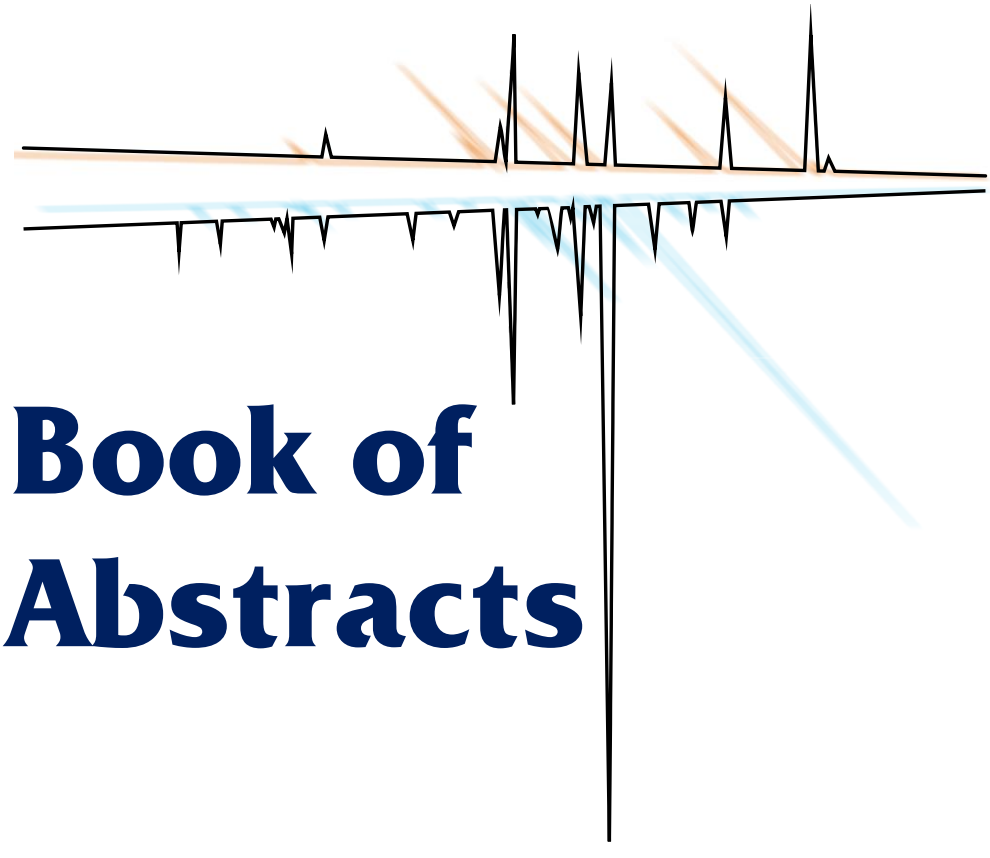
6th IAPC Meeting

Sixth World Conference on Physico-Chemical Methods in Drug Discovery

&

Third World Conference on ADMET and DMPK



Book of Abstracts

Zagreb, Croatia, September 4-7, 2017

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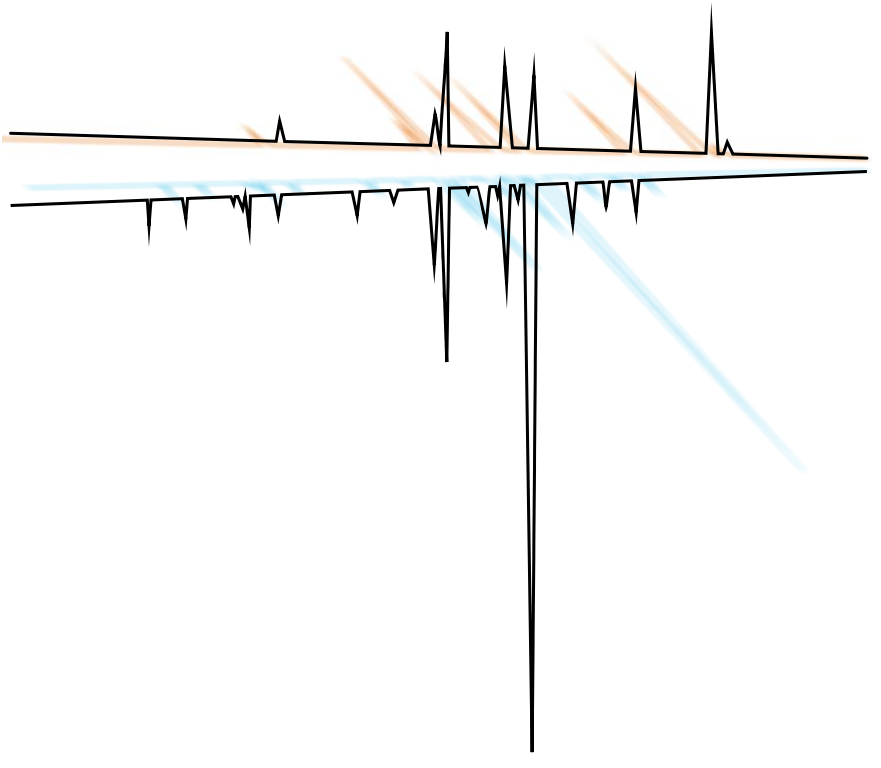

O 01

The environment in the lower intestine and its impact on drug absorption

Christos Reppas

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Luminal environment in the lower intestine is important for the performance of orally administered active pharmaceutical ingredients with extended and/or delayed absorption kinetics. Due to the limited water content in the transverse colon, regions of primary interest in regard to drug/dosage form performance in the lower gut are the distal small intestine, the cecum, and the ascending colon. In recent years various groups have focused to the environmnet in the lumen of lower intestine by applying imaging and direct sampling techniques. This presentation is divided into two parts.

In the first, the physicochemical characteristics of the environment in the lower gut of adults will be summarized. Emphasis will be given to luminal volumes (total and of the water), size of non-liquid particles, type of colloidal particles, and, also, to specific components such as biles salts, short and long chain fatty acids, cholesterol, lecithin, total protein content and total carbohydrate content [1]. Regional differences and impact of food components on the reductase activity along the lower gut will also be discussed [2].

In the second part of the presentation, recent work on the in vitro evaluation of dissolution in the lower intestine and its impact on the absorption process of high dose low solubility drugs will be discussed. Specifically, the usefulness of in vitro data collected under conditions simulating the environment in the upper gastrointestinal lumen and in the lower gut in predicting the average plasma profile will be evaluated by applying physiologically based pharmacokinetic modelling procedures [3].

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O 02

Strategies for the development of liquid, semisolid and solid lipid-based drug delivery systems

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In recent years, there has been much interest in the pharmaceutical field on lipid-based drug delivery systems for the oral bioavailability enhancement of poorly water-soluble drugs. They are usually developed as solutions with drugs dissolved in mixtures of lipids, surfactants and/or co-surfactants that form microemulsion with particle size <250 nm upon dilution with aqueous media of the gastrointestinal tract. The formulations are often referred to as microemulsion preconcentrates or self-microemulsifying drugs delivery systems (SMEDDS). In this presentation, a systematic approach of selecting lipid-surfactant mixtures (preconcentrates) that would spontaneously form microemulsions upon dilution with water will be discussed. One major disadvantage with the lipid-based drug delivery systems is that the preconcentrates used as formulations are generally liquids and have to be encapsulated in soft gelatin capsules (for example, Neoral, a Novartis product). Various strategies to convert microemulsion preconcentrates into solids, yet maintaining all attributes of liquids, will be discussed. The solidified formulation can be filled into hard gelatin capsules. There has also been much interest on the conversion of liquid lipid-based formulations into tablets. However, there are only limited studies on the development of tablet dosage forms for lipid-based drug delivery systems reported in the literature. Our recent work on the development of tablet formulations of lipid-based systems showed that, among many commercially available silicates studied, Neusilin® US2 is the only one that is able to produce tablets with acceptable tensile strength in presence of the lipid component as high as 1:1 w/w ratio. However, there are conflicting reports on the ability of porous silicas, like Neusilin® US2, to release lipid formulations completely, especially after long-term storage. We studied the release of a model drug, probucol, from different microemulsion preconcntrates containing medium chain lipids and a surfactant (Cremophor EL or polysorbate 80) after adsorbing them onto Neusilin® US2. Complete drug release (>80%) was obtained from all formulations on Day 1; however, the extent of drug release decreased progressively with time. The decrease was dependent on the relative hydrophilicity of the formulations. The maximum decrease (<10% drug released on day 60) was seen from formulations containing the highest amount of lipid (70% in the SEDDS preconcentrate) and lowest decrease was seen in formulations containing the drug dissolved in the neat surfactant only (ca. 65% drug released on day 60). By synthesizing silicas with different pore sizes, we demonstrated that the drug release is also dependent on pore size; the lower the pore size, the less was the drug release. Precoating Neusilin® US2 and other porous silicas with polyvinylpyrrolidone (PVP), by treating them with an alcoholic solution of PVP and then drying, eliminated or minimized the decrease in drug release upon storage, possibly by blocking the mesoporous region of the silicate and improving hydration, thus allowing emulsification of the formulations within the larger pores. Formulations containing PVP K-90 precoated on Neusilin® US2 exhibited complete drug release (>80%) even after 6 months of storage.

O 03

In vitro dissolution/release methods for novel mucosal drug delivery systems

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In vitro dissolution/release tests are an important tool in the drug product development phase as well as in its quality control and the regulatory approval process. Mucosal drug delivery systems are aimed to provide both local and systemic drug action via mucosal surfaces of the body and exhibit significant differences in formulation design, as well as in their physicochemical and release characteristics [1]. Therefore, it is not possible to devise a single method which would be suitable for release testing of such complex dosage forms. This presentation is aimed to provide a comprehensive review of both compendial and noncompendial methods used for *in vitro* dissolution/release testing of novel mucosal drug delivery systems aimed for ocular, nasal, oromucosal, vaginal and rectal administration. Different apparatuses and techniques for *in vitro* release testing are designed and developed for mucosal delivery systems considering the specific conditions at the administration site [2–4]. In order to avoid unnecessary proliferation of equipment and method design, compendial apparatuses and methods should be used as a first approach in method development when applicable. However, to assure adequate simulation of conditions *in vivo*, novel biorelevant *in vitro* dissolution/release methods should be developed enabling the prediction of formulation performance *in vivo* and, preferably, the establishment of an *in vitro/in vivo* correlation. Equipment set up, the selection of dissolution media and volume, membrane type, agitation speed, temperature, and assay analysis technique need to be carefully defined based on mucosal drug delivery system characteristics. All those parameters depend on the delivery system and physiological conditions at the site of application and may vary in a wide range, which will be discussed in details.

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O 04

How to increase the success of bioequivalence studies in the generic drug industry

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Generic drugs are important options that allow greater access to health care for all countries in the world. While all countries implement their own health policies, mostly they adopt the general guidelines publisned by FDA, EMEA, WHO and ICH for generic drug licensing regulations.
According to BCS (Biopharmaceutics Classification System) rules, it is possible that some groups of classified drugs and dosage forms can obtain biowaive without perform clinical trials, but most of the generic drugs must go comparative clinical studies with the reference product.
The biggest problem in bioequivalence studies is that the generic product developed by spending almost 2 years can be failed.
In silico and in vitro and cell culture studies can give us opportunity to improve our generic drug formulations from the begining of the project. These studies can be accepted as the check points for the development process of the generic drugs.
It can possible to utilize some rules appeared in the guidelines for waivine of bioequivalence studies of generic drugs. Moreover, by using in silico methods, the generic formulation can be improved under the QbD and in silico or in vitro and cell culture methods can be used for simulation of bioavailability studies. The most important points in these studies are which groups should be added to the study, which conditions should be selected, which method should be used and what sampling time should be and which parameters obtained from the study would be used for the comparison of clinical study's parameters.
We are going to explain these issues given above, by presenting our generic drug study results. We are going to present how to waiver clinical studies by evaluating rules of the authorities and how to improve the generic drug formulations by using in silico and in vitro and cell culture studies.

O 05

Design of colloidal drug delivery systems for controlled release of non-steroidal anti-inflammatory drugs

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Novel nano- and microparticle-based drug delivery systems has become one of the most fascinating research areas in modern pharmaceutical developments. Several biodegradable macromolecules, proteins and inorganic materials are used as drug carrier in order to achive a targeted drug delivery and also the controlled drug release process.
This work clearly demonstrates the preparation and possible application of different nano- (100-400 nm) and microparticles (5-20 µm) as potential drug delivery systems using several carriers like cross-linked and hydrophobized hyaluronic acid (HyA), biocompatible polymer (polyethyleneglycol (PEG)), bovine serum albumin (BSA), mesoporous silica nanoparticles (NPs) and layered double hydroxide (LDH) with one- and two-layered polymer shell(s). The size and the structure of the synthesized ibuprofen (IBU)-, ketoprofen (KP)- and kynurenic acid (KYNA)-containing systems have been determined by DLS, TEM, BET, SAXS, FT-IR and CD techniques. The increasing cross-linking ratio (50, 75, 100 %) of HyA results in the increase in the average particle size and a well-defined microgel network were formed proved by reological studies and the KP release was also slightly decreased with increasing cross-linking ratio. Due to the stoichiometric hydrophobization of HyA backbone with ionic surfactants (decylamine, CTAB) nanosized (500-600 nm) aggregated systems have been formed and according to this process the amount of the released KP was further decreased (only 30 % after 8h). Biocompatible PEG microparticles and BSA-based NPs containing IBU, KP and KYNA were also prepared by spray-drying and precipitation techniques which resulted in the formation of particles with core-shell structure. The 5-20 µm polymer-based particles with varied thickness of polymer shell and constant amount of drug as core have been fabricated where the thickness of the shell strongly influences the release profile. The interaction between the polymer/protein carriers and drugs have been investigated by surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) to provide quantitative (adsorbed amount of drug on polymer/protein surface), kinetic (k_D) and thermodynamic (ΔG, $\Delta H°$, $\Delta S°$) data on the drug binding process. Based on the preliminary results (determined k_D values by release investigations) the most applicable carrier for binding of IBU and KP and for the controlled drug release is the hydrophobized HyA system (only 30% after 8h) but th PEG- and BSA-based micro- and NPs with two-layered oppositely charged polymer shells also suitable for encapsulation of IBU and KP. (40-45 % after 8h).

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O 06

Spray-dried melatonin-NLC loaded chitosan microspheres: development, optimisation and physicochemical characterization

Marieta Duvnjak Romić, Drago Špoljarić, Biserka Cetina-Čižmek, Jelena Filipović-Grčić*, Anita Hafner*

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Nanostructured lipid carriers (NLCs) are over the last decade one of the most investigated lipid delivery systems for various application routes [1]. NLCs represent an interesting and suitable strategy for the treatment of chronic wounds [2], especially in combination with chitosan, a well known wound dressing material [3]. As a continuation of previous research [4], the aim of this study was to develop an innovative drug delivery platform comprised of melatonin-loaded NLCs incorporated within chitosan based microspheres as dry powder formulation. Melatonin-loaded NLCs were prepared by hot homogenisation technique, while NLC-loaded microspheres were produced by spray-drying method. To understand the effect of formulation and critical process parameters for both, nano and micro systems, a novel screening design of experiment, called definitive screening design [5] was employed.

Investigated parameters for NLCs were homogenisation time, liquid lipid and total lipid content. Within the same design, parameteres lipid to chitosan ratio, feed flow rate and inlet air temperature that impact on the characteristics of melatonin-NLC loaded microspheres was also studied. The particle size of NLCs and production yield, as well as microspheres particle size, moisture content and zeta potential were investigated as responses. Melatonin-loaded NLCs were characterised by small particle size, negative charge and high melatonin entrapment efficiency. Optimized melatonin-NLC loaded chitosan microspheres were characterised with high production yield, positive surface charge, low moisture content, good flowing properties and controlled drug release. These results suggest that, under appropriate conditions, spray-drying technique represents a suitable approach for manufacturing of melatonin-NLC loaded chitosan microspheres as dry powder formulation.

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O 07

Exploring *in vivo* fate of drug nanocrystals

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Formulating a poorly water-soluble drug substance into nanocrystals offer many advantages. Understanding of the in vivo fate of drug nanocrystals is however very limited. In this study, we utilized the hybrid nanocrystal concept and studied the kinetic process of dissolution in cancer cells. The idea of hybrid nanocrystal is to physically integrate guest substances directly into the crystal lattice of drug nanocrystals. The existence of foreign substances in a host is typically insignificant, having little effect on the structure and integrity of the hosting crystal structure but capable of casting drastic influence on the optical appearance and other physical properties of the host. Our current study is to take advantage of aggregation-induced emission (AIE) and produce hybrid paclitaxel (PTX) nanocrystals with an AIE dye integrated. When the fluorophore is entrapped in a nanocrystal, fluorescence is emitted when the nanocrystal is optically excited. When an entrapped dye molecule is released to a liquid medium due to the dissolution of the nanocrystal, its fluorescence is quenched. By monitoring the change in fluorescence, it is possible to quantify the dissolution of nanocrystals in a biological environment. Cellular uptake studies of hybrid nanocrystals were conducted with KB and HT-29 cell lines and characterized by confocal microscopy, flow cytometry, and HPLC. The results suggest that drug nanocrystals were taken up directly by the cells, and subsequently dissolved in the cytoplasm. The extent to which drug nanocrystal dissolved was estimated according to the fluorescence measurement. The cellular uptake and intracellular dissolution could be influenced by drug concentration, incubation time, and surface coating, as well as the type of cell line. The study opens the door for further understanding how drug nanocrystals behaves kinetically in the body, leading ways to optimize the design of drug nanocrystals for improved treatment outcome.

O 08

Drugs as materials: processing, structure, properties

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To become a drug, a pharmacologically active compound must be prepared in a specific form. It is in this form that the drug must be manufactured, packaged, stored, transported, administred and delivered to a target in the body. Whereas drugs move around the body in solution and act on the cellular and molecular level, the properties of their physical form need to be identified and optimized for in vivo performance, reliable manufacture and the protection of intellectual property. To successfully prepare a drug form that will be robust through manufacturing, stable before administration and then active with high bioavailability after administration, one needs to produce solid forms with a specific crystal structure and controlled particle size and shape, often as a multi-component composite. From a legal standpoint, it is important to note that in addition to active pharmaceutical ingredients (APIs), drug forms are also patentable. The development and control of drug forms is therefore crucial to many issues in intellectual property.

Considering drugs as materials, one can apply the knowledge of solid-state chemistry and materials science to obtain solid forms with optimized properties. The methods can include, among others, different types of mechanical and ultrasonic treatment, hydro-static compression, high-temperature or cryogenic spray-drying, and crystallization from supercritical solvents. Solid-state transformations (*e.g.* dehydration or clathrate decomposition, as well as polymorphic transitions) can be efficient in accessing metastable polymorphs or in micronizing the sample uniformly. To achieve control over drug forms and the processes used for their robust manufacturing, one needs to take into account both the thermodynamic and kinetic aspects of their transformations.

In the present contribution, I give a general introduction into the problem and illustrate it using several selected examples.

See more: *Boldyreva E.V. Non-ambient Conditions in the Investigation and Manufacturing of Drug Forms. Current Pharmaceutical Design, 2016, 22, 4981-5000 and references therein*.

International Association of Physical Chemists

6th IAPC Meeting, Physico Chemical Methods Drug Discovery Development
4-7 September 2017, ZAGREB, Croatia

Monday, September 4 - Afternoon & Evenning Sessions

Pharmaceutical Cocrystals – Physicochemical Properties and Formulations

Interest in cocrystal (CC) formulations continues to grow in the pharmaceutical industry (1). The session on cocrystal formulations at the IAPC-6 meeting in Zagreb will highlight issues related to the measurement of physicochemical properties, particularly dissolution and solubility as a function of pH, in support of formulation development of oral drug products with improved bioavailability (2,3).

In 2016, the US FDA provided revised guidance (4), defining cocrystals as "…crystalline materials composed of two or more different molecules within the same crystal lattice that are associated by nonionic and noncovalent bonds." Drug products containing a new CC are considered analogous to a new *polymorph* of the active pharmaceutical ingredient (API).

Poorly-soluble oral drugs ordinarily show low intestinal absorption. Most often, such drugs are formulated as salts. However, a more recently employed strategy is based on CCs. The altered solid state properties in CCs are generally expected to enhance the effective solubility, as the API is released in the dissolution process. In the supersaturated state, the increased solubility potentially can lead to increased oral absorption of the drug. Also, the formation of CCs presents an opportunity to diversify the number of crystal forms of an API and extend potential intellectual property protection.

