FACULTY OF PHARMACY
IN HRADEC KRÁLOVÉ
Charles University

10th Postgraduate and Postdoc Conference

22. - 23. January 2020

Abstracts

Session 3 - Pharmaceutical Technology

EFSA-CDN

This conference is supported by the EFSA-CDN project (Reg. No. CZ.02.1.01/0.0/0.0/16_019/0000841)
co-funded by the European Union

EUROPEAN UNION
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Development and Education

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

FT1

IMPROVING THE DISSOLUTION RATE OF POORLY SOLUBLE MELOXICAM BY CO-MILLING

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The aim of this work was to study the possibility of increasing the dissolution rate of the model drug meloxicam (MLX, BCS class II, solubility in water is 4.4 mg / ml at 25 °C[1]) using solventless co-milling with a hydrophilic drug carrier chitosan (CHIT) of 85 % deacetylation degree. Firstly, the properties of both substances were estimated. MLX particles are very fine with a mean particle size x_{50} = 3.7 μm and a relatively narrow distribution (span = 1.94). On the other hand, CHIT has platelet particles with a mean size x_{50} = 59.3 μm and a wider distribution (span = 2.30). The melting point T_m = 262.4 °C was detected for MLX (crystalline substance). CHIT shows glass transition temperature T_g at 122.5 °C (amorphous substance). Subsequently, the binary powder mixtures were prepared at 1:1 and 1:8 ratios in a planetary ball mill. The effects of ball size, rpm and milling time on properties of co-milled mixtures were investigated. The changes in the particle size and particle size distribution were monitored by laser diffraction using a dry cell. Flow-through cell type of dissolution (USP 4, phosphate buffer pH 6.8) with open loop was used to evaluate the dissolution rate of the drug in the co-milled powder mixtures. The detected dissolution rate r (mg × dm^{-3} × s^{-1}) of MLX in the MLX:CHIT mixture co-milled in 1:8 ratio for 15 minutes was approximately four times higher than the dissolution rate of MLX within the first ten minutes. The reason is presumably an increase in specific surface area of the micronized drug particles together with deagglomeration during the co-milling/mixing with the hydrophilic carrier with larger particles.

The study was supported by the Funding Agency of Charles University under Grant No. 1286218/201, by SVV 260 401 and Zentiva, k. s.

References

1. AMBRUS, R. et al.: Int. J. Pharm., 381, 2009, 153-159.

AVALANCHE TESTING AND ITS RELATIONSHIP TO COHESION OF POWDERS

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Powders and granular materials are two-phase systems where individual solid particles touch each other and are diffused in gaseous surroundings; the interparticle contacts influence the behaviour of the powder bed. Powder properties depend on many parameters such as particle size, shape, density, cohesive forces as well as on the air humidity and temperature.[1] More cohesive materials can cause flow difficulties during pharmaceutical processes. Therefore, shear testing and new methods of avalanche measurements studying dynamic powder behaviour are used to describe these materials.

It was observed previously that some portion of powder microcrystalline cellulose (MCC) in mixtures with pellets made of microcrystalline cellulose (C100) improved compressibility of mixtures into tablets. In this work, the influence of MCC on the interparticle cohesion and flow properties were studied using the automated shear cell (ShearScan) and dynamic flow measurements (Revolution powder analyser). The more MCC in the mixture the higher cohesion was observed following with worsening in avalanche behaviour from slumping (C100 and M90) through cascading (M80-M60) to cataracting when 50 % or more of MCC was added. The relationship between the cohesion and avalanche angle was described with the linear regression with the coefficient of regression R = 0.9242. The results show the importance of careful choice of mixture composition to achieve the optimum composition for both good compression and flowability.

The study was supported by the Funding Agency of Charles University under Grant No. 1286218/2018 and by SVV 260 401.

