORKSHOP

NEW NOSE-TO-BRAIN LIPOSOMAL THERAPY FOR THE TREATMENT OF ALCOHOL ADDICTION

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Introduction. Alcohol Use Disorder is an important Public Health problem that it is very difficult to treat. The pharmacological toolbox available has shown little efficacy and new therapeutic target such is the Dynorphin/Kappa Opioid Receptor (Dyn/KOR) system have shown promising preclinical and clinical data. However, targeting systemically this system can increase the occurrence and the severity of unwanted side effects. Nose-to-Brain delivery of drugs for CNS disorders treatment could be an option to reach the central nervous system (CNS) avoiding collateral effects. **Objective.** To develop and test efficacy of a intranasal formulation that allows a gradual release and delivery to the CNS of norbinaltorphimine (NorBNI), a KOR-antagonist. **Materials and methods.** Liposomes were prepared by the Thin Film Method technique and their size and Z-potential were studied by Dynamic Light Scattering. Liposomes were included in an in situ-forming gel that was developed to guarantee the desired properties. The gelation temperature was studied by the Modified Miller-Donovan Method and its viscosity at different temperatures was measured using a rotary viscometer. Following, Franz Cells were used for the drug release assay. Finally, efficacy to block the effect of a KOR agonist on dopamine release in the nucleus accumbens (NAc) was tested by microdialysis *in vivo* in rats. Rats were surgically implanted with two microdialysis probes targeting the nucleus accumbens (NAc) and daily treated with our formulation. **Results.** The Liposomes obtained were homogeneous with a 2,62 Z-potential and 109 nm size. They were adequately incorporated into the in situ-forming gel, which increased its viscosity at 27ºC. Drug-release studies show that liposomes achieve a gradual release of the norBNI from the pharmaceutical form, establishing equilibrium at 48h with a maximum release of 60%. Finally, our microdialysis data revealed that the treatment with our formulation blocked the effects of the KOR agonist administration. **Conclusión**. Our pharmaceutical formulation containing NorBNI-loaded liposomes reached key structures of the CNS providing the expected pharmacological effect.

Key words: Alcohol use desorder (AUD), KOR-antagonist, mucoadhesive intranasal formula and liposomes.

COMPARISON OF PURE ELLAGIC ACID OR POMEGRANATE EXTRACT RELEASE BY EUDRAGUARD® COLON TARGETED DELIVERY SYSTEMS (CTDSs)

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Introduction: The targeted delivery of active substances to the colon has several advantages for both topical and systemic action. The colon has a large surface area for absorption. The targeted delivery allows a reduction of the payload of active compounds because the release occurs directly at the site of action/absorption. In addition, CTDSs protect the loaded actives from pH or enzyme degradation in digestive fluids. In this comparative study, ellagic acid and pomegranate extract (titrated at 20% ellagic acid) were chosen for the widely known antioxidant and anti-inflammatory activities useful against inflammatory bowel diseases (IBD).
Objectives: The purpose of this research is to compare ellagic acid release from pomegranate phytoextract with the pure compound release from food-grade CTDSs.
Materials and Methods: Eudraguard® Biotic (EUGB) and Control (EUGC) copolymers were chosen as carriers. They are approved by EFSA as food additives (with code E1207 and E1206 respectively). Both have been tested in previous studies [1-3] to realize food-grade CTDSs for potential nutraceutical application. The formulation technique chosen is the Solvent Evaporation (SE). An ethanol solution, containing the lyophilized Eudraguard commercial dispersion and the active compound is dripped, under magnetic stirring (300 rpm), into an equal volume of water. Ethanol is then evaporated by rotary extractor. The formulation is frozen at -80 °C and then freeze-dried. The systems were formulated with matrices consisting of 1) EUGB; 2) EUGC; 3) 90% (w/w) EUGB and 10% (w/w) EUGC; and, 4) 70% (w/w) EUGB and 30% (w/w) EUGC. The preformulation study showed the formation of aggregates during ethanol extraction as the concentration of EUGC increased. The problem was overcome by adding AEROSIL® 200 F, a chemically synthesized SiO_2 approved as a food additive (E551). It provides effective protection of microparticle integrity during solvent extraction. The release test was performed on a modified model of the dissolution test for gastro-resistant tablets (apparatus 2 and method A) according to European Pharmacopoeia 10th Edn: the procedure consisted in a pH-change assay (i.e., 2 h at pH 1, 4 h at pH 6.8 and then up to 24 h at pH 7.4).
Results and Discussion: The obtained systems appear as highly hygroscopic brownish powders. The release profiles were compared and the degradation process was supported by a SEM study. The systems showed a gastric release less than 15%. The purê EUGB system showed the maximum peak release in the simulated colon environment (pH = 7.4) after 6 h from the beginning of the test. Pure EUGC microparticles or mixed matrices showed a prolonged release of the active compound. These profiles are in agreement with the copolymer properties shown in the fuctional coating of solid dosage forms [4, 5]. Thus, by modulating the ratio of copolymers it is possible to regulate the release of loaded actives in different regions of the intestinal tract.
Conclusions: The proposed systems ensure the release of ellagic acid from the phytoextract with a profile overlapping that of the pure compound. This can be na economic advantage in the development of oral nutraceuticals based on natural products and using targeted delivery technology.