Since polymorphs of cocrystals can form, it is important (but not generally easy) to identify the most stable form early in development (5). Along with solid state characterizations of the CC (1,5), dissolution studies play an important role in examining the time-dependent concentration of the released API from a CC form (6-10). Mechanistic insights into dissolution studies benefit from the measurement of solubility-pH of CCs. The Rodríguez-Hornedo laboratory has been at the leading edge in the measurement of CC solubility as a function of pH (11-14). Only a handful of other laboratories have tackled the challenge (15-17). New computational procedures are emerging to augment existing methods in the analysis and simulation of solubility-pH data of cocrystals (18). Consensus-driven suggestions for improvements in equilibrium solubility measurement are expected to further raise the quality of solubility data reported in the future (19).

SPEAKERS

· **Nair Rodríguez-Hornedo** (Univ. of Michigan, USA) – "Approaches to the development of cocrystal formulations" · **Rafel Prohens** (Univ. of Barcelona, Spain) – "Application of surface site interaction points calculation to cocrystal screening" · **Rolf Hilfiker** (Solvias AG,Switzerland) – "Co-Crystals: The role of phase diagrams in optimizing experimental strategies" · **Guang J. Choi** (Soonchunhyang Univ., Asan, S. Korea) – "Preparation and Characterization of various Aripiprazole Cocrystals" · **Abu Serajuddin** (St. John's Univ., USA) – "How feasible is it to form cocrystals by acid-base interaction?" · **Nikoletta Fotaki** (Bath Univ., UK) – "Dissolution of pharmaceutical cocrystals for oral administration" · **Alex Avdeef** (*in-ADME* Research, USA) – "Cocrystal Dissolution-pH Mechanistic Models: Aqueous Boundary Layer Convection-Diffusion-Reaction in Rotating Disk/Powder Methods"

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O 09

Cocrystal thermodynamic and kinetic properties: Mathematical relationships and insights

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Cocrystals are an important class of pharmaceutical materials with remarkable potential to fine-tune solubility. The stoichiometric nature of cocrystals predisposes them to huge, yet predictable, changes in solubility and thermodynamic stability as solution conditions change such as drug solubilizing agents and pH. This property differentiates them from other supersaturating drug delivery systems. Cocrystal thermodynamic properties, while scarce in the literature, provide an unexploited spectrum of cocrystal behaviors that up till now may only show up inadvertently – sometimes to the point of cocrystals appearing risky compared to other solid-state forms. This talk will present the mathematical and chemical basis of simple relationships that quantifiably predict cocrystal solubility and supersaturation, and provide a conceptual basis for identifying conditions for desired behavior. Without this knowledge, selecting and developing cocrystals becomes an empirical exercise based on trial and error.

O 10

Application of Surface Site Interaction Points calculation to cocrystal screening

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In the pharmaceutical industry there is an increasing interest for discovering new crystal forms of drug compounds. In this sense, cocrystal formation has been recognized as a successful strategy for improving delivery of poorly soluble drugs. However, to engineer new cocrystals of an API presents many challenges and requires not only a deep knowledge of the intermolecular forces present in the structure and crystal packing, but to find the right set of experimental conditions. In this talk, I will present several case studies with different drug compounds of how experimental and virtual approaches can be efficiently combined to improve the discovery of new cocrystals.

O 11

Preparation and characterization of various aripiprazole cocrystals

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CANCELLED

For the last decade, enormous efforts and attention have been given to the study of pharmaceutical cocrystals primarily aiming at the enhanced stability as well as the modified solubility/dissolution behavior of drug substances.
In this study, aripiprazole, one of the most successful active substances as the atypical antipsychotic Abilify® but with poor solubility, was investigated. A new cocrystal of aripiprazole with orcinol was synthesized and fully characterized. Various processes were employed and compared; neat grinding, liquid-assisted grinding, and s[illegible]oration. Based on single-crystal x-ray diffraction measurement, it was determined that the crystal structure of aripiprazole-orcinol cocrystal is monoclinic. The melting point of the cocrystal was determined to be around 185 °C, which is higher than aripiprazole and cocrystals created with five dihydroxy- and trihydroxy benzene conformers as reported in previous study.(1) In addition, the aripiprazole-orcinol cocrystal showed a better dissolution rate behaviour than those aripiprazole cocrystals a[illegible]
It was examined how the differences in the crystallization and dissolution behavior could be correlated with various [illegible]ethanol such as the conformer moiety (in terms of the number of hydroxyl [illegible] the chemical structure) and the cocrystal structure. The [illegible]ween aripiprazole and phloroglucinol via grinding (either [illegible]or liquid [illegible]required a relatively high activation energy primarily due to the high melting point of phloroglucinol. It was speculated that the superior dissolution rate of the [illegible]ripiprazole-orcinol cocrystal to others could be closely associated with the bond angle in the cocrystal superlattice structure.
The stability of various aripiprazole cocrystals was compared by aging those powders in a controlled oven at 80 °C and 98 % RH. All cocrystals but the aripiprazole-catechol did not show noticeable degradation or physicochemical change up to a full week. Based on all data and observation, the aripiprazole-orcinol cocrystal can be considered as a potential drug substance form for drugs with improved stability and dissolution behavior.

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O 12

Co-Crystals: The role of phase diagrams in optimizing experimental strategies

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If common solid forms (i.e. salts, polymorphs, hydrates) of a pharmaceutical ingredient do not meet the required properties and quality attributes in order to be usable in the drug product, co-crystals may represent an attractive alternative.
In the context of co-crystal screening it is of utmost importance to understand what the phase diagrams of co-crystals can look like, so that optimal screening strategies can be defined. For that reason, a mathematical model was developed to predict the topology of ternary phase diagrams of systems that are forming salts, co-crystals, solvates, or hydrates. The considerations include the thermodynamic stability of the multi-component crystal relative to the individual components, the type of interactions in solution, the effect of relative solubility of the components, the formation of multi-component crystals with different stoichiometry, and the competition between co-crystals or salts and solvates. Based on the characteristics of the phase diagrams, recommendations are provided as to an efficient design of salt formation and co-crystallization experiments.
Finally, a case study will show how physical properties can be improved by choosing a suitable co-crystal.

O 13

Dissolution of pharmaceutical cocrystals for oral administration

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Solubility and dissolution studies are used to characterise the behaviour of cocrystals. Dissolution data for cocrystals would represent many complex processes occurring simultaneously, such as the change of the solid form and of the surface area of the particles as cocrystals undergo solution-mediated phase transformation. The relationship between the transformation rate and the dissolution rate is critical. Therefore, an appropriate dissolution design, in terms of the media, the hydrodynamics, the time scale and their relevance to the in vivo conditions would provide important information relevant to their transformation and their absorption. Dissolution studies of cocrystals in conditions relevant to in vivo conditions can improve the understanding of their behaviour, their precipitation kinetics and the prediction of their in vivo performance.
In this presentation dissolution studies of cocrystals based on a small-scale dissolution assay and based on an open flow through cell apparatus system mimicking the in vivo conditions will be discussed. Case studies showing that the improved dissolution of poorly soluble compounds from the cocrystal form can be revealed by appropriate selection of in vitro conditions and that dissolution of the cocrystal strongly depends on the type of coformer and the media selected will be presented.

O 14

How feasible is it to form cocrystals by acid-base interaction?

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In recent years, there has been much interest on the preparation of cocrystals of poorly water-soluble drugs to improve their dissolution rate and bioavailability. Cocrystals are multicomponent systems, and in the majority of examples of cocrystal formation reported in the literature, interactions between basic and acidic components are involved. For many basic compounds, carboxylic acids were used as coformers. However, cocrystals are different from salts as no proton transfer between the two components is involved. They are connected by hydrogen bonds or other non-covalent bonds. It has been reported in the literature that a continuum exists between salts and cocrystals. If the difference in pKa between the basic and acidic components (ΔpKa) is greater than 3, it is generally considered that a salt would be formed, while a cocrystal would be formed when ΔpKa is less than 0. In the ΔpKa range of 3 to 0, either a salt or a corystal may be formed. Following these rules, it is expected that a cocrystal may be formed in case a salt is not formed. To test the proposed continuum principle and the ΔpKa rule, we have recently attempted to synthesize salts and/or cocrystals of haloperidol (pKa 8), cinnarizine (pKa 2.55, 7.69) and ritonavir (pKa 2.5) with different carboxylic acids (maleic acid, pKa 1.9, 6.07; malic acid, pKa 3.25, 4.68; tartaric acid, 2.79, 3.90; citric acid, 2.91, 4.34, 5.68; succinic acid, 4.2, 5.6; fumaric acid, 3.03, 4.54). Several methods, such as crystallization from organic solvents, dry grinding, solvent-assisted grinding, and, in some cases, melt extrusion were used to prepare possible salts or cocrystals. Among the acids used, only maleic acid formed salt with haloperidol by solvent crystallization, dry griding and solvent assisted grinding. Despite favorable pKa difference, no salts were formed with other acids. No haloperidol cocrystals were also formed. Cinnarizine salts with maleic acid, malic acid and succinic acid were prepared by the mechanochemical methods, and salts/cocrystals were not formed with other acids. There was also no salt/cocrystal formation by ritonavir with any of the acids. Thus, the ΔpKa rule and the salt-cocrystal continuum may not serve as general guidelines for the feasibility of cocrystal formation. The structural features of the components ("synthon approach") may serve as the better guidance for the selection of coformer.

O 15

Cocrystal dissolution-pH mechanistic models: Aqueous boundary layer convection-diffusion-reaction in rotating disk/powder methods

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Interest in cocrystal (CC) formulations continues to grow in the pharmaceutical industry.[1] Poorly-soluble oral drugs ordinarily show low intestinal absorption. The altered solid state properties in CCs are generally expected to enhance the apparent solubility, as the API is released in the dissolution process. In the supersaturated state, the increased API concentration potentially can lead to increased oral absorption of the drug. The Rodríguez-Hornedo laboratory has been at the cutting edge in the measurement of CC solubility and dissolution as a function of pH.[2-6] New computational procedures are emerging to augment existing methods in the analysis and simulation of solubility-pH data of CCs.[7] The presentation will highlight the analysis of dissolution-pH profiles of cocrystal drug compounds, based on the convection-diffusion-reaction theory of dissolution, using the rotating disk and the Wang-Flanagan spherical particle models. A new software tool, *p*DISOL-X, will be applied to assess departures from the theoretically expected dissolution profiles as a result of supersaturation and/or re-precipitation effects. Mechanistic insights into dissolution processes potentially could lead to improved methods for formulation assessment.

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O 16

How to optimize the physicochemical and biomimetic properties of putative drug molecules?

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Potent putative drug molecules often fail at later stages of the drug discovery process due to poor absorption, distribution and pharmacokinetic properties. Physicochemical properties of molecules may be responsible for the distribution and nonspecific binding of molecules *in vivo.* The *in vivo* experiments are resource intensive and can be carried out only for a small number of compounds.

Biomimetic HPLC platform with immobilized artificial membrane (IAM), human serum albumin (HSA) and alpha-1-acid glycoprotein (AGP) stationary phases provide a high throughput measure of protein and membrane binding of discovery compounds[1]. Several models have been developed using the biomimetic properties of compounds to predict volume of distribution and unbound volume of distribution[2]. Thus, the volume of distribution can be estimated from the difference between the IAM and HSA binding. The sum of these two types of binding can be used to estimate the *in vivo* drug efficiency max (DE_{max}). Brain tissue binding, lung tissue binding, mucus binding, plasma protein binding can be estimated by various combinations of the measured HSA, AGP and IAM binding. It has been observed that the cellular concentration of compounds is proportional to their IAM binding[3]. When the potency data are available (pIC50) the Drug Efficiency Index, DEI can be calculated that also enables early dose estimation[4].

The structure – biomimetic property studies revealed that compounds bearing a positive charge bind strongy to IAM and tissues, while the negatively charged compound bind strongly to plasma proteins (HSA) and have low volume of distribution. The octanol/water log D is not suitable to model these differences as it is lower for both the positively and the negatively charged compounds. Biomimetic binding properties are therefore suggested replacing the octanol/water partition coefficients in early drug discovery compound optimization.

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O 17

Industrialisation of physicochemical measurements in early drug discovery

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It has long been recognised that poor physchem profiles are a major contributor to the high attrition rate of drug candidates[1]. During the early phase of drug discovery it is becoming increasingly important to acquire the full physicochemical profile of molecules. For this purpose there is a strong interest in developing efficient and cost effective platforms for fast and reliable measurements of physchem properties. Progressing compounds with good physicochemical properties is fundamental to GlaxoSmithKline's aspirations for Lead and Candidate Molecule Quality. An industrialised physchem platform ensures that consistent, comprehensive, and high quality physicochemical property measurements and derived property information are available at the right time to guide compound progression decisions.

High throughput determination of the aqueous solubility, lipophilicity, biomimetic binding and artificial membrane permeability of novel compounds is required to aid the selection of suitable compounds for progression and developability. High performance liquid chromatography (HPLC) offers great potential to determine distribution coefficients of biologically active compounds between an aqueous mobile phase and various non polar and bio-mimetic stationary phases via the measurement of retention times[2]. Fully automated fast gradient HPLC methods have been developed to measure compound-lipophilicity (using C-18 stationary phase[3]), plasma protein binding (using Human Serum Albumin[4] and Alpha-1-acid-glycoprotein stationary phases) and membrane partition (using Immobilised Artificial Membrane[5] stationary phase). HPLC with charged aerosol detection (CAD)[6] without the need for analyte reference standards has recently been developed to measure aqueous kinetic solubility. A high throughput technique for measuring membrane permeability is used to assess the rate of passive permeation across a phospholipid membrane-like barrier[7]. Extensive application of automated platforms and parallelised chromatography has enabled efficient and cost effective high throughput physicochemical profiling of compounds in early drug discovery.

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O 18

Phys chem tool box for drug design in beyond rule of five chemical space

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The new drug targets characterized as “difficult” and “flat” require large binding surfaces between the protein and an active ligand, thus leading to large molecules in Bro5 chemical space poorly adopted for oral drug delivery. The new concepts and methods are being developed for evaluation and modulation of properties of Bro5 compounds to achieve acceptable PK/PD in drug candidate. An application of ElogD, EPSA and PLRP-S methods toward balancing permeability and solubility is described. In particular, the PLRP-S [1] assay in combination with pKa by MCE [2] is used to asses lipophilicity-ionization patterns of lipophilic, low solubility Bro5 compounds, while EPSA [3] and ElogD [4] are used to drive permeability and efficiency (lipE).

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O 19

A new approach for HT permeability screening based on a biomimetic barrier

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The use of computational methods, combinatorial chemistry along with high-throughput (HT) screening of binding affinities for drug discovery have fostered large libraries of potent drug candidates. Such assays are typically very sensitive and prioritize highly binding compounds, which typically have lipophilic properties. Thus, commonly experienced challenges during development of these candidates into medicines are poor solubility and/or insufficient oral bioavailability. To tackle these challenges, solubility and permeability assays in the microtiter plate format have gained increasing interest over the past decade. HT approaches for solubility and partioning have become an indispensable part of drug discovery, the latter being used to estimate passive permeability through cell membranes. Furthermore, cellular assays - typically in the microtiter plate format - are employed to screen biochemical and biological activity and toxicity prior to the first animal experiments. As a mile-stone in the further refinement of HT-tools, Caco-2 cell-based permeation assay has for many years been used to estimate absorption of drug compounds from the human intestine, and thereby predict oral bioavailability. However, practical and analytical restrictions apply for screening of poorly water-soluble compounds. The results of Caco-2 studies, like many other cellular screens, may be compromised if the use of co-solvents or surfactants is inevitable with regard to the solubility of the drug compounds. Furthermore, to improve bioavailability so-called “enabling formulations” are commonly employed, of which many different and promising approaches is known [1]. With these formulations, however, traditional permeability screens may be overstrained and novel screens have been suggested to cope with formulation ingredients, so-solvents, surfactants and biomimetic media.
Commonly used platforms for permeation screening comprise Caco-2 cell assay, artificial assays PAMPA (including its variants) [2], PVPA (not commercially available, but possible to automation) [3]. In the presence of enabling formulations, the integrity of these barriers may be compromised by the composition (solubilizing excipients) or due to the use of bio-mimetic digestion media. The ultimate biomimentic artificial barrier would keep its integrity in the presence of such media over the time interval of the experiments (hours). At this stage of development, it may be sufficient to classify formulations coarsly into “good”, “medium”, or “bad” to estimate the potential of a drug candidate to be developed. An approach using a new biomimetic barrier is presented and discussed [4-6].

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O 20

A mechanistic intestinal cell permeation model

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A mechanistic intestinal cell model was derived from the first principle of passive diffusion and the Michaelis Menten equation considering the anatomy of the intestinal epithelial cells. The shape of the intestinal epithelial cells is columnar with an aspect ratio of 1: 10. The tight junction of the paracellular pathway is located near the apical surface. In addition, the microvilli structure expands the apical surface area by 15-fold. All in all, the apical area becomes about 1/3 of the basal area. This causes the difference of apparent Km values of an efflux transporter between apical to basal (A to B) and B to A directions. The concentration of unbound undissociated molecular species in the cytosol becomes 1/3 of that in the luminal side due to concentration gradient. Taken together with the difference of pH values (6.5 and 7.0), the concentration of unbound drug in the cytosol becomes about 1/10 of that in the luminal side for base drugs, coinciding with the regulatory criteria for drug – drug interaction empirically derived from the clinical data. A mechanistic model for Fg calculation will also be discussed in this presentation, especially focusing on the effect of passive diffusion in the sub-epithelial space.

O 21

Measuring, modelling and predicting pH-profiles of membrane permeability

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The biopharmaceutical classification system (BCS) classifys drugs into four classes based on their permeability and solubility, and which affect the intestinal absorption. The permeability for drug candidates is in majority described with the in vitro measurement at pH 7.4, while in the gastrointestinal tract the range of pH is ~2 to ~9. Therefore for describing absorption properties of new drug candidates and modelling of membrane permeability it is logical to take into account influence of pH. So far very few studies explicitly consider and/or take into account pH-dependence of membrane permeability, which can lead to the false conclusions during new active compounds discovery and may abandon potential new drug candidates.

The purpose of current research is to experimentally determine the influence of pH to intestinal absorption and develop *in silico* models to predict pH-profiles of membrane permeability. For this membrane permeability values were measured using the parallel artificial membrane permeability assay (PAMPA) for 238 drugs and drug-like compounds at pH 3, 5, 7.4 and 9 with in-house experimental setup. These original experimental values were used to develop the descriptive and predictive multi-linear regression models at selected pH-s to describe regional based absorption, for the highest membrane permeability over selected pH-s to describe maximum permeability in the intestine and for the intrinsic membrane permeability to describe permeability for uncharged compounds. The descriptor set included calculated logarithmic octanol-water distribution coefficients at exact pH-s and molecular descriptors calculated with the CodessaPro software. The models are derived *via* stepwise forward selection of descriptors.

The results show that membrane permeability at pH 7.4 is not sufficient to estimate intestinal absorption. Considerable improvement in estimation of intestinal absorption was obtained using the highest membrane permeability over the wide pH-range. All developed models are statistically significant and are blind tested (first predicted and then measured) for separate set of compounds while estimating the pH-profile of membrane permeability for these compounds. The molecular descriptor content of derived prediction models for selected pH-s indicates that the permeability process at acidic environment is influenced by the different molecular properties compared to the basic environment. Proposed models allowed to obtain more accurate information of absorption properties for drug candidates in the early stage of drug discovery, make easier to select suitable biowaivers for the bioavailability experiments based on BCS and also give valuable information for the formulation studies.

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O 22

Surrogation of ADMET processes by chromatography and capillary electrophoresis

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The up-take and effectiveness of a pharmaceutical compound within the organism is strongly dependent of its ADMET properties. Accurate prediction of ADMET properties is crucial in the early steps of pharmaceutical drug development. However, direct measurement of ADMET properties is long, complex and expensive.

Liquid chromatography and capillary electrophoresis are well stablished, fast, simple and high-throughput techniques that can be used to surrogate some ADMET processes. Parameters of ADMET interest (*e.g.* blood-brain partition, skin permeation, toxicity) can be easily estimated from the drug retention in the surrogating chromatographic or electrophoretic system.

