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Abstracts

Section 2 - Pharmaceutical Technology

PT1

INTRANASAL DELIVERY OF GALANTAMINE VIA ENZYMATICALLY CROSSLINKED HA-BASED HYDROGELS.

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Galantamine has been approved and used in oral dosage forms for the treatment of Alzheimer's disease. It functions through the inhibition of acetylcholinesterase, the enzyme which impedes cholinergic neurotransmission. However, the oral dosage forms are characterized by gastrointestinal disorders such as vomiting, nausea and diarrhea[1]. This preliminary work seeks to mediate these side effects through the intranasal administration of galantamine via enzymatically crosslinked hydrogels. Hyaluronic acid tyramine derivative (HATA) was used as a matrix and formulated into hydrogels through tyrosinase crosslinking. Precursor solutions of the hydrogels were characterized for their viscosity and wettability[2]. Hydrogels were then characterized for rheological parameters such as kinetics of gelation and the linear viscoelastic properties. Swelling and in vitro dissolution studies were conducted. The results depict a successful synthesis of hydrogels and subsequent rapid release of galantamine within three hours which is dependent on polymer concentration.

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PT2

ADVANCED THERAPY FOR LIVER INFLAMMATION: TARGETING MACROPHAGES WITH CORTICOSTEROID-LOADED NANOSPHERES

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Corticosteroids are widely use highly effective anti-inflammatory drugs. Nonetheless corticosteroid therapy relates to serious side effects.[1] Loading corticosteroids into polymeric nanoparticles offers a promising approach for safer and more effective inflammation treatment. Our project focuses on liver inflammatory diseases. Macrophages play a crucial role in the inflammation process, making them an interesting target for corticosteroid-loaded nanospheres (NSs).[2] In our project dexamethasone acetate-loaded NSs (DA NSs) were prepared using the nanoprecipitation method. These NSs were designed to be selectively attractive for macrophages, using passive targeting for macrophage uptake. Two types of DA NSs, ranging in size from 100 to 200 nm, were synthesized, showing a DA loading efficiency approximately 20 %, with a prolonged release of the active substance within 3 days. *In vitro* tests using murine bone marrow-derived macrophages demonstrated promising outcomes, showing a significant reduction in pro-inflammatory cytokine levels (*Il-1β* and *Tnf- α*) without obvious signs of toxicity. The internalization of fluorescent dye-loaded NSs by macrophages, particularly the pro-inflammatory M1 macrophages, was confirmed by flow analysis. Subsequent *in vivo* experiments on murine models examined accumulation of our NSs after intravenous administration. The accumulation in the liver was confirmed by IVIS imaging system, flow analyzer and microscopic examination of various organ slides.

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PT3

STUDY OF DRUG-SILICA INTERACTIONS IN SIMULATED MEDIA

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It has been proven that mesoporous silica materials have the potential to increase the solubility of poorly water-soluble drugs. This is achieved by nanoconfinement of the amorphous drug form in pores, hence hindering its recrystallization[1]. However, the clinical use of mesoporous silica-based systems is still limited, mainly due to the often-observed incomplete drug release. This may be connected to the drug-silica interactions and potential readsorption of the drug onto the silica surface during the dissolution process. The type and strength of such interactions (e.g., hydrogen bonding, electrostatic, and nonspecific hydrophobic interactions) may be affected by the drug and silica surface chemistry, which varies under different physiological conditions as a function of the pH and ionization state[2]. These interactions might lead to the drug molecules absorbing back into the silica after being released, which can result in incomplete release[3]. The drug adsorption from simulated media to the silica can be investigated with adsorption isotherms. In this study, the poorly soluble drug flufenamic acid and two non-ordered mesoporous silica carriers, Aeroperl® 300 Pharma and Neusilin® US2 were used for the adsorption isotherms. The obtained results suggest that flufenamic acid has an adsorption tendency to mesoporous silica which may play a role in the incomplete drug release from such systems.

