SHORT PRESENTATION

Biocompatibility and dissolution measurements of different 3D printed drug delivery systems

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Background: 3D printing is a modern technique which can be used in numerous fields like in the pharmaceutical industry. This technique enables to manufacture complex, personalized products on-demand.
Aims: Our aim is to manufacture different drug delivery systems which can be used for personalized medication or manufacturing of orphan drugs.
Methods: For the 3D printing we use Fused Deposition Modelling (FDM) technology with PLA, PMMA and PET polymers. The printed samples material structure is characterized by SEM, FTIR, PALS and contact angle measurements. Biocompatibility is a compulsory examination through the development so with our colleagues we developed a long-term modified MTT assay which is a high efficacy screening test. For the final interpretation we also performed biofilm formation with crystal violet assay to gain extensive information. The samples dissolution profile is examined with an Erweka USP type I apparatus with automatic sampling system at pH=7.4 for 24 hours.
Results: Based on the biocompatibility results we determined the samples non-cytotoxic but with the results of biofilm formation we can sort out the most proper polymer for further examinations. The material structure was determined and the FTIR spectra proves the side-chain modification of the polymers. The samples dissolution profile can be affected by the variation of the diameter and the infill percentage through the 3D printing technique.
Conclusion: Based on the determined data we have successfully printed 3D samples in runs up to 50 units which are non-cytotoxic and has good material structure properties. Based on the results the applicability of the different samples can be determined and it can give us information for the scale enlargement.

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References: 1 Arany, P. et al.; Pharmaceutics 2019;11:277; 2 Boetker, J. et al.; Eur. J. Pharm. Sci. 2016;90:47-52.

Synthesis of twin-nucleosides

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Background: Natural nucleoside derivatives play pivotal role in many biological processes, while synthetic nucleoside analogues are used in several areas of medicine, e.g. in the anticancer or antiviral chemotherapy [1]. Therefore, the design and synthesis of nucleoside analogues is an important topic of chemistry. Morpholinos, containing a morpholine ring instead of the furanose, represent a valuable class of nucleoside analogues [2]. These compounds are obtained by the reaction of the corresponding 2',3'-dialdehyde derivative of nucleosides with ammonia or alkylamine (R-NH2) under reductive conditions.
Aims: We planned to synthesize new nucleoside dimers, consisting of a nucleoside and a morpholino, creating a direct linkage between the morpholine nitrogen and the 5'-carbon of the nucleoside, to produce so called „twin-nucleosides".
Methods: Two types of monomeric units were prepared for the synthesis of dimers. On the one hand, the appropriately protected nucleosides were oxidized into 2',3'-dialdehydes with NaIO4. On the other hand, 5'-deoxy-5'-aminonucleosides were synthesized. The amines and aldehydes were coupled in various combinations in EtOH, using NaCNBH3 as reducing agent.
Results: Purine and pyrimidine nucleosides: uridine, ribothymidine, thymidine, adenosine, and inosine were involved into the reactions. Seven new dimers were obtained (including homo- and heterodimers), and also a trimer and a tetramer were synthesized, by elongation of the shorter oligomers at position 5' of the morpholino unit.

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Conclusion: A new type of oligomeric nucleoside analogues were synthesized, creating a tight bond between the monomers. We are planning the synthesis of further dimers using other nucleosides or other combinations of the monomers, incorporation of a twin-nucleoside into normal oligonucleotide for hybridization studies, as well as the biological evaluation of the obtained dimers in cytotoxicity and antiviral assays.

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References: 1 Jordheim, L., P., et al. Nat. Rev. Drug Discov. 2013;12:447-464; 2 Khym, J., X., Biochemistry, 1963;2:344-350.

Investigation of the permeability and cytotoxicity in novel topical ophthalmic formulations using in vitro and ex vivo models

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Background: Research and development are big challenge in the field of ocular drug delivery. Suitable drug penetration is a key factor, meanwhile negative effects of reflex mechanisms and complex anatomical structure limit the therapy. Besides the therapeutic efficacy, the antimicrobial stability must be ensured during the application and storage. Novel preservative alternatives are required, considering the toxic attributes of widely applied benzalkoniumchloride (BK). Prednisolone (PR) containing eye drops were developed by our research group, where cyclodextrin (CD) derivatives and antimicrobial biopolymer were applied. The results of increased in vitro drug diffusion, acceptable viscosity, mucoadhesion and antimicrobial effectiveness test were previously published [1].

Aims: Our aim was to confirm the compliance of developed eye drop formulations by investigation on cytotoxicity and permeability models. By application of preservative biopolymer and CD additives, a biocompatible, non-toxic composition is created which can be an innovative approach in the therapy of inflammatory eye diseases.

Methods: In vitro permeability and toxicity were investigated on transformed human cornea epithelial cell line (HCE-T). The cytotoxicity was tested by impedance measurement and immunohistochemistry methods. Ex vivo permeability was tested on porcine cornea model. The quantitative analysis of samples from permeability studies was performed by HPLC.

Results: The results of impedance measurement show that BK containing formulations have significant cytotoxic effect on the HCE-T cell culture model, meanwhile in the case of antimicrobial polymer containing compositions have no cell damage. That was confirmed by the immunohistochemistry assay. As the in vitro permeability test on HCE-T shows, significantly higher permeability of PR was seen in the case of CD and biopolymer additives containing solutions in comparison with the PR suspension. In the case of ex vivo permeability test, no significant change was obtained by applying the CD, although slower drug release is expected from delivery systems with biopolymer.

Conclusion: Considering the results of in vitro and ex vivo assays, the developed ophthalmic formulations could be innovative approaches, where favourable drug permeability and non-toxic attributes are expected. Taking into account the mucoadhesion and solution form, suitable therapeutic effect, less irritation and better patient-compliance can be achieved.

References: 1 Bíró et al., Drug Des Dev Ther 2018;12:2529–2537.

Rheological investigation of human sputum in cystic fibrosis

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Background: Cystic fibrosis (CF) is one of the most common progressive genetic life-limiting disease, in which the defect in cystic fibrosis transmembrane conductance regulator proteins (CFTR) leads to an imbalance ion-water equilibrium resulting in dehydration of the sputum; increased concentration of mucus and thus increased viscosity. The high viscosity mucus contributes to the low clearance of the surface liquid, which leads recurrent infections and a chronic inflammation and subsequent progressive lung damage.

Aims: The aim of our study was to work out an adequate protocol to measure the rheological properties of human sputum (HS) and analyse the effect of the locally administrated mucolytic agents in vitro.

Methods: Rheological oscillatory tests were applied to investigate the mechanical properties of the human sputum samples: time sweep tests in order to determine a holding time before the measurement; and then frequency sweep tests were performed the present the real structure of the mucus gels.

Results: The time sweep investigation indicated a structure build-up during the measurement which can be explained by the destruction effect of the sample insertion into the instrument and/or the thermogelling of the samples at body temperature in the instrument. Addition of mucolytic agent before the measurement this structure build-up was elongated, or absolutely missing, but in some cases any differences could not be observed compared with the non-treated sputum. On the basis of the average G' value of the non-treated samples during the frequency sweep tests the human sputum samples can be categorized into 3 categories: high elastic samples; viscoelastic gels and viscoelastic liquids. The effect of the mucolytic agents (ΔG') were calculated from the G' values of the treated and the not-treated samples, and it was established there is no statistical differences among the additive types.

Conclusion: The constant handling of the sputum sample before the measurements is very important. The in vitro treatments indicated the liquefactions of the samples due to the treatment, but statistical differences could not be stated among the different additives, which suggest the dilution effect of the solution dominated.

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Historical evaluation of Transylvanian Ethnobotanical data in herbal books and manuscripts

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Background: Regions of Transylvania inhabited by Székely and Csángó people have been studied for ethnobotanical and ethnomedicinal data. Our team surveyed settlements in the Homorod Valley, Úz Valley, and Covasna County focusing mostly on plants' use in human and veterinary ethnomedicine (2007-2019). Based on these collections, plants were listed and compared to data obtained from databases to select species for historical and medicinal study.