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MICROENCAPSULATION OF LIVE PROBIOTICS FOR GALT ACTIVATION

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Introduction: Vaccines' efficacy can be challenged by a wide variety of factors. In patients with a dysbiosis, vaccine responses are usually lower, as shown by several studies[1]. On the other hand, probiotics can help restore a perturbed microbiota. WHO/FAO defines probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host"[2]. Some strain-specific probiotics can exert immune modulation, by activating the Gut-associated Lymphoid Tissue (GALT)[3]. However, industrial development of orally safe and viable probiotic formulations faces multiple challenges, especially the loss of bacterial viability along the intestinal tract. Therefore, multiple techniques for probiotic vehiculation have been developed with the aim to protect them, such as microencapsulation in organic particles[4].

Objectives: We aim to characterize the microparticles obtained by encapsulating two immunomodulatory probiotic strains, in order to promote the interaction with the GALT.

Materials and Methods: Strains of *L. rhamnosus* and *A. muciniphila* were selected and cultured under specific conditions to overexpress an immunomodulatory phenotype. Then, separately, they were microencapsulated with a cosolvation method[5]. Encapsulation efficacy and bacteria viability were studied, as well as the main physicochemical characteristics of the microparticles, such as size, distribution, and morphology.

Results and Discussion: Several prototypes of probiotic loaded microparticles were developed, obtaining a final product consisting in a spray-dried white powder with good rheological flow properties. The particles showed a size of approx. 15 µm and a rounded shape, reaching a final concentration of $>10^7$ CFU/mL in the final product.

Conclusions: We have successfully encapsulated two immunomodulatory bacterial strains, reaching a high concentration in the final product. Further methods to accurately quantify bacterial viability should be carried on, such as flow cytometry. The final goal is to deliver them safely to the GALT, located in the large intestine, where they could activate the innate immune system.

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OPTIMIZATION OF TRANSETHOSOMES FOR HYALURONIC ACID DELIVERY

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Introduction: Hyaluronic acid (HA) is a biopolymer with a key role in tissue regeneration and effective anti-ageing due to its properties like skin hydration, wound healing, skin wrinkling defence and UV radiation-protective ability.[1] Decreased amounts of HA in ageing skin are associated with loss of skin moisture.[2] The skin penetration of HA is limited by low permeability and large molecular size.[3] Hence, we decided to encapsulate HA in transethosomes (TEs), which are nanocarriers with ultra-deformability and enhanced skin permeability properties.
Objectives: This work aims to obtain stable HA-loaded transethosomes with enhanced skin penetration properties and low permeability for improved local action.
Materials and Methods: TEs composed of egg yolk phosphatidylcholine and surface-active agents (jojoba oil or tween 80) containing HA were prepared by the thin-film hydration method. HA of two molecular weights (20-60 kDa and 650 kDa) were used. HA was added in the aqueous component, and after ethanol addition, the dispersions were sonicated to produce nanoparticles. The vesicle size, size distribution and morphology of TEs were characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM). Quantification of HA incorporation efficiency (IE) was measured by high-performance liquid chromatography (HPLC) with a UV detector. Evaluation of storage stability of HA-loaded TEs was done by DLS and HPLC.
Results and Discussion: Produced HA-loaded transethosomes showed storage stability up to 10 weeks based on DLS measurements and HA content. Effective diameters show the size of TEs are suitable for transdermal delivery and depending on the composition of TEs, namely small molecular mass of HA (HA20) and the presence of tween 80 gives smaller sizes of TEs. The polydispersity index (PDI) is slightly higher than 0.2, which indicates an almost homogenous population of TEs. Zeta potential values verified the stability of formulations. HA content was determined on week 1 and 10 weeks later. The quantification of HA incorporation efficiency showed that the formulations have more than 87% HA inclusion in the TEs, except composition with jojoba oil and HA20.
Conclusions: Stable delivery systems containing HA were produced. Most of the studied formulations show high loading capacity and good potential for skin application.