References

1. PRESCOTT, J. K., BARNUM, R. A.: Pharm Technol., 24, 2000, 60-84.

FT3

CHARACTERIZATION OF RHEOLOGICAL PROPERTIES OF POLYMERS FOR FORMULATION OF LIQUISOLID SYSTEMS TARGETED TO THE COLON

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Targeting drugs directly to the colon is beneficial for the local treatment of inflammatory bowel diseases and other colonic pathologies. Drug delivery systems which can control or sustain drug release in the colon is needed in order to achieve satisfactory drug levels for a longer duration, thereby minimizing dosing frequency and adverse effects. A possible approach to control drug release is by the formulation of liquisolid systems by incorporating hydrophilic polymers as matrices. Such polymers become hydrated in aqueous medium to form a gel layer which controls the drug release. The ability of the polymer to control drug release is partly influenced by viscosity of the hydrated gel layer. Therefore, this study aims to characterize a range of polymer candidates (guar gum, sodium alginate, sodium carboxymethyl cellulose and various grades of hydroxypropyl methylcellulose) by rheological measurements. Biorelevant media; Fasted State Simulated Gastric Fluid (FaSSGF) and Fasted State Intestinal Fluid (FaSSIF) of pH 1.6 and 6.5, respectively, were used to prepare polymer dispersions simulating the gel layer formed at the surface of hydrated matrices in the gastrointestinal tract. Flow rheology measurements were carried out on a rheometer. Flow curves were obtained, using a shear rate ramp at shear rate 0.01–100 s^{-1}. Ostwald-de Waele (power law) model was applied to describe the rheological flow behaviour. The apparent viscosities of the polymers at shear rates of practical importance were reported. Although other formulation parameters will influence the drug release, viscosity data from the rheological studies will serve as a useful basis for further studies with regards to the formulation of liquisolid systems for colon-targeted drug delivery.

The study was supported by Project No. 70119/2019 of Grant Agency of Charles University and by SVV 260 401

IN VITRO MODELS OF SKIN LIPID BARRIER

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First-line protection of the human body is carried out by the outermost skin layer, the stratum corneum (SC), which is composed of corneocytes embedded in a lipid matrix of ceramides, cholesterol and fatty acids.[1] Skin diseases are associated with modified skin barrier functions which result from alterations in the skin barrier composition. In this regard, the use of model lipid membranes is a very useful tool to mimic healthy or diseased skin states.[2]

The aim of this study is to create model lipid membranes and investigate the polymorphic nature of the lipids at different annealing conditions. Initially, skin isolated lipids were annealed at different temperatures (from room temperature to 90° C) in the presence or absence of water. Preliminary results did not show major differences for the lengths of long periodicity phase or cholesterol phase between the different annealing conditions, while short periodicity phase starts to be visible when annealing temperature is 70° C in presence of water. Moreover, even if annealing of lipids was considered an essential step for the creation of model lipid membranes, our results indicate that lipids are also able to spontaneously arrange.

The study was supported by SVV: 260401 and Czech Science Foundation (GACR 19-09135J)

References

1. JACKSON S.M., WILLIAMS M.L., FEINGOLD K.R., *et al*.: West J. Med., 158(3), 1993, 279-285.
2. ČUŘÍKOVÁ B.A., PROCHÁZKOVÁ K., FILKOVÁ B., *et al*.: International Journal of Pharmaceutics., 534, 2017, 287–296.