Aims: According to our collections, the goal was to sum historical and (ethno) medicinal data of four selected species namely *Anthyllis vulneraria* L., *Lathyrus tuberosus* L., *Lysimachia nummularia* L., and *Tanacetum balsamita* L. in earlier Transylvanian and other Central European herbal books.

Methods: Ethnomedicinal records of the selected four species were collected from works between 16-19th century (Olosz 2002). In Transylvania, printed (Melius: *Herbarium*, 1578; Beythe: *Fiveskönyv*, 1595; Pápai Páriz: *Pax corporis*, 1690; Juhász: *Házi különös orvosságok*, 1761), and handwritten works (Lencsés: *Ars medica*, 1577 k./Varjas, 1943; *Orvosságos könyv*, 1677; Gellen G. 1680; Gellen I. 1714) were studied for the historical background of Hungarian prescriptions of plant origin. These records were compared to those of Béla Radvánszky's collection on books published about old Hungarian human and veterinary medicine (Hoffmann ed. 1989).

Results: Some earlier examples for the selected species: data of *A. vulneraria* and wounds occur from 1807 for kidney disorders and diabetes, and in plant list in Pápai's 1690) and Benkő's work (1770-1790). Lathyrus genus is described from 1471. *L. nummularia* was mentioned as *fillérfű* (1824) by Béla Práter for sprain. *T. balsamita* was documented in the 17th century (Hoffmann ed. 1989), and as *lapos menta* (Lexicon Budense 1825) and *gilisztahajtó* for endoparastites as a tea in Székely Land.

Conclusions: Historical relationship of ethnomedicine and medical science can support the interpretation of their different and similar aspects. Historical evaluation confirms the significant influence of official medicine to ethnomedicine in many centuries. Based on the interaction of Transylvanian (Hungarian, Romanian, and Saxon) traditional elements, several analogue treatments can be mentioned these days.

Acknowledgement: This work was supported by a grant from the OTKA (Hungarian Scientific Research Fund, K 127944).

References: 1 Olosz, K. Kriza János Néprajzi Társaság Évkönyve, 2002;10:31-41; 2 Hoffmann, G. (ed) Medicusi és borbélyi mesterség. MTA, 1989.

The mode of action of thyme (*Thymus vulgaris* L.) essential oil in an endotoxin-induced acute airway inflammation in vivo model

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Background: Respiratory tract diseases (RTDs) affect a large number of people worldwide. One of the traditional application modes of essential oils is the

inhalation. RTDs generally are associated with infection and inflammation at the same time. However, the anti-inflammatory effect of essential oils is poorly studied in vivo. Transient Receptor Potential Vanilloid1 (TRPV1) and Ankyrin1 (TRPA1) ion channels are expressed on the sensory neurons and epithelial cells of the airways and play a role in sensory-immune interactions.

Aims: Therefore, we aimed to examine the chemical composition and effects of thyme oil (TO) inhalation in the endotoxin (LPS)-induced acute airway inflammation mouse model and the potential role of TRPA1/V1 ion channels in mediating TO effect. The essential oil was selected on the basis of its potent antibacterial activity.

Methods: The chemical composition of TO was determined by GC-MS. Lung inflammation was evoked by the intratracheal administration of 60μL LPS (*E. coli* 083) in female *TRPA1/V1*+/+ (WT) and *TRPA1/V1*-/- (KO) mice. TO or the control oil was inhaled 3 times for 30 min during the 24-h period of the experiments. Airway function was measured in awake, spontaneously breathing animals by unrestrained whole-body plethysmography. Lung myeloperoxidase (MPO) activity was determined by spectrophotometry. The histopathological alterations were evaluated from hematoxylin-eosin stained lung sections by semiquantitative scoring.

Results: Thymol (46.3%) and p-cymene (22.1%) were the two main components of TO. TO inhalation significantly decreased airway hyperreactivity in WT, but aggravated it in KO mice. Histological parameters were not affected significantly by TO inhalation in either WT or KO mice. LPS treatment induced a remarkably increased MPO activity, which was significantly reduced by TO inhalation in WT, but not in KO mice.

Conclusion: Therefore, thyme oil can be considered as a potential treatment in airway inflammation, and its protective effect is potentially mediated by TRPA1/V1 ion channels.

Stability study of nasal powder formulation containing nanosized lamotrigine

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Background: The nose offers a great possibility to avoid adverse events and increase the patient compliance. Due to its advantageous properties local, systemic and central nervous (CNS) system effects are also available. The application of innovative and efficient products, that are containing nanoparticles, may lead to the improvement of different therapies. The quality insurance of the pharmaceutical products has received considerable attention in the past few years. That is why the stability of the formulations has become extremely important and therefore, quality influencing parameters need to be kept near constant during the transport, storage and application. Generally, solid dosage forms (e.g. nasal powders) have better stability than liquid formulations.

Aims: The aim of our study was to carry out the long-term stability study of a previously developed nasal powder (NP) formulation, which contained nanosized lamotrigine (LAM).

Methods: Stability tests were performed in Binder KBF 240 (Binder GmbH, Tuttlingen, Germany) equipment, with a constant-climate chamber. The long-term stability test was performed according to the ICH Q1A guideline, at 25 ± 2 °C with 60 ± 5% relative humidity. To justify the unchanged parameters, different properties were investigated. The particle size and morphology were examined by SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan). Structural investigations (XRPD, DSC) were also carried out. The in vitro dissolution profiles were determined spectrophotometrically at 307 nm with an ATI UNICAM UV–VIS system. A self-developed, horizontal diffusion apparatus was used for in vitro permeability testing.

Results: The particle size of LAM in the NP (97 ± 60nm vs. 239 ± 116nm) formulation was near constant. The results were confirmed by SEM pictures, which did not show particle aggregation. The structural investigations showed that the LAM remained partially amorphous. The in vitro dissolution rate was around 80% after 5 mins both at the beginning and at the end of the study.

Conclusion: During the test period no considerable change occurred in the powder formulation, as the examined parameters were nearly unchanged according to the results of the investigations. This is beneficial, because it means that the patients could receive their medication in proper quality.

References: 1 Gieszinger P et al. Drug Dev Ind Pharm, 2018;44(10):1622-1630

Formulation and characterization of a verapamil containing low-density gastroretentive dosage form

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Background: Gastroretentive drug delivery is useful

when the active ingredient has narrow absorption window or gastric targeted release is aimed. One of the retentive mechanisms is the floating by low density. If the density is below 1g/cm^3, the formulation floats on the gastric-fluid, and avoids the elimination from the stomach.

Aims: We aimed to develop a verapamil containing drug delivery system based on its high-porosity and low-density by foam formation after melting the components [1].

Methods: We used the following materials for our work: PEG 4000, stearic acid and verapamil for formulation. We prepared different compositions by continuous production and determined the basic parameters such as weight and density. We made micro-CT images to get information about the pore size and distribution. We also tested the in vitro drug release in 1.2 pH buffer and texture changes during dissolution. Finally, we made animal experiments to prove the modified release and gastro-retention of the formulation.

Results: We reached less, than 0.9g/cm3 density in all of the formulations. The micro-CT images showed homogeneous distribution of the cavities in the matrix and closed cell structure. The pore size was between 200 and 300μm. The prepared foams had hard structure even at body temperature that was measured by texture analyzer. The dissolution studies showed prolonged drug release up to 10 hours, when 80% of the API was dissolved. At the end of the dissolution test the water uptake reached 75% of the total mass and the weight of the formulation decreased to 25% of the initial mass. Following the process, we detected dry, hard core until 5 hours of dissolution. After that, we aimed to prove the gastro retention ability of formulations in rats, so we created small samples with 30% $BaSO_4$ as a contrast material. The sample stayed in the rat stomach for 2 hours until the total erosion, that was measured by texture analyzer also.

Conclusion: In summary, we prepared and characterized a solid and low-density drug delivery system with zero floating lag-time. The prepared samples showed prolonged verapamil release and hard structure with the ability to remain in the stomach for several hours.

