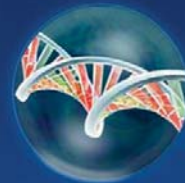
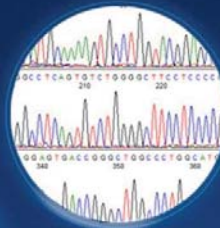
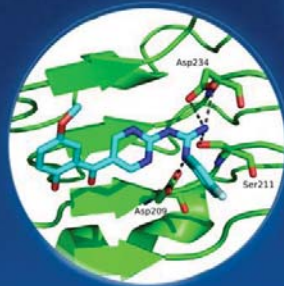




Research

Faculty of Pharmacy, University of Ljubljana, Slovenia



*Imagination is more important
than knowledge.*

Albert Einstein



ABOUT THE UNIVERSITY OF LJUBLJANA

University of Ljubljana (UL, www.uni-lj.si) has the longest tradition in the university education and the highest number of students in the Republic of Slovenia. The UL is an institution with a strong international profile, innovative and reform oriented. It was established in 1919 with five faculties; today it integrates 3 art academies and 23 faculties (the UL Members) with 45.607 students and 5920 higher education teachers, researchers, assistants and administrative staff (in the academic year 2013/2014). The UL is a comprehensive and integrated university, covering all the ISCED and FRASCATTI disciplines and therefore covers diverse individual interests and goals. The UL implements higher education activities based on research in all fields of science and art, such as humanities, social sciences, biomedicine, natural sciences, technology and engineering and arts. However, in addition to the reformulation of study programmes during the Bologna reform, the UL Members implemented a number of new programmes, which were created as a result of environmental needs and new scientific discoveries. Being fully aware of the importance of knowledge and skills in new global environment, the UL is increasingly and systematically developing innovation oriented activities. The UL has close ties with the Slovenian and with foreign enterprises. Additionally, the UL is preparing for the future challenges with intra- and international partnerships, exchange of good practices, introduction of new teaching and learning methods, including information and communication technologies, strengthening the quality culture, all with the aim to achieve higher number of innovations, higher degree of creativity and sustainability. The University of Ljubljana is listed amongst the top 500 universities in the world according to the ARWU Shanghai, 550-600 on the Times THES-QS and 192 on the WEBOMETRICS rankings.



ABOUT THE FACULTY OF PHARMACY

University of Ljubljana, Faculty of Pharmacy (UL FFA, www.ffa.uni-lj.si) is the leading research and higher educational institution in the field of pharmacy, clinical biochemistry, toxicology and cosmetology. Education and study offers broad spectrum of theoretical and applied subjects, whereas the research policy of the faculty focuses on interdisciplinary approach in academic disciplines representing the major future challenges. Medicinal chemistry, clinical biochemistry, and in particular pharmacogenomics, pharmaceutical technology with pharmacokinetics, and pharmaceutical biotechnology together with the broad field of nanotechnology and toxicology, represent key disciplines shaping current education and research profiles. The research of advanced drug delivery systems with novel active ingredients for the treatment of different diseases (inflammation, cancer, and cardiovascular, infectious and autoimmune diseases), drug transport through biological barriers, toxicological methods, and diagnostic markers markedly increase reputation of the pharmaceutical science at the UL and broader. The academic research occupation is closely related with economy and industry policy providing an important platform for novel development and cooperation. Research is based on current EU-GMP and FDA guidelines and other international standards. The objective of the UL FFA is to aggregate and improve the current fragmental research work; to initiate activities to enhance pharmaceutical competence on the main topics related to developing and offering new medicines; to build the capacities for facilitation knowledge transfer related on specific strategies and scalable services; to find integrated solutions in pharmacy, clinical laboratories, and industry.

ISOLATION AND IDENTIFICATION OF PLANT SECONDARY METABOLITES

Plant secondary metabolites are responsible for therapeutic activity of medicinal plants, for their side effects and also for their nutritional, aromatic and toxicological properties.

For assuring the appropriate quality of herbal medicinal product (and food supplements and food), it is important to know the identity of these secondary metabolites and to employ the appropriate analytical methods. In our group, we use various chromatographic techniques to isolate and analyse these substances, spectroscopic techniques to identify them and bioactivity assays to characterise them (antimicrobial, enzyme inhibitory, antioxidative, organoleptic):

1. isolation and structure elucidation of phototoxic fagopyrins from buckwheat herb (*Fagopyrum esculentum*),
2. isolation and structure elucidation of antioxidative polyphenols from white fir (*Abies alba*) bark extract,
3. identification and quantification of aroma compounds of tartary and common buckwheat (*Fagopyrum tataricum* and *Fagopyrum esculentum*),
4. identification of herbal samples by multivariate statistical analysis of IR spectra obtained with direct transmission, KBr tablet, diffuse reflectance and ATR.