In our approach for searching the best surrogating systems, ADMET and chromatographic processes are characterized by the same LFER model, commonly the solvation parameter model of Abraham. The similarity of the chromatographic and biological processes is later parametrized by the euclidean distance between the normalized coefficients of both models. The best surrogating chromatographic systems for a particular ADMET process are selected from the analysis of the distances between them by dendrograms or principal component analysis plots. This method combined with the estimation of the precision in the surrogation process allows us to select the most promising chromatographic systems for surrogation of the ADMET process. These systems are later validated and tested.

Some examples of ADMET processes we have surrogated by chromatographic/electrophoretic systems are: skin permeation, blood-brain partition and permeation, and aquatic toxicity.

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O 23

Behaviour of ionisable drugs after precipitation from aqueous solution in response to pH change

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The aqueous solubilities of 173 ionizable small-molecule drugs were measured by experiments in which the drug was first dissolved in ionized form in aqueous 0.15M KCl solution after adjustment to low or high pH; water-miscible solvents were added during some experiments to facilitate dissolution. The pH of the solution was then adjusted in small steps until the drug precipitated, after which the solubility was measured by CheqSol or Curve-fitting methods. As well as measuring solubility, the data generated during these experiments could also be analysed to determine the behaviour of the drug during and after precipitation. For most drugs, the solubility reached a stable value within a few minutes after precipitation. Most of these became supersaturated to 5-50 times their intrinsic solubility before precipitation but some did not supersaturate before precipitation. A third class precipitated and quickly reached a stable solubility value, but after periods ranging between a few minutes up to several hours, they suddenly converted into a form with lower solubility. Attempts were made to predict the tendency of drugs to to behave in one of these three ways. Precipitates were studied by microscopy to determine their degree of crystallinity. The results suggest that drugs that become supersaturated before precipitation rapidly crystallize after precipitation. Those that do not supersaturate form liquid or amorphous structures after precipitation, while the third class converts from amorphous to crystalline form during the experiment. Structures of the 173 drugs were analysed to determine their physchem descriptors. Those that precipitated in liquid or amorphous form tended to have low numbers of hydrogen bond donors and acceptors and melting points below 150 deg. C, and their solubility was inversely proportional to their lipophilicity. Those that precipitated as crystals had higher numbers of hydrogen bond donors and acceptors and higher melting points, but there was no simple relationship between solubility and lipophilicity. Yalkowsky's General Solvation Equation was used to predict the solubilities of these 173 drugs and was found to work well for those that precipitate in amorphous form. A modified form of the equation was derived that predicted solubility better for all classes.

O 24

Mechanical properties of Venlafaxine HCl solid mini matrices prepared by hot-melt extrusion and hot or ambient compression and effect of plasticizer

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Aim: Direct compression (DC) tableting is the established method for the preparation of slow release pharmaceutical solid matrix systems due to its simplicity. However, hot-melt extrusion (HME) is attracting interest as it is continuous and solvent-free and has been applied for the modification of the release of drugs [1]. In this study matrices of pellets and mini tablets with the same composition and 2mm diameter were prepared by HME and by hot (HC) or ambient (AC) compression and tested for their mechanical properties.
Methods: Batches of the experimental powder mixtures containing 15 % Venlafaxine HCl, 65 or 75 % Eudragit® RSPO and 10 or 20 % w/w plasticizer (citric acid or Lutrol® F127) were prepared by blending in a Turbula mixer (W.A. Bachofen, Switzerland). Half of each mixture was hot-melt extruded and the other half used for HC and AC compression. HME was performed on a bench-type vertical single-screw extruder (RCP-0250 Randcastle Extrusion Systems) connected to a pelletizer. For the preparation of mini tablets 10 mg were compressed on an instrumented tablet press (D-Series Press, Gamlen Tableting Ltd., UK) fitted with 2-mm flat-faced punches, at 120 MPa pressure above which there was no decrease of thickness. In the case of HC compression, the samples in the die were preheated to 110-115 ^{o}C, the same temperature range as that applied for extrusion. Force-displacement compression/ejection profiles were recorded from which the maximum ejection pressure was obtained as an estimate of frictional forces to die wall.
Results: HME pellets and mini HC and AC matrices exhibited differences in tensile strength due to the different contribution of the two formation mechanisms: a) coherent solid solution/solid dispersion matrix held together by short range intermolecular forces occurring mainly in HME and partly in HC and b) weaker interparticle bonding occuring mainly in AC and partly in HC. Therefore, remarkably higher tensile strength resulted by HME pellets, followed by the HC and AC tablets, and there was significant statistical interaction of the effects of the matrix preparation method and type of plasticizer on tensile strength ($p=0.033$). Both citric acid and Lutrol® F127 were effective HME plasticizers with Lutrol® F127 showing greater plasticization and also good lubrication ability for both HC and AC tablets. HME pellets containing Lutrol® F127 were softer and exhibited lower tensile strength due to its high miscibility and plasticization ability with Eudragit® RSPO but stronger HC and AC tablets by acting as thermal binder in HC compression or compressible binder in AC compression.
Conclusions: Both polymers were effective plasticisers for the extrusion of venlafaxine HCl with Eudragit® RSPO and Lutrol® F127 also showed lubrication and binding ability during HC and AC compression**.**

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O 25

Use of Low field NMR for the characterisation of gels and biological tissues

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Despite the very common use of High Field Nuclear Magnetic Resonance (7 – 37 T), Low Field Nuclear Magnetic Resonance (LF-NMR; 0.37 – 2.4 T), typically applied in food science for the characterisation of edible fluids and solids, is much less common. However, the works of Brownstein and Tarr [1], Mitra *et al.* [2], Chui *et al.* [3] and Scherer [4] clearly demonstrated that the use of LF-NMR can be profitably extended to the study the micro- and nano structure of polymeric systems such as gels [5] and scaffolds [6]. In addition, also biological tissues such as bones [7] and sputum of patients affectd by cronic pulmonary diseases such as cystic fibrosis paptients (CF) [8], can be characterized by LF-NMR. Whatever the system considered, the leading principle allowing the LF-NMR characterization relies on the effect of solid surfaces (polymeric chains, bones and so on) on the relaxation process of water hydrogens subjected to a sudden variation of an external magnetic field. The higher the ratio between system solid surface and system volume, the faster the hydrogens relaxation process. Based on this information, it is possible to obtain interesting information on the three-dimensional architecture of gels network (mesh size distribution) and pores size distribution of porous materials.
The focus of this presentation will be on the characterization of polymeric gels network, on the determination of scaffold pores size distribution and on the use of LF-NMR to monitor CF patients.

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O 26

Automated LC and SFC method development for new APIs: The power of selectivity and the strength to choose

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During API (active pharmaceutical ingredient) development, drug stereoisomerism is recognized as an issue having clinical and regulatory implications. Enantiomers have essentially identical physical and chemical properties, while potentially showing large differences in toxicity. Therefore, the stereoisomeric composition of a drug with a chiral center should be well documented. To evaluate the pharmacokinetics of a single enantiomer or any mixture of enantiomers, manufacturers must develop quantitative assays for individual enantiomers early in drug development.

But what technique do we choose, what will we compromise? Obtaining the best choice, the most selectivity surely requires compromise? Chromatography has recently developed a new quality, it now offers more, by being both more selective and more flexible. A new class of hybrid SFC-LC switching systems enables chromatographers to utilize both, the selectivity of liquid chromatography as well as supercritical fluid chromatography with only one system, allowing more complex achiral and chiral separations. Switching from SFC to LC is a seamless and automated process and method screening using both techniques was performed in one overnight sequence.

Fast and simple SFC and LC methods for the separation of chiral compounds were developed within two days, using a column screening system with 6 LC and 6 SFC columns and 3 different organic modifiers for each technique. SFC and LC switching was shown to be a reliable, robust and simple alternative to routine LC screening. Use of the system in terms of complexity of the instrument and method development was very similar to the HPLC approach. However, since SFC is known to be often superior to HPLC for chiral separations, screening with both techniques has proven advantageous.

All of this achieved with no compromise. With a new back pressure regulator design of the direct injection through to the MS without the need to split offers increased sensitivity, compared to older models in the market. The switch from SFC to LC is seamless and automatic, making this a simple-to-use and highly efficient approach to modern method development.

O 27

Measurements of plasma protein binding – variety of experimental techniques

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Drug molecules *in vivo* may be bound to proteins and lipids in plasma and/or in tissues, or free (unbound) in diffusion among the aqueous environment of the blood and tissues. Data from *in vitro* plasma protein binding experiments that determine the fraction of protein-bound drug are frequently used in drug discovery [1].

Human plasma proteins contain around 40 % albumin (HSA), α_1-acid glycoprotein (AGP) in much lower concentration (1-3 %) and immunoglobulins [2]. Methods used for drug – plasma protein binding (PPB) studies are numerous and can be divided into two main groups: separation methods (enabling the calculation of binding parameters, *i.e.* the number of binding sites and their respective affinity constants) and non-separation methods (describing predominantly qualitative parameters of the ligand-protein complex) [3]. Sometimes, results of PPB measurements obtained by different techniques are not consistent. High binding affinity to plasma proteins is not necessarily a crucial limiting factor for further delivery of compound to the target organ [1]. As an example, we show the study of the interactions between HSA/AGP and an "in-house" synthesized steroidal derivative that showed remarkable inhibitory potency against BoNT/A holotoxin in mouse embryonic stem cell derived motor neurons [4]. A variety of experimental techniques (ITC, HPLC, spectrofluorimetry, FTIR, and equilibrium dialysis) were used and the results were compared highlighting the advantages and disadvatages of various techniques.

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O 28

The role of pharmacology in European cancer drug development

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Drug development consists of many sequential and parallel steps; failure in each step can lead to discontinuation of the process. The process is time-consuming and very costly, especially when the clinical phase has been reached. Medicines agencies require extensive safety testing. In order to enhance cancer drug development, the National Cancer Institute (NCI) adopted a new screening system in the 1980ies using 60 different cell lines from various cancer histologies (the so-called NCI-60 panel), in which all standard drugs were tested and which was (and still is) open for testing of any new chemical entities (NCEs) of potential interest. Since that time hundreds of thousands of drugs have been submitted to the NCI and tested in their system yielding an enormous amount of data. Interesting compounds were planned to move to xenograft testing or to the intermediate hollow fiber system. In the early nineties, it was recognized that an enormous backlog threatened to be formed, and since a large part of the submitted NCEs was from European origin a collaboration was established with the Cancer Research Campaign (CRC; now CRUK) and the drug development groups of the EORTC (European Organization on Research and Treatment of Cancer), the Pharmacology and Molecular Mechanism Group (PAMM) and the Screening and Pharmacology Group (SPG). These groups (EORTC, NCI and CRUK) formed a collaboration the so-called European NCI compounds programme, in which considerable expertise in drug development was represented: chemists, pharmacists, biologists, pharmacologists, physicians, oncologists. A CRC/EORTC review committee evaluated potentially interesting European compounds coming from the NCI screen in order to determine whether they would be worthwhile for further testing. Selection criteria included novelty of the NCE, in vitro activity, when available in vivo and hollow fiber activity and COMPARE negativity. COMPARE is a comparison tool developed by the NCI in which, based on the sensitivity profile, a NCE could be listed as belonging to a group of compounds that often showed the same mechanism of action. Hence an unknown compound could be listed as a tubulin binder, which was usually confirmed upon a molecular analysis. However, since many NCEs appeared to be tubulin binder, this became a criterion to dismiss such a compound. NCEs selected by the committee were subsequently tested by CRC and EORTC members of the group for several parameters: a suitable formulation to administer the NCE to mice, an HPLC assay (or another suitable assay), limited toxicology, specific assays (e.g. for angiogenic properties, migration, tubulin binding) and additional in vitro and in vivo testing. With a suitable formulation limited pharmacokinetics were performed to determine whether the NCE would reach sufficiently high plasma concentrations (> IC50 value). Using this approach about 2000 NCEs were evaluated. The first screening eliminated the majority of the NCEs for further testing, leaving 95 for further evaluation. From these a substantial number were formulated and tested in animals, initially at one bolus injection, but depending on the in vitro profile, daily injections were also evaluated. Using this approach many additional NCEs could be eliminated because of a poor pharmacological and/or activity profile. However, several compounds survived this process and were selected for further more extensive in vivo testing and entered the clinical to be included in Phase I and Phase II evaluation[1]. Of utmost importance, the work resulted in fruitful multidisciplinary interactions in Europe and increased the understanding of knowledge of structures and mechanism of actions of drugs that made it to the clinic. The programme transformed from pan-European multidisciplinary collaborations into an academic platform of excellence for anticancer drug discovery and development. It fosters the collaborations with the NCI and is open for any initiative in the field of cancer pharmacology.

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O 29

In silico ADME in drug design – aiming at the decision-to-make

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Drug design is a multi-parameter optimization process, with ADME properties being important to consider early on. Parameters such as lipophilicity, solubility, permeability and metabolic stability can be measured in a high throughput manner *in vitro* and are thus often used as early ADME screens. During lead identification and lead optimization phases a substantial number of compounds is synthesized and characterized in such screening assays for selection towards further, more costly *in vitro* and *in vivo* experiments to be able to finally select a single candidate drug. However, a compound needs to be physically available to be subjected to such assays. Thus, drug industry generates thousands of compounds per month out of which many do not show good enough ADME properties.
In silico models for such ADME endpoints, on the other hand, have been available for a long time, though with varying quality and usability. Ideally, such models would be used before synthesis and, together with any potency estimation for the specific case, influence the decision to make a compound. Thus, the number of compounds made with inadequate properties should be reduced considerably. In practice, it seems that most often only one (or two) predicted properties are really considered at this stage, usually including a prediction of lipophilicity. While it is understood that all available knowledge should be used to select which compound to make, it is not easy to define this knowledge, for example to combine the outcome of various predictions unambiguously. Lately, the use of multiparameter optimization and scoring tools has been proposed. However, the scoring functions used in these tools need to be defined and can be debated. Predicting dose to man early on, especially based on *in silico* data only, is maybe too speculative to be of real use before synthesis.
The purpose of the present work is to investigate, whether a combination of *in silico* ADME models can be used to define a minimum potency level required for an acceptable dose, for example 100 mg per day, with high enough confidence. This approach could then also be used for an estimate of the likely maximum concentration reached at that dose thereby give an indication of the expected therapeutic window. Using a set of project compounds [1] *in silico* ADME models will be utilized, to rank the compounds, based on both the resulting minimum potency level and an estimation of the therapeutic window, and to compare the outcome with the actually selected lead compound.

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O 30

Automation of ADME *in vitro* binding assays using HTDialysis device on Tecan Freedom EVO

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During early stages of drug discovery newly synthetized compounds undergo tiered *in vitro* ADME profiling in order to characterize their solubility, permeability, metabolic stability, binding, as well as potential for drug-drug interactions. Automation of *in vitro* ADME assays has facilitated an increased throughput with respect to the number of compounds screened, as well as a reduction in turnaround time from compound submission to results.

The process of semi-automation and validation of such an assay will be described here. Correction for microsomal binding for the prediction of unbound intrinsic clearance has been shown to improve the prediction of *in vivo* metabolic clearance from *in vitro* microsomal assays, as well as the prediction of *in vivo* drug-drug interactions. The microsomal binding assay measures the extent of nonspecific binding following incubation in a HTDialysis device. This method has been semi-automated on a Tecan Freedom EVO workstation with a MCA96 head. Validation was performed with 12 commercially available compounds and 44 unknown compounds using human liver microsomes.

Results show correlation with manual testing methods for all tested compounds, as well as day-to-day repoducibility for the 12 commercial compounds which also correlate well with literature data.

In conclusion, a semi-automated and robust assay has been validated with significantly increased thoughput.

O 31

Venom-based peptide drug discovery: enhancing specificity in cancer treatment

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In the last decade, many of the venom-based bioactive peptides demonstrated with a broad and diverse spectrum of pharmacological activities, which help to enlarging the current drug-screening library for searching of new specific biomarkers and novel prototype drugs for diagnosis of diseases such as cancer. Moreover, it paved a new insight to overcome the current drug discovery issues, which including drug resistance, side effect and so on.

In cancer, serine proteases mediate a variety of events relevant to fundamental processes of cancer progression in cancer, and serine protease inhibitors have been introduced that target these enzymes. Bowman-bark proteinase inhibitor (BBI) is one of the well-known serine proteinase inhibitor families. Recent evidences have shown that BBI can function as tumour suppressor. Through our in-house developed high throughput screening techniques and bioinformatics analysis, we successfully isolated a panel of novel peptides from scorpion venoms and frog secretions including one of our lead peptides -- BBI-like cyclic peptide QUB 1813. We performed a series of anti-proliferative assays to demonstrate that these peptides could inhibit the growth of a broad spectrum of tumour cells, especially in human colon cancer cell line; while almost have no effect on normal human epithelial cells. Haemolysis assay was also performed to show our peptides do not cause any harm on normal human red blood cells, which point to the direction of bringing those peptides into the preclinical trial studies.

Here we report the discovery of a novel BBI-like cyclic peptide QUB 1813, which is a moderate trypsin inhibitor with strong bradykinin-like mycotrophic activity. At the same time, our results demonstrate that QUB 1813 can significantly inhibit the growth of human colon cancer without any cytotoxic effect on normal human epithelial cells. Taken together, this finding could lead to further clinical investigation for the new strategy of peptide mono-/combination therapy.

O 32

4-Isopropyl-3-nitro-dichloroacetophenone (INDAP) targets pyruvate dehydrogenase kinase 1 and inhibits cancer cells proliferation

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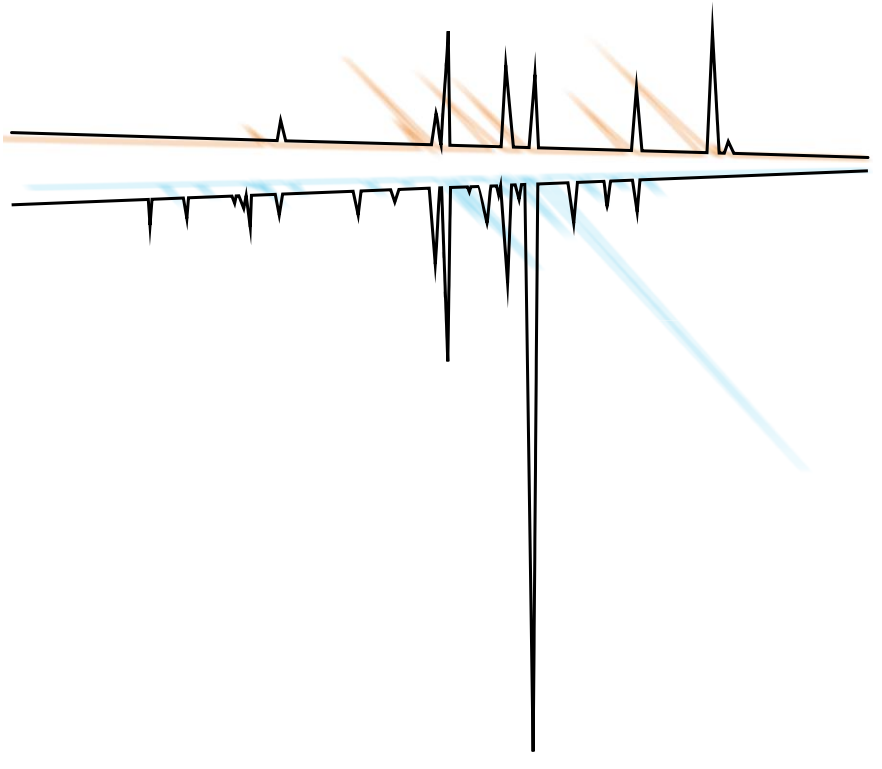
We describe herein the discovery of a novel potent pyruvate dehydrogenase kinase (PDK1) inhibitor, namely 4-isopropyl-3-nitro-dichloroacetophenone (INDAP). INDAP bound to PDK1 (K_d = 0.97 μM), activated pyruvate dehydrogenase complex (EC_{50} = 0.49 μM), and reduced the proliferation of NCI-H1975 cells (IC_{50} = 2.75 μM), but exhibited little effect against non-cancerous 293T cell (IC_{50} > 20 μM). Moreover, we observed that INDAP decreased the extracellular acidification rate and lactate formation as well as increased reactive oxygen species production, which could serve as a potential modulator to reprogram the glucose metabolic pathways in cancer cells. Furthermore, INDAP was found to depolarize the mitochondrial membrane potential of NCI-H1975 cells. Collectively, this evidence suggested that INDAP could be a useful probe compound to explore the pharmacology of PDK1.