Sample	Effective diameter, nm	PDI	Zeta potential, mV	IE, %
TEs JO HA20	188 ± 6	0.29 ± 0.01	-17.1 ± 4.3	0.00
TEs JO HA650	179 ± 8	0.33 ± 0.01	-25.5 ± 5.8	88.7 ± 2.4
TEs tween 80 HA20	74 ± 6	0.27 ± 0.01	-16.8 ± 4.4	87.5 ± 0.1
TEs tween 80 HA650	187 ± 12	0.32 ± 0.01	-26.9 ± 2.6	98.5 ± 0.1

Data expressed as mean ± standard deviation (n = 9 for DLS data and n = 2 for IE quantification)

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New strategies in targeted therapy for scleroderma fibrosis

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Introduction: Systemic sclerosis or scleroderma is a systemic autoimmune disorder characterized by vasculopathy, immune dysfunction, and fibrosis of the skin and other organs. Several studies have been performed to investigate potential benefits of statins in scleroderma[1]. Specifically, the effect of statins to prevent both skin thickness and pulmonary fibrosis in a murine model of scleroderma has been demonstrated being statin administration performed by s.c. injection[2]. Fluvastatin, a synthetic statin used for the treatment of hyperlipidemia has been found to exert an antioxidant effect and to prevent apoptosis due to lipid peroxidation induced by hydrogen peroxide in endothelial cells. The possibility to use fluvastatin to treat the dermal disorders associated to scleroderma has never been studied. The current study converged on investigating dermal and transdermal route for fluvastatin administration. For the purpose we designed a formulation based on nanostructured lipid carriers (NLC) for transdermal delivery of fluvastatin, in a combined *in vitro/in vivo* development approach.
Objectives: The aim of this work is to develop a non-invasive approach to deliver fluvastatin on the skin.
Materials and Methods: NLC were prepared by emulsification followed by ultrasonication technique, using glycerol distearate as solid lipid, oleic acid as liquid lipid and Tween 80 as surfactant[3]. Fluvastatin was dispersed in the oil phase. The developed system was characterized in terms of size, charge, thermal stability, and permeation through newborn pig, using Franz diffusion cells.
Results and Discussion: Fluvastatin was successfully encapsulated in NLC. The influence of increasing amounts of fluvastatin in particle size, polydispersity index, entrapment efficiency and drug loading values was also studied. The incorporation efficiency for fluvastatin was higher than 95%, supporting the NLC as efficient carriers for drug encapsulation. In order to investigate the effect of temperature on particle size, thermal analysis was performed and shown that the effects caused by temperature on drug-loaded nanoparticles are reversible. *Ex vivo* permeation studies were done to assess the amount of fluvastatin found in skin tissue after 24h. The results revealed no fluvastatin permeation but high drug skin deposition was achieved.
Conclusions: The encapsulation and skin retention parameters achieved were very satisfactory, and encouraged us to use of the developed formulation on a mice model of chronic oxidant stress that will evaluate skin and lung fibrosis. The effect of permeation enhancers and of the vehicle viscosity on the permeation of the drug formulated as NLC must be further investigated.

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ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL POMPIA LOADED IN LIPOSOMES

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Introduction: Some natural compounds can be used as alternative to synthetic drugs to prevent and treat skin diseases. However, the use of natural compounds, such as plant derivatives and essential oils, is often limited because of their volatility and poor stability.[1] To solve it, essential oils are incorporated in liposomes that presents several advantages as high biocompatibility, self-assembly properties, the ability to carry large amounts of the payload, and a wide range of physico-chemical properties that can be modified in favor of their biological characteristics.[2]

Objectives: This study was conducted with the aim of evaluating the antimicrobial activity of pompia essential oil, alone and loaded in liposomes, against a yeast (*Candida albicans*) and a gram-positive bacterium (*Staphylococcus aureus*).