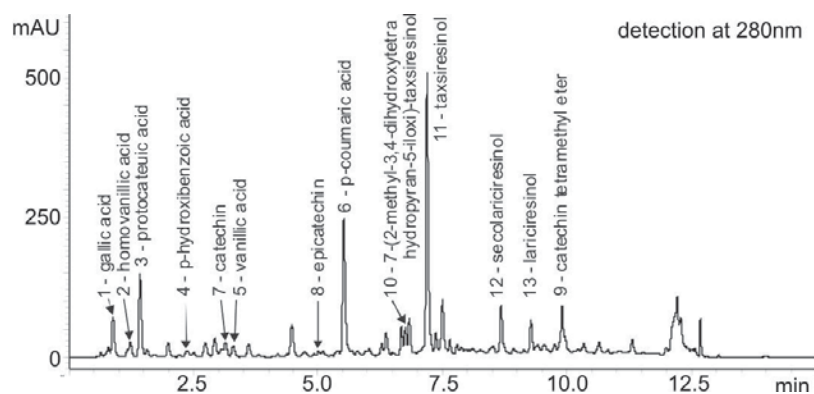


Figure 1: Polyphenols from white fir

Key words: herbal medicinal products, phytochemistry, capillary electrophoresis, GC-MS

Capacities:

- Development, optimisation and validation of analytical methods
- Isolation and structure elucidation of plant secondary metabolites
- Optimisation of herbal extract production for industry

PHARMACEUTICAL BIOTECHNOLOGY

Pharmaceutical biotechnology is rapidly expanding scientific and professional discipline in pharmacy, drug development, and medicine diagnostics. Our group is focused on the screening and selection of peptides with affinity to target proteins by means of phage display technology. One of the important parts of our research covers gene cloning and development of surface-expressed recombinant probiotics for therapy against irritable bowel syndrome, including Crohn's disease and ulcerative colitis. In addition, different screenings of pharmacologically interested natural products, peptides and proteins with the activity on various physiological targets are performed:

1. peptidic ghrelin and leptin modulators by phage display technology,
2. screening for natural inhibitors of alpha-glucosidase and alpha amylase,
3. novel peptide inhibitors of DPP4 protease,
4. recombinant probiotics with anti TNF-alpha, anti IL12 and anti IL-23 activity.

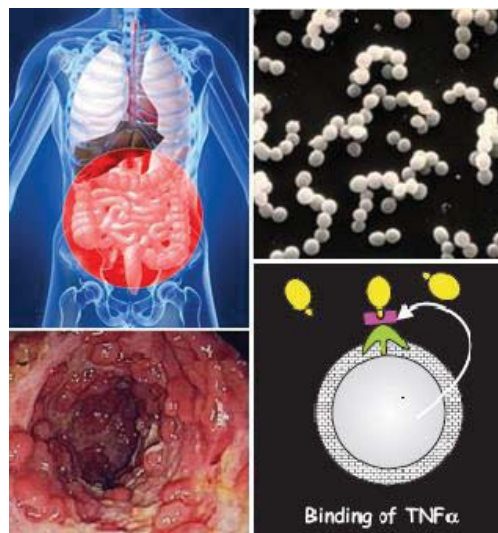


Figure 2: GIT; recombinant *Lactococcus lactis*; irritable bowel; and schematic presentation of mechanism of TNF α binding by recombinant probiotic (from left to right, from up to down)

Key words: recombinant proteins, phage display, recombinant lactic acid bacteria

Capacities:

- Gene cloning and expression
- Development of recombinant *E. coli*, recombinant lactic acid bacteria
- Isolation and identification of natural products (fungal, plant, animal)

PEPTIDASE SIGNALLING IN CANCER, NEURODEGENERATIVE AND IMMUNE DISORDERS

Peptidases are key molecules in regulation and turnover of proteins. We investigate the role of cysteine peptidases in development and progression of cancer, neurodegenerative diseases and immunological disorders. By determining natural substrates and molecular mechanisms involving particular peptidase, we search for the targets being relevant for therapeutic and diagnostic interventions. In our research, we apply advanced methods of molecular and cell biology including in vitro and in vivo models of tumour migration, invasion, angiogenesis, metastasis, neuritogenesis, and neurodegeneration. Additionally, we develop inhibitors and systems for their delivery to targeted cells. Research:

1. characterisation of cysteine proteases and their endogenous inhibitors in normal and pathological cells and tissues,
2. regulation of cysteine proteases by endogenous and exogenous inhibitors,
3. development of immunoassays for quantification of proteases and inhibitors in clinical samples,
4. delivery systems for active cell targeting.

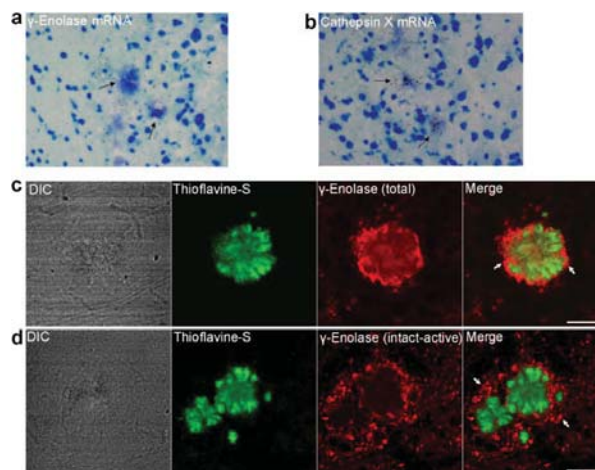


Figure 3: Cysteine peptidase Cathepsin X and its target γ -enolase in amyloid plaques in brain. a,b in situ hybridization of γ -enolase and cathepsin X; c,d co-localisation with Thioflavine marker (Aging Cell, 2013,12,604-614)