Poster Presentations



P 01

Simultaneous Lipolysis/Permeation *in vitro* Model for the Estimation of Bioavailability of Lipid Based Formulations

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Lipid based formulations (LBF) are a promising approach to increase the solubility of poorly water-soluble drug compounds, e.g. BCS Class II [1].
The most common *in vitro* evaluation tool for LBFs is lipolysis where digestion of the lipids of the formulations is carried out enzymatically. The dissolution and solubility of the compounds is tested in the expectation that the formed mono- and diglycerides solubilize the drug molecules. However, in the case of highly permeable drugs, lipolysis studies tend to overestimate precipitation of the drug compounds with respect to the *in vivo* situation where simultaneously absorption of the drug takes place. This leads to poor *in vitro in vivo* correlations (*IVIVCs*) [2].
The aim of the current work was to set up an *in vitro* model which combines lipolysis of LBF with an absorption (permeation) step, using a biomimetic barrier that has proven to be resistant to surfactants and solvents commonly used in enabling formulations [3]. The functional stability of the barrier against a self-nano emulsifying drug delivery system (SNEDDS), lipolysis medium, and pancreatic enzymes was evaluated and an *in vitro* lipolysis/permeation model proposed.
The compatibility of the barrier with a SNEDDS formulation and pancreatic enzymes in lipolysis studies were shown using calcein, a hydrophilic marker, qualifying the barrier to be used in lipolysis/permeation studies [4]. Furthermore, the permeability of the model drug cinnarizine dissolved in a SNEDDS formulation was studied, carrying out both a normal permeation study without lipid digestion of the formulation and the proposed lipolysis/permeation model. The permeability of cinnarizine increased significantly (50 times increase in flux) when lipid digestion was included.
The proposed lipolysis/permeation model was successfully set up, and results showed that the permeability of cinnarizine increased 50 times when digesting the SNEDDS formulation as compared to the non-digested formulation.
This emphasizes the importance of introducing an absorption step in *in vitro* lipolysis studies, and such estimation of the bioavailability of LBFs may lead to better *IVIVC*.

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P 02

Small Scale Biphasic Dissolution testing of Dipyridamole Suspension

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Precipitation *in-vivo* can occur for a multitude of reasons, including the shift of pH from the acidic gastric environment to the more neutral intestinal environment. Predicting *in-vivo* supersaturation and precipitation is key to understanding variability in oral bioavailability. Biphasic dissolution tests use an organic layer, which is immiscible with the dissolution media, to act as an "absorptive sink". This could be particularly advantageous for BCS class II drugs (low solubility, high permeability) as sink conditions can be generated as drug is removed by uptake into the organic layer. The effect of a pH shift from an acidic gastric environment to the more neutral intestinal environment on the dissolution profile of dipyridamole suspension was investigated with a small-scale biphasic dissolution test, using Sirius-Analytical's inForm platform.

An aqueous suspension of 10 mg/mL dipyridamole was prepared using 0.5 % methylcellulose as the suspending agent. Simple acetate-phosphate buffered media (40 mL) was used as the aqueous layer, whereas decanol (40 mL) was used as the organic layer. Decanol was introduced after the pH of the aqueous layer was adjusted to pH 6.8. Dipyridamole 10 mg/mL suspension (1 mL) was added into the aqueous compartment using an automatic aqueous handler needle. To examine the effect of the pH shift, the experiment was carried out with and without an initial thirty-minute sector at pH 2. Testing at pH 6.8 was carried out over four hours.

Significantly higher dipyridamole concentrations were recorded in both the aqueous and organic layers in the experiment incorporating an initial thirty-minute period at pH 2. Dipyridamole did not appear to significantly precipitate out of solution upon transition to pH 6.8, which correlated well with previous *in-vivo* studies [1]. Dipyridamole seemed to readily partition from the the aqueous phase into the organic phase.

This experiment highlights the need to incorporate a pH shift from acidic to intestinal pH when analysing weakly basic drugs. Carrying out dissolution experiments at a single pH could lead to a large underestimation of oral bioavailability of weakly basic drugs. Further research is ongoing to investigate the utility of biphasic dissolution tests among a wide range of drugs and novel formulations.

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P 03

Estimation of the octanol-water distribution coefficient of acidic compounds by microemulsion electrokinetic chromatography measurements

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Lipophilicity, which is defined as the ability of a compound to be dissolved in lipids or non-polar solvents, is a key factor in drug development. This property is related to the capacity of a substance to go through different biological membranes, which are formed by a lipid bilayer. Therefore, the effectiveness of a compound as a drug candidate will be clearly linked to its lipohilicity, the higher the value of this property, the better. In order to estimate the lipohilicity of a substance, different parameters can be evaluated. Among these, the most widely used is the octanol-water partition coeficient. However, the direct evaluation of this parameter (shake-flask method) is time-consuming and it is not automated. Because of these drawbacks, other alternatives have been developed.
Recently, a method to estimate the octanol-water partition coefficient of neutral substances at differents pH values using microemulsion electrokinetic chromatography (MEEKC) measurements has been developed [1]. Considering that most part of drugs from the pharmaceutical industry are acids or bases, a fast determination of this parameter for ionic compounds is of great interest. Therefore, the aim of this work is to estimate the distribution coefficient of acidic solutes at several degrees of ionization through MEEKC measurements. To this end, the retention factor of six ionizable compounds has been determined at different pH values. For these six substances, good correlations between the logK and logD values have been observed at several pH values and at different ionization degrees. Finally, the logD at pH value of 7.4 (pH of blood) of some compounds at different degrees of ionization has been predicted. Great predicted values have been obtained, especially for partially ionized acids.

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P 04

Physicochemical classification analysis of hERG inhibitor specificity

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Drug-induced inhibition of hERG potassium ion channel that may result in a life-threatening Torsades de Pointes arrhytmia has become a major obstacle in drug discovery. Broad ligand specificity of hERG has attracted significant interest from computational studies, but the majority of published QSAR approaches focus on maximizing the goodness of fit, and sacrifice interpretability in favor of statistical performance. As a result, basic physicochemical principles often remain overlooked. In this study, we attempt to overcome these issues by constructing a large and thoroughly curated hERG inhibition database spanning a range of >6700 diverse chemicals, and using these data to develop a purely physicochemical classification model of hERG inhibition based on a minimal set of descriptors such as lipophilicity, ionization, TPSA, aromaticity, molecular size and flexibility. The predictive model was built using Gradient Boosting Machine (GBM) statistical method that is well-suited to describe complex nonlinear relationships between properties and provides wide possibilities for visualization of parameter contributions. The obtained model was able to assign correct activity classes for 75 to 80 % of validation set compounds. Since most QSAR studies that utilize full information on molecular structures report only slightly higher accuracies of about 80-85 %, our results suggest that at affinity levels close to the 10 µM threshold, ligand interactions with hERG are mostly-driven by non-specific effects. The observed descriptor-response profiles are consistent with common knowledge about hERG binding site, but also reveal several important quantitative trends, as well as slight inter-assay variability in hERG inhibition data. According to our model, even weakly basic groups might substantially contribute to hERG inhibition propensity, whereas the impact of lipophilicity depends on the compound's ionization state, with its effect decreasing in the following order: bases > zwitterions > neutrals > acids. In addition to evaluation and visualization of the physicochemical property contributions, the obtained model can be used as a baseline predictive tool for more detailed analysis, e.g., exploring the potential of discrete structural modifications to further attenuate hERG liability of candidate compounds.

P 05

Prediction of physico-chemical properties of illegal drugs using the conformational analysis

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Methylone, MDPV (methylenedioxypyrovalerone), AM 2201 (1-(5-fluoropentyl)-3-(1-naphthoyl) indole), BZP (benzylpiperazine), 25I-NBOMe, 25C-NBOMe, 25B-NBOMe, AH-7921 and mephedrone were banned in many countries, and they are regarded as illegal drugs. Unfortunately, there are no collected and unified data on the toxicity of these drugs, a cause of serious social and medical problems, particularly in youngsters. There are published papers on the interactions of these drugs with corresponding receptors: mephedrone, 25B-NBOMe, 25C-NBOMe, 25I-NBOMe with serotonine receptor 5-HT_{2A} [1,2], AH-7921 with micro-opioid receptor, and AM 2201 with cannabinoid receptor. However, the conformational behaviour of these drugs was not previously investigated. Conformational analysis using Macromodel and molecular mechanics gave global minimums with the following energies, expressed in kJ/mol: 243.09 (methylone), 265.43 (MDPV), 345.15 (AM 2201), 307.50 (BZP), 244.16 (25I-NBOMe), 252.04 (25B-NBOMe), 153.56 (AH-7921) and 276.15 (mephedrone). Results of this work can be important for the further investigations regarding the toxicity of these substances and the prediction of their biochemical parameters.

Acknowledgement: *This work was supported by the Ministry of Education, Science and Technological Development Republic of Serbia, Project No. TR34025 and TR37016.*

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P 06

pH-dependent solubility profile of desipramine hydrochloride

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Desipramine hydrochloride (Ds·HCl; **Figure 1.**) is a known surface-active molecule, which may form sub-micellar aggregates in slightly acidic solutions. If a neutral or slightly basic solution is prepared from Ds·HCl, it may remain supersaturated for a very long time, as aggregates form. Appearance of aggregates might lead to slow sedimentation. Furthermore, at high pH values oils might form that are more soluble than crystalline form; this was already observed for surface-active compounds [1]. There are many other druglike molecules with similarly challenging properties, which have not been adequately characterized. Thus, much attention must be paid to set up the experimental procedure for precise solubility determinations [2].

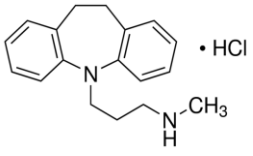

Figure 1. Structure of desipramine hydrochloride

Although solubility data for Ds·HCl can be found in the literature [3], in this study pH-dependent solubility profile of Ds·HCl was studied using slightly different method: pH-ramp shake flask. First, the pH value of Ds·HCl stock solution in 0.15 M phosphate buffer was adjusted to 11.7 in order to minimize supersaturation effect. Then, the pH value in separate samples was adjusted downwards with HCl, to prepare solutions in the pH 1.7-11.7 region. After stirring (6 h) and sedimentation (18 h), PTFE (hydrophobic, pore size 0.22 µm) filters or centrifugation were used for phase separation. Concentration was measured using HPLC with UV/Vis detection. The computer program *p*DISOL-X was used for data processing and refinement of equilibrium constants. Different techniques were used for solid phase characterization.

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P 07

Protolytic equilibria of rupatadine in micellar solutions of differently charged surfactants

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Rupatadine is selective second-generation H_1 antagonist, used in seasonal allergic rhinitis and chronic urticaria, and reported to be an antagonist to platelet-activating factor. Rupatadine contains three ionizable basic centers, two aromatic and one cyclic aliphatic amine. The pharmaceutical dosage forms for oral administration contain rupatadine fumarate as an active substance. A complex system of protolytic equilibria establishes in the solution of rupatadine fumarate which includes three basic centers of rupatadine and two acidic groups of fumaric acid. The data on the physicochemical properties of drugs determined in aqueous solution is not sufficient for the prediction of solubility and bioavailability in physiological conditions that are significantly more complex. For a better understanding of pharmacological behavior of ionizable drugs their physicochemical properties should be investigated under conditions more similar to physiological. As the biomembrane mimetic systems micellar solutions of surfactants can be used. The aim of this study was to investigate the effect of micellar solutions of differently charged surfactants sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and 4-octylphenol polyethoxylate (TX-100), as biomembrane mimetic systems, on protolytic equilibria of rupatadine.

The solutions (5×10^{-4} M) with and without of the 0.01 M surfactants were titrated with standard NaOH solution (0.0983 M) at a constant ionic strength (0.1 M NaCl) and temperature 25°C. Experimental data obtained by potentiometric titration were analyzed in the program Hyperquad.

The pK_a values of rupatadine (pK_{a1} = 3.45, pK_{a2} = 4.72, pK_{a3} = 6.75) were determined and the ionization was defined in aqueous media. The shift in protolytic equilibria was observed based on the pK_a values of rupatadine determined in the presence of surfactants, anionic SDS (ΔpK_a up to +1.44); cationic CTAB (ΔpK_a up to -1.99) and nonionic TX-100 (ΔpK_a up to -0.69). Different types of interactions between rupatadine and micelles were assumed.

Rupatadine ionizable groups participate in electrostatic interactions with the ionic SDS and CTAB micelles and are involved in the interactions with a hydrophilic layer of nonionic TX-100 micelles. Observed shift in protolytic equilibria at biopharmaceutically significante pH values can be considered in the presence of biomolecules with various charge and polarity in physiological conditions.

P 08

Protolytic equilibria and stability of a pair of novel pentaaza macrocyclic chelators

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Polyaza macrocyclic ligands have drawn much attention because of their higher stability towards lanthanide complexes and their potential application as MRI contrast agents. To this end, two novel pentaaza macrocyclic ligands L_1 and L_2 have been prepared (**Figure 1.**) and their protolytic equilibria and complexation ability toward Cu(II) and Eu(III) ions have been studied. Acidity constants of L_1 and L_2 were determined by potentiometric acid-base titration in aqueous media at t=25±1°C and I=0.1 M (NaCl). pK_a Values observed within pH range 2-12 (L_1: pK_{a4}=5.53±0.06, pK_{a5}=6.63±0.07 and L_2: pK_{a3}=3.45±0.05, pK_{a4}=5.34±0.05, pK_{a5}=6.33±0.06) are in good agreement with theoreticaly calculated values [1] and verify L_1 as a more basic compound. The complexation ability of the ligands was studied by mole ratio method with UV/Vis spectrophotometric detection at two pH values. The results proved that the complex stoichiometry is always M:L=1:1; the corresponding logK_s values are shown in **Table 1**. Considering pK_{a5} values, it can be calculated that both ligands are mostly present in molecular form at pH 7.3 (85 % L_1 and 95 % L_2), thus obtained logK_s values might be considered as real, not conditional constants. On the other hand, only complex
L_1–Eu was formed at pH 8.5, likely due to the formation of hydroxide species.

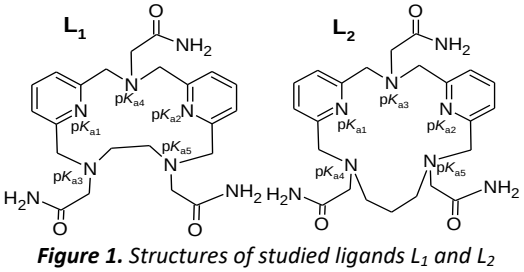

Figure 1. *Structures of studied ligands L_1 and L_2*

Table 1. *Determined logK_s values*

	logK_s±δ	
	pH 7.34	**pH 8.55**
L_1–Eu	5.84±0.15	6.11±0.09
L_2–Eu	5.93±0.12	/
L_1–Cu	6.73±0.17	/
L_2–Cu	7.63±0.14	/

Ackowledgement: *Ministry of Education, Science, and Technological Development of the Republic of Serbia supported this work, Grant No. 172035. RNP acknowledges UGC, Delhi, India for fellowship.*

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P 09

Solid forms screening of fentanyl citrate

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Fentanyl citrate is a synthetic opioid analgesic first developed in early 1970's which is 50 to 100 times more potent than morphine but with a greater margin cardiovascular safety. Industrial crystallization batches of Fentanyl citrate obtained in plant showed a unexpected turbidity profile with a XRPD diagram containing traces of an unknown crystal phase which suggested the presence of an insoluble form. Thus, and with the objective of characterizing this potential form a polymorph screening has been performed. As a result, six crystal forms (three anhydrous polymorphs, two hydrates and one toluene solvate [1]) have been isolated and the crystal structures of polymorphs I and V have been solved by single crystal X-ray diffraction. Once the comprehensive characterization of the solid-state landscape of Fentanyl citrate has been conducted, a deep analysis of the X-ray powder diffractograms has led us to discard a polymorphic impurity. A further comparison with a database of materials suggests that the insoluble traces correspond to Cr_2O_3 which can be assigned to small particles detached from the Chromium reactor used in the industrial process.

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P 010

Solid dispersions as expanded multicomponent systems

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Solid dispersions are multicomponent systems, where poorly water-soluble drug is dispersed in an inert carrier. Due to the arrangement of a drug and hydrophilic carrier, the drug release profile is improved compared to conventional dosage forms. Hence, solid dispersions are one of the most promising strategies to enhance the oral bioavailability of poorly soluble drugs [1].

In most cases, solid dispersions are binary systems which are comprised of a drug and and carrier. However, the system can be expanded by other component (another carrier or surfactant) [1,2]. Our aim was to prepare solid dispersions incorporating other excipient. For this purpose, we used a hot-melt extrusion technology.

In the standard the hot-melt extrusion procedure, the drug and carrier are fed into an extruder and extruded under defined thermal and mechanical conditions [3]. The obtained extrudate is milled and subsequently mixed with other excipients (filler, binder, disintergrant, lubricant *etc.*) to the final dosage form. In this study, the filler (corn starch, microcrystalline and amorphous cellulose, or the mixture of these compounds) was added to poorly water-soluble drug (candesartan cilexetil) and the carrier (PVP) before extrusion. The filler was added to the mixture drug-carrier in order to cut out the mixing step after extrusion. The extrudates, which contained the drug, carrier, and filler, were milled but no futher excipient was added to the powder. The formulation of the solid dispersion was verified by XRD, DSC and FTIR analyses. The analyses showed no evidence of the crystalline form of candesartan cilexetil and only single glass transition temperature which indicates forming a solid solution, where the drug is molecularly dissolved. The drug release profiles of candesartan cilexetil from tablets or capsules were measured by dissolution testing.

The characterization of the multicomponent solid dispersions determined that the added excipients were not only bulking agents, whereas the filler had a fundamental influence on the character and behavior of prepared extrudates. Therefore, this procedure represent an efficient manufacturing technology to drug delivery systems.

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P 11

Quantification of residual amorphous content, by Dynamic Vapor Sorption, in predominantly crystalline drugs

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Solid state characterization of active pharmaceutical ingredients (APIs) and excipients is crucial in the pharmaceutical industry because the manufacturability and performance of a final dosage form greatly depends on the physicochemical, mechanical and biopharmaceutical properties of its constituents [1]. For example, very small differences in the amorphous/crystalline state of compounds have significant impact on the hygroscopicity, wettability, dissolution, flowability and compaction of the formulation. Therefore, it is critical to determine and quantify residual contents of amorphous/crystalline material in pharmaceutical dosage forms and in their individual constituents.

Many analytical techniques exist that are capable to detect and quantify small amounts of crystalline material in predominantly amorphous material. Some of these techniques are DSC, mDSC, XRPD, Raman or infrared spectroscopy. On the other hand, only a few techniques are able to detect small amounts of amorphous material in a predominantly crystalline matrix: isothermal microcalorimetry, solution calorimetry, solid state NMR and dynamic vapour sorption analysis (DVS).

The work presented herein will explore the use of DVS to quantify very small amounts of amorphous content in a crystalline drug (minocycline free base) by using one of the three vapour sorption methods described in the literature [2]: the equilibrium moisture uptake method. In this method, the equilibrium moisture sorbed by pure crystalline and amorphous forms and physical mixtures of both is determined at a specific relative humidity (RH). Good linearity was observed within a wide range of percent amorphous content and the method was able to quantify amorphous contents as low as 1%.

A comparison between the results obtained at different relative humidities will also be discussed and considerations on the thermodynamic activity of the solids and its determination from DVS data will be presented.