References: 1 Vasvári et al. AAPS PharmSciTech, 2019;20:7

Alteration of the cell membrane – a novel method in the pain modulation

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Background: The Transient Receptor Potential ion channels, such as Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1/A1) are expressed in nociceptive primary sensory neurons. These channels are widely studied, but there are some missing information about their modulation. Noxious heat, capsaicin (CAPS), resiniferatoxin (RTX), fatty acid metabolites activates TRPV1. TRPA1 can be activated by noxious cold, mechanical stimuli and formaldehyde. Lipid rafts are specific membrane domains, which are rich in cholesterol, sphingomyelin and gangliosides, and create functional complexes with TRP channels. Sphingomyelinase (SMase) is an enzyme, which hydrolyzes the membrane sphingomyelin content, while myriocin (Myr) an enzyme inhibitor, which blocks the de novo sphingolipid synthesis. Our group previously described, that the treatment with SMase or Myr inhibits the function of TRP receptors in vitro [1-2].

Aims: We examined the potential antinociceptive effect of SMase and Myr in different in vivo mouse models, and also tested some in vitro properties.

Methods: Capsaicin-evoked acute nocifensive ("eye-wiping") test, formaldehyde-evoked hyperalgesia, and RTX induced thermal allodynia and mechanical hyperalgesia model were performed to investigate the effect of SMase and Myr. The in vitro properties were tested by fluorescent spectroscopy.

Results: Both SMase and Myr decreased the CAPS-evoked "eye-wiping" movements, and Myr has a prolonged effect in this model. SMase largely reduced the formaldehyde-caused nociceptive behavior in the second phase, while Myr did not have any effect. In the RTX-model both compounds reduced the thermal allodynia, and SMase had significant effect also in the mechanical hyperalgesia. In the fluorescent spectroscopy SMase did not modify the Laudran spectras.

Conclusion: On the basis of our results, we assume that the hydrophobic interactions between the TRP channels and lipid rafts might be a promising drug target in the future.

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Implementation of digital pharmaceutical technology in the practical education of graduate pharmaceutical students at Semmelweis University

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Background: The essential characteristics of a pharmacy student among others is the openness and curiosity towards digital technology. In the age of information, when breakthrough technologies and approaches turn up within a few years, the education system and universities have a great responsibility in this respect. The lecturers at the cathedra face day by day a noble challenge: somehow they have to prepare students not just for the questions of "today" but also for the answers of "tomorrow". 3D printing is gaining more and more attention and have a great potential in the future of pharmaceutics. Deploying the benefits of this technology (e.g. flexible, patient-based medication) in a community/hospital pharmacy, could help to satisfy the diversified demands of patients through the added-value of pharmacists.
Aims: The aim of the study was to check the knowledge of 4th year pharmacy students at Semmelweis University about digital technology and 3D printing and to introduce them the theoretical and practical fundaments of 3D printing. The investigation of attitude, opinion and knowledge before and after the 135 min long practice was also put into the focus.
Methods: The theoretical knowledge and attitude of students regarding 3D printing were screened with a questionnaire on voluntary basis. The questionnaire involved 8 questions (single or multiple choice) and had to fill before and after practice. The information was collected anonymously, online via a QR code or paper-based. The designing, slicing and printing steps were demonstrated by an FDM printer. Conventional, round, polylactic acid-based tablets were printed.
Results: Willingness on filling out the questionnaire was 96.8%. Approx. 60% of participants had no experience with 3D printing and 25% of students estimated moderately useful to learn about 3D printing. After the practice 96% regarded it as very worthy. More than 50% believed that the formulation of patient based medicines at a hospital pharmacy is feasible within 5 years. One of three students considered the completion of GMP requirements as a great challenge.
Conclusion: The 4th year pharmacy students at Semmelweis University were satisfied with the implemented practice and judged it as useful. The mysterious nature of this technique has been dissolved and they see a great potential in 3D printing but some limiting barriers were also revealed.

Polymeric micelles as promising tools to substitute surfactants in the dissolution of poorly water soluble drug

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Background: In the recent years the application of surfactants to increase the wetting properties and therefore to overcome the hurdles of drug dissolution and absorption in oral drug delivery is decreasing because of their disadvantageous properties. Soluplus® (BASF GmbH, Germany) a novel solubilizing amphiphilic graft co-polymer, offers the possibility of a good solubility enhancement in combination with a fast dissolution through polymer micelle forming. Melt technology is an appropriate green technology, to produce solid dispersions involving these surfactants in an inert carrier or matrix in the solid state.
Aims: Our aim was to develop a solid drug delivery system (DDS) using melt technology to design such carrier systems which are based on crystalline eutectic and additives with various solubility enhancing agents.
Methods: In the present work two solubilizers (Cremophor® RH 40 and Soluplus®) were tested for its capability to improve drug dissolution. As model drug the lipophilic, poorly water solu-ble megestrol acetate (MEGA) was chosen. The basis of the carrier were two sucrose alcohols, xylitol (XYL) and mannitol (MAN), which form eutectic mixture in certain ratio with PEG 6000. The solubilizers were dispersed with the MEGA in the eutectic carrier by fusion meth-od. The phase diagram of eutectic compositions of the carrier was determined using DSC. In vitro dissolution studies were carried out on gastric pH to investigate the release kinetic of the compositions with different solubilizers in comparison with pure MEGA containing carrier.
Results: DSC measurements showed that the XYL and the MAN formed eutectic mixture with each other, as a result the melting process could be carried out on decreased temperature [1]. The dissolution studies of the developed formulations showed increased dissolution rate in case of solubilizing agent containing products, even after tenfold scale up process.
Conclusion: We developed a carrier from XYL-MAN eutectic and PEG 6000 ternary system. It was found that the Soluplus® had a high solubilization capacity, thanks to form polymeric micelles, which encapsulates partly the MEGA during dissolution. Increas-

ing quantity of graft copolymer resulted in higher amount of dissolved drug, therefore it is suitable to have DDS with controlled drug release. We can conclude an innovative "value added" solid formula of MEGA, which can be suggested for the patient for ex tempore sus-pension preparation before administration.

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Microstructural distinction of antiemetic drug-loaded nanofibrous orally dissolving web formulated with different excipients

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Background: The hydrophilic polymer-based electrospun fibrous orally dissolving webs are promising candidates for rapid drug release, which is due to the high surface area to volume ratio of the fibers and the active ingredient can be incorporated in the polymer matrix in amorphous state. However, the enhanced molecular mobility of these materials is responsible for their physical and/or chemical instability.
Aims: The aim of the project was to prepare poly(vinyl alcohol)-based, metoclopramide hydrochloride-loaded electrospun nanofibers using either polysorbate 80 (PS80) or hydroxypropyl-β-cyclodextrin (HP-β-CD) and tracked how the excipient influences the fiber formation process, the mechanical properties, macro- and microstructures of the electrospun samples and the drug release from the nanofibers.
Methods: The electrospun samples were subjected to several imaging techniques: Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), and complex physicochemical characterization (with Positron Annihilation Lifetime Spectroscopy (PALS), X-ray Diffractometry (XRD) and solid-state Nuclear Magnetic Resonance (NMR) spectroscopy) the fibrous delivery systems was carried out which were enabled the better understanding the supramolecular interactions of multicomponent systems.
Results: SEM verified that clearly fibrous structures were obtained, without any beads and film-like areas. The mechano-manipulation of AFM revealed that the usage of PS80 led to about two times stiffer, less plastic fibers than the addition of HP-β-CD. The cross-polarization build-up curves of 1H-13C NMR spectroscopy verified that CD is an inner plasticizer, while PS80 acts as an outer plasticizer and can migrate in the polymer matrix, which is due to its "liquid-like" behavior. PALS measurements also showed the enhanced mobility of the PS80 containing formulation and the molecular packing enhancer properties of the HP-β-CD. XRD method suggested that as a result of the fiber formation process the active pharmaceutical ingredient incorporated into the fibers in a purely amorphous state, but the ssNMR pointed out that usage of the examined additives enabled the development of a molecularly dispersed system of different homogeneities.
Conclusion: In case of both formulations clearly fibrous structure and molecularly dispersed system were formed. It was also showed that PS80 and HP-β-CD render distinct mechanical properties of the fibers, as PS80 advances elastic behaviour and HP-β-CD promotes plastic features.