Key words: peptidases, cathepsins, cystatins, inhibitors, cancer, neurodegenerative disorders, immune disorders

Capacities:

- Molecular and cell biology
- Design, preparation, isolation and characterisation of recombinant proteins
- Development of immunoassays
- Development of ligand-based delivery systems for active targeting of cells and tissues
- Live cell imaging and fluorescence confocal microscopy

CUSTOM SYNTHESIS OF BUILDING BLOCKS, BIOLOGICALLY ACTIVE SMALL MOLECULES, FLUORESCENT/SPIN LABELS AND PROBES

Our group has long experience in the development of novel synthetic pathways and organic synthesis of small molecules (building blocks, intermediates and biologically active compounds). Compound classes belong to heterocycles, peptides, peptidomimetics, glycomimetics, steroidomimetics, lipidomimetics, stable nitroxide radicals, natural products and their analogues, which are prepared using classical as well as modern organic synthesis methods (microwave-assisted, parallel, solid-phase synthesis). We also provide custom synthesis of fluorescent/spin labels and probes (pH-sensitive probes, redox dependent probes, probes sensitive to the polarity of the environment, labels for polymers, membranes and proteins). Classical isolation and purification methods (TLC, column chromatography, HPLC, size-exclusion chromatography) in combination with characterisation by spectroscopic methods (NMR, MS, IR, EPR, UV-VIS, fluorescence spectroscopy, polarimetry) are used to provide compounds of desired structure and purity.

Recent examples of our research include:

1. process for the synthesis of **ezetimibe** and intermediates,
2. preparation of **sitagliptin** intermediates,
3. process for synthesis of **pramipexole** and its pharmaceutically acceptable salts,
4. total synthesis of **clathrocin**, **oroidin** and **hymenidin** alkaloids from *Agelas* sponges,
5. nitroxide-fluorophore **double probes** as tools for studying membrane heterogeneity by ESR and fluorescence.

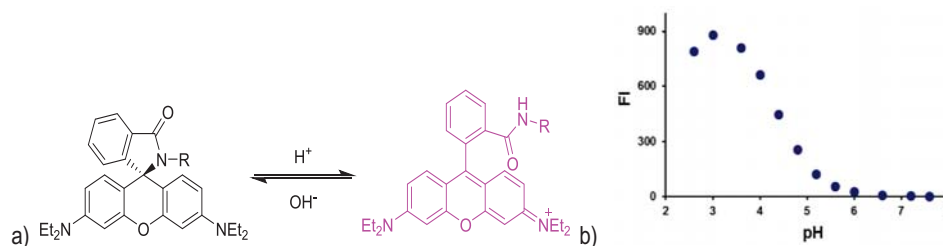


Figure 4: Example of a pH-dependent probe (a) and the effect of pH on fluorescence (b).

Keywords: building block, fluorescent/spin probe, glycomimetic, heterocycle, microwave-assisted synthesis, organic synthesis, peptidomimetic, steroidomimetic

Capacities:

- Optimisation of particular chemical reaction
- Checking of described (patented) synthetic procedure
- Proposal of patent-independent synthetic pathways
- Synthesis of standards or intermediates in short time
- Synthesis of natural products
- Custom made fluorescent/spin labels and probes
- Structure determination

COMPUTER-AIDED DISCOVERY AND DESIGN OF NOVEL BIOLOGICALLY ACTIVE COMPOUNDS

Computer-aided discovery and design is a rapidly evolving and increasingly utilised methodology in rational drug design. It is commonly used for identification of hit compounds possessing desired biological activity, optimisation of affinity and selectivity of hit compounds to leads, and optimisation of other pharmaceutical properties (physicochemical properties, off-target activity) while maintaining affinity of lead compound. We have a broad experience in molecular modelling ranging from database preparation, virtual screening and molecular docking, to molecular dynamics simulations and homology modelling using up-to-date software and hardware.

Recent examples of our computer-aided discovery and design include:

1. homology modelling, ligand- and structure-based virtual screening for human voltage-gated sodium channel modulators,
2. novel toll-like receptor 4 (TLR4) antagonists identified by structure- and ligand-based virtual screening,
3. selective inhibitors of aldo-keto reductases AKR1C1 and AKR1C3 discovered by virtual screening of a fragment library,
4. virtual screening identifies antibiotic nitroxoline as cathepsin B inhibitor,
5. discovery of novel inhibitors of peptidoglycan biosynthesis (Ddl, MurC-F, penicillin-binding proteins) by structure-based virtual screening.

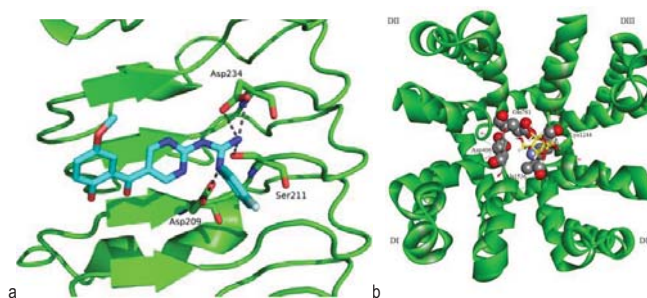


Figure 5: a) Novel TLR4 antagonist identified by virtual screening (*Eur J Med Chem*, 2013, 70, 393); b) Homology model of human voltage-gated sodium channel 1.4 used for structure-based virtual screening (*J Chem Inf Model* 2013, 53, 3223).