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P 12

New cocrystals of bexarotene with pyridinecarboxamide isomers sustained by the acid···aromatic nitrogen supramolecular heterosynthon

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Pharmaceutical cocrystals are homogenous crystalline structures made up of two or more components in a definite stoichiometric ratio, where at least one of the components in the crystal lattice is an active pharmaceutical ingredient (API).[1] Pharmaceutical cocrystals have opened the opportunity for engineering solid-state forms designed to have tailored properties to enhance drug product bioavailability and stability, as well a enhance processsability of the solid material inputs in drug product manufacture.[2]
In this work the cocrystallization of bexarotene, an approved API by the U.S. Food and Drug Administration that belongs to Biopharmaceutics Classification System Class II (low solubility–high permeability), with pyridine carboxamide isomers (picolinamide, nicotinamide and isonicotinamide) was successfully undertaken.
The synthesis was achieved by liquid assisted grinding (LAG) and the solids obtained were characterized by differential scanning calorimetry (DSC), infrared spectroscopy (FTIR-ATR), powder X-ray diffraction (XRPD), single crystal X-ray diffraction (SXD), and polarized light thermomicroscopy (PLTM).
For bexarotene : picolinamide and bexarotene:isonicotinamide, 1:1 cocrystals were obtained directly from milling. SXD data of bexarotene : isonicotinamide cocrystal reveal acid···aromatic nitrogen supramolecular heterosynthon and that the homosynthon amide···amide in isonicotinamide is preserved. The bexarotene : nicotinamide mixtures prepared by ball milling give rise to simple binary solid–liquid phase diagram with an eutectic point, well described by the Schröder-van Laar equation. Melt crystallization of the 1:1 mixture gives rise to a cocrystal for which a complex phase behaviour is observed.

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P 13

Obtaining of cocrystals of meloxicam with carboxylic acids Study of their structure and properties

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The production of molecular cocrystals with unique properties is an advanced direction in the modern engineering of crystals. As a result of the fact that the transformation of already known drugs is more beneficial than the creation of new active pharmaceutical ingredients (API), one of the most important approaches in the modification of the crystalline form is the obtaining of API-based crystals. A cocrystal may be defined as a crystalline material that consists of two or more electrically neutral molecular (both components are solids at room temperature) species held together by non-covalent forces [1], including hydrogen bonding, pi-stacking, and van der Waals forces. There has however been much debate about the use of the term cocrystal. Since structures of cocrystals of medicinal substances and structures of the starting compounds differ in their structure, then their physicochemical properties also differ. Accordingly pharmaceutical cocrystals can be used to address physicochemical property issues such as solubility, stability and bioavailability in pharmaceutical development.

The subject of this research was chosen 4-hydroxy-2-methyl-N-(5-methylthiazol-2-yl)-2H--1,2-benzothiazine-3-carboxamide-1,1-dioxide (meloxicam). Meloxicam is non-steroidal anti-inflammatory drug (NSAID) which used for the long-term treatment of chronic rheumatic diseases such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. From the point of view of physicochemical properties, high permeability and low solubility characterize this drug [2]. Unfortunately, low solubility of drugs can lead to side effects.

The purpose of this work was to obtain and characterize (study melting points, recording of IR spectra and powder diffractograms, structure of the obtained single crystals has been determined by single-crystal X-ray diffraction analysis) new cocrystals of meloxicam with carboxylic acids. All the measured properties of the cocrystals were compared with the properties of the original individual substances.

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P 14

Behaviour of the new API-API Co-Crystal of Tramadol Hydrochloride-Celecoxib (ctc) in preclinical formulation solvents

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Co-crystals, incorporating two different active pharmaceutical ingredients (APIs) in the same crystal lattice, represent a new alternative approach that can overcome some of the problems associated wih traditional fixed-dose combinations (FDCs), including stability, solubility differences and chemical interactions between the individual APIs. API-API co-crystals are, therefore, novel and unique solid forms which display physical and chemical properties different from their parent APIs, which in the end may also confer unique pharmacokinetics and improved formulation capacity.

ctc is a co-crystal consisting of rac-tramadol hydrochloride and celecoxib in a 1:1 molecular ratio that forms a unique 3-dimensional structure and can be classified as an ionic co-crystal, since charge-assisted hydrogen bonds sustain it[1]. **ctc** has been shown to provide a differential intrinsic dissolution rate in water compared with individual APIs [1], as well as supra-additive antinociceptive effects in rats [2] and is currently in Phase III clinical trials for the treatment of pain.

We present here the study of the behaviour of **ctc** in different preclinical formulation solvents in comparison to the open combination of tramadol hydrochloride and celecoxib. This study was carried out in order to assure the physical stability of the co-crystal in solvents used for preclinical studies (pharmacological, pharmacokinetic or toxicological) and also to establish to which extent the co-crystal effect provides a differential performance. The remaning solids of the suspensions of **ctc** in different solvents were analysed, after incremental stirring times, using DSC and NMR and were compared to the individual APIs. The co-crystal **ctc** showed a clear differential behaviour over the open equimolar tramadol-celecoxib mixture, in one of the most used preclinical formulation solvents (0.5% HPMC/water), highlighting the co-crystal effect in its dissolution profile.

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P 15

Dissolution rate of cocrystals: effect of pH

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The synthesis of cocrystals has gained importance in the last years. The crystallisation of a drug with a suitable coformer results in a new compound that may have improved physical properties compared to the drug, such as melting point, moisture sorption, compressibility, solubility, and dissolution rate. This work evaluates the improvement of the dissolution rate of cocrystals of norfloxacin, ciprofloxacin and adefovir dipivoxil compared to the dissolution rate of the free drugs.

Cocrystals were prepared with different coformers using evaporation or slurry crystallisation method. Then, the dissolution rate of the drugs and the cocrystals were measured at different pH values. Because the studied drugs and some of the coformers are ionisable compounds, the dissolution behaviours are strongly dependent on the pH. Determinations were done at pH values typically encountered in the gastrointestinal (GI) tract (1.5, 4.0, 5.5, and 7.4). First, dissolution rates were measured individually at each pH sector. Then, dissolution rates were recorded in a sequential experiment in which pH was gradually increased from 1.5 to 7.4, staying 30 minutes at each of the selected pH values. Finally, biphasic dissolution studies were performed with adefovir pividoxyl adding a lipid layer (decanol) to mimic the absorption in the GI tract. In all cases the solid state of the remaining disks was analysed using PXRD.

Dissolution rate of cocrystals were higher than the ones of the parent drugs, and the difference became clearer at pH values where drug was in the neutral form (zwitterionic form for norfloxacin and ciprofloxacin). Instead, only a slight improvement was observed when drugs were ionised. The full pH experiment did not reflect the dissolution rate of the compounds at each pH sector because of the high dissolution rate at acidic pH value. Thus, performing the dissolution rate measurements at separated pH values allowed a better comparison of the behavior of the drugs at each pH. As regard biphasic experiments, adefovir dipivoxil migrated to the organic phase, which indicated good passive permeability. The analysis of the remaining solid after dissolution experiments evidenced that in some occasions transformations occurred in the outer surface of the tablet, which might modify dissolution behaviour of the cocrystals or drugs.

P 16

3D QSAR and target fishing docking studies of novel aryldiketo acids with promising antibacterial activity toward MDR strains

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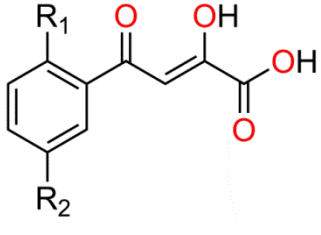
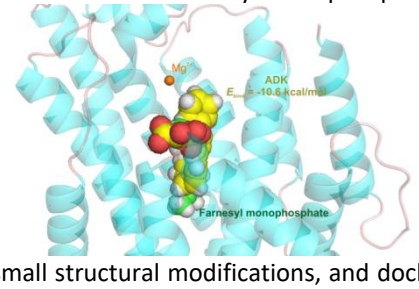
Antimicrobial resistance (AMR) is a major health problem worldwide, because of ability of bacteria, fungi and viruses to evade known therapeutic agents used in treatment of infections. Aryldiketo acids (ADK) exerted antimicrobial activity against several resistant strains of Gram-positive *S. aureus* bacteria. Our previous studies revealed that ADK analogues having bulky alkyl group in the ortho position on a phenyl ring have comparable activity of norfloxacin against the same strains [1].

Our current efforts led to development of ADK analogues with ten-fold increase of activity. Using an alignment independent 3D QSAR model based on descriptors derived from molecular interaction fields (MIFs), structural features important for antibacterial activity of ADK were found. Obtained model was statistically significant and could be used to guide the design of more potent derivatives as well as in virtual screening of novel antibacterial agents.

In order to elucidate a mechanism of action for these potentially novel classes of antimicrobials, several bacterial enzymes were identified as possible targets according to literature data and pharmacophoric similarity searches for potent ADK analogues. Among the seven bacterial targets chosen, the strongest favorable binding interactions were observed between the most active analogue and *S. aureus* dehydrosqualene synthase (CrtM; PDB entry: 4F6V), in the binding site of natural substrate farnesyl monophosphate.





In conclusion, 3D QSAR model based on MIFs, small structural modifications, and docking studies highlight the importance of diketo moiety for the antibacterial activity of ADK. *S. aureus* dehydrosqualene synthase is a potential target for ADK and we plan to experimentally verify this hypothesis.

Acknowledgement: *Ministry of Education, Science, and Technological Development of Serbia (Grant No. 172035) supported this work.*

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P 17

Lysosomal accumulation of tyrosine kinase inhibitors in relation to molecular physico-chemical parameters

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Small molecule tyrosine kinase inhibitors are orally admininstered anticancer agents. These molecules have sensitivity towards a wide variety of solid tumor cancers. However, many of these drugs failed to move to clinical practise because of poor animal bioavailability, while several failed to achieve efficacy at clinical trials. To endeavour to account for lack of efficacy we investigated the gut absorption pharmacokinetic parameters of selected TKI's in a $CaCO_2$ model system and the uptake of individual molecules into tumor cells. It was observed that systemic uptake was controlled by the concentration levels in the epithelial – gut boundary layer. High concentration increased absorption by diffusion whereas at lower concentration active transport mediated elimination back into the gut was the predominant process. TKIs such as erlotinib or gefitinib had permeability efflux ratios of between 1-2 whereas dasatinib had an efflux ratio of greater than 10.

Previously sunitinib was demonstrated conclusively to be sequestrated in the lysosomes of tumour cells. The extent of the accumulation was greater than 90 % of the total measured concentration. This sequestrated portion of the drug is isolated from its intended target, and hence can be considered to be inactive. We investigated a series of tyrosine kinase inhibitors both biomimetically and translationally to determine the extent of lysosomal accumulation. The biomimetic membrane and protein binding were measured using the methodology developed by Valko *et al.*[1] and demonstrated two clear groups of molecules those with a theoretical volume of distribution greater than 2 l/kg and those of 1 l/kg or less. Correlation to the % translational lysosomal accumulation determined *via* cell accumulation with and without a lysosomal inhibitor was performed using statistical analysis.

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P 18

ADME-Tox profiling of some water soluble chitosan derivatives used to obtain nanomaterials

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Chitosan is a nontoxic and biodegradable polysaccharide used as a biomaterial for the production of drug delivery systems. Chitosan has a poor solubility in neutral or alkalinized media and it restricts its pharmaceutical and biomedical applications. To improve the solubility in aqueous media, different derivatives of chitosan are obtained and they may be used for producing biomaterials and in the development of biomedical nanodevices and controlled release drug formulations. Within this study we use a few computational tools for predicting absorption, distribution, metabolism, excretion and toxicity (ADME-Tox), pharmacokinetics profiles, biological activity spectra, toxic/adverse effects, carcinogenety, cardiotoxicity, endocrine disruption and environmental toxicity of some water-soluble chitosan derivatives used to obtain chitosan-based nanomaterials related to pharmaceutical applications.
The pharmacokinetics profiles of these derivatives reveal poor gastrointestinal absorption and consequently, low oral bioavailability and low skin permeation. Chitosan derivatives cannot pass the blood-brain barrier and they are not able to inhibit the enzymes of the cytochrome P450 that are involved in the metabolism of xenobiotics. They do not reflect carcinogenity and cardiotoxixity and are only moderate potent endocrine disruptors. The main side effects of chitosan and its water-soluble derivatives in humans are: weight loss, acidosis, gastrointestinal toxicity. They reflect different degrees of environmental toxicity.

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P 19

Computational assessment of pharmacokinetics and biological effects of some anabolic and androgen steroids

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Most data concerning the adverse effects of anabolic and androgen steroids in humans come from few case reports and it may indicate that the incidence of their adverse effects is low. Taking into account the high occurrence of illicit use of steroids, serious adverse effects may not be reported or may be under recognized and computational assessment of these effects becomes important. Within this study, the predictions of the pharmacokinetic profiles, the molecular targets, the biological activity spectra and side or toxic effects of 31 anabolic and androgen steroids in humans are obtained using computational approaches. Investigated steroids usually reveal a high gastrointestinal absorption and consequently a good oral bioavailability, they may inhibit many of the human cytochromes involved in the metabolism of numerous xenobiotics (CYP2C9 being the most affected) and they reflect a good capacity for skin penetration. There are predicted numerous side effects of anabolic and androgenic steroids in humans, such as hepatotoxicity, teratogenicity, embryotoxicity, endocrine disruption and reproductive dysfunction, some of them being confirmed by case studies reported in specific literature and such as enhancing the accuracy of predictions. These results are important to be known as an occupational exposure to anabolic and androgenic steroids at workplaces may occur and because there also is a deliberate human exposure to steroids for their performance enhancement and anti-aging properties.

P 20

Enterovirus Inhibitory Activity of substituted Urea and Thiourea derivatives of *p*-Benzene sulfonamide

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Member of a series of substituted urea and thiourea derivatives of *p*-Benzene sulfonamide were prepared with one or two methylene group/s as linker between them. These were tested for their inhibition property against human rhinovirus-A (HRV-A) and human rhinovirus-B (HRV-B). Some of the compounds synthesized have sub-micro molar range of activity against HRV 21 and HRV 71 and moderate activity against HRV 14 with low cytotoxicity and high selectivity index values. Initial time of addition experiments shows that this might be a capsid binding inhibition.

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P 21

The relationship between the antioxidant and hepatoprotective activity of oil of *Thymus mugodzharicus*

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To identify prospective objects in developing of medicines, an antioxidant activity of *in vitro* of thyme (*Thymus marschallianus*, *Thymus roseus*, *Thymus rasitatus*, *Thymus lavrenkoanus*, *Thymus petraeus*, *Thymus crebrifolius*, *Thymus serphullum*, *Thymus mugodzharicus*) has been investigated. Analysis of o-phenanthroline and FRAP (Ferric reducing/antioxidant power) assays revealed a pronounced antioxidant effect for the etheric oil *Thymus mugodzharicus* antioxidant capacity, which allows to consider this essential oil of thyme growing in Kazakhstan, as an antioxidant, which can be used as a new phytodrug. Folin-Ciocalteu test analysis showed an increased content of polyphenolic compounds, in particular flavonoids, which caused the antiradical activity of the extract in the analysis using DPPH (1,1-diphenyl-2--picrylhydrazyl radical). Ascorbic acid, BHA (butylhydroxyanisole) and gallic acid were used as controls [1]. The manifestation of pronounced antioxidant activity by etheric oil *Thymus mugodzharicus* was as the basis for the investigation of hepatoprotective properties *in vivo*, although the mechanism of the latter may not be at the level of free radicals, but is caused by the membrane-stabilizing action of the exogenous object. Hepatoprotective activity of the drug is reflected in decreasing activity of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) to the level of intact animals, and for alkaline phosphatase (ALP), bilirubin, malondialdehyde is even lower in comparison with intact white rats. Similar dynamics of these enzymes is established for essential oil of *Thymus mugodzharicus*. It has been established that for the enzymes ALT, AST and ALP for essential oil of *Thymus mugodzharicus* there is a pronounced pattern reflecting the hepatoprotective property of the object, which indicates the advisability for further research using other methods to assess a hepatoprotective activity. In the group of intact animals, the level of AST was 2.04 ± 0.19 µmol / l h, and in animals with a control pathology caused by intoxication with carbon tetrachloride (CCl_4), it is increased to 20.53 ± 0.13 µmol / l h. Under the influence of the drug carsil and essential oil of the mugodzhar cytolysis of the syndrome declined, which manifested itself in a decrease in the activity of this enzyme, also a similar pattern found for ALT. The activity of ALP in toxic hepatitis also increases. Conclusion: the studied hepatoprotective activity *in vivo* of essential oil of *Thymus mugodzharicus* by the method of modeling the acute hepatic pathology of laboratory animals shows the positive influence of this substrate on the dynamics of peroxide processes. It opens the possibility of further study as a potential heaptoprotector based on plant raw materials of Kazakhstan. Thus, it can be considered that the expressed antioxidant and antiradical activity *in vitro* of the investigated object causes a positive hepatoprotective property in case of intoxication of CCl_4 in laboratory animals *in vivo*.

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P 22

Biogenic synthesis of silver nanoparticles from labisia pumila and its antioxidant efficacy

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Labisia Pumila is one of the medicinal plants which is traditionally been consumed by the rural and indigenous population in Malaysia and is and also well-known as Kacip Fatimah. Green synthesis of nanoparticles using medicinal plants as biological sources has become one of the prominent areas in nanoparticle research. The current study was focused on the synthesis silver nanoparticles using *Labisia Pumila* leaves and roots in different concentrations. DPPH scavenging assay was used to determine the antioxidant efficacy. The higher concentration of plant extracts led the higher generation of silver nanoparticles. The formation of AgNPs from leaf and root extracts was confirmed by UV-Visible spectra. The presence of functional groups such as flavonoids, terpenoids, and phenolic compounds in the AgNPs was analyzed by the FTIR spectroscopy. The highest percentage of Ag content in AgNPs from both extracts was 92.3 % and 94.3 % respectively was detected in XRF. Scanning electron microscopy revealed that most of the AgNPs from leaf and root extract were spherical in shape (45.01 nm) and cuboidal shaped (55.95 nm) respectively. The average particle size of AgNPs from both extracts was higher due to the aggregation of particles. Zeta potential revealed that AgNPs from root was more stable compared to AgNPs from leaf. The synthesized silver nanoparticles from *Labisia Pumila* showed strong antioxidant efficacy compared to the plant extracts. The current findings would be advantageous for future studies in investigating the potential health benefits of these biogenically synthesized nanoparticles and their mechanisms of actions at molecular levels.

P 23

Liver protective effects of KGSP in ethanol-induced liver damage model

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Polysaccharides isolated from plants have been reported to have antiinflammatory and antioxidant effects in various models [1]. *Triticum aestivum* L.(Kumgang) sprouts derived polysaccharide (KGSP) has anti-diabetic properties [2], but its effect on the protective effects against ethanol-induced hepatic injury is unknown. In the present study, we investigated the effect of KGSP on in ethanol induced liver injury in mice. Male C57BL/6 mice were administered ethanol with or without KGSP for 10 consecutive days by oral gavage. Silymarin was administered in the same manner as a control medicine. KGSP reduced ethanol-induced hepatic lipid accumulation and serum levels of alanine amino-transferase (ALT) and aspartate aminotransferase (AST). KGSP also prevented glutathione (GSH) depletion and increased the superoxide dismutase (SOD) in liver tissue. In addition, KGSP significantly inhibited ethanol-induced cytochrome P450 2E1 (CYP2E1) activation, and up-regulated the expressions of nuclear factor erythroid 2-related factor 2 (Nrf2) and hemeoxygenase-1 (HO-1), and down-regulated NADPH oxidase genes in ethanol fed mice. Furthermore, the up regulation of Nrf-2 was found to be regulated by a phosphati-dylinositol 3-kinase (PI3K)/Akt pathway. KGSP also, attenuated hepatic injury by modulating of caspase-3 and apoptosis associated mitochondrial proteins like B-cell lymphoma 2 (Bcl-2) and Bcl-2-associated X (Bax) in liver tissues of mice. Taken together, KGSP treatment could protect against ethanol-induced hepatic injury via multiple pathways by inhibiting steatosis and improving antioxidant marker levels during hepatic injury.

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P 24

Inhibitory effect of *Euphorbia supina* extract and its fractions on tyrosinase activity and melanin formation in B16F10 melanoma cells

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Melanin protects the skin from ultraviolet rays, but if overproduced, pigmentation may build up causing several skin problems [1]. *Euphorbia supina* Rafin. (ES), an herbaceous plant belonging to Euphorbiaceae is characterized with a dot in the leaves. It is widely distributed in temperate and tropical regions, such as Korea, China, Japan and Southeast Asia. In traditional oriental medicine, ES has been used as anti-inflammatory [2], anti-viral, anti-oxidative [3] and anti-allergic agents. Therefore, this research is aimed to determine the role of natural products in whitening property of skin. In this study, ES was extracted with ethanol and then the ethanol extract (ESEE) was fractionated to give dichloromethane (ESDM), ethyl acetate (ESEA) and water (ESH_2O) fractions. The anti-melanogenesis effect of ethanol extract and its fractions were studied for mushroom tyrosinase assay, cellular tyrosinase assay and melain content assay, and the expression of proteins involved in melanogenesis were assayed by western blot in B16F10 murine melanoma cells. Our results revealed that ES extract and its fractions inhibited mushroom tyrosinase activity in cell-free system. Also, ES extract and its fractions significantly decreased melanin content and inhibited the cellular tyrosinase activity in α-MSH-induced B16F10 cells. Morever, extract and fractions of ES significantly inhibited also the expression of MITF, JNK and p-ERK proteins in melanoma cells. These results suggest that ES extract and its fractions may be a potential skin-whitening and anti-melanogenic agent useful in cosmetic product.