Materials and Methods: Liposomes were prepared mixing 360 mg of Lipoid S75 and 60 mg of pompia essential oil. Mixtures were hydrated with water and sonicated with 15 cycles (5 seconds "on" and 2 seconds "off", 13 microns of probe amplitude) with a high intensity ultrasonic disintegrator. Vesicle formation and morphology were checked by transmission electron microscopy (TEM). The antimicrobial activity was evaluated using the disk diffusion test and the microdilution method. In the case of pompia essential oil, traditional turbidimetric assays using a 96-well plate were performed. However, liposomes were studied using a colorimetric method due to their self-turbidity.

Results and Discussion: Morphology of pompia essential oil in liposomes was checked by TEM and the obtained micrographs confirmed the formation of spherical vesicles. The results obtained in the agar diffusion tests showed that pompia essential oil exhibited antimicrobial and antifungal activity against both tested microorganisms (inhibition halos of 25 ± 6 mm and 16 ± 3 mm, respectively). By contrast, inhibition was not observed when liposomes were assayed. MIC_{50} and MFC/MBC (minimum fungicidal and bactericidal concentration) values demonstrated a similar antimicrobial activity of pompia essential oil against both microorganisms. However, liposomes were more active against *C. albicans* than against *S. aureus* (MIC_{50} 2.5 and 10 mg/ml, respectively). The antimicrobial activity of liposomes was less potent than the reference product, but this decrease was not very intense, making these formulations adequate for the administration of the assayed essential oil.

Conclusions: The essential oil pompia can be suitably loaded in liposomes and it could be useful for the treatment of skin or mucosal infections caused by microorganisms such as *C. albicans* and *S. aureus*.

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SYNTHESIS AND EVALUATION OF SULFOSUCCINATE-BASED SURFACTANTS AS COUNTER IONS FOR HYDROPHOBIC ION PAIRING

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Introduction: Hydrophobic ion pairing (HIP) describes the conversion of charged hydrophilic molecules into hydrophobic complexes via interaction with counterions, typically surfactants. Thus, the encapsulation efficiency of peptides and proteins into lipid-based formulations can be significantly increased[1]. Despite encouraging *in vitro* and *in vivo*-studies[2], no oral formulation based on HIP has yet reached clinical trials. Systematic studies and an enlargened variety of counterions could enable a rational design of hydrophobic complexes, revealing the yet untapped potential of HIP.
Objectives: This study aims to establish a facile synthesis protocol that allows researchers to design various mono- and dialkyl sulfosuccinates with saturated and unsatured linear, as well as branched alkyl tails. Comparison of the counterions, including the gold standard docusate, gives systematic insights which hydrophobic tails are beneficial and might result in the formation of complexes with superior hydrophobicity.
Materials and Methods: Synthesis of 13 sulfosuccinate mono- and diesters was confirmed via FT-IR, ^{1}H-NMR, and ^{13}C-NMR. A broad screening of all counterions was conducted with the model protein hemoglobin as it allows sensitive detection via spectrophotometry. Precipitation efficiency and logP were determined. Lead counterions were identified and further examined with the therapeutic peptides and proteins vancomycin, insulin, and horseradish peroxidase.
Results and Discussion: LogP-values of the hemoglobin-complexes increased with increasing cLogP of the respective counterions. Formation of diesters led to counterions of higher hydrophobicity. Branched and unsaturated linear alkyl tails outperformed saturated linear alkyl tails in terms of hydrophobicity of the formed complexes. Monostearyl sulfosuccinate, dioleyl sulfosuccinate, and bis(isotridecyl) sulfosuccinate were identified as lead counterions. Hydrophobic complexes formed between the therapeutics and dioleyl sulfosuccinate provided an up to 8.3-fold higher partition coefficient and up to 26.5-fold higher solubility in 1-octanol than DOC, whereas bis(isotridecyl) sulfosuccinate resulted in an up to 6.7-fold improvement in the partition coefficient and up to 44.0-fold higher solubility in 1-octanol (Fig. 1).
Conclusions: A synthesis template to yield sulfosuccinate mono- and diesters with various hydrophobic tails was established. Based on this template, novel counterions were synthesized that were superior to the current gold standard docusate. Thus, these lead compounds are promising candidates for future studies on HIP.