Keywords: database filtering, database preparation, homology modelling, ligand docking, molecular dynamics, virtual screening

Capacities:

- Discovery of hit compounds using ligand- and/or structure-based virtual screening of compound libraries
- hit-to-lead optimisation of affinity and selectivity
- studying protein-ligand interactions by molecular docking
- homology modelling of proteins with unknown structure
- molecular dynamics of proteins in water and/or lipid environment

STRUCTURE-BASED DESIGN OF NOVEL ENZYME INHIBITORS AND RECEPTOR MODULATORS

In structure-based design of biologically active compounds three-dimensional structure of the biological target (determined by X-ray crystallography, NMR spectroscopy, homology modelling) is used to predict, which candidate molecules will bind to target with high affinity. Iterative cycles of design, synthesis and biological evaluation of selected compounds are used to optimise affinity, selectivity and physicochemical properties of compounds. Our group has recently been successful in the design, synthesis, optimisation and biological evaluation of several structural classes of compounds acting on enzymes, receptors, ion channels and membranes. A part of the results was achieved by fruitful collaboration with partners from EUR-INTAFAR (6th Framework Programme), ORCHID and MAREX (7th Framework Programmes) and other projects.

Recent examples of our research include structure-based design of novel:

1. antibacterial agents (inhibitors of peptidoglycan biosynthesis, type IIa topoisomerases, InhA)
2. voltage-gated sodium and potassium channel modulators,
3. anti-adhesion agents (DC-SIGN and FimH antagonists),
4. anti-Alzheimer's agents (cholinesterase inhibitors),
5. inhibitors of enzymes involved in blood coagulation cascade (thrombin, factor Xa inhibitors),
6. inhibitors of enzymes involved in steroid hormones transformation (17 β -hydroxysteroid dehydrogenase type 1 inhibitors).

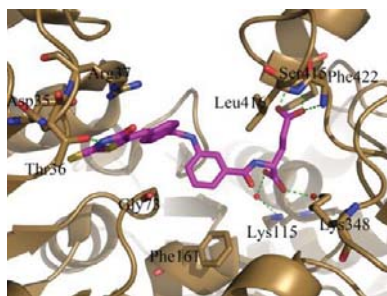


Figure 6: Crystal structure of MurD-inhibitor complex obtained during structure-based design of novel inhibitors of intracellular steps of peptidoglycan biosynthesis (ACS Med Chem Lett, 2012, 3, 626).

Keywords: agonist, antagonist, inhibitor, ion channel, protein, receptor, structure-based

Capacities:

- Structure-based design of enzyme inhibitors, receptor agonists and antagonists, ion channel modulators
- Hit-to-lead optimisation of affinity and selectivity
- Optimisation of physicochemical properties while maintaining affinity
- *In vitro* assays on various enzymes and receptors
- Molecular toxicology

MECHANISTIC TOXICOLOGY AND THE ROLE OF METABOLISM IN TOXICITY

Mechanistic toxicology describes the processes of how xenobiotics exert their toxic effects in biological systems and aims at identifying the underlying molecular events that lead from initial exposure to the chemical to the ultimate manifestation of toxic injury in an organism. The aim of our group is to understand how xenobiotics enter an organism or a cell, how are they metabolized, interact with target molecules, and exert their toxic effects at the molecular level and what are the downstream biological consequences for the organism.

Recent examples of our mechanistic toxicology studies include:

1. reactive metabolites study of bisphenol A analogs and bazedoxifene,
2. bisphenol S glucuronidation; the central role of UGT1A9,
3. genome-wide gene expression profiling of low-dose, long-term exposure of human osteosarcoma cells to bisphenol A and its analogs bisphenols AF and S,
4. estrogenic and androgenic activities of novel brominated flame retardants, and selected bisphenols, using the XenoScreen XL YES/YAS assay,
5. reactivity of bisphenol A-3,4-quinone with DNA, a quantum chemical study,
6. molecular docking of potential disruptors of the glucocorticoid receptor-dependent reporter gene expression,
7. Endocrine Disruptome – an open source prediction tool for assessing endocrine disruption of nuclear receptors.

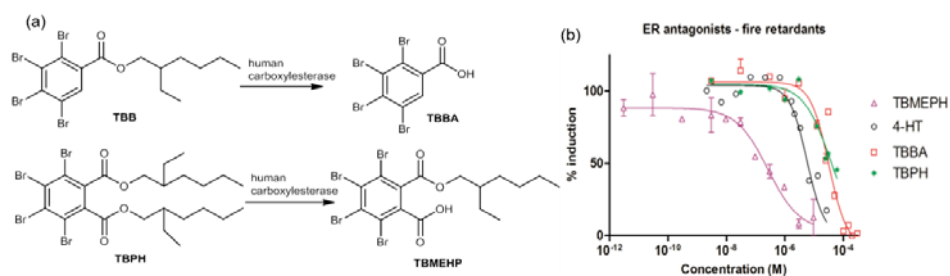


Figure 7: (a) Structures of flame retardants TBB, TBPH and their metabolites. (b) Anti-estrogenic activities of TBPH, TBMEPH, and TBBA using XenoScreen XL YES/YAS assay.