Determination of enantiomeric purity of esomeprazole by capillary electrophoresis

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Background: Esomeprazole, as a proton pump inhibitor, is one of the most effective agents used in gastric hiperacidity-related disorders. It is a pyridinyl-methyl-sulfnyl-benzimidazole derivative with an asymmetric sulfoxide group in its structure. It was the first proton pump inhibitor introduced as enantiomerically pure compound in the therapy.
Aims: The aim of this study was to develop and validate a suitable separation method for the determination of the enantiomeric purity of the compound of interest using cyclodextrin-mediated capillary zone electrophoresis.
Methods: Different native and derivatized cyclodexrins were screened as chiral additives, using phosphate buffer at pH 2.5 as background electrolyte, in order to find the most suitable chiral selector for the separation of omeprazole enantiomers. Apparent complexation constants of the enantiomer-cyclodextrin complexes were determined in order to investigate the possible mechanism of enantiomeric separation. The effect of analytical conditions as temperature, applied voltage and buffer concentration was also evaluated in order to find optimal separation conditions.

Results: Baseline chiral separation of the two enantiomers with favorable migration order (R-omeprazole migrates first) was achieved in less than ten minutes using the following conditions: a 50mM phosphate buffer at pH 2.5 as background electrolyte, 20mM randomly methylated β-CD as chiral selector, +20kV applied voltage and 20°C system temperature, and UV detection at 210nm. The method was validated according to current guidelines and proved to be reliable, linear, precise, and accurate for the determination of 0.2% distomer as chiral impurity in esomeprazole samples.

Conclusion: A rapid and cost-effective capillary electrophoresis method was developed for the separation of omeprazole enantiomers. The optimized and validated method proved to be suitable for the determination of enantiomeric purity of esomeprazole from pharmaceutical preparations and could represent an alternative for the available compendial methods.

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A study of drug response to cisplatin, erlotinib and (E)-2- (4-methoxybenzylidene)-1-benzosuberone in human lung adenocarcinoma

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Background: Lung cancer is the main cause of cancer-related mortality in Hungary. Most of the patients are diagnosed at an advanced stage and referred for chemotherapy. The platinum-based drug, cisplatin still gives the backbone of therapy but in combination with other drugs. Clinical application of targeted therapy e.g. tyrosine kinase inhibitors affecting epidermal growth factor dependent signalling offers better tumour control in the presence of mutation. Chalcones intermediary precursors of flavonoid biosynthesis are also effective against human malignant cells, therefore chalcone analogues could be promising for drug development.

Aims: Our primary aim was to investigate cytotoxity as well as metastasis associated cytokine expression and cellular invasion during mono- and combined administration of cisplatin and erlotinib in human lung adenocarcinomas with known mutations. It was also the aim to study the cytotoxic effect of (E)-2-(4-methoxybenzylidene)-1-benzosuberone in lung adenocarcinoma cell lines.

Methods: Cytotoxic effect of the compounds were tested in primary lung adenocarcinoma derived cell cultures, in 2D monolayer and 3D in vitro cultures using luminescent cell viability assay. Drug-induced changes in mRNA and protein levels were measured by quantitative real-time PCR and cytometric bead array-based assays. For cellular invasions, scratch assays were performed. Cell survival after chalcone treatment was analysed by flow cytometry using 7-AAD staining.

Results: Primary NSCLC derived cell cultures respond to therapeutic drugs similarly in 3D in vitro cultures as detected in clinical therapy. Cisplatin induced an increase in IL-6 and IL-8 cytokine mRNA levels in all patient samples, while erlotinib only increased IL-6 expression in the presence of EGFR mutation. Both cytokine production was increased at mRNA and protein level in EGFR mutant cells in 3D cultures exposed to mono- or combination treatment but erlotinib could reduce cisplatin induced IL-6 protein expression. Cellular migration and proliferation were increased in the presence of IL-6. Cytotoxic effect of the cyclic chalcone analogue and a marked decrease of cyclin D1 proliferation marker expression was detected in a dose-dependent manner.

Conclusion: As the firstly applied drug can determine the clinical response to the second drug, the sequence of drug administration can have a great impact on therapeutic success.

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The investigation of the in vitro penetration of 3 types of model API using modified horizontal diffusion cells

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Background: The nasal administration can be an alternative choice of intravenous intake thanks to the rapid absorption. The residence time and the administered nasal powder dose can be higher than in the liquid formulations. The development of in vitro models can accelerate pharmaceutical development by reducing the resources of the studies. There are numerous diffusion models but they are less suitable for investigating nasal powders [1].

Aims: Our goal was to investigate the in vitro diffusion of different APIs using modified horizontal dif-

fusion cells. 3 types of model APIs were used for this purpose with different hydrophobicity: the hydrophobic meloxicam (MEL, logP = 3.43), the moderately hydrophobic lamotrigine (LAM, logP = 2.50) and the hydrophilic levodopa (LEV, logP = -2.39). The inline results were compared to the offline ones.

Methods: Our setup can be divided into a donor and an acceptor chamber. The offline measurements were performed spectrophotometrically, the inline ones with UV-Vis immersion probe. 3 different membranes and 2 impregnation liquid (isopropil-myristate – IPM and pH=7.4 phosphate buffer) were used (Isopore, Metricel, Whatman). The results of the diffusion studies were analyzed according to the dissolution rate and the arimethric mean of the relative standard deviations (SDmean).

Results: MEL diffusion studies: the inline measurements were more precise than the offline ones. The IPM-impregnated Metricel membrane was the most effective membrane in inline measurements because of the high extent of diffusion and the low SDmean among all setups. LAM diffusion studies: the results of the IPM-impregnated membranes were better-reproducible (low SDmean) in inline measurements. Whatman is suggested based on its high diffusion and high precision. LEV diffusion studies: IPM-soaked Whatman behaved comparable to Metricel but the SDmean in case of Metricel was higher, therefore it is suggested in inline measurements.

Conclusion: Precise inline horizontal setups were constructed to model the in vitro diffusion of model APIs. The hydrophobicity of the API was proportional to the efficient penetration through the IPM-impregnated membrane.

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Effects of acute (S)-ketamine treatment on EEG power spectra

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Background: (S)-ketamine is an N-methyl-d-aspartate (NMDA) receptor antagonist with a rapid and long-lasting antidepressant activity. Recently, the U.S. Food and Drug Administration and the European Medicines Agency have approved (S)-ketamine as adjunctive therapy for treatment-resistant depression.

Aims: Most studies have examined the effects of (S)-ketamine on the electroencephalogram (EEG) in anesthetic doses or in a short-term (≤1 h) time scale. Therefore, the aim of our study was to investigate the acute and long-term (≤30 h) effects of the rapid-acting antidepressant (S)-ketamine on EEG spectra.

Methods: Male Wistar rats were equipped with EEG and electromyography (EMG) electrodes. On the day of the experiment, 10 or 30mg/kg (S)-ketamine or 1ml/kg physiological saline was administered intraperitoneally at the beginning of passive phase (at light onset), and EEG, EMG and motor activity were recorded for 30h.

Results: Quantitative EEG (qEEG) analysis revealed that the acutely administered 30mg/kg (S)-ketamine enhanced gamma (30-60Hz) power in the first 3h, and decreased delta (1-4Hz) power in the 2nd h, that was followed by a rebound during passive phase. Both doses reduced alpha (10-13Hz) and theta (5-9Hz) power in the 1st h. In contrast, tendencies to increase alpha and theta frequency band power were observed during active phase, 12-24h post administration.

Conclusion: These findings suggest that (S)-ketamine has a robust effect on gamma activity in the passive phase, and also raise the possibility that subanesthetic doses may have phase-dependent long-term qEEG effects. Future work could involve investigating sleep-wake stage-dependent qEEG effects of (S)-ketamine.

Analytical investigation of organic solvent-free co-grinding technique in terbinafine hydrochloride cyclodextrin complexation

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Background: Recent scientific publications have already demonstrated that co-grinding appears as an efficient, solvent-free technique for preparing cyclodextrin complexes and improving physicochemical properties of active ingredients. Terbinafine hydrochloride (TER), an antifungal BCS II drug was chosen as a model drug, which has poor water-solubility.