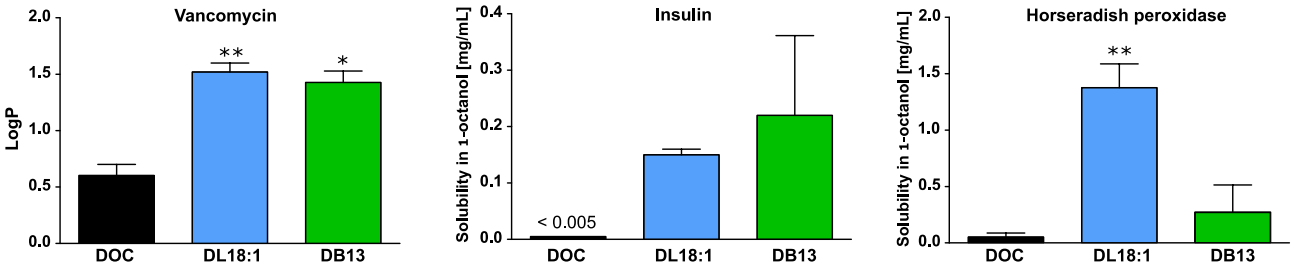

Figure 1: Hydrophobicity of complexes formed between docusate (DOC), dioleyl sulfosuccinate (DL18:1), and bis(isotridecyl) sulfosuccinate (DB13) and vancomycin, insulin, and horseradish peroxidase, respectively. Solubility in 1-octanol is given as measure for hydrophobicity, when the amount of drug in the water phase was below the limit of detection ($n \geq 3 \pm SD$). Significant differences to docusate are marked with * ($p < 0.05$) and ** ($p < 0.005$).

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DESIGN OF DIOSMIN-LOADED NANOSTRUCTURED LIPID CARRIERS BY RESPONSE SURFACE METHODOLOGY: CHARACTERIZATION AND IN VITRO EVALUATION ON ARPE-19 CELLS

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Introduction: Inflammatory response is a typical feature of the most common degenerative diseases of the posterior segment of the eye. Diosmin, a natural flavonoid, is considered a protective agent widely used in the treatment of vascular diseases. Its use in the treatment of eye diseases is due to its anti-inflammatory and free-radical scavenging properties. Despite its benefits, like many other flavonoids, it is characterized by a poor water solubility that impair its pharmacological application. The encapsulation of Diosmin in nanostructured lipid carriers (NLC) intended for ocular delivery could represent a promising strategy to overcome this issue and improve its pharmacological activity.
Objectives: To the best of our knowledge, the design of diosmin-loaded NLC (D-NLC) for ophtalmic route represents a novelty that has not been yet explored. Thus, Box Behnken design (BBD) was exploited to optimize NLC suitable for diosmin delivery. After diosmin encapsulation, the optimized formulation was investigated for particle size, polydispersity, zeta potential, pH and osmolarity. Finally, in vitro studies were carried out on retinal ARPE-19 cells in order to assess the cell tolerability of the carrier and the anti-inflammatory effect of D-NLC.
Materials and Methods: NLC were prepared by a melt emulsification method followed by ultrasonication. The design was composed of four independent variables (solid lipid concentration, liquid lipid concentration, surfactant concentration and type of solid lipid). The effect of the factors was evaluated on NLC size (response). Analysis of variance (ANOVA) was used to assess the statistical significance of the model and the impact of the independent variables on the response. The optimized formulation was selected according to the desirability function. Diosmin was encapsulated in the optimized NLC (at 160 μM). The viability test was performed on ARPE-19 cells after 48 h of treatment. The effect of D-NLC was evaluated in an *in vitro* model of retinal inflammation, after exposure of ARPE-19 cells to TNF-α (20 ng/mL) and 160 μM D-NLC for 48 h.
Results and Discussion: BBD revealed that the model used (2FI) was significant (p value <0.0001) and that the type of solid lipid exerted the most significant effect on particle size. The optimized NLC was composed of 10% (w/v) Softisan® 100, 3% (w/v) Capryol® 90 and 1% (w/v) Tween® 80. The size of the optimized NLC were around 70 nm, that can be considered highly suitable for ocular drug delivery. After Diosmin encapsulation, mean particle size remained homogeneous (PDI 0.20 ± 0.07) and almost unchanged (74.5 ± 0.88 nm) compared to the empty NLC; a negative Zeta potential (-12 ± 0.2 mV) was registered. *In vitro* studies revealed the cytocompatibility of empty NLC on ARPE-19 cells at all the tested concentrations (from 0.5 to 0.0025 %, v/v) and the cytoprotective effect of D-NLC on an *in vitro* model of retinal inflammation at 0.01, 0.075, and 0.005 % v/v.
Conclusions: BBD was found to be a rieliable model to optimize NLC for Diosmin encapsulation. D-NLC were found to be safe and well-tolerated by ARPE-19 cells and could be a promising candidate for the treatment of ocular tissues inflammation. However, additional studies are ongoing to further assess and confirm D-NLC anti-inflammatory activity.