Keywords: hormone disruptors, bisphenol A analogs, novel flame retardants, reactive metabolites, toxicity, microarrays

Capacities:

- *In vitro* evaluation of (anti)estrogenic, (anti)androgenic, (anti)thyroid, and (anti)glucocorticoid activities
- Mechanistic toxicology studies
- Use of alternative methods for finding new endocrine disruptors and explaining their mechanisms of action
- *In vitro* determination of reactive metabolites formation using LC-MS/MS
- Toxicity studies in the early stages of the drug discovery process

PHARMACEUTICAL ANALYSIS

Quality of medicines is a goal that requires continuous efforts for improvements and knowledge from modern manufacturers and is supported by vast amount of data obtained by diverse analytical methods. The paramount of successful analytical method development and implementation lies in a strong scientific knowledge about substance(s) chemistry, structural features, reactivity, routes of preparation (chemical synthesis, isolation from natural sources,...) and physical properties. This must be accompanied by *in depth* familiarity with analytical techniques, their scopes and limitations, as well as understanding method's purpose and relevant regulatory requirements that it must fulfil.

With all of the above and a broad portfolio of analytical techniques ranging from chemical methods (derivatization reactions, volumetric analysis), physical methods (optical rotation, pH), spectroscopy (UV/VIS, IR, NMR) to chromatography (GC, (U)HPLC, prep LC,) we can provide analytical support to all aspects of pharmaceutical discovery, development and manufacturing. The key to successful cooperation lies in strong communications with clients throughout the project to understand their needs and provide adequate solutions.

Our track of successful cooperation with pharmaceutical industry includes:

1. development of analytical methods, examples include assay of drug substance, impurities in various product forms by HPLC, control of genotoxic impurities by derivatization and HS-GC, *in-process* control of impurities during API manufacture,
2. validation of HPLC analytical methods for assay of drug substance and impurities
3. Investigating *Out-Of-Specification* (OOS) results of product batches: detailed ^1H and ^{13}C NMR studies of different batches and different vendors of polymeric coating agent
4. deformulation of multidrug tablets: quantitation and structure of multiple excipients,
5. quantitative study of API polymorphism by ATR FT-IR,
6. implementation of Raman spectroscopy for fast identification of raw materials,
7. isolation and structural characterization of new impurity from drug substance which emerged after new HPLC method for impurity analysis was introduced.

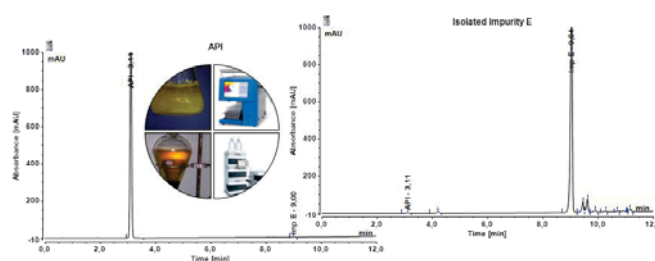


Figure 8: Example of successful industrial project: novel impurity that was present in API at about 0.5% level was isolated, identified and used as a reference for HPLC method validation

Key words: analytical method development, identification, assay, impurities, specifications

Capacities:

- Development of reliable, robust and cost-effective analytical methods: all aspects from sample preparation to setting system suitability criteria
- Validation of novel analytical methods and verification of compendial methods
- Preparation and determination of structure and physicochemical properties of substances, support in setting specifications
- Scientific approach towards OOS investigation with full attention and absolute priority
- In line with current trends most skills from pharmaceutical analysis can be adopted to quality assurance of nutritional supplements and cosmetic

PREFORMULATION STUDIES: TAILORING AND ENGINEERING OF DRUGS INGREDIENTS

Design and production of optimal drugs require detailed knowledge about all relevant chemical, physical and mechanical properties of included active pharmaceutical ingredient (API) and excipients. When needed a tailoring approach for modifying of these properties is used: liquid or melt (co)crystallization, (co)milling, (co)spray drying, lyophilization and spherical agglomeration. For these modifications, modern technologies, which are environmentally acceptable and industrially applicable, i.e. spray drying technologies, particle precipitation in the microreactor, melt granulation, melt congealing, mechano-chemical methods, supercritical fluids are used and have an impact on the solubility, dissolution rate, physical and mechanical properties.

Objectives:

1. Preparation of amorphous and crystalline API forms and quantitative characterisation by ATR FT-IR
2. Modification of APIs and excipients particle properties
3. Preparation of co-processed materials from APIs and excipients
4. Determination of correlation between relevant physicochemical properties of (co)processed components at the level of particles with those at the bulk level, which allows faster development of medicinal products.
5. Establishment of solids surface properties
6. Establishment of solids mechanical characteristics (hardness, elasticity) relevant for the technologies used (milling, mixing, compression)

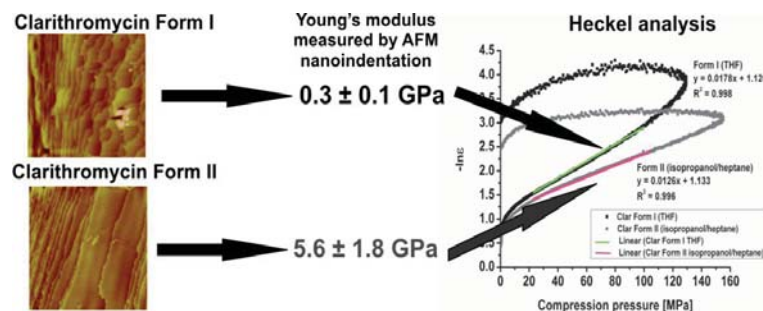


Figure 9: Mechanical properties of active pharmaceutical ingredient obtained by atomic force microscopy nanoindentation and Heckel analysis