Aims: The aim of this study was the analytical characterization of cyclodextrin-TER inclusion complexes prepared by co-grinding used different grinding time (thermoanalytical behaviour, detection of occured changes in crystalline properties, molecular relationships between the components).

Methods: Prospects of preparation and characterization of co-grinded cyclodextrin complexes were studied. TER and amorphous cyclodextrin derivatives (hydroxypropyl-β-cyclodextrin (HPBCD), heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB)) were used for the preparation of products, which were analysed by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), hot-humidity stage X-ray powder diffractometry (HOT-XRPD), Raman spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and dissolution studies.
Results: Cyclodextrin-TER complexes in the 1:1 molar ratio were prepared. DSC and XRPD studies suggested that crystallinity of products gradually decreased by the increasing grinding time, and after 75 minutes of co-grinding the products were completely amorphous. HOT-XRPD studies revealed that product containing HPBCD remained amorphous with the increasing temperature, while in the case of DIMEB complex recrystallized in a different crystalline phase. Raman and FT-IR spectroscopy were used to confirm the molecular interactions between the components. Dissolution studies showed that dissolution rate of complexes improved, and the solubility of TER increased both in simulated gastric and intestinal fluid, depending on the pH of dissolution medium.
Conclusion: The improvement of solubility and dissolution ratio could enhance the biopharmaceutical properties of the active ingredients in solid pharmaceutical products. Furthermore, stable, amorphous cyclodextrin-containing, organic solvent-free products could be formed efficiently in industrial environment.

High-dose of salicylic acid treatment increases the activity of MMP-2 and 9 in the gastrointestinal and cervical smooth muscle in rats

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Background: Salicylic acid (SA) is a well-known cyclooxygenase enzyme (COX) inhibitor can cause gastric damage and intestinal ulceration already in therapeutic dosage. In our preliminary study, we found that the high-dose of SA induced gastric diverticulum and block of GI passage. Beyond cyclooxygenase enzymes, salicylates may influence the action of other target proteins such as TNFα, NF-κB, PPARγ. The gelatinases like MMP-2 and 9 are expressed in gastrointestinal tract and have an important function in cervical ripening.
Aims: We hypothesized that the GI passage block might be associated with the alteration of activities of MMP-2 and 9. Therefore, the aim of our study was to investigate the effect of high-dose salicylic acid oral treatment on GI smooth muscles motility in vitro and in vivo, and to determine the changing of cervical resistance in rats.
Methods: Non-pregnant female Sprague-Dawley rats were treated to 3 days with high-dose 400 mg/day salicylic acid suspension by oral gavage. The alterations of smooth muscle contractions were determined in GI (intestine, stomach, coecum) and cervical tissues with in vitro isolated organ bath and in vivo electromyography. The activity of MMP-2 and 9 were measured with gelatin zymography and IVIS Lumina imaging system.
Results: After SA treatment, the spontaneous contractions of stomach, ileum and coecum were significantly reduced, furthermore the cervical resistance was also decreased. The intensity of GI electromyographic signal was increased on each day of treatment, the highest values were reached on 2nd day in all samples. Both gelatin zymography and IVIS Lumina imaging system demonstrated that the activity of MMP-2 and 9 were increased by high-dose SA administration.
Conclusion: Our results suggest that the high-dose SA can provoke gastric hypermotility at the start of the treatment while at the end of that, the motility is reduced, and the GI passage is blocked. Furthermore, it can decrease the cervical resistance. It seems that both in GI and cervical tissues the high-dose SA treatment can increase the MMP-2 and 9 activities which may lead to the gastrointestinal and cervical tissues transformation and impaired smooth muscle function.

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Micro- and macrostructural comparison of pH modifier- or solubilizer-containing furosemide-loaded electrospun nanofibers

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Background: Electrospinning technique enables the formulation of novel fibrous drug delivery systems

of improved bioavailability. The selection of proper excipients is the prerequisite of the required functionality-related characteristics of the fibrous system. For this purpose, it is essential to better understand their influence on the fiber formation and functional characteristics of the formulated fibers, including the stability of the drug-loaded electrospun product. Thus an in-depth physical-chemical and morphological study is necessary to define the final composition.

Aims: The present study is focusing on the comparison of solubilizer (triethanolamine) or pH modifier (sodium hydroxide)-containing furosemide-loaded electrospun nanofibers from the point of their micro- and macrostructural properties.

Methods: Two hydroxypropyl cellulose and poly(vinylpyrrolidone)-based formulations were prepared with the addition of triethanolamine or sodium hydroxide to improve the solubility of the furosemide. For the morphological characterisation scanning electron microscopic (SEM) images were performed, and statistical analysis was carried out for the comparison of the distribution of fiber diameters. X-ray diffraction (XRD) spectroscopy and Fourier-transform infrared spectroscopy (FTIR) were applied to investigate the amorphous or crystalline nature of furosemide in the fibrous samples. For further microstructural characterisation positron annihilation lifetime spectroscopy (PALS) was carried out.

Results: The solid-state characterization measurements revealed similar morphology with similar fiber diameter distribution. XRD and FTIR measurements confirmed that the examined formulations contained the furosemide in amorphous salt form, but the PALS measurements indicated different microstructure of the two formulations.

Conclusion: The results may predict different long-term stability features.

Proteomic analysis of human blood derived osteoclast cells

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Background: Osteoclasts have essential role in certain rheumatological inflammatory disorders, however the detailed pathomechaism of these diseases is not yet fully understoodWe have lack of data about the molecular changes during osteoclast differentiation, and the proteome of human blood derived osteoclasts is still unknown.

Aims: Our aims were to optimize a sample preparation and analytical method using nanoHPLC-MS/MS for the proteomic analysis of osteoclasts and their differentiation. After optimizing the methods using healthy samples, we planned to identify and compare the expressed proteins of monocytes, preosteoclasts and osteoclasts; and to determine the differences in osteoclasts proteome of healty population and diseased individuals.

Methods: First, we collect blood samples from healthy donors and isolate monocytes by positive magnetic separation. After this, preosteoclasts and osteoclasts are differentiated from monocytes in vitro using M-CSF and RANK-L growth factors. Next, monocytes, preosteoclast and osteoclast samples are lysed, proteins are reduced, alkylated and digested with trypsin. After C18 clean-up, peptides are separated with nanoHPLC and analyzed with tandem mass spectrometry. The evaulation of raw data is performed using different proteomic databases and softwares.

Results: We have successfully optimized the proteomic sample preparation and analysis on healthy osteoclast samples for maximizing the number of identified proteins and minimizing false positive results. We have determined the proteome of healthy donors' monocytes, preosteoclast, as well as osteoclast samples. Based on our preliminary results there are significant differences among the expressed proteins during osteoclast differentiation.

Conclusion: The developed sample preparation protocol, the applied chromatographic and mass spectrometric conditions, and the properly adjusted evaluation methods are fully appropriate for the analysis of proteomic dynamic changes during osteoclast differentiation. Results obtained with these set methods may promote the better understanding of the molecular pathomechanism of rheumatological diseases.

Antiproliferative and antimetastatic properties of a 17-exo-heterocyclic derivative of androstadiene in vitro

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Background: Cancer is one of the leading causes of death wordwide. Therefore, one of the main goals of

drug research is to find new and effective antitumor molecules. Nowadays, D-ring modified, exo-heterocyclic androstadien analogs are used in cancer therapy (abiraterone). Accordingly, further modifications of this steroid skeleton may result additional effective compounds.

Aims: Our aim was to characterize the anticancer and antimetastatic properties and mechanism of action of 3β-hydroxy-17-[1′-(4′′-cyanophenyl)-4′-hydroxymethyl-1′H-pyrazol-3′-yl]androsta-5,16-diene. Antiproliferative effects of this modified androstadiene derivative have proven in a previous study on human breast cancer cells [1.].

Methods: Antiproliferative effects and tumour selectivity of our test compound were determined by standard MTT-assay on a panel of human gynecological cancer cell lines. Cell cycle disturbances were recorded by flow cytometry after 24h and 48h incubation on SiHa, C33a and MDA-MB-231 cells. Changes in activity of caspase 3 enzyme were detected by a colorimetric assay on SiHa cell line after 24, 48 and 72h treatment. Furthermore, mitochonrial membrane potency of treated and untreated cells were recorded by JC-1 staining. Finally, inhibitory effect of the compound on the early stage of metastasis was investigated by 3D co-culture Circular Chemorepellent Induced Deffects (CCIDs) assay.