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P 25

Evaluation of the interactions between some drugs and human serum albumin (HSA) using fluorescence

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Albumin, the most abundant protein in plasma and serum, is a water-soluble macromolecule with high biological significance. It can maintain the plasma oncotic pressure and modulate the fluid distribution among body compartments. Native albumin, that is the one without ligands or bound molecules, is built up from three homologous domains (I, II and III), showing each one two distinct subdomains, named A and B. There are numerous binding sites on albumin, but drugs and other exogenous compounds bind, mainly, to two of them: Sudlow I or acidic drug binding site, placed on subdomain IIA, and Sudlow II or benzodiazepine binding site, located on subdomain IIIA. Thus, albumin plays a relevant role in binding and transport functions and, therefore, on the pharmacokinetics of drugs.

Drug-albumin interactions can be evaluated using several complementary techniques such as isothermal titration calorimetry, fluorescence, frontal analysis capillary electrophoresis or equilibrium dialysis. In the case of fluorescence, the albumin is considered as the fluorophore. This is because it contains three fluorescent aminoacids (tryptophan, tyrosine and phenylalanine). When the complex drug-protein is formed, the albumin fluorescence can be whether quenched or enhanced. There are some equations to evaluate the fluorescence quenching/enhancement that allow calculating the binding parameters (binding constant, stoichiometry, change of enthalpy, *etc.*). Those equations consider the albumin as the unique fluorophore and assume that the concentration of the free drug is much higher than the bound fraction. However, there often exist other fluorophores in solution (such as the drug or a drug-albumin complex) that force to work under more selective but less sensitive conditions. Another strategy to evaluate the binding constants is to consider the whole spectrum, considering all the possible species in equilibrium; in this case we have used an extended version of the STAR program [1], which can deal with 300 spectra, each containing up to 300 data points.

The aim of this work is to evaluate the interaction between warfarin (Sudlow I), ibuprofen, flurbiprofen, naproxen and diflunisal (Sudlow II) and HSA using both approaches and compare the results obtained.

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P 26

Antiviral activity of hydrocarbon-stapled peptides against HIV-1 predominantly circulating in China

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The *i, i+4* or *i, i+7* hydrocarbon-stapled peptides [1,2], which were converted from CAI [3] by using hydrocarbon stapling technique [4], targeted caspid and inhibited HIV-1 assembly. These peptides haver been shown to have antiviral activity against HIV-1 subtypes circulating in Europe and North America [1,2]. We evaluate the antiviral activities of the *i, i+4* hydrocarbon-stapled peptides (NYAD-1, 15 and 23) and the *i, i+7* hydrocarbon-stapled peptides (NYAD-36, -67 and -66) against HIV-1 subtypes predominantly circulating in China including CRF07_BC, CRF01_AE and subtype B' isolates in PBMCs.

All stapled peptides were effective in inhibiting infection against all HIV-1 isolates tested with inhibition of 50 % viral replication (IC_{50}s) at low micro-molar concentrations. NYAD-1, NYAD-36 and NYAD-67 showed better antiviral activity than others, corroborating the findings of Zhang [1,2]. It is noticeable that antiviral activity against CRF07_BC and CRF01_AE isolates was slightly lower than that against subtype B' isolates. Meanwhile, the antiviral activity of these peptides was different in the same subtype. As previously reported, natural polymorphism among HIV-1 variants might be associated with the drug resistance and drug susceptibility of viruses [5]. Hydrocarbon-stapled peptides appeared to have broad antiviral activity against the predominant HIV-1 viruses in China, which would provide the impetus to the rational design of peptides for future antiviral therapy.

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P 27

Estimation of skin permeation by chromatography

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Dermal absorption is a key process in drug delivery studies of pharmaceutical and cosmetic industries as well as in the fields of dermal toxicology, risk assessment and the exposure of environmental pollutants. It is tipically described by skin-water permeability coefficients (K_p, units in $cm \cdot s^{-1}$) and can be determined using experimental techniques, both in vivo and in vitro. These methods usually are laborious, costly and ethically questionable. Previous works [1] have shown that retention in a HPLC system with a common C18 column in combination with McGowan solute's volume is a good surrogate of skin permeability. This system has been chosen in the present work to develop a methodology to predict K_p values, based on easy, fast and cheap experimental measurements. For the establishment of the model 65 solutes of different chemical nature have been selected, and the experimental K_p values of the neutral species have been extracted from Abraham Database [2]. Next, experimental logarithmic retention factor (log *k*) values have been measured in the chromatographic system, and correlated together with McGowan's volumes to log K_p values. In some way, the retention factor is related to the lipophilicity of the compound and McGowans's volume represents the size influence in the ability of the compound to penetrate through the skin. The established model has been validated internally in relation to robustness and externally in relation to predictive ability. Finally, the method has been applied to estimate K_p values of a different set of 29 compounds and they have been compared to experimental [3,4] and calculated values [2,5] available from literature. A good concordance is observed between the estimated and some experimental values and the calculated values. Finally, estimated values of permeation by chromatography for 4 compounds of pharmaceutical interest (capsaicin, oxycodone, tramadol and warfarine) have been obtained for the first time and have shown to correlate well with the calculated values.

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P 28

Estimation of biological properties of pharmaceutical interest using lecithin preparations as pseudostationary phases for capillary electrophoresis or liquid chromatography

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The pharmaceutical and chemical industries devote many efforts for the development of new active compounds. Bioactive and toxicological actions often depend on the partition of a solute between two phases with different polarity (*i.e.* blood and a biological membrane). The partition can be studied by means of *in vivo* methods or, alternatively, with predictive methods that use physicochemical measurements and/or structure-activity relationships.

In the present work, we have studied the partition of the compounds between an aqueous solution and lecithin-based liposomes or microemulsions using liposome electrokinetic chromatography (LEKC), microemulsion electrokinetic chromatography (MEEKC) and microemulsion liquid chromatography (MELC). The aim of this study is to evaluate the similarities between the partition in a lecithin-based system and the one in a biological membrane. In this way, biological properties of pharmaceutical interest can be predicted in a fast, simple, automated and economic manner.

First, the chromatographic systems were characterized with the solvation parameter model (SPM) [1] and compared between them. Next, they were compared with several biological systems of pharmaceutical interest, also characterized with the same model (skin partition, skin permeation, intestinal absorption, blood brain distribution, blood brain permeation). Mathematical evaluation of the model coefficients' similarity showed that the three lecithin-based systems were promising in the prediction of the human skin partition (K_{sc}) and permeation (K_p) coefficients.

Next, the prediction ability of the chromatography was checked experimentally. The retention of different drugs in the three systems was measured and it was correlated to the corresponding biological property value. Good correlation, and thus a good ability to emulate a biological property, was found for skin partition using the MEEKC system. After applying a model correction, it was also possible to predict the skin permeation using the LEKC system.

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Vibrational spectroscopy coupled with chemometrics used in monitoring reaction for pharmaceutical and chemical industries

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Reaction monitoring by vibrational spectroscopy (VS), is being increasingly used during process development in pharmaceutical and chemical industries.[1,2] An efficient, versatile and non-destructive in situ method in reaction monitoring using VS is described. The Suzuki cross-coupling reaction was used as a model system.
The reaction was monitored using near-infrared (NIR) and Raman spectroscopy coupled with chemometric tools such as principle component analysis and partial least squares to anticipate how far the reaction had proceeded. We investigated which spectroscopic method can be used to monitor reactions that can be very useful in pharmaceutical applications. To confirm the presence of the desired product, offline analyses were performed using gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. Results demonstrate that Raman spectroscopy is superior over NIR in detecting the formation of the product in real time. These technique allow us to obtain a huge amoung of information abouth the reaction with minimum effort and time.

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P 30

ACD/Portal technology – bringing ACD/Percepta capabilities to a new level

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ACD/Percepta Portal is a new platform that builds upon the well-established components of ACD/Percepta software – reliable predictive algorithms for a multitude of physico-chemical, ADME, and safety-related properties, powerful data mining, visualization, compound profiling and risk assessment capabilities, as well as ACD/Structure Design Engine for generating libraries of virtual analogs compatible with the desired characteristics. ACD/Percepta Portal combines these features with flexible network-based deployment, raising software interactivity to a new level and offering some exciting features. This work brings particular focus to the components of ACD/Percepta Portal that leverage the power of high performance computing in a server environment. The server-side architecture of ACD/Percepta Portal relies on multiple calculation units (kernels) that enable parallel processing of very large amounts of data in real time. These capabilities paved the road for new developments in several key areas. The addition of a quick exploration of the predicted property values for a multitude of structural analogs of a compound enables on-the-fly liability checking, *i.e.* identifying the areas of the molecule potentially responsible for unfavorable ADME/Tox characteristics. Adaptation of the ACD/Structure Design Engine to the employed architecture gave rise to a new generation of this tool that enables extensive enumeration of substituent property space in accordance with specific user-defined constraints at up to four independently varying substituent positions at the same time. Along with a built-in database of more than 10^4 building blocks, this leads to exploration of up to 10^{16} virtual analogs, which is actually feasible in real time inside ACD/Percepta Portal. Such broadened scope of the chemical space investigated greatly enhances the potential of encountering new compounds with the most favorable property profiles.

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Confocal Micro Raman Spectroscopy approach to characterize content uniformity in pharmaceutical development

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Confocal Raman microscopy was used in this study as a non-destructive tool to characterize the content uniformity of a poorly water-soluble drug incorporated in a solid dispersion [1]. Fenbendazole (FBZ) was selected as a model drug of Benzimidazole (BZ) broad-spectrum anthelmintic class, widely use for the prevention and treatment of parasitic diseases in veterinary and human medicine. BZs are classified as class II in the Biopharmaceutical Classification System, showing poor water-solubility and concomitant low bioavailability. In this work, we prepared binary solid dispersions (SDs) of FBZ, with the aim of improving its dissolution rate. Poloxamers were used in different proportions as polymeric carriers. Furthermore, physical mixtures (PMs) of the components were prepared in the same ratios of the SDs to evaluate the influence of the formulation process. Solid state characterization included X-Ray Powder Difraction (XRPD), FT-IR Spectroscopy and Confocal Micro Raman Spectroscopy (CMRS), among others. Particularly, CMRS was used to understand the difference between the SDs and PMs dissolution profiles. Different statistical approaches as principal component analysis (PCA) and peak to peak normalization were used to analize the collected Raman mappings in order to explore content uniformity and drug distribution. In vitro dissolution results showed that all SDs markly improved dissolution rate of FBZ compared to PMs and pure FBZ (a mean of 85%, 55% and 6% of FBZ dissolved respectively, within 60 minutes). SDs dissolution profiles were statistically similar, indicating that the different polymer ratios and types did not influence the release of FBZ. When comparing SDs profiles with the PMs, within 15 minutes of the dissolution test the percentage of FBZ dissolved in the SDs double those of the PMs. XRPD and FT-IR results showed no interactions between components, and particularly XRPD showed that the drug crystalline structure remain unaltered after the SD process. CMRS allowed obtaining mappings of the distribution of the drug within the polymeric matrix. These results showed the drug homogenoulsly dispersed in the SDs while areas containing pure polymer or pure FBZ were predominant in the PMs. This difference in the distribution of FBZ is produced by the manufacturing process and explains the observed differences in dissolution rate between SDs and PMs. In conclusion, we obtained SDs of FBZ with markly improved dissolution rate and relate it to the benefits of the SD process thanks to chemical imaging techniques as CMRS mapping.

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P 32

Revisiting blood-brain barrier: a chromatographic approach

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The blood–brain barrier (BBB) plays a fundamental role in the pharmacological activity of drugs targeting the central nervous system. Two different *in vivo* BBB experimental models have been considered in the present work, the plasma-to-brain distribution ratio (log BB) and the permeability-surface area product (log PS). log BB was extensively studied by Abraham and coworkers [1] by means of linear free energy relationships (LFER), and in the present work log PS has been comprehensively characterized using the database compiled by Avdeef [2] from *in situ* rodent brain perfusion measurements.

Beyond ethical concerns in animal experimentation, in early stages of the drug discovery process an accurate *in vivo* determination of biological activity for a large number of potential candidates is unaffordable. Alternatively, microemulsions (ME) can be used as physicochemical surrogate models of biological processes, such as lipophilicity or BBB [3], since ME mimic, to some extent, the properties of cell membranes.

Chromatographic systems consisting of a Gemini C18 column as stationary phase and MEs made of 50 mM phosphate buffer pH 7.4, SDS, 1-butanol, and heptane have been characterized and compared to blood brain transport by the Abraham model. The most relevant factor for solute retention is the molecular volume, suggesting a high affinity of large compounds for the C18 stationary phase. In contrast, dipolar/polarizable analytes and those with hydrogen-bonding basicity interact preferably with the ME mobile phase, decreasing retention times. The oil concentration seems to have a minor effect on interactions through π- and *n*-electrons and solute acidity by hydrogen-bonding, reducing retention as well but to a much lesser extent.

Finally, a chromatographic system consisting of 3.3 % w/v SDS, 6.6 % w/v of 1-butanol, and 0.8 % w/v of heptane as mobile phase is proposed as surrogate model for the rate of BBB penetration, particularly the logarithm of the passive permeability surface area product. Chromatographic retention factors (log *k*) of neutral and ionized drugs are directly and linearly related to log PS, without the need of any additional correction parameter [4].

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P 33

New derivatization approach for TLC/MALDI-TOF mass spectrometry analysis of mixtures of sterols and steroids

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Thin-layer chromatography (TLC) occupies an important place in the qualitative and semi-quantitative studies of low molecular weight, pharmaceutical, and synthetic chemical samples. The main advantages of TLC are its versatility, low cost of equipment and rapidity of analysis. However, this method posses disadvantages for the identification of the separated components: normally identification is carried out by different chemical and optical methods or using Rf (retardation factor) values, which rarely are informative. In the other hand, due to a high sensitivity and the possibility of analyte conversion into an ionized state directly from a TLC plate, MALDI mass spectrometry seems to be a suitable method in the combination with TLC for separation and identification of mixtures of different analytes. Earlier [1], we have shown that application of composite matrices containing mixture of glycerol, graphite and tradicional MALDI matrices allows one to record the mass spectra of a wide range of compounds directly from the TLC plate. At the same time, some non-polar or low polar compounds are not capable of undergoing ionization reactions in MALDI conditions. One of the main methods to solve this problem is derivatization of analytes yeilding readily ionizable compounds. In the present work, we report the results of development of a simple and convenient method for chemical modification of steroids and sterols (cholesterol, sitosterol, stigmasterol, androsterone, estrone, β-estradiol, testosterone) directly on TLC plates for their analysis by MALDI MS. The proposed derivatization approach is based on the introduction of a fixed charge into the analytes molecules by their acylation with halo-substituted acyl halides followed by quaternization with nitrogen-containing bases on the TLC plates.
For the derivatization reaction, 2 µL of pyridine (Lewis base) and 2 µL of 3-bromopropionyl chloride were applied to each spot of mixtures of analytes separated on TLC plates. The formed derivatives showed high desorption / ionization efficiencies in MALDI MS conditions and this allows recording their TLC/MALDI mass spectra, which contain intense peaks of cationic parts of the molecules. In the case of β-estradiol, bearing two hydroxyl groups, the recorded mass scpectrum contained intense peak corresponding to the product of double acylation followed by single quaternization. The obtained results prove the potential of the proposed approach for analysis of low polar compounds by TLC/MALDI.

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Fixed-charge derivatization of carbonyl compounds for their analysis by MALDI mass spectrometry

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Reagents allowing the introduction of fixed-charged group in analyte molecules are widely used for the analysis of carbonyl compounds (Girard's reagents T and P, for example) [1]. However, they have one drawback: it is not easy to get their isotopically labeled analogs if isotope dilution quantitation is required. Herein we describe derivatization of carbonyl compounds by α,ω-N,N-dimethylaminoalkylamines followed by the quaternization by alkyl halide. The suggested reagents for primary modification of carbonyl compounds contains two reactive centers – primary and tertiary amine groups. The former provides the condensation with carbonyl group to form Schiff base and the latter one can undergo the quaternization in the presence of alkyl halides. As a result, fixed-charged fragment can be introduced in analyte molecule providing high desorption/ionization efficiencies in MALDI conditions. At the same time, one can also use deuterium labeled alkyl halids to prepare the most suitable standards for quantitation.
The proposed approach was tested using various types of carbonyl compounds (aliphatic, cyclic and steroid ketones), aminoalkylamines (2-dimethylaminoethylamine (DMAEA), 3-dimethylaminopropylamine (DMAPA), 4-dimethylaminobutylamine (DMABA)) and quaternization agents (methyl, ethyl and trideuteromethyl iodides). According to GC/MS data, all agents (DMAEA, DMAPA and DMABA) readily react with the analytes quantively yielding Schiff bases. Further interaction of resulting imines with alkyl halids gives rise to quaternary ammonium salts whose MALDI mass spectra contain the peaks for corresponding cationic parts of the derivatives. The intensities of ion peaks of derivatives synthesized using methyl and trideuteromethyl iodides are equal. CID tandem mass spectra of the derivatives contain peaks mainly corresponding to elimination of trialkylamine group. These peaks are common and predictable for these compounds and could be easily used for MRM determinations.

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LC-MS/MS assessment of metabolic switch in cancer cell after treatment with PDK1 inhibitors

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Cancer cells are more dependent on cytoplasmic glycolysis rather than mitochondrial respiration even in the presence of adequate oxygen, which is one of the most significant difference between normal cells and cancer ones. Pyruvate dehydrogenase kinase 1 (PDK1) is a key enzyme in glucose metabolism to regulate the pyruvate dehydrogenase (PDH) activity via phosphorylation (1). PDH catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, which is a main substrate for tricarboxylic acid (TCA) cycle. The deficiency of acetyl-CoA may lead to attenuated oxidative phosphorylation and accordingly augment lactate fermentation. Therefore, PDK1, by controlling PDH, dominates the critical switch between mitochondria-based respiration and cytoplasm-based glycolysis. Several reported PDK1 inhibitors, such as dichloroacetate (DCA) and AZD7545, could inhibit PDH phosphorylation and reactivate it selectively (2). Accordingly, these compounds might affect the metabolic pathway and influence the glycolytic intermediates in cancer cells. To investigate what metabolic alterations are induced, liquid chromatography-based tandem mass spectrometry (LC-MS/MS) method is employed to analyze the metabolites after treatment with by PDK1 inhibitors in cancer cells. To our knowledge, this is the first paper which use chromatographic method to detect the effect of PDK1 inhibitors on cancer cell metabolic pathway.

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2D inkjet printing for nanogram-scale formulation micro-array testing

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Pre-formulation screening of potential new drugs can be costly in terms of materials and time, as well as financially. Historically, gram quantities of drug have been necessary. Efforts to address this have mainly aimed at miniaturised versions of existing large-scale processes, often with a high-throughput approach using parallel pathways. An example of this approach would be the use of well-plate technologies for solid-form screening. The resultant assays typically require several milligrams of drug per well, and around 100 mg in total [1]. However even this sample quantity can be challenging when a limited supply of drug is available, and is especially problematic when the drug is highly soluble. For a typical 96-well plate the minimum solvent volume required for a single well-plate may be around 200 µl (total for 96 wells ca. 20 ml). A drug with a solubility of 100 mg/ml would therefore need to be available in 2 g quantities to undertake a basic crystallisation screen, with any serious exploration of parameter space (*e.g.* temperature, solvent) requiring significantly more material. For early-stage compounds this sample quantity is typically not available. Reduction of scale via further miniaturisation of screening assays is therefore desirable.