Key words: physicochemical properties of active ingredients and excipients, thermal analysis, surface free energy, particles mechanical properties, dissolution rate

Capacities:

- Solid surfaces characteristics (IGC, wettability, AFM)
- Solubility and dissolution improvement
- Polymorphism and amorphous state research (DSC, ^{14}N NQR, SEM)
- Mechanical properties of APIs and excipients (nanoindentation, AFM)
- Compressibility and compactibility studies of APIs and excipients (Heckel, Walker, Kawakita)
- Supporting industry with integrated process solutions

SOLID DOSAGE FORMS AND COMPUTATIONAL SIMULATIONS

Incorporation of active pharmaceutical ingredient (API) into final solid drug delivery systems is an important approach to achieve optimal clinical outcomes. Modification of conventional dosage forms represent a way in solid dosage form design mainly aimed to overcome some therapeutic obstacles such as controlled release, impaired swallowing and polypharmacotherapy. These systems offer some therapeutic benefits such as dose flexibility and combined release patterns. Computational fluid dynamics (CFD) is a branch of fluid mechanics that uses numerical methods and algorithms to solve and analyze problems that involve fluid flows. The research group is using CFD for simulation of pellets' coating in Wurster chamber. With PAT approaches (picture analysis, FBRM) pellets' film thickness increase in Wurster and granulating process in FBD are monitored in line.

Objectives:

1. Preparation of granules, pellets, capsules and tablets
2. Mini and (oro)dispersible tablets, flexible dosing, controlled release and taste modification
3. Modified release dosage forms for oral and time controlled drug delivery (chronotherapeutics, floating systems)
4. Medicated wafers and films for oral/oromucosal application
5. Microcapsulation
6. CFD simulation for getting the detailed knowledge of the process
7. PAT approaches for controlling the process

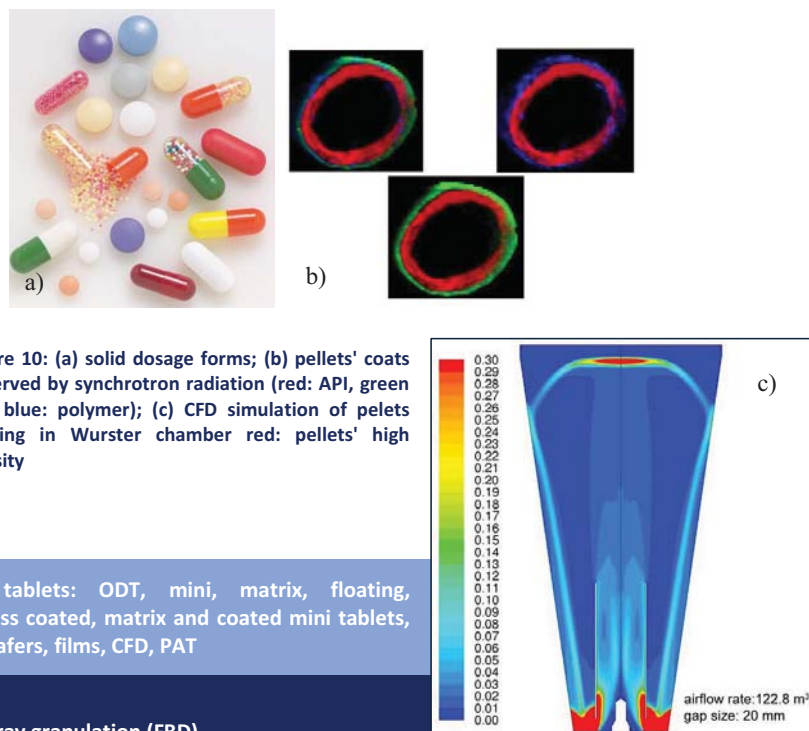


Figure 10: (a) solid dosage forms; (b) pellets' coats observed by synchrotron radiation (red: API, green and blue: polymer); (c) CFD simulation of pellets moving in Wurster chamber red: pellets' high density

Key words: tablets: ODT, mini, matrix, floating, multilayer, press coated, matrix and coated mini tablets, pellets, Oral wafers, films, CFD, PAT

Capacities:

- Top spray granulation (FBD)
- Bottom spray (Tornado) granulation (FBD)
- High shear granulation
- Swirl Wurster coater(FBD)
- Tornado chamber coater(FBD)
- Rotor chamber coater (FBD)
- Spray congealing (spray dryer)
- IMA instrumented single punch tablet machine
- Pan coater
- Microcapsulator

LIPID BASED FORMULATIONS AND MICROEMULSIONS

Poor water solubility of the API is a growing challenge to the development of new pharmaceutical products. One of the most promising novel approaches for solubility enhancement are lipid based formulations such as self-micro- or self-nano-emulsifying drug delivery system (SMEDDS / SNEDDS). Lipid based formulations are principally liquid dosage forms. The transformation of liquid SMEDDS / SNEDDS into solid dosage forms combines the advantages of lipid based formulations such as enhanced solubility and bioavailability, with those of solid dosage forms, such as low production cost, convenience of process control, high stability and reproducibility, and improved patient compliance.