Results: Our test compound shows lower IC50 values than 3μM and moderate tumour selectivity compared to cisplatin. Significant changes in the hypodiploid subG1, G1 and S phases and caspase 3 activity were recorded at 2μM and higher concentrations. Therefore, JC-1 staining improved the mitochondrial origin of induced apoptosis. Size of cell –free areas induced by treated tumor spheroids decreased by 30% at 8μM in CCIDs assay.

Conclusion: This study provides experimental evidence that 3β-hydroxy-17-[1′-(4′′-cyanophenyl)-4′-hydroxymethyl-1′H-pyrazol-3′-yl]androsta-5,16-diene has potent antitumor and antimetastatic properties. This compound can be regarded as promising structure in design of new anticancer agents.

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The effect of L-theanine on the D-serine uptake of SH-SY5Y neuroblastoma cells

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Background: D-serine (D-Ser) is a co-agonist of the N-methyl-D-aspartate receptors (NMDAr). Decrease in its extracellular concentration in the brain has been connected to NMDAr hypofunction and the deficit of cognitive functions. Extracellular D-Ser levels in the central nervous system are modulated by neutral amino acid transporters, ASCT1 and ASCT2. L-theanine (L-The), a neutral amino acid and a major component of green tea leaves has been found to improve memory and cognitive functions.

Aims: Our aim was to examine the possible inhibitory effect of L-The on the D-Ser uptake of SH-SY5Y neuroblastoma cells. The cell line has been previously found in our laboratory as a good model of D-Ser uptake into cortical astrocytes.

Methods: Cells were incubated with 25μM of D-Ser and various concentrations of L-The or S-ketamine (S-Ket) for 15min. The intracellular D-Ser concentration was then determined by a capillary electrophoresis laser induced fluorescence detection method. Presence of ASCT1 and ASCT2 in SH-SY5Y cells was confirmed by Western blot.

Results: Although ASCT2 reportedly contributes to a minimal L-The transport, in our experiments L-The significantly inhibited D-Ser uptake into SH-SY5Y cells suspended in Hank's solution. Sixty % inhibition was observed albeit only when high concentration of L-The was used. The experiments were then repeated in cell culture medium abundant in neutral amino acids that are known ligands of the ASC-type transporters. Under this condition a reduced D-Ser uptake was measured possibly due to the competitive effect of these amino acids. L-The was able to significantly inhibit D-Ser uptake even in the medium though only a 22% inhibition was achieved. Having previously described as an inhibitor of ASCT2 we used S-Ket as a positive control. Surprisingly, no inhibition was observed even at several times higher concentrations compared to the previous publication. Its possible long-term regulatory effect on D-Ser uptake was also examined by 72h culturing cells with 25 or 50μM S-Ket. Since no significant change was observed between D-Ser uptake of control and S-Ket-treated cells we concluded that S-Ket does not affect the activity of ASCT1 and ASCT2.

Conclusion: Though reported as an inhibitor, S-Ket failed to inhibit D-Ser uptake or modify the uptake kinetics after a long-term incubation period. L-The, however, was found to be a competitive inhibitor of the ASC-type transporters and it is taken up considerably by SH-SY5Y cells.

Biocompatibility and antimicrobial studies of food and pharmaceutical preservatives

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Background: Preservatives are not highlighted by current scientific research, yet, they are present in everyday life, as their role in the antimicrobial protection of food, drugs and cosmetics is unquestionable. Cheap to use compounds, with a wide range of antimicrobial effect, these chemicals did not change during the last decades, for new molecules applied by industry are few. Publications revealed several limitations in case of preservatives, such as their cytotoxicity, pH dependent inhibitory effect or interaction with other excipients or APIs.
Aims: Our research group is focused on the biocompatibility and antimicrobial investigation of preservatives. Four individual experiments presented at the conference include the structure-activity study of ten p-hydroxybenzoic acid esters; the study of parabens in different complex co-solvent systems; the comparison of sorbates and sorbate esters and formulation of essential oil emulsions as preservatives.
Methods: Our primary technique for assessing cytotoxicity are MTT and NR assays, carried out on Caco-2 human colon adenocarcinoma cell line. In vivo experiments involved *Galleria mellonella* larvae. Antimicrobial tests of different kinds used Candida spp., *E. coli, S. aureus* and *P. aeruginosa* as pathogen model organisms.
Results: Our experiments revealed, that the relative toxicity of parabens is highly modified by the other excipients applied in the product and the non-linear toxicity of the homologous series of parabens. Sorbate esters were more effective against microbes, than sorbic acid and potassium sorbate and the essential oil emulsions proved to be a viable alternative for preservatives.
Conclusion: Innovative compounds and advantageous interactions between preservatives and other excipients are promising new methods for ensuring microbial safety of pharmaceutical products. Meanwhile these new techniques require lower concentrations than the currently recommended molecules which increase their safety.

Risk Assessment based therapy development in cystic Fibrosis

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Background: Cystic Fibrosis (CF) is an autosomal recessive disorder, where the cellular transport's defect results in viscous secretions in different exocrine tissues (respiratory tract, pancreas GI tract, sweat glands, etc). The therapy of this chronic and progressive disorder is complex, lasts a lifetime and significantly influences the Quality of Life (QoL) of patients. The adherence to medical treatment is crucial in this case for the patient's QoL expectations [1]. The Quality by Design (QbD) approach of the pharmaceutical developments is a holistic, systemic, knowledge and Risk Assessment (RA) focused method with profound previous target product design. QbD is a complex process with several steps, by the International Council on Harmonisation (ICH) guidelines and can be applied to each phase of the pharmaceutical research and development and also can be extended [2].
Aims: The aims of this study were the analysis of the early development phase of the pharmaceutical development in CF therapy and the application of the QbD guided risk-based approach in the medicinal product-design by the extended QbD methodology [2] in order to improve patient adherence and quality of life.
Methods: Collection, analysis and summary of all the relevant factors of CF therapy development, with special attention to patient expectations, adherence related factors, therapy and regulatory-specific elements and application of modern quality management tools in visualization, for better evaluation. Implementation of the RA by means of LEAN QbD® Software (QbDWorks LLC, Fremont, USA) in order to determine the factors with most highly risk on therapy development.
Results: After collection, systematization and evaluation of all the therapy-related factors, the RA of the potential critical factors was performed and the ranking of the factors has been determined. Based on the RA results, the recommended intervention points in the CF therapy-management are: increased social support, increased patient education, and increased monitoring.
Conclusion: These interventions stated by the risk-based evaluation of therapy development or management which can improve the patients' quality of life can be the alternative of the costly and time-consuming new drug developments.

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Sleep-wake stage-dependent effects of acute $5HT_{2C}$ receptor antagonist SB242084 treatment on EEG gamma band activity

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Background: Gamma activity of the brain is associated with cognitive and sensory processes and might provide a signature of cognitive state and also network dysfunction. Moreover, alterations of these oscillations have been implicated as biomarkers of depression. On the other hand, several lines of evidence suggest a role of serotonin 2C receptors ($5HT_{2C}Rs$) in the pathomechanism and treatment of depression and anxiety. Numerous drugs used in the therapy of psychiatric disorders, such as most atypical antipsychotics and several antidepressants, possess $5HT_{2C}R$-blocking property. In addition, selective $5HT_{2C}R$-antagonists have been proposed as putative fast-onset antidepressants.
Aims: As we hardly could find any data about the effects of selective 5HT2CR-acting drugs on gamma oscillations in freely moving animals, our aim was to investigate the acute effects of the 5-HT2CR-antagonist SB242048 on the 3060Hz gamma band of the electroencephalogram (EEG) during wakefulness, rapid eye movement (REM) sleep, and non-REM sleep.
Methods: Wistar rats were equipped with EEG and electromyography (EMG) electrodes. Following their recovery and habituation, we administered 1.0mg/kg SB242084 or vehicle intraperitoneally, at light onset (beginning of passive phase), and recorded frontoparietal EEG, EMG and motor activity for the subsequent 3h.
Results: Quantitative EEG analysis performed by means of Fast Fourier transformation revealed that the acutely administered SB242084 markedly enhanced power density in the gamma band (3060 Hz) during non-REM sleep. In contrast, no change was observed during REM sleep and during wakefulness in this frequency band.
Conclusion: These findings indicate that the selective $5HT_{2C}R$-antagonist affected gamma activity, moreover, in a sleep-wake stage-dependent manner (reflecting different brain functions related to gamma oscillations), that may provide further evidence for the therapeutic potential of these compounds in the therapy of depression and/or anxiety.