We present some recent advances made by our group using a piezoelectric inkjet dispensing/printing approach, adopted from high-throughput screening approaches [2–4]. In this approach, pico-litre volume droplets are deposited onto a substrate and allowed to dry, to create a printed micro-array. Presented will include: 1) crystallisation screening of pure drugs, and determination of the concentration threshold below which bulk-like behaviour is not observed; 2) screening of drug-loading amphiphilic materials and determination of critical micelle concentration (CMC) using nano-grams of material; 3) monitoring phase segregation and recrystallisation of amorphous solid dispersions [5], again using only nano-grams of material, typically printed from DMSO solution (other solutions can be used). This represents an order of magnitude miniaturisation in terms of sample quantity compared to current state of the art, and may enable (for example) rapid pre-formulation screening of lead compounds, for which only micro-grams are typically available.

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P 37

Prolonged release system with paliperidone: micro-NIR real-time preparation process monitoring

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Due to the widely known potential of NIR-infrared spectroscopy in pharmaceutical product and process monitoring, considerable attention has been given to the miniaturisation and portability of spectroscopic devices. The main goal of the study was to investigate the performance of micro-NIR in the evaluation and prediction of blend uniformity.

Hydrophilic matrix tablets with 7.5 % paliperidone were prepared by direct compression, using hydroxypropyl methylcellulose K100M, sodium carboxymethyl cellulose and lactose as major excipients. A micro-NIR PAT-W was used to collect the spectral data of the powder blends. In the blending stage, a set of 5 off-line static calibration samples with 60-80-100-120-140 % paliperidone were used to develop a multivariate partial least squares (PLS) calibration model for the prediction of paliperidone content and blend uniformity. The validation set included three active content levels (from 80 % to 120 %). The model was generated for the spectral range of 1350-1550 nm, applying 1st derivative pre-treatment processing. Data analysis revealed a number of PLS factors of 3, R2 of 0.993 and RMSECV of 3.78. Using these calibration models, the method was fully validated according to the ICH guidance.

The performance of the off-line calibration model was evaluated in real-time laboratory scale blend experiments. To confirm the robustness of the off-line calibration model, different batch sizes and revolution speeds were used. The PLS calibration model developed in off-line experiments was successfully employed to predict the API content in the powder blend and establish the blend uniformity. The NIR-chemometric method showed good reproducibility and satisfactory accuracy and linearity profiles, indicating that it could be used for direct determination of paliperidone in powder blends for tableting and tablets.

The micro-NIR device showed good robustness for pharmaceutical process monitoring, despite of the reduced wavelength range and the combination with non-linear modelling yields a reliable tool for in-line monitoring.

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Exploitation of griseofulvin chloroform solvate incorporated inside polymeric matrix to improve aqueus solubility

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The poor solubility of hydrophobic drugs has been a challenge to develop new medicines in pharmaceutical research and industry. This class of drugs often has limited bioavailability and irregular absorption profiles [1]. Various formulation approaches have been studied in order to obtain the best formulation that attains highest solubility without compromising the safety and stability aspect of the drug. Solid dispersions has been one of the successful approaches to achieve high solubility of the drug [2]. In fact, there are already solid dispersion formulations that have been marketed, *e.g.* Gris-PEG®, a griseofulvin-PEG crystalline solid disperion. It is often difficult to avoid preparing solid dispersions without the use of solvents such as the common use of spray drying to prepare drug-polymer dispersions using organic solvents. In this study, the effectiveness of potential polymers in enhancing the solubility of griseofulvin solid dispersions was invesitiagted. The polymers include silica, microcrystalline cellulose (MCC), hydroxypropyl methylcellulose (HPMC), hydroxypropyl methylcellulose acetate succinate (HPMCAS) and polyvinylpyrrolidone (PVP). The effect of different solvents on the solubility of resulting solid dispersion was also investigated. Three solvents, *i.e.* acetone, chloroform and methanol, were used to prepare the drug polymer mixtures. The formed solid dispersions were then further analysed using DSC, FTIR, XRPD, DVS, solution calorimetry, solid and liquid state NMR and SEM. The results showed that HPMCAS was the best polymer and chloroform was the best solvent in promoting drug release. It was concluded that the extensive hydrogen bonding between GF and HPMCAS plus the formation of chloroform solvate significantly enhanced the dissolution of GF. While solvaes and hydrates were traditionally known to reduce solubility, it was clear from the results that formed solvate maintained its structure within the polymeric matrix, which implies potential use of this approach to improve solubility. It is acknowledged that chloroform may not be the optimum solvent, but using this principle would allow use of hydrates for similar purpose.

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Development and optimization by experimental design of an in vitro method for prediction of drug buccal absorption

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Drug administration via the buccal route is receiving growing attention, due to the advantages it presents with respect to the classic oral route [1]. Moreover, the continuous advances in drug delivery technology are progressively increasing the variety of drugs, including biological agents, that can be delivered across the oral mucosa for systemic diseases treatment as an alternative to parenteral administration [2]. Therefore, considering the great potential applications of this administration route, it is important to have a reliable in vitro method available for an easy and rapid evaluation of permeability through the oral mucosa of possible candidate drugs. Buccal drug delivery is commonly evaluated in vitro by diffusion studies across freshly excised animal mucosa [3], or using oral epithelial cells grown on filters [4]. However, both methods present several drawbacks (difficult handling and maintenance of fresh excised tissue, high biological variability and consequent poor reproducibility, the first one; long cell growth cycles, risks of microbial contamination, high costs and rather elevated inter-experimental and inter-laboratory variability, the second one) which limit their use for a high throughput screening approach. In vitro methods based on the use of artificial membranes could represent an interesting alternative to the above methods, due to their greater simplicity, lower cost, shorter times and better reproducibility [5]. In this light, the aim of this work was to develop an in vitro method for drug buccal absorption assessment, based on the use of a special artificial membrane purposely optimized. Naproxen was selected as reference model drug in this preliminary study. Its apparent permeation coefficient (P_{app}) through freshly excised pig buccal mucosa, determined using a suitably modified Sartorius apparatus, was considered as the target value to be obtained with the artificial membrane. An experimental design methodology was used to optimize the composition of the lipid mixture used for support impregnation. An initial screening phase provided indication on P_{app} variation trend as a function of the kind of phospholipid component, allowing selection of the most suitable one. The following response surface study, performed by a mixture design, enabled to define a design space where each combination of the lipid mixture components fulfilled the desired P_{app} target value within the prefixed confidence interval with a failure risk < 1%. Further studies with other model drugs have been planned to confirm the actual predictive ability of the method, which could offer a useful tool for a quick and effective screening in the early stages of drug discovery and/or in preformulation studies.

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Mucoadhesive microspheres for vaginal administration of Cefixime: development and evaluation

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Vagina is becoming a preferred route of drug administration, not only for local treatment of vaginal diseases, but also for systemic delivery, due to the benefits offered compared to the oral route, such as need of lower doses, less frequent administration, elimination of gastro-intestinal disturbances and first-pass effect [1]. Different innovative formulation strategies like the use of bioadhesive and/or thermosensitive polymers, micro- or nano-particulate systems have been proposed to improve drug vaginal delivery and absorption [2]. Among these, mucoadhesivemultiparticulate systems offer several advantages compared to conventional vaginal dosage forms, covering a wide mucosa area, assuring a prolonged in situ drug permanence and allowing a controlled and sustained drug release [3]. We recently investigated the effectiveness of different mucoadhesive polymers for the development of microspheres (MS) for vaginal delivery of metronidazole [4]; among the different tested formulations, chitosan (CS) coated Ca-alginate MS covered gave the best results in terms of drug entrapment, mucoadhesion and antibacterial activity [5]. Based on these premises, the goal of this work was the development of muco-adhesive CS-coated Ca-alginate MS for vaginal administration of cefixime (CFX), a third-generation cephalosporin antibiotic used in the treatment of urinary-tract infections and gonorrheia. Ca-alginate MSprepared by iono-tropic gelation technique were submitted to different coating methods with CS and loaded with increasing CFX concentration. Each batch was characterized for drug entrapment, mean weight and diameter, morphology (by ESEM and optical microscopy), and mucoadhesion properties. Swelling and drug release studies showed a relationship between drug content in the beads and MS water uptake, allowing the choice of MS with the optimal drug/polymer ratio endowed with the best swelling and drug release profile. Microbiological studies showed a close relation between reduction of metabolic activity of E. Coli (taken as model strain) and drug release rate from MS, confirming the 300 mg CFX-loaded MS as the best formulation and proving its suitability for local treatment of vaginal diseases.

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P 41

Nanostructured lipid carriers (NLC) as a new drug delivery system for potential hydrochlorothiazide oral administration in the paediatric therapy

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Hydrochlorothiazide (HCT), a diuretic drug belonging to the thiazide class, is widely used in paediatrics for the treatment of hypertension. HCT is classified as a Class IV drug of the BCS, due to its low aqueous solubility and low membrane permeability, thus leading to oral bioavailability problems. There is still no oral liquid formulation of HCT available on the market. Extemporaneous preparation of suspensions or solutions of the drug made from crushed tablets is a widespread used technique in paediatric pharmacy practice. However, no stability or dosing accuracy is known for these oral liquid preparations [1].
Therefore, the aim of this work was to develop a low-dosage liquid oral paediatric formulation of HCT endowed with high stability and tolerability, able to assure a complete and sustained drug release, by designing a suitable Nanostructured Lipid Carrier (NLC) formulation. The performance of different syntethic and natural liquid lipids was examined and two different preparation methods were employed, *i.e.* the homogenization-ultrasonication method (HU) and the microemulsion technique (ME), in order to evaluate their influence on the NLC properties in terms of particle size, PDI, Z-potential, entrapment efficiency, gastric stability and drug release properties.
Precirol®ATO5 was used as solid lipid and Tween®80 and Pluronic®F68 as surfactants, formerly selected in a previous study [2]. An initial screening study allowed selecting the most suitable liquid lipid type and solid/liquid lipid ratio, based on the solubilizing ability toward HCT and gastric stability. In the case of NLC-ME, a further screening study was performed to select the best Co-Surfactant able to form microemulsions in the presence of the selected solid and liquid lipids and surfactants. The presence of Pluronic®F68 did not allow in any case the microemulsion formation. On the contrary, when using Tween®80 as surfactant, the ME method allowed a higher entrapment efficiency than the HU technique, and provided a prolonged release, which lasted for 6 h. In particular, NLC-ME containing Tween®20 as Co-Surfactant gave rise to a complete drug release. In vivo studies confirmed these results, displaying the best diuretic profile of such a formulation, which significantly increased the urine volume at 4 and 6h after treatment with respect to the reference HCT suspension. Stability over time of the NLC formulations has been evaluated for 3 months, by monitoring particle size, PDI and Z potential.

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P 42

pH and temperature dependent release of diclofenac from wound dressing material

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Pain was proven to cause a delay in wound healing, therefore effectively alleviating it, as well providing a less painful wound-healing environment, should attract significantly more attention in future development of novel wound care solutions.
In this study, a nonsteroidal anti-inflammatory drug diclofenac (DCS) was included into a novel wound dressing material. Generally, it is of great importance that the drug release from any material is adjusted to the specific therapeutic needs of the patients. It is no different in the treatment of wounds. Controlled delivery systems are used to improve therapeutic efficacy and safety of drugs by delivering them at a rate dictated by the need of the physiological environment over a period of treatment to the site of action [1]. Several factors affect the kinetics of drug release , among them also the pH value [2] and the temperature (T) [3] of exudate in the wound. The pH value in the wound is often changed due to an underlying infection, while T can be significantly affected by local and systemic inflammation (it can rise even to 41 °C). This study provides the proof of direct corelation of pH and T change of exudate on the DCS release from the wound dressing material to the wound site (**Fig. 1**).

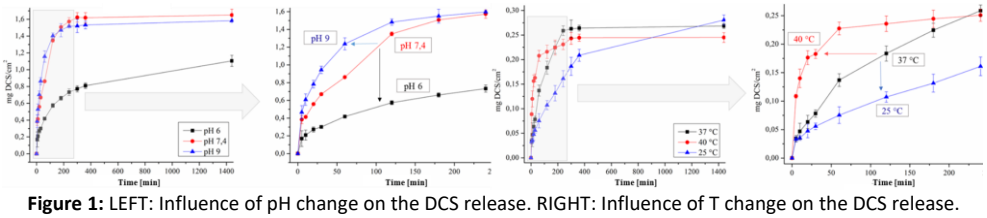

Figure 1: LEFT: Influence of pH change on the DCS release. RIGHT: Influence of T change on the DCS release.

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P 43

Benzocaine and pH sensitive dye in electrospuned nanofibers for painless wound care and fast detection of wound infection

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There is an increasing clinical evidence that pain significantly slows down the wound healing process [1]. Strategies that could diminish the sensation of pain for various wound types are therefore in high demand. One of such, which is also the focus of this study, is the preparation of novel wound dressings with incorporated pain-relieving drugs.
Controlled delivery of the pain reducing drug bezocaine (a local anesthetic) into wounds was achieved by its *in situ* incorporation during electrospinning. The controlled release was achieved through a tailored morphology and porosity of the electrospunn nanofibers [2]. An important factor, affecting the drug release kinetics, is the pH of the exudate in the wound bed, which can vary from 6 to 9 [3]. The actual pH of the wound bed is often also an indication for a possible infection.
Based on all mentioned, the goal of this study was to prepare a novel cellulose acetate nanofibrous wound dressing material with the incorporated local anesthetic benzocaine and a pH sensitive dye, bromocresol green. This novel bifunctional material was characterized in regard of its physico-chemical, structural and morphological properties, as well as it capability for controlled drug release, pH sensing and biocompatibility towards human skin derived fibroblasts.
The prepared material was proven biocompatible and was shown to effectively sense the pH in the desired range (from 6 to 9). As expected, the drug release was affected by the varying pH, making the color change of the wound dressing, when exposed to different pHs, useful in two regards. Firstly, it can serve as a direct indication for a possible infection and secondly, it can visually alert on the change in drug release kinetics, possibly leading to a change in the incorporated drugs therapeutic effectiveness. Preparation and use of such wound dressings could be interesting in treatment of infected and painful wounds. In response to the simple coloration upon pH change, the physician can efficiently respond to the underlying infection, which can also affect the drug release rate.

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P 44

Liposomal co-encapsulation of DOX and CURC: a nanotechnological approach to increase antitumor activity against colon cancer

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A promising strategy to increase the therapeutic efficiency in colon cancer is combination anticancer treatment, which offers the possibility of synergism. Nanotechnological approaches, i.e. the use of drug delivery systems, can increase the accumulation of anticancer agent in tumor cells, thus increasing the antitumor activity. The aim of this study was to develop doxorubicin (DOX) and curcumin (CURC) co-loaded PEGylated liposomes (LCL-DOX-CURC) and to assess their effect on tumor growth *in vivo*.
Liposomes were prepared by the film hydration method, as previously described [1]. The liposomes were characterized through size, polydispersity, Zeta potential, DOX and CURC entrapment efficiencies, morphology, and the release of the drugs was also evaluated. The effects of different experimental treatments (free DOX or LCL- DOX 2.5 mg/kg; free CURC or LCL-CURC 5mg/kg; free co-administered, liposomal co-administered and liposomal co-encapsulated DOX and CURC at 2.5 mg/kg and 5mg/kg, respectively) on tumor growth, were evaluated in a C26 murine colon carcinoma model in BALB/c mice [2], by measuring the tumor volume at day 11 and AUTC until day 11.
Liposomes were nanosized, on average 180 nm, showed good stability and a prolonged release profile of DOX and CURC over 72h. Both LCL-DOX and LCL-CURC were very effective in suppressing tumor growth compared to the control treatment (PBS), while empty LCL had no such effect on this parameter. Notably, liposomal co-encapsulation strongly augments both DOX and CURC antitumor activity, demonstrated by the reduction with more than 50% of tumor volumes and AUTC, compared to control. Based on the *in vivo* findings, this liposomal formulation co-delivering DOX and CURC, which is currently under mechanistic evaluation regarding its molecular tumor targets, exhibits an enhanced antitumor activity, which can be further exploited for the treatment of colon cancer.

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Study on the synthesis and Characterization of a novel Icariin/Bone powder/Polylactic acid (IBP) hybrid scaffold

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Polylactic acid (PLA) is a popular material in the field of biomedical engineering. However, because of the lack of active functional groups, it is difficult to produce specific recognition signals, which is not conducive to cell adhesion, proliferation and differentiation. Therefore, it is an urgent problem to introduce the specific bioactive components or functional regulation factors in the substrate to effectively improve the growth behavior of cells within the PLA based hybrid scaffolds. In this study, PLA scaffold was uniformly mixed with bone powder with a method of ultrasound-assisted and *in situ* polymerization. On this basis, the bone powder was used as the carrier of Icariin (ICA), which was premixed in ICA ethanol solution, and then dissolved in PLA solution. In this way, the hybrid scaffolds were then prepared, which the physical and chemical properties and cell compatibility were focused on. The results showed that, the porosity of the bone powder/PLA hybrid scaffolds with different ICA concentrations was more than 85%. With the increase of the ICA loading, the porosity decreased, but there was no statistical difference ($P > 0.05$), which showed that loading amounts of bone powder and ICA had no significant effect on the porosity of PLA scaffolds during the preparation. hADSCs were cultured with PLA and bone/PLA hybrid scaffolds separately, and the cell adhesion rates were 47.71 % and 53.22 %, which showed that cell adhesion performance of bone powder/PLA hybrid scaffolds was better than that of PLA. In addition, the cells grown on the drug loading scaffolds were changed into short spindle shape, and the extracellular matrix secretion was significantly increased. In some areas of the culture hole, the aggregation growth could be found, around which structures like the calcified nodule were naturally formed. Amongst them, ALP staining of 10^{-7}M ICA-bone powder/PLA hybrid scaffolds was strongly positive. This indicated that the addition of ICA could promote the differentiation of hADSCs into osteoblasts by a local slow release, and 10^{-7}M ICA-bone powder/PLA hybrid scaffolds had the best effect on inducing bone formation.

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P 46

Particle properties and water content homogeneity influence on glass transition phenomenon

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Amorphous Solid Dispersions (ASD) have become an established formulation strategy to increase solubility and bioavailability of poorly soluble drug substances. In a pharmaceutical ASD the drug substance is molecularly dispersed into an amorphous carrier, usually a polymer(s), resulting in a one-phase system. Despite this apparent simplicity, these two-components systems (or multiple component systems) can form multiple structures depending on their composition and sample processing history. The manufacturing of this type of formulations have inherent high risk of phase separation into drug-rich and polymer-rich regions, which can ultimately lead to crystallization of the drug substance and consequently loss of its solubility advantage. The hygroscopicity/water content of ASDs may also play an important role in physical stability since the plasticizing effect of water on the glass transition of amorphous solids promotes molecular mobility and, ultimately, may lead to crystallization. So, the understanding of the physical structure and phase behavior is of paramount importance for the estimation of the stability of the produced ASD.

Thermal analysis using Differential Scanning Calorimetry (DSC) is often used to characterize ASD and to evaluate its stability. However, phase separation in these types of systems may be difficult to detect in case samples contain very small domains with different molecular arrangements. In addition, the complexity of thermal events occurring near the glass transition region can sometimes lead to wrong conclusions regarding phase separation phenomenon. Differences in the particle size and/or water distribution in the samples may increase the complexity of interpreting calorimetry results. For example, in cases where the distribution of water in the sample is not homogenous, the thermal events occurring for water-rich regions will show a plasticizing effect on T_g that will not be observed in the dry regions. This can lead to multiple glass transition events that will make phase separation evaluation more challenging.

The work presented herein refers to a study by DSC in various ASD in order to increase knowledge on how the glass transition phenomenon is afffected by the water content homogeneity and particle size. Further, this study will and how can this be applied in the pharmaceutical industry to further characterize and predict physical stability of amorphous solid dispersions.

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Anti-carcinogenic capacity of microcapsules loaded with NaF through coaxial ultrasonic atomizer

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A new method to deliver fluoride using biodegradable poly(lactic-co-glycolic acid) microcapsules to suppress cariogenic bacterial growth during orthodontic treatment was investigated. A coaxial ultrasonic atomizer was used to encapsulate NaF solution into microcapsules. The orthodontic adhesive resin disk containing fluoride loaded microcapsules was prepared by LED light curing. The microstructure of microcapsules, successful loading of NaF, fracture strength, and shear bonding strength were assessed by FE-SEM, confocal laser scanning microscope, and general purpose testing machine, respectively. Fluoride release from the orthodontic adhesive resin disk containing fluoride loaded microcapsules orthodontic adhesive resin disk containing fluoride loaded microcapsules in phosphate buffered saline and pH changes were measured after different periods of soaking time. Antibacterial activity of the orthodontic adhesive resin disk containing fluoride loaded microcapsules was assessed in tryptic soy broth (TSB) containing mutant streptococci. The starting inoculum and the orthodontic resin disk containing microcapsules not loaded with NaF were used as negative and positive controls, respectively. As results, the cumulative amount of NaF after 49 days was about 90% of the initial amount of fluoride contained in the microcapsules. The fracture and shear bonding strengths of the orthodontic resin disks with and without the microcapsules were similar to each other. The orthodontic adhesive resin disk containing fluoride loaded microcapsules showed lower bacterial growth than the control groups, whereas no statistically significant differences were found between the negative and positive controls. It can be concluded that the microcapsules loaded with NaF solution prepared by a coaxial ultrasonic atomizer have good potential for application as an antibacterial agent due to their excellent cariogenic antibacterial activity when incorporated into orthodontic adhesive resin.