FT5

DELIVERY OF SKIN BARRIER LIPIDS TO RECONSTRUCTED HUMAN EPIDERMIS

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Skin barrier is found in the outermost part of the skin, called *stratum corneum* (SC), which consists of flattened dead cells (corneocytes) embedded in a lipid matrix. A lipid species specific for epidermis, ω-O-acylceramides (ω-O-acylcer), are essential for the organization of the two SC lipid structures: the highly organized intercellular lipid lamellae and the monolayer of lipids covalently bound to the surface of corneocytes. Both are essential for our survival on dry land.[1] One of the key enzyme in the synthesis of ω-O-acylcer is PNPLA1, which was found deficient in a subset of patients suffering from Autosomal Recessive Congenital Ichthyoses.[2] The available treatment is only symptomatic, thus, we attempted to restore skin barrier by delivering ω-O-acylcer to the SC. In this study, delivery systems for ω-O-acylcer were tested for their ability to deliver ω-O-acylcer to reconstructed human epidermis (RHE) deficient in PNPLA1. Effect of treatment on permeability (Lucifer yellow assay), ultrastructure of the SC (electron microscopy) and lipid content (LC/MS) was evaluated. Furthermore, two different models of RHE were compared for their ability to mimic diseased epidermis and protocol for application of delivery systems on RHE was developed.

The study was supported by the Czech Science Foundation (GAČR 19-09135J) and the Charles University (SVV 260401).

References

1. ELIAS, P. M.*, et al.:* Biochim. Biophys. Acta - Mol. Cell. Biol. Lipids, 1841, 2014, 314–31.

2. PICHERY, M., *et al.*: Hum. Mol. Genet., 26, 2017, 1787-180

FT6

SNEDDS FOR TARGETED OLIGONUCLEOTIDE DELIVERY TO INFLAMED INTESTINAL TISSUE

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Self-nanoemulsifying drug delivery systems (SNEDDS) have potential to locally deliver an anti-inflammatory acting oligonucleotide (OND) to inflamed intestinal tissue. A non-specific OND was complexed with a cationic lipid to increase its lipophilicity[1]. This allowed to load the complex (OND-CL) into SNEDDS containing medium-chain fatty acids that enhance tight junction (TJ) opening[2]. Caco-2 cells were pre-treated with Compound 48/80 (C48/80), an inhibitor of TJ opening, and subsequently incubated with OND-CL loaded in SNEDDS, blank SNEDDS (bSN) and the cationic lipid dissolved in SNEDDS both dispersed in a solution of OND, and a solution of OND. Size of dispersed nanodroplets between 180 and 215 nm together with zeta potential ~ - 8 mV are properties enabling passive targeting to the inflamed area. All treatments containing SNEDDS showed permeation of OND unlike in the case of the OND solution. The highest amount of OND permeated after treatment with OND-CL in SNEDDS. C48/80 lowered and delayed the transport of OND-CL, however, there was no significant difference in the case of bSN in presence and absence of the inhibitor. When treated with SNEDDS, TEER dropped to ~ 50% of initial values. The inhibitor C48/80 modulates opening of TJs and suggests their role in transport of OND through Caco-2 monolayer.

The study was supported by Czech Ministry of Education and Sports, project no. SVV 260 401 and Czech Grant Agency, project. No.GA17-06841S.

References

1. LOBOVKINA, T., JACOBSON, G., GONZALEZ GONZELEZ, E. *et al*.: ACS Nano, 12, 2011, 9977-9983.
2. HAYASHI, M., SAKAI, T., HASEGAWA, Y. *et al.*: J CONTROL RELEASE, 62, 1999, 141-148.

POLYMERIC PARTICLES: A TOOL FOR TARGETED INFLAMMATION MANAGEMENT

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The aim of this work is to prepare biodegradable polymeric nanoparticles with encapsulated anti-inflammatory substance. Polymeric particles are actively scavenged from systemic circulation by phagocytic cells of the reticuloendothelial system. Macrophages such as Kupffer cells play an important modulatory role in the development of inflammation. Therefore, such particles could be suitable for liver targeting.[1] The encapsulation of the active substance into nanoparticles has undeniable advantages, such as reduced side effects, site specific action or reduced active substance dose.[2] A number of different variants of poly (lactic-co-glycolic) acid (PLGA) (several linear variants and a branched with polyacrylic acid) were tested for preparation of nanoparticles. These co-polymers are sufficiently stable, biodegradable and do not induce stimulation of the immune system.[3] PLGA nanoparticles of desired size (80 – 300 nm) with low polydispersity were prepared and assayed *in vitro*. Particles with size under 100 nm were prepared by emulsification solvent evaporation method. Nanoprecipitation yielded nanoparticles ranging between 150 and 200 nm. Formulations of such parameters are attractive to target cells, non-toxic and also with high cell entry demonstrated *in vitro* (more than 50 %).