Formulation and evaluation of BGP15 loaded topical drug delivery systems

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Background: BGP15 is a novel insulin sensitizer drug candidate that also has protective effects in oxidative stress related diseases and may have a protective role in inflammatory diseases.
Aims: The aim of our research was to investigate the anti-inflammatory effect of this molecule by formulating o/w emulsion type ointments, perform biocompatibility assays, check in vitro dissolution of the formulated ointments with the help of Franz diffusion chamber apparatus and formulate nanospheres of the pharmacon.
Methods: Sodium alginate nanospheres were formulated to increase the efficacy of transdermal drug delivery. Formulation has been performed by controlled polimerisation method and the role of the nanospheres has been evaluated. During the formulation we used Büchi B395 Pro Encapsulator. The bead formation is based on the fact that a controlled, laminar liquid jet is broken into equally sized beads, if vibrated at an optimal frequency. To enhance penetration different excipients were incorporated. Since safety is an important case of pharmaceutical formulations, citotoxicity of applied excipients had been evaluated. As in vitro model of human skin, HaCaT cell line was selected. To determine the biocompatibility of these materials MTT cell viability test had been performed. After the ointment formulation membrane diffusion and permeability studies were performed with Franz-diffusion chamber apparatus.
Results: The results of MTT experiments demonstrated that the selected excipients are safe under in vitro conditions. Based on the results of the ointments' dissolution we can conclude that all four compositions resulted very similar dissolution of the pharmacon, so all four surfactants can be used in the future for further investigations.
Conclusion: Based on the results it can be proven that BGP-15 is a versitale molecule and in the future it can be an increadibly useful therapeutic choice for many diseases.

Development and evaluation of ibuprofen rectal gels

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Background: Rectal route represents an alternative to oral administration in pediatrics, a possibility to avoid gastro-intestinal adverse reactions. Ibuprofen is a non-steroidal anti-inflammatory agent, used in all age groups to reduce pain, fever and inflammation.
Our study aims the formulation and characterization of ibuprofen containing rectal gels.
Methods: For the formulation of gels ibuprofen was used as active substance, Carbopol 940, sodium alginate and low viscosity HPMC as gel formers. Propylene-glycol and glycerin were used as permeation enhancers. 5% of ibuprofen was incorporated in all formulations. Three gels have been prepared containing 1 % Carbopol, 0.5 % Carbopol and 5 % HPMC, and 5 % sodium alginate. The influence of formulation variables on the pharmaco-technical parameters were studied: bioadhesion was recorded as the force required to detach the sample from the surface, pH, and diffusion. Diffusion of ibuprofen was measured in phosphate buffer (at a pH of 7.4) with Franz cells. Mathematical models were applied to model the release of active substance.
Results: pH of gels was 4.2-5.0 viscosity varied between 165 and 212 mPas. Detachment force was from 0.02 to 0.12N.
Conclusions: Three gels containing ibuprofen were formulated for rectal used, suitable for children and adults. The obtained experimental results showed a superior release of ibuprofen (10%) from the 5 % sodium alginate gel.

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Investigation of the cellular effects of beta-cyclodextrin derivatives on intestinal epithelial cells

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Background: Cyclodextrins are widely used excipients for increasing water solubility, delivery and bioavailability of lipophilic drugs. We showed previously, that cyclodextrins are able to enter Caco-2 intestinal cells by endocytosis, but the different fluorescent labelling has not been compared on the same cyclodextrin derivative. On the other hand, the consequences of the cellular internalization of cyclodextrins have not been revealed yet.
Aims: Our aim was to compare the cellular internalization of fluorescein and rhodamine labelled hydroxypropyl-, (HPBCD) and randomly-methylated-beta-cyclodextrins (RAMEB) and to reveal the appropriate mechanism of the endocytosis. We also wanted to investigate the effects of these derivatives on the NF-κB pathway and autophagy and to examine the involvement of the lysosomal pathway in the cells.
Methods: The endocytosis of the cyclodextrin derivatives was investigated by fluorescence microscopy, the more accurate mechanism of endocytosis was investigated by flow cytomtery using various inhibitors. The effect of cyclodextrins on NF-κB pathway was examined bythe detection of the p65 subunit nuclear translocation by fluorescence microscopy. The effect of cyclodextrins on autophagy was investigated qualitatively by immuno labelling the LC3B molecule in the autophagosomes membrane and quantitatively, whereby the autophagosomes membrane was stained with fluorescent dye. Lysosomes present in Caco-2 cells were examined by fluorescence microscopy and the membrane of the lysosomes was stained by LysoTracker®.
Results: Both fluorescein and rhodamine labeled derivatives are able to enter the intestinal Caco-2 cells by endocytosis. Cooling perfectly inhibited endocytosis, while rottlerin inhibited significantly the uptake. Cyclodextrin pretreatment did not activate the NF-kB pathway. After HPBCD and RAMEB treatments the presence of autophagosomes is detectable, similar to control samples. Using flourescence microscopy we revealed, that these cyclodextrin derivatives are able to enter lysosomes.
Conclusion: The type of fluorescent labelling does not influence the internalization of HPBCD and RAMEB cyclodextrin derivatives. FITC and rhodamine conjugates showed similar intracellular localization. The endocytosis of cyclodextrin does not activate NF-κB pathway and does not increase the formation of autophagosomes compared to the control sample. At the same time these derivates can be detected in lysosomes after internalization.

The effect of alpha-lipoic acid on the antitumor effect of bortezomib in melanoma and myeloma cells

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Background: Bortezomib (BOZ) is a proteasome inhibitor chemotherapeutic agent utilized to treat multiple

myeloma and recently offered to cure melanoma. Bortezomib-induced peripheral neuropathy is one of the most significant and dose-limiting side-effects, which can be treated with antioxidants (e.g. alpha-lipoic acid – ALA and vitamin B_1 – vit B_1) as a part of cancer supportive care. We hypothesized that these antioxidants may counteract the antitumor activity of BOZ.

Aims: The objectives of our experiments were: (i) to verify the cytotoxicity of BOZ; (ii) to test and compare the influence of the antioxidants on the antitumor effect of BOZ in melanoma (A2058) and myeloma (U266) cells as clinically relevant target cells.

Methods: The cell viability was determined by xCELLigence® RTCA SP instrument and by a cell based CellTiter-Glo® Luminescent Cell Viability Assay. Then the possible molecular pattern was characterized by the analysis of phospho-p53 (S15) by flow cytometry and the cell cycle by NucleoCounter ® NC-250™. Cell-based assays were also assessed on the proteasome activity and on the ROS generation.

Results: At first, the cytotoxicity inhibiting effect of alpha-lipoic acid was proved in melanoma cells. Analysis of p53 phosphorylation and the cell cycle progression revealed that ALA failed to counteract the effects of BOZ on these processes. Nevertheless, a good correlation was found between the inhibition of the cytotoxicity, the anti-proteasome activity and the oxidative stress level after the co-treatment with 20ng/mL BOZ + 100μg/mL ALA.

Conclusion: The antagonizing effect of ALA on the antineoplastic activity of BOZ in melanoma cells draw the attention to the proper application of cancer supportive care.

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Characterisation of phenolic profile and antioxidant activity of *Carpinus Betulus*

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Background: Plants are still potential sources of new drugs, however, the ligneous flora is rarely explored in this regard. Hofmann et al. evaluated phenolic compounds and total antioxidant effect of European hornbeam (*Carpinus betulus*) leaf methanol extract [1]. However, analysis of other plant parts has not been performed and diarylheptanoids described in the Betulaceae family, have not been identified in *C. betulus*. Diarylheptanoids have gained interest due to their remarkable anticancer and antioxidant activity [2].