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PT4

ENHANCING ENCAPSULATION AND PROLONGING RELEASE OF HYDROPHILIC ANTIBIOTICS WITH PLGA NANOPARTICLES THROUGH ION PAIRING

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Drug loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) have significant potential in managing bone infections. Antibiotics used for local treatment of bone infections are highly hydrophilic, so they interact weakly with PLGA, leading to reduced encapsulation efficiency (EE), drug loading (DL), and high initial burst release. The method of hydrophobic ion pairing (HIP) could overcome these limitations and optimally achieve sustained antibiotics release for 2-3 weeks.[1,2]

The study aimed to optimize the hydrophobic ion pairing (HIP) method, in which an ionically charged molecule is paired with counterionic compound containing a hydrophobic component. Selection of suitable HIP agent and its molar ratio to antibiotics were investigated. The size, polydispersity index, and zeta potential of prepared PLGA NPs were determined using a Zetasizer Nano ZS. The EE and DL of antibiotics and their HIP complexes in PLGA NPs were determined using HPLC and UV-vis spectroscopy. The complexes' charge was established using Particle Charge detector. The results showed that the use of HIP method significantly increased the EE and DL of highly hydrophilic antibiotics in PLGA NP.

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PT5

SKIN BARRIER MODELS: FROM BARRIER FORMATION TO POSSIBLE TOPICAL APPLICATION OF BARRIER LIPIDS

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The skin barrier, located in the *stratum corneum* (SC), is an essential component in the existence of land mammals, including humans. The structure is composed of the corneocytes, free (ceramides, free fatty acids and cholesterol) and covalently bound lipids.[1] The amount of barrier lipids and also their arrangement in the intercellular space of the SC can change and that change is typical for some skin diseases and for damage of the barrier function as well. Understanding the process of the arrangement of lipids into lamellar structures is essential for finding new more effective approaches to treating skin diseases. The aim of this work is to develop a simple and valid procedure for delipidization and then incorporation of lipids into damaged skin barrier as well. For this purpose, skin barrier models were used, *i.e.,* extracted human SC with topically applied barrier lipids. With the lipid replenishment, there was emphasis placed on the control of the time and physico-chemical conditions during the lipid lamellae formation, especially on the change in pH of the aqueous phase. The barrier lipids extracted from healthy human skin were transferred to an aqueous alkaline environment (HEPES buffer solution; pH around 8) to form lamellar lipid vesicles, so-called liposomes/cerosomes. In this work, the amount of hydrochloric/acetic acid, which acidifies the lipid vesicles to a physiologically acidic pH, was monitored. Dialysis membranes for controlled acidification were used to achieve the slow arrangement of barrier lipids. The prepared models were evaluated by X-ray diffraction (repeated distance of lipid lamellae) and infrared spectroscopy (orthorhombic vs. hexagonal packing and phase separation).

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PT6

HYALURONIC ACID GRAFTED WITH CERAMIDE NP AS PROMISING MACROMOLECULES FOR SKIN APPLICATION

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In this work, amphiphilic hyaluronan (HA) was synthesized by grafting succinylated N-oleoyl-phytosphingosine (known as ceramide NP) via esters bonds. Succinylated N-oleoyl-phytosphingosine (sCER) was first synthesized by esterification of hydroxy moieties of the ceramide with succinic anhydride. Secondly, the esterification of hyaluronan was carried out using oligomeric, low, and medium molecular weight HA. The oligomeric HA-sCER derivatives exhibited a strong self-aggregation as evidenced by a very low critical aggregation concentration (1.9 μg m^{-1}), higher pyrene binding constant, and the particle's smallest size in solution. The self-aggregation properties were demonstrated to be a function of the substitution degree, the molecular weight of HA, and the substitution pattern. The prepared derivatives were non-cytotoxic towards cell lines NIH-3T3. A significant inhibition of the pro-inflammatory cytokine interleukin-6 was observed in vitro using macrophages differentiated from THP-1 cells. Regardless of their size, nanoparticles prepared using amphiphilic HA-sCER derivatives improved penetration of the hydrophobic Nile red dye through the porcine stratum corneum. Interestingly, the fluorescence intensity localized at the stratum corneum was higher for oligomeric HA-sCER. These findings showed that HA-sCER are promising vehicles for use in transdermal medical or cosmetic agents for skin drug delivery.