The study was supported by SVV 260 401, GAUK 1586119, GAČR 19-14497S

References

1. TAMMAM, S. N., AZZAZY, H. M., LAMPRECHT, A. J. Biomed. Nanotech., 11, 2015, 555–577.
2. WANG, M. T., JIN, Y., YANG, Y. X., *et al.* Int. J. Nanomed., 5, 2010, 487–497.
3. BARTNECK, M., PETERS F. M., WARZECHA K.T., *et al.* Nanomedicine, 10, 2014, 1209–1220.

FT8

IMIQUIMOD CONTAINING LIPOSOMES FOR DERMAL DELIVERY

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Over the past years, (trans)dermal delivery has gained extensive popularity due to its advantages over other routes of administration.[1] Dermal delivery has been improved by different methods and vehicles, of which, liposomes seem to be the most studied.[2] Imiquimod (IMQ) is a topically-applied imidazoquinolon, used for the treatment of several skin diseases, like actinic keratosis and basal cell carcinoma.[3] Traditional formulations restrict IMQ's efficiency for dermal delivery because of the drug's poor solubility and low cutaneous permeability [3] The aim of the present study is, to prepare IMQ loaded liposomes and evaluate their ability to deliver the active substance to the desired skin layers. Different liposomal formulations with IMQ were prepared by using the thin film method. The physicochemical properties of the prepared formulations were determined in terms of particle size, polydispersity index, zeta potential, and entrapment efficiency. Ex vivo experiments on human tissue showed that liposomes have a potential as carriers of IMQ for dermal application

The study was supported by SVV: 260401 and Czech Science Foundation (GACR 19-09600S).

References

1. PRAUSNITZ, M., LANGER, R.: Nat. Biotechnol., 26, 2008, 1261–1268.
2. NOUNOU, M.I., EL-KHORDAGUI, L.K., KHALAFALLAH, N.A., *et al*.: Recent Pat. Drug Deliv. Formul., 2, 2008, 9-18.
3. MA, M., *et al*.: J. Mater. Sci. Mater. Med., 26, 2015, 191-198.

FT9

OPTIMIZATION OF RIFAMPICIN-LOADED NANOPARTICLES PREPARATION

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In past two decades poly lactic-co-glycolic acid (PLGA) has been among the most attractive polymeric candidates used to fabricate devices for drug delivery and tissue engineering applications.[1] Using different branching agents, direct melt polycondensation without addition of catalyst led to group of PLGA with diverse architecture.[2] These polymers were used for the preparation of rifampicin-loaded nanoparticles by the nanoprecipitation method. The aim of this work was to optimize this method by selecting a suitable polymeric carrier and its concentration, the ratio of organic phase to aqueous phase, the total amount of rifampicin added, and the concentration of the previously verified stabilizer didodecyldimethylammonium bromide (DDAB). Prepared formulations were evaluated and compared by testing of their parameters such as stability, particles size, polydispersity, zeta potential, and encapsulation efficiency. Two suitable PLGA derivatives were found, star-shaped PLGA branched on tripentaerythritol appeared to be the most appropriate. The effect of the amount of stabilizer on the encapsulation efficiency was significant. Encapsulation efficiency increased with decreasing amount of stabilizer to maximum at 0.02 %, but further decrease in concentration led to the encapsulation efficiency drop, even in the case of stable formulations with low polydispersity. Dissolution tests were performed in phosphate buffer pH 7.4, and showed sustained release of rifampicin.

The study was supported by SVV 260401.