Aims: Our aim was to analyse and compare the phenolic composition of *C. betulus* extracts made from distinct plant parts with various solvents, with special regard to its diarylheptanoid profile. In addition, we aimed to characterise the contribution of individual constituents to the total antioxidant capacity of the extracts.

Methods: Dried bark, leaf, male and female flowers of *C. betulus* were extracted with solvents of increasing polarity (chloroform, ethyl acetate, methanol) in ultrasonic bath. For the phytochemical analyses an LC-ESI-MS/MS method was applied. Diarylheptanoids were isolated by a combination of reversed-phase chromatographic techniques and identified by HR-MS and NMR methods. Total antioxidant activity of the extracts was assayed with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. We compared the contribution of each compound to the antioxidant effect against DPPH using an LC-MS method.

Results: Methanol extracts of *C. betulus* were dominated by the presence of gallic acid derivatives and ellagitannins. Ethyl acetate extracts contained gallotannins, hydroxycinnamic acids, flavonol-glycosides and diarylheptanoids. Five diarylheptanoids were isolated from the bark for the first time with carpinontriols A and B being the most abundant. The methanol extract of the leaves showed the highest antioxidant effect, due to its high tannin content. Galloyl-hexahydroxydiphenyl glycosides and other galloyl esters contributed to the greatest extent to the total antioxidant activity.

Conclusion: Phenolic fingerprints of different parts of *C. betulus* were compared and cyclic diarylheptanoids were identified for the first time. Gallotannins were described as constituents being responsible for the antioxidant activity of hornbeam.

References: 1 Hofmann, T. et al. Ind Crops Prod, 2016;87:340-349; 2 Alberti, Á. et al. J Pharm Biomed Anal, 2018;147:13-34.

HPLC-DAD-MS investigation of the carotenoid composition of flowers collected in transylvania

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Background: Ethnobotanical studies play an important role worldwide nowadays. The local knowledge of rural people possesses many valuable elements related to drugs of plant origin, such as in the isolated regions in Transylvania, Romania. These field trips are of pivotal importance to preserve the archa-

ic data, and after comparison with data obtained from scientific databases, plants can be analysed for the phytochemical characters of the ethnomedicinally used parts, e.g. for the carotenoid content of flowers and fruits, as well.

Aims: This paper focuses on the HPLC investigation of the carotenoid composition of flowers of *Telekia speciosa* (heartleaf oxeye, Asteraceae) and six Verbascum species (mullein, also known as velvet plant, Scrophulariaceae), collected in Transylvania, Romania.

Methods: By HPLC-DAD-MS systems based on their UV-VIS and mass spectrum as well as co-chromatography with authentic samples, some main and minor carotenoids were identified in the studied plants.

Results: The flower of *Telekia speciosa* contained lutein as main carotenoid while the minor components seemed mainly carotenoid 5,6-epoxides (neoxanthin, (9Z)-neoxanthin, violaxanthin, antheraxanthin and beta-cryptoxanthin 5,6-epoxide). Carotenes occurred only in traces. In the selected populations some quantitative differences were detected in the identified components. In the flower of Verbascum species lutein and beta-carotene were identified as main compounds. In addition to zeaxanthin, alfa- and beta-cryptoxanthin, alfa-carotene, gama-carotene and delta-carotene were detected. The content of gama- and delta-carotene and their (9Z)-isomers differed significantly (from 3 to 20 %) in the different species. Carotenoid epoxides (except violaxanthin) were detected in traces in the plants.

Conclusion: The yellow flowers usually contain lutein and beta-carotene as main compounds and some epoxides (neoxanthin, violaxanthin) as minor carotenoids. The investigated species show two different but complex carotenoid profils. Unfortunately, at the moment some compounds remained unidentified. The isolation and identification of these compounds are in progress.

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Development and characterisation of solid gastroretentive dosage form based on melt foaming

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Background: Several drugs display site-specific absorption in GI tract. Dosage forms with increased gastric residence time are promising tools to increase bioavailability of drugs with narrow absorption window. Low-density floating formulations could avoid gastric emptying; therefore, sustained drug release can be achieved [1].

Aims: Our aim was to develop a simple, new technology, based on melt-foaming, which can be easily filled into the final dosage form, namely hard gel capsules. After filling, the foam quickly solidifies upon cooling and keeps its structure. Excipients were selected carefully, with the criteria of low gastric irritation, melting range below 70°C and well-known use in oral drug formulations.

Methods: PEG4000, stearic acid, Labrasol and metronidazole were used for experiments. A novel, inhouse apparatus was built to mechanically dispersing air into the melt. Densities were determined by the pycnometer method. SEM and MicroCT was performed to characterize the foam and cell structure. Dissolution and floating properties were investigated in pH 1.2 hydrochloric acid media. Dissolution data were also analysed by fitting to kinetic models and by model independent approach. Texture analysis was chosen to monitor the hardness changes of the foams during dissolution.

Results: 53°C was found as an optimal temperature for gas entrapment in the molten dispersion. Stearic acid was necessary to improve the foamability and to achieve density values below 1.0g/mL. The lowest density reached was 0.82g/mL. The cell structure was homogeneous and the smooth outer surface did not form a shell. The shape of the cavities was typically spherical or spheroidal. Cavities formed by the merging of bubbles were present, as well. During the dissolution tests, all samples were proven to possess zero floating lag time. Composition with 10% stearic acid was found to release 86% its drug content in 10 hours. All formulations fitted best to the Korsmeyer-Peppas model and none of them fitted to zero-order or first-order model. Texture analysis confirmed the presence of an unwetted and solid core in the formulation with 10% stearic acid even after 5 hours.

Conclusion: A novel technology was developed to foam hot and molten dispersions on atmospheric pressure, which is applicable to produce floating, low-density moulded dosage forms. Drug was released mainly by the erosion of the foamed matrix.

References: 1 Vo, A.Q., et al. Eur J Pharm Biopharm 2016;98:108–21.

Updating the European Pharmacopoeia Impurity Profiling Method for Terazosin

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Background: This work was motivated by the demand of European Directorate for the Quality of Medicines and HealthCare (EDQM). A new liquid

chromatographic (LC) method was developed for terazosin impurity profiling to replace the old European Pharmacopoeia (Ph. Eur.) method. This new method is published as part of the new Ph. Eur. monograph proposal of terazosin in PharmEuropa issue 32.2.

Aims: The aim of the method renewal was to cut the analysis time from 90 minutes (2 x 45 min) down to below 20 minutes. The Ph. Eur. monograph method is based on two different chromatographic separations to analyze the specified impurities of terazosin. The reason for the two methods is that two of the impurities are not sufficiently retained in reversed phase (RP) conditions, not even with 100% water as eluent. Therefore, next to RP, an ion-pair (IP) chromatographic method has to be applied to analyze those two impurities.

Methods: With our new proposed method it was possible to appropriately increase the retention of the two critical compounds using alternative stationary phases (instead of a C18 phase which is suggested by the Ph. Eur. method). Applying a pentafluoro-phenyl (PFP) stationary phase, it was feasible to separate and adequately retain all the impurities. The detection wavelength was also changed compared to the Ph. Eur. method and is now appropriate for the detection and quantification of all impurities using perchloric acid in the mobile phase at low pH.

Results: Another goal of the present study was to develop a generic workflow and to evaluate the chromatographic resolution in a wide range of method variables and suggest some replacement columns for terazosin impurity profiling. Retention modeling was applied to study the chromatographic behavior of the compounds of interest and visualize resolution for the different columns, where a given criterion is fulfilled. A zone (set of chromatographic conditions) of a robust space could be then quickly identified by the overlay of the individual response surfaces (resolution maps).

Conclusion: It was also demonstrated that two columns from different providers (Kinetex F5 and SpeedCore PFP) can be used as replacement columns, providing sufficient resolution at the same working point and a high degree of robustness.

References: Enesei D, et al. J. Pharm. Biomed. Anal. 2020;187:113371