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PT7

OLIGONUCLEOTIDE LOADED HYBRID NANOPARTICLES FOR CHRONIC LIVER INFLAMMATION MANAGEMENT

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Therapeutic oligonucleotides such as siRNA are currently one of the most potent therapeutics for a wide variety of diseases. However, naked siRNA undergoes non-specific distribution, fast degradation by endonucleases and has a poor cell-uptake. The suitable carrier ensuring siRNA protection and ensuring site-specific distribution is of utmost importance. This project's aim is the preparation and biological evaluation of a fully biodegradable, biocompatible and macrophage specific nano-drug delivery system for anti-inflammatory siRNA as a potential platform for chronic inflammation therapy. Creation of a suitable delivery system consists of preparation of a oligonucleotides and cationic lipid (DOTAP) complex by Blight-Dyer technique. This approach gives the naturally hydrophilic oligonucleotide molecule overall hydrophobic properties. Consequently, oligonucleotide-loaded nanoparticles (NPs) based on poly(lactic-co-glycolic) acid were in this case prepared by simple and rapid nanoprecipitation method. Two methods have been used for encapsulation efficiency (EE) estimation. Direct method, where the oligonucleotide is extracted directly from NPs and indirect method using supernatant for measuring the non-encapsulated oligonucleotide. In both cases plate reader has been used for oligonucleotide quantification. The comparison was evaluated on various oligonucleotides and NPs prepared by different methods, and it showed approximately 40 % difference. The direct method shows lower EE compared to indirect one, but the results show, that the direct method is probably more accurate and has a benefit of measuring the EE after purification steps and preparation for cell experiments.

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PT8

PREPARATION OF ORODISPERSIBLE TABLETS BY SELECTIVE LASER SINTERING

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Orodispersible tablets (ODTs) are fast-dissolving solid dosage forms that represent a possible solution for easier administration in children, elderly, and people with an affected mucosa of the upper gastrointestinal tract.[1] In terms of individualization of medicine preparation, selective laser sintering is a promising method for ODT preparation due to the possibility of exact dosing and proper regulation of tablet porosity.[2]

In this work, printing parameters such as laser speed, chamber and powder temperature, layer height were studied along with powder flow properties. Two polymers with different characteristics, Kollidon® VA64 and Kollicoat® IR, were used to create the tablet matrix. The results from differential scanning calorimetry revealed amophous nature of Kollidon® VA64 with glass transition point of 104°C, whereas Kollicoat® IR had semicrystalline structure and melting point at 214°C. For this reason, the printing temperatures were selected as folows: chamber temperature 90°C and powder temperature 100°C. At these temperatures, Kollicoat® IR did not melt and therefore functioned as pore-forming agent and flow-enhancing substance. Formulation containing Kollidon® VA64 and 10% of Kollicoat® IR resulted in improved flow properties and high-quality tablets with high values of hardness, mass, and fast disintegration time suitable for orodispersible tablets.