References

1. MAKADIA, H., SIEGEL, S.: Polymers, 3(3), 2011, 1377–1397.
2. ŠNEJDROVÁ, E., PODZIMEK, Š., MARTIŠKA, J., *et al*.: Acta Pharm., 70, 2020, 63–75.

A STUDY OF COMPRESSIBILITY AND COMPACTABILITY OF TABLETING MATERIALS WITH CHITOSAN

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Natural polysaccharides find use as excipients for the formulation of oral controlled drug release systems. Some of these systems can target drug release to the distal part of the gastrointestinal tract. One of these polysaccharides is cationic chitosan, which is safe, non-toxic and biodegradable. Its very important property is the ability to adhere to mucous, In the presence of an anionic polysaccharide (sodium alginate) it forms a polyelectrolyte complex.

The aim of this work was to study the compressibility, compactibility and lubricant sensitivity of directly compressible tableting materials containing chitosan, sodium alginate and two viscous types of hypromellose. Tableting materials with silicified microcrystalline cellulose Prosolv SMCC 90 in proportion to 1: 3 chitosan were tested as well. Compressiblity was evaluated by energy profile of compression, compatibility by tensile strength of the tablets, lubricant sensitivity by values of lubricant sensitivity ratio.

The highest total energy of compression values schowed the mixture chitosan and Prosolv SMCC 90 in the ratio 3:1. Prosolv SMCC 90 increased the values of total energy of compression at all formulations excluding the mixtures with HPMC 100M. The adding of retarding components decreased them, the most in the case of alginate sodium. Tensile strength of tablets was incresed by Prosolv SMCC 90 at all formulations. Alginate sodium and magnesium stearate decresed strength values. The highest strength values showed formulations with HPMC 100M. The mixture of chitosan and Prosolv SMCC 90 had the lowest LSR value, the highest LSR value showed this mixture with 50 % sodium alginate.

References

1. BADWAN A. A., RASHID I, et al..: *Chitin and chitosan as direct compression excipients in pharmaceutical applications.* Mar. Drugs 2015, 13, 1519-1547.

2. LIANG LI, LINLIN WANG, YANG SHAO, et.al., *Drug release characteristics from chitosan-alginate matrix tablets based on the theory of self-assembled film,* Int. J. of Pharm. 2013, 450, 197-207.

INTRODUCTION INTO THE MAIN IDEAS OF PCA WITH ILLUSTRATIONS FROM PHARMACEUTICAL TECHNOLOGY AND PHARMACOLOGY

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Principal component analysis (PCA) is a mathematical algorithm that helps to reduce the dimensionality of the data while retaining most of the variation in the data set. It accomplishes this reduction by identifying directions, called principal components, along which the variation in the data is maximal.[1] In the context of drug design PCA is used to identify the two principal components and to indicate by visual assessment of the plotted point the influence of the factor on a principal component or on a subset of outcomes. In this presentation we would like to demonstrate the main ideas of PCA on the example of its use in pharmaceutical technology and pharmacology. The first example illustrates the application of PCA in tablet formulation in order to find the influential process formulation parameters and describe their optimal settings.[2] The second example related to pharmacology demonstrates gene expression depending on the clinical/pathological conditions of the placenta. The study was conducted on 177 placenta tissues (39 healthy and 138 pathological divided into subgroups). The parameter of interest is the expression of 16 genes directly involved in placental homeostasis of tryptophan and serotonin. The main aim of the study is to indicate a difference in gene expression between the pathological vs healthy placentas, taking into account the main patient characteristics such as markers of inflammation, fetal sex, fetal weight, BMI etc.

References

1. RINGNER, M., NATURE BIOTECHNOLOGY VOL.26 (3), 2008, 303-304.
2. KURHAJEC, S., FRANC, A., DOLEZEL, P., SABADKOVA: Pharmaceutical Development and Technology, 342, 2017, 22(7): 881–888.