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MAGISTERIAL FORMULATION IN VETERINARY MEDICINE: ORAL ITRACONAZOLE FOR CATS

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Introduction: Itraconazole is an azolic drug used for the treatment of differents dermatophytoses, the most usuals in cats, generated by fungi of *Malasezzia spp* family. But it has also been studied in the treatment of blastomycosis and histoplasmosis in dogs, or even in Aspergillosis or Coccidiomycosis in penguins and sea lions respectively.
In Spain, for the treament of different dermatophytoses, the autorized medication for veterinarian use is Itrafungol®. This is an oral suspensión which contains itraconazole 10mg/mL, but it's only autorized for cats. The dosage recommended in the information leaflet is 0.5 mL/kg/day or 5 mg/kg/day, administered in three periods alternating seven days of treatment, followed by another seven days without treatment. It is recommended to administer this drug on an empty stomach since food can interfere with the absorption of the drug.[1-3]
In Spain there are serious problems of shortages of the medicine. In addition, the commercial formulation contains 52 ml of itraconazole 10mg/mL, which in case of need for high doses may be insufficient to finish the treatment. [1-3]
Objectives: To design, obtain and characterize new magisterial formulations of itraconazole and compare the stability and pharmacokinetics parameters in order to solve shortages and dosage problems.
Materials and Methods: Five suspensions of itraconazole were obtained including different solubility enhacers and gelling agentes. Organoleptic and phisicochemical parameters were measured and compared with the reference. Stability assays, disolution assays and permeability assays were carried out for all the new formulations. Dissolution profiles for the reference product and the different formulations were performed at pH 1.2, 4.5 and 6.8. For the analysis of itraconazole concentrations, two techniques were used, mass spectrometry and the reverse phase high-performance liquid chromatography (HPLC) technique with fluorescence detection. Permeability assays were carried out using Doluisio technique.
Results and Discussion: Five formulations were obtained with itraconazole and different excicipients. The five formulations have different organolpetic and phisicochemical parameters. In fact, pH and osmolarity depend on the composition.
Stability assaays reveled that there is an initial precipitation process and the expiration date of the different formulations of itraconazole was low compared with free itraconazole. However at 4ºC stability was acceptable for magisterial formulations
Dissolution profiles for the reference product and formulations at pH 1.2, 4.5 and 6.8 show that the magisterial formulations dissolution processes were different from reference but little modifications allow to improve the dissolution profiles. Dissolution profiles of modified formulations and the reference product obtained using the dumping test technique revealed that one of the formulations exhibit an interesting dissolution profile comparable with reference formulation
Permeability assays indicate that the itraconazole permeability remains equal or increase in all the assayed formulations compared with itraconazole permeability of the reference formulation

Conclusions: The inclusión of solubility enhancers and gelling agents in the elaboration of the itraconazole in syrup suspensions has a great influence on phisicochemical and biopharmaceutics parameters. However, it is possible to design a formulation with similar parameters than the reference one

The preparation of magisterial itraconazole syrup is useful for both to solve shortages and to elaborate medicines at different doses and volumes in order to have enough for all the treatment.

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