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Evaluation by PXRD and vibrational spectroscopy of spironolactone solid dispersions prepared by co-spray drying with polymers

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Objective: Solid dispersion is a formulation strategy for enhancing the bioavailability of highly lipophilic poorly-soluble drugs and spray drying is suitable for their preparation [1]. Recently, increased interest has been shown for the use of drug carriers with enhanced solubilizing power alone or in combinations. Spironolactone is a BCS Class II drug used for heart failure and hypertension which due to poor water-solubility exhibits incomplete oral bioavailability. Therefore solubility improvement by formulating into solid dispersions is highly desirable. The aim of this work was to evaluate the applicability of spray drying to prepare solid dispersions of spironolactone with Soluplus® and PVP K30.
Methods: Spray drying was performed with a lab scale spray drier using hydro-ethanolic (1:1) solutions of different compositions of spironolactone (1%), Soluplus® and PVP (total polymer 0-4% w/w). The products were characterised for crystallinity by PXRD and for drug-polymer chemical interactions by vibrational spectroscopy (FTIR & Raman).
Results: Amorphous drug dispersion in the spray-dried products prepared with both polymers was evidenced by the presence of humps only in the PXRD diffraction patterns and by changes in their FTIR spectra showing great reduction of the intensity of the peaks around 2900-3000 cm^{-1} (C-H vibrations) and 1600-1800 cm^{-1} (C=O vibrations) and merging of the drug peaks at 1690 cm^{-1} (C=O of lactone) and 1670 cm^{-1} (C=O of thioacetyl) into one. In the Raman spectra amorphicity was demonstrated by the absence of peak at 1690 cm^{-1} (C=O) and the merging of the double peak at 582 and 600 cm^{-1} (C-S vibration). Formation of molecular solution of drug with Soluplus® was indicated by the appearance of the characteristic peaks of both drug and Soluplus® in the FTIR spectra of their products but not in their corresponding physical mixtures. Additionally, formation of hydrogen bonding between the thioacetyl group of drugs (C=O acceptor) and the -OH of Soluplus® (donor) was indicated by the absence of the peak of drug at 1190 cm^{-1} in the Raman spectrum.
Conclusions: Cospray drying of spironolactone with PVP K30 and Soluplus® resulted in amorphous solid dispersions. Solid solution formation and hydrogen bonding interaction was observed when cospray drying with Soluplus®.

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Microencapsulation of oregano essential oil by spray drying: antimicrobial activity of the spray-dried product

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Objective: To study the antibacterial activity of essential oil of Oregano (EO) cultivated in Greece and having high carvacrol content encapsulated by spray drying using Arabic gum, modified starch and maltodextrin as suitable wall materials. These materials were chosen because of their high oil retention capacity [1]. Properties of the final encapsulated product were compared with that of non-encapsulated EO (in 10 % Tween 80).
Method: Emulsion of EO in water with the wall materials as stabilisers was spray-dried with a small bench type spray dryer. The quality of the final product was examined using GC-MS, vibration spectroscopy and, its antibacterial activity was tested using disc diffusion and broth dilution methods.
Results: The final spray-dried product (EOSD) consisted of particles of narrow size distribution, showed high oil retention (74.9 %), encapsulation efficiency (98.30 %) and retained the high carvacrol content. FT-IR and Raman spectroscopy demonstrated only minor chemical interaction of the EO with the wall materials indicated by a small shift of the peak at 1260 cm^{-1} (trisubstituted aromatic ring). Dissolution of EO into aqueous medium, in the first 15 min, was high from the non-encapsulated form whereas a lag time was noticed for the encapsulated form. However, from 30 min onwards, the release was significantly higher from the latter. Unprocessed EO showed slower dissolution and, in all cases the release was completed within 2h. Dissolution efficiency of EO from EOSD was higher (339.6 mg.h) than that from non-encapsulated (311.6 mg.h) or unprocessed EO (292.4 mg.h). Antibacterial activity of the encapsulated EO after re-emulsification of EOSD for Gram positive (*S aureus*) and Gram negative (*E coli, P mirabilis* and *Klebsiella sp*) bacteria was comparable with that from a non-encapsulated emulsion at high EO. Interestingly, at a low EO concentration, the activity was greater with EOSD which is possibly related to the extended release shown by the dissolution profiles. Furthermore, Minimum Inhibitory Concentrations (mg L^{-1}) for the antibacterial activity of the final product obtained by fitting Gompertz equation to the data (R^2>0.995) ranked as follows: *E coli* (119.8) < *S aureus* (129.0) < *P mirabilis* (132.2) < *Klebsiella sp* (193.0).
Conclusion: Spray drying is a suitable and efficient method for the microencapsulation of Oregano essential oil for protection from oxidization, retention of its volatile constituents, improvement of dissolution efficiency and effective antibacterial activity.

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Anti-inflammatory activity of TAS associated with activation of suppressor of cytokine signaling 1 in human HaCaT keratinocyte cells.

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Keratinocytes have been shown to be involved in skin inflamation associated with the production of pro-inflammatory chemokines [1]. *Triticum aestivum* Lamarck sprouts (TAS) are reported to provide a variety of health benefits and used as a dietary supplement [2]. In this study, we investigated anti-inflammatory activity of TAS on tumor necrosis factor-alpha / interferon-gamma (TI)-stimulated human keratinocyte HaCaT cells. TAS reduced TI-induced expression of chemokines, such as regulated on activation, normal T-cell expressed and secreted (RANTES), macrophage-derived chemokine (MDC) and IFN-γ-induced protein 10 (IP-10). TI-induced phosphorylation of signal transducer and activator of transcription 1 (STAT1) also decreased in the cells by TAS treatment. Furthermore, TAS increased suppressor of cytokine signaling 1 (SOCS1), encoding a member of the STAT-induced STAT inhibitor [3]. In conclusion, these results showed that TAS inhibits expression of inflammatory chemokines, such as RANTES, MDC and IP-10, through inhibition of transcription factor STAT1 phosphorylation by activating SOCS1. Our data indicate that TAS inhibits TI-induced chemokines in HaCaT cells and might be useful as an anti-inflammatory reagent for inflammatory skin diseases.

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P 51

The role of protein tyrosine phosphatases in hepatocellular carcinoma

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Protein-tyrosine phosphatase (PTP) family is involved in multiple cellular functions and plays an important role in various physiological and pathological processes. In many diseases such as cancer PTPs are potential pharmaceutical target for treatment. Dozens of PTP inhibitors were developed in the past twenty years. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and the third most deadly cancer worldwide. Recent studies have unveiled both oncogenic and tumor suppressive functions of PTPs in HCC. Here, we review the current knowledge on the involvement of PTPs in HCC and discuss the possibility of targeting PTPs in HCC.

P 52

Anti-neuroinflammatory effects of SLOH in Aβ-induced BV-2 microglial cells and 3xTg-AD mice involve the inhibition of GSK-3β

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Purpose SLOH, a carbazole-based fluorophore has been shown to inhibit the amyloid-β (Aβ) aggregation via binding with Aβ. In the current study, we aimed to investigate the therapeutic effects of SLOH on Aβ-induced neuroinflammation in BV-2 microglial cells and triple transgenic AD (3xTg-AD) mice.

Methods BV-2 cells were incubated with Aβ in the absence or presence of SLOH (0.3, 1 and 3 μM) for 24 hr. The levels of pro-inflammatory tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and anti-inflammatory cytokine IL-10 were determined using western blot assay. The mRNA levels of TNF-α, IL-1β, IL-10, cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) were measured by qPCR. The protein expressions of iNOS, COX-2, glycogen synthase kinase-3β (GSK-3β) and phospho-GSK-3β (Ser 9) were detected via western blot assay. Moreover, 3xTg-AD mice were administrated with SLOH (0.5, 1 and 2 mg/kg) or vehicle control for one month. Then the mice were sacrificed and the brains were used for the immunofluorescence study of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1).

Results Our ex vivo results showed that SLOH treatment could reduce the activation of GFAP and Iba1 in 3xTg-AD mice. Results from the BV-2 cell studies suggested that the SLOH treatment reduced the production and the mRNA levels of pro-inflammatory TNF-α, IL-1β, and increased IL-10. Moreover, SLOH treatment also decreased the protein expressions and mRNA levels of COX-2 and iNOS. Furthermore, SLOH inhibited the activity of GSK-3β by increasing the expression of phospho-GSK-3β at Ser 9 site.

Conclusions The results demonstrated that SLOH significantly attenuated the neuroinflammation through down-regulating the activity of GSK-3β.

P 53

Neuroprotective effect of linagliptin in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced mouse model of Parkinson's disease

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Parkinson's disease (PD) is the second most common degenerative disorder characterized with reduced dopamine content in the substantia nigra (SN) and striatum. Type 2 diabetes (T2D) is considered as a risk factors that leads to PD, which suggests that drugs useful to treat T2D might be a therapeutic avenue for PD. In our previous attempt, Linagliptin, a dipeptidyl peptidase-4 inhibitor shows neuroprotective activity in a mouse model of Alzheimer's disease. With this context, the present study was designed to elucidate the neuroprotective activity of linagliptin in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. PD was induced in male C57BL/6 mice by five intraperitoneal injections of MPTP and treated with linagliptin. Linagliptin improved the glucagon-like peptide-1 and glucose dependent insulinotropin levels in the brain of MPTP injected mice. Linagliptin treatment for 21 days in MPTP treated mice improved the locomotor activity in open-field test and swim test. Linagliptin treatment restored tyrosine hydroxylase and synaptophysin levels in the brain of MPTP injected mice. Linagliptin treatment also reduced the apoptosis signaling Bcl-2 in the brain. Altogether, linagliptin improves locomotor activity, protects dopamine neurons and inhibits the apoptosis by improving GLP-1 and GIP levels in the SN. The present study shows a promising PD therapy with linagliptin.

P 54

Identification of Novel M1 – selective muscarinic receptor modulator using virtual screening

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Alzheimer's disease (AD) is of serious health concern across the globe. Owing to the complexity in its pathogenesis current line of treatment only provides symptomatic relief. M1 agonists not only provides the relief, but are known to target the three major hallmarks of AD, *i.e.* Aβ, τ, and cognitive debilities. However, co-activation of other mAChR subtypes poses a major challenge towards its development. Targeting allosteric site has therefore spurred research interest in this direction. In the absence of the active state crystal structure, we developed a homology model of the active M1-mAChR based on M2-mAChR PDB id 4MQT as the template using web server Swiss-Model. Acetylcholine and LY2033298 (a known allosteric modulator) were docked at the orthosteric and allosteric sites. Approximately 3, 00,000 molecules (downloaded randomly) from the ZINC database and 30,000 molecules from the ChemBridge diverse set library were docked using LibDock to the allosteric site. Top forty molecules from the above screening were further docked to 4MQT. Herein, we selected the molecules with the least affinities for the M2-mAChR to rule out its co-activation. Five novel classes of molecules were hence identified as M1-selective Muscarinic receptor modulator. Molecular dynamics simulations were further performed to access the affinity of these molecules and its effect to the acetylcholine binding site.

P 55

Value of intermediates in drug design – example of novel *L*-ascorbic acid derivatives

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In our search of new hybrids of *L*-ascorbic acid and diverse pharmacophoric scaffolds as potential anticancer agents, novel conjugates of 1,2,3-triazole and *L*-ascorbic acid are synthesized using Cu(I) catalyzed click reaction (CuAAC) [1,2]. Herein considered dibenzylated compounds are obtained as intermediates in the synthesis of target derivatives of vitamin C. The library of compounds with reasonable ADME/Tox profile is designed in a way to span physicochemical space by varying a substituent at position R^1 of 1,2,3-triazole ring and by elimination of C5-OH group and introduction of C4=C5 double bond (Fig. 1).

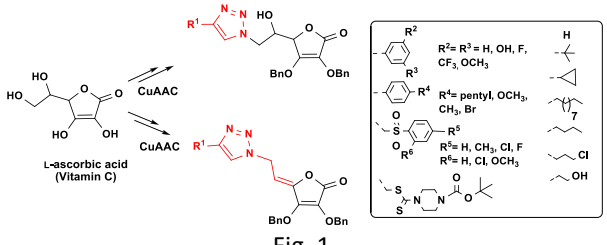

Fig. 1.

The lipophilicity, aquaous solubility and ADME/Tox properties of novel compounds are predicted by using freely available algorithms. Most of the dibenzylated intermediates show antiproliferative activities. The effects of structural modifications on their MTT IC_{50} values on several cancer cell lines are analysed by multivariate statistical analysis. The results of *in vitro* screening and *in silico* analysis will be presented.

Acknowledgement: *The studies were supported by the Croatian Science Foundation Project No. 5596.*

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P 56

Synthesis, structure, antibacterial and anticancer activity of new macrolide antibiotics analogs

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Macrolide antibiotics are large group of natural products produced by various *Streptomyces* strains. They are used against various infectious diseases. Macrolides can be classified by a lot of different criteria. One of them is type and size of the macrolide ring[1] as for e.g. lactone macrolides 14-membered erythromycins (fig. 1), 15-membered azithromycins (fig. 2), and 16-membered spiramycins (fig. 3), or leucomycins (fig. 4), or lactam macrolides as 26-membered rifamycines as rifampicin (fig. 5). Apart from these criteria, semisynthetic and natural macrolide antibiotics, especially lactone ones, have aglycone joined to the different saccharide moieties as *e.g.* mycaminose, mycarose, cladinose, forosamine, desosamine.[2] The macrolide lactone antibiotics' mechanism of action is based on the inhibition of bacterial protein biosynthesis at different stages by reversible binding to the bacterial 50s subunit at the ribosome[3] whereas macrolide lactam antibiotics mechanism of action as rifamycins depends on inhibition of bacterial RNA polymerases dependent on DNA[4]. Macrolide antibiotics are mainly bacteriostatic, but at higher concentrations they reveal bactericidal properties. In our laboratory, we work on new modifications of lactam and lactone macrolide antibiotics, of an improved binding profile to biological target and of increased antibacterial potency. Our modifications are performed using cascade and click approaches to enable better matching between antibiotic and target enzyme/protein. For example, thorough changes at aglycone ring *via* complete reconstruction of saccharides parts using regio- and diastereoselective cascade combination of intramolecular esterifications followed by tandem E1cB eliminations and subsequent 1,2-addition to carbonyl followed by 1,6-conjugate addition α, β, γ, δ-unsaturated aglycone led to entirely new series of macrolide antibiotics of antibacterial and anticancer potential.[5,6] We try to apply this approach to modification of another group of natural macrolide antibiotics like 14-membered lactone erythromycins to obtain efficient alternatives to the currently used antibiotics in clinical therapy.

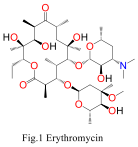

Fig.1 Erythromycin

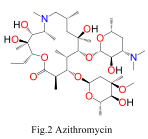

Fig.2 Azithromycin

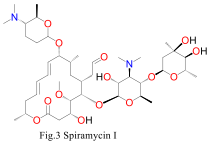

Fig.3 Spiramycin I

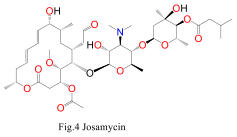

Fig.4 Josamycin

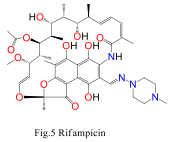

Fig.5 Rifampicin

Acknowledgements: *The project is financially supported by Polish National Science Centre (NCN), No. UMO-2015/19//B/ST5/00231.*

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P 57

The influence of the plant food fibers and "MILKYBOOM-PLUS" on the children's reactivity of nursery schools from 3 till 6 years old

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The goal of the work is to study an influence of the food product "MILKYBOOM – PLUS" and «BAPOL» to reactivity of the preschool children aged 3-6 years old.
137 children aged 3-6 years old have been investigated at the kindergarten № 41, Astana. The ration of the children was enriched by foods "MILKYBOOM-PLUS" and "BAPOL". All investigated children were selected by sex and age, and the kind of the food product in their ration. The parameters of the physical health, adaptation system, reactivity, and nutrition practice were examined. The following modern investigation methods like as questionnaires for morbidity rate, nutrition practice; clinical and laboratory, anthropometric, and statistic methods were used.

Theoretical and practical value:

The sourmilk product "MYLKYBOOM – PLUS" lead to improvement of physical development, reactivity, adaptation mechanisms due to stabilization of the respiratory, cardiovascular systems, and also increasing of energetic, biologic value of ration.
On the background of "BAPOL" consuming the content of food fibers, mineral substances (potassium, natrium), and vitamin E was increased. Food fobers in ration lead to improving gastrointestinal tract functions, adaptation potential, immunity.

- Within research work the following was established:
- Low health rate, disadvantage of physical growth, reduction of functional and immunologic reactivity of the children in the kindergartens of Astana city are associated with the deficiency of quality provisions in food ration.
- Children (3 to 6 years) administered with "MILKYBOOM-PLUS" were found to have an increased physical development balance; children administered with dietary fibers "BAPOL" were found to have stabilized congruence of physical development, improved cardiovascular and respiratory systems and their adaptation potential, as the result the reactivity of an organism had been improved.
- Baby foods enrichment (children at the age of 3 to 6 years) with dietary fibers "MILKYBOOM-PLUS" and "BAPOL" improves dietary status, metabolism and reduces level of morbidity.

So, application the sour milk product "MYLKYBOOM – PLUS" and food product "BAPOL" to improve children's health and alimentary dependent diseases prevention is an essential and suitable at the kindergartens, children's oup-patient clinics, and hospitals.

P 58

Rapid biomimetic screening of drug-membrane affinity using IAM HPLC

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Bio-Mimetic Chromatography Consultancy
**Regis Technologies, Inc.*

As phospholipids are major components of tissues and cells, drug interaction with phospholipids is an important contributor to distribution. Immobilized Artificial Membrane (IAM) chromatography can be used to quickly measure drug -phospholipid interactions via retention times.

P 59

Late arrived

Solubility-pH profiles of a free base and its salt: sibutramine as a case of study

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**Polymorphism and Calorimetry Unit, Scientific and Technological Centers, Universitat de Barcelona, Spain*

The crystalline racemic compound sibutramine hydrochloride monohydrate was used as the active pharmaceutical ingredient of medicines for the management of obesity from 1999 to 2010. Its withdrawal from European and US markets was due to cardiovascular side effects. However, the physicochemical properties of sibutramine make it a very interesting model for the solubility study of basic compounds.

In the present work the solubility-pH profiles of the free base and its hydrochloride salt have been determined at 25°C in the pH range between 2.0 and 9.5 by means of the shake-flask method and using the recommended protocol [1]. Briefly, minimalist universal buffer (MUB) consisting of 25 mM acetic acid, 25 mM ethylenediamine, and 75 mM trifluoroacetic acid was used, incubation was carried out for 48 h (24 h stirring and 24 h sedimentation), pH was measured after 4h of stirring and readjusted if necessary with sodium hydroxide or hydrochloric acid, and finally the pH was measured again before the phase separation by centrifugation. Obtained solids were dried and studied by X-ray diffraction in order to elucidate their free base or salt structure. With the aim of evaluating the effect of the MUB components on the precipitated solid, simple buffers prepared from acetic acid, trifluoroacetic acid, and hydrochloric acid were used as well.

The Henderson-Hasselbalch profile was constructed from the potentiometrically determined pK_a and the intrinsic solubility measured by the CheqSol method, showing a good agreement with the shake-flask profile in the pH range above pH_{max} (or *Gibbs* pK_a) for both sibutramine species (free base or hydrochloride salt) initially solved. As expected, in this pH region the solid collected was always identified as free base. However, in the pH range below pH_{max} two different solids were isolated depending on the buffers employed, the hydrochloride and the TFA salt.

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