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PT9

INCREASING OF MELOXICAM DISSOLUTION RATE BY SPRAY DRYING FROM MIXTURE OF ETHANOL AND ACETONE

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The aim of this study is to increase the dissolution rate of the poorly water-soluble drug meloxicam (MX) by preparation of co-processed products via spray drying from an organic solvents mixture of ethanol: acetone 1:1. Three spray dried products were prepared: the spray dried model drug MX (MX SD); MX with a chitosan carrier (CHIT; MX-CHIT SD), and the mixture of MX, CHIT, and surfactant sodium lauryl sulfate (SLS; SDM). The constant ratio of MX, SLS, and CHIT 1:0.1:5 was used based on the solubility assessment in the ethanol/acetone (technical grade) mixture and in compliance with the critical parameters connected with the spray drying process (Mini Büchi spray dryer B-290). The dissolution rate of MX for the obtained products was characterized primarily by a flow through cell (USP 4). Furthermore, the resulted spray dried materials were analysed for particle size (laser diffraction analyser), shape and morphology (scanning electron microscope), and crystallinity (X-Ray diffraction, (modulated) differential scanning calorimetry, Fourier transform infrared spectroscopy). MX SD reached 3.35 % dissolution and the maximum release rate 0.04 min^{-1} in the 5-minutes dissolution test while MX-CHIT SD reached 53.35 % of the dissolved MX and SDM reached the complete dissolution. Therefore, it can be concluded that all spray dried mixtures increased the amount of dissolved MX and its release rate compared to MX SD. The highest dissolution rate was observed particularly within the first 60 seconds due to the MX deagglomeration and the improved availability for the liquid medium to access the increased surface area. Moreover, the detected partial amorphization and the change of MX polymorph contributed to the improved dissolution. In conclusion, co-processing by spray drying of MX from organic solvent in the presence of SLS and CHIT is a promising method to improve MX dissolution rate.

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PT10

EARLY SCREENING OF APPARENT DRUG INTERACTIONS TO MESOPOROUS SILICA

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Drug delivery systems based on supersaturation provide an encouraging way to improve the bioavailability of poorly water-soluble drugs that remain a challenge in formulation development[1]. The use of mesoporous silica formulations (MSF) represents one of the most promising supersaturating formulation strategies. However, there is a lack of screening approaches at the drug discovery stage regarding candidate (drug molecule) selection for the intended use in a mesoporous silica-based formulation. Therefore, this study aimed to address both aspects, the efficient screening of drugs and the formulation aspect with mesoporous silica. Within this study, an efficient experimental method for screening of drug molecules regarding the interaction with silica surfaces (retention time) was developed. 52 active pharmaceutical ingredients were screened in an automated way using a high-pressure liquid chromatography (HPLC) system and a 25cm long column which was packed with hydrophilic porous silica as stationary material simulating the mesoporous carrier surface. By simple detection of the retention time of the eluting drug peaks, it is proposed to obtain a ranking of the drug's apparent interaction with silica. This is very useful since it is expected that a balanced affinity of the drug molecules to the silica surface is desirable regarding drug loading and release from mesoporous silica formulations.[2] Therefore, there is great potential in the introduced screening method which may help formulation scientists to perform efficient screening tests to choose optimal drug candidates for MSF at the drug discovery stage with very low drug amounts.

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PT11

THE INTERPLAY BETWEEN WATER PHASE PH AND CHARACTERISTICS OF OBETICHOLIC ACID-LOADED PLGA NANOPARTICLES

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PLGA (poly(lactic-co-glycolic acid)) is an FDA-approved, biodegradable and biocompatible copolymer. PLGA nanoparticles can encapsulate a wide range of substances, from small-molecule drugs to macromolecules with high encapsulation efficiency. The physicochemical properties of the water phase has a crucial significance in determining the final characteristics of nanoparticles prepared by nanoprecipitation method. The aim of the work was to investigate the effect of water phase pH during nanoprecipitation on the parameters of obeticholic acid (OCA) loaded PLGA nanoparticles. Buffers with various pH ranges were used as a water phase. PLGA nanoparticles size decreased from 189 nm to 42 nm while using buffered water phase of pH from 2.5 to 10. The most significant change in PLGA nanoparticles size occurred around pH corresponding to pKa of PLGA of 3.85[1]. This suggest that PLGA ionization plays an important role in particles formation. The phenomenon of pH-dependent size reduction can be explained by two possible mechanisms: decreased hydrophobicity and at the same time enhanced repulsion forces between PLGA chains at different pH. Our findings also demonstrated that encapsulation efficiency of OCA into PLGA nanoparticles was pH – dependent: with the increasing pH of water phase from 2.5 to 10, the encapsulation efficiency of obeticholic acid decreased from 88,7% to 14.8%. It can be explained by the OCA ionization and corresponding increased solubility with the increasing pH. The nonionized form of OCA is preferably incorporated into PLGA.

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