Upon *in vivo* conditions in GIT (SMEDDS / SNEDDS) are transformed into O/W microemulsions. The latter are also considered as advanced drug delivery systems for dermal/transdermal application representing non-invasive approach for systematic action and being especially appropriate for paediatric and geriatric population. Microemulsions are dispersions with high solubilisation capacity and ability to incorporate lipophilic, hydrophilic and amphiphilic drugs. They are thermodynamically stable, showing increased permeability of cell membrane due to presence of surfactants and they are easy to prepare. However, their characterisation is far away to be simple.

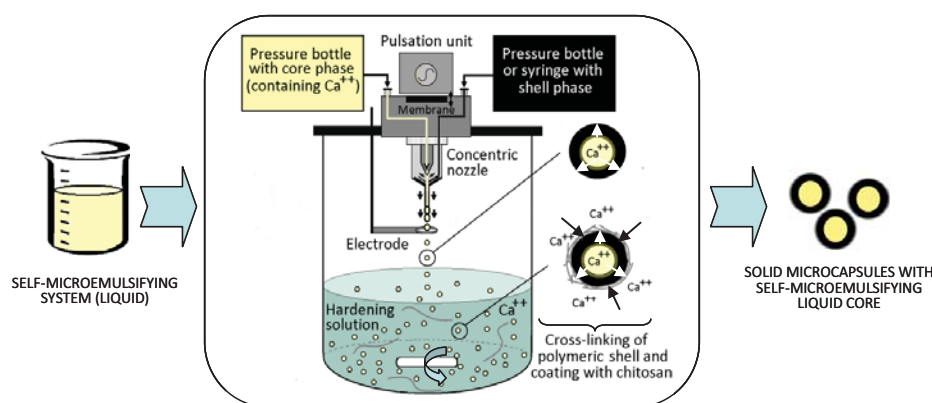


Figure 11: Preparation of solid microcapsules from liquid SMEDDS by vibration nozzle method

Key words: lipid formulations, SMEDDS, SNEDDS, phase diagram, structure characterization

Capacities:

- Development and evaluation of SMEDDS/SNEDDS including *in vitro* lipolysis testing
- Transition of liquid SMES into solid dosage forms (microencapsulation, coextrusion of liquid jet by vibrating nozzle device, adsorption on solid carriers and granulation/tableting, spray drying)
- Development and characterization of micro (nano) emulsions and lyotropic liquid crystal including release/permeation (using pig ear skin/skin constructs) and *in vitro* cell toxicity studies
- Instrumental support for microemulsions characterizations (DSC, SAXS, surface tension, density, electron microscopy – TEM, SEM, AFM)
- Development of patent non-infringement formulation

NANOTECHNOLOGY AND NANODELIVERY SYSTEMS

Nanotechnology has been gaining interest within drug formulation and drug delivery to serve specific therapeutic purposes. This approach creates opportunities to tailor the functionality of a wide range of delivery systems and devices for various administration routes. Nanodelivery systems (NDS) can, due to their nanometer size, reach the cellular level and act directly on cells and their subcellular structures, thus can realize drug delivery challenges, which cannot be resolved using the classical approaches.

The main objectives of our research group are the development and evaluation of:

1. NDS (nanosuspensions, liposomes, polymeric and solid lipid nanoparticles, nanofibers) for passive and active targeted therapy
2. Thermoresponsive hydrogels with nanocomplexes for prolonged drug delivery
3. SPION-based nanotheranostics
4. Nanofibres for chronic wound treatment and tissue regeneration
5. NDS transport inter- and intracellularly and localization on cell or organel level
6. Mild condition based technologies for production of NDS with biopharmaceutics
7. Industrially applicable and environmentally friendly technologies

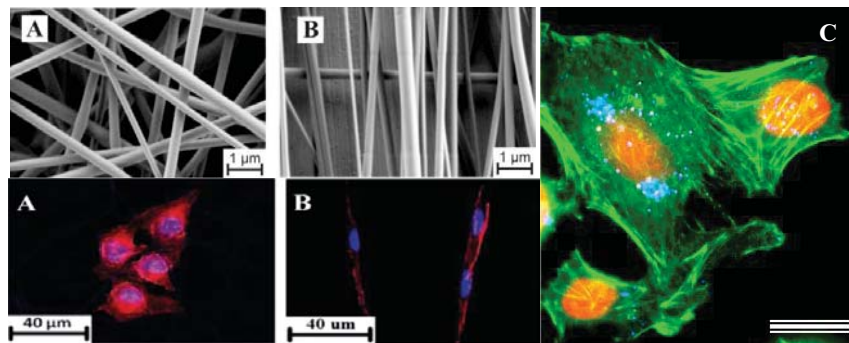


Figure 12: Nanofibers - Randomly -A and aligned -B nanofibers and keratinocytes morphology grown on them (EJPB 2013; 84: 401-411). C - Imaging of intracellular localization of solid lipid nanoparticles (Int J Pharm 2011, 416, 384)

Key words: nanomedicine, nanoparticles, nanofibers, electrospinning, theranostics, tissue engineering

Capacities:

- Formulation and characterization of different nanocarriers (nanoparticles, liposomes, nanofibers, thermoresponsive micelles) with certain drugs and therapeutic classes
- Development of new analytical methods for evaluating the incorporation and release of drugs from nanomedicines and for determination of their safety or toxicity
- Development of *in vitro* assays for live cell imaging and drug response monitoring
- Investigation of carrier transport and their distribution into mono- or co-cultured cells

SAFE AND EFFECTIVE COSMETIC PRODUCTS

Cosmetic formulations are nowadays very sophisticated products, exploiting many advanced technologies to develop innovative cosmetic products. According to Cosmetic Regulation EC 1223/2009 they have to be safe; it means they cause no damage to human health when applied under either normal or reasonably foreseeable conditions of use. Efficacy claims such as “anti-aging”, “moisturizing” and “wrinkle-reduction” can be substantiated scientifically using state-of-the-art measurement techniques either by clinical testing or by alternative *in vitro* methods.

The aim of such activities is to optimise and rationalise design, formulation and evaluation in order to achieve satisfied consumer outcomes:

1. Development and evaluation of active skin care products
2. Formulation and evaluation of cosmetic delivery systems: liposomes, lipid nanoparticles, nanoemulsions, liquid crystals
3. Characterization of physicochemical properties of formulation (pH, viscosity, surface tension)
4. Testing the release/permeation profile of cosmetic active using pig ear skin/skin constructs)
5. *In vitro* cell toxicity studies and irritancy evaluation by patch test
6. Clinical efficacy studies measurement of skin hydration, transepidermal water loss, elasticity of the skin, skin pH, sebum content, skin gloss.

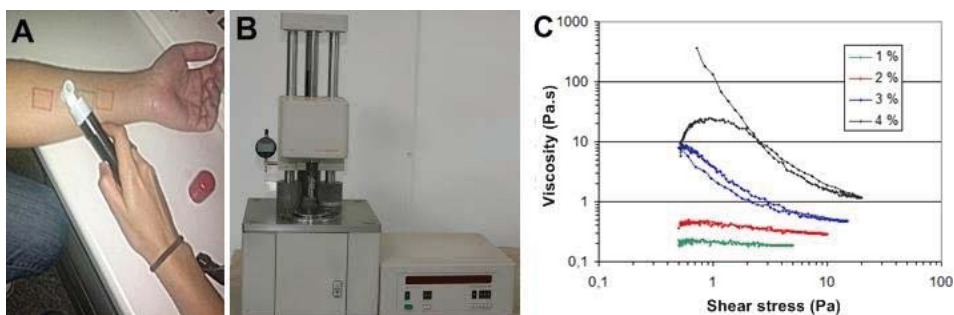


Figure 13: A- Transepidermal water loss measurement on forearm skin, B-Oscillatory viscometer for rheological evaluation of the products and C - typical viscosity curve of hydrogel

Key words: cosmetic products, cosmetic delivery systems, safety, cytotoxicity, clinical studies, biophysical measurements

Capacities:

- Design and formulation particular cosmetic products
- Characterization and evaluation of different cosmetic products for relevant sectors

BIOANALYTICS

The key to successful *in vitro* and *in vivo* research are good and reliable data. Therefore, analytical procedures must be carefully chosen and developed to meet the commonly accepted criteria for selectivity, accuracy and precision in order to produce useful data.

Our analytical techniques span from the simplest ultraviolet detector to the most sophisticated and modern high-end LC-MS-MS system.

Sample preparation

All sorts of samples, especially the ones of biological origin, such as blood, plasma, urine, and tissue extracts often need a careful planning and development of sample treatment before the analysis.

Most commonly, various extraction or separation methods are used:

- centrifugation and filtration,
- liquid-liquid extraction with various organic solvents and their mixtures,
- solid-phase extraction, solid phase clean-up,
- extraction from dried blood spots.

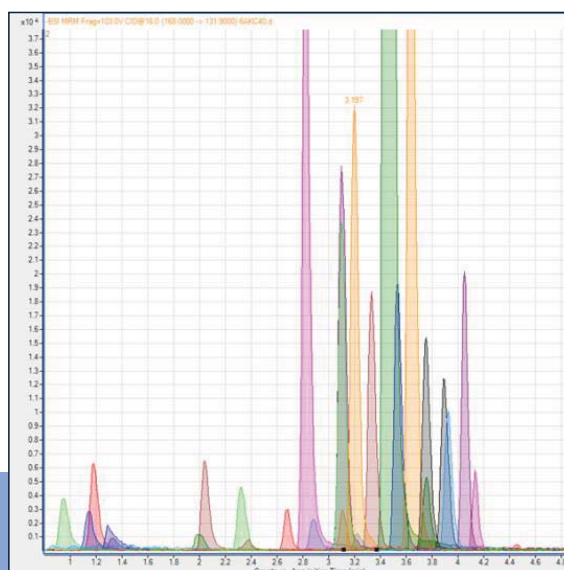
Chromatographic separation

The chromatographic method development is always targeted to achieve good and reproducible separation in minimal required time and to produce robust and reliable methods. Ultra high performance or rapid resolution chromatography is often employed to gain as much separation power in the shortest amounts of time and to save solvents.

Chromatographic detectors

A large amount of *in vitro* samples are analysed by simple UV or photodiode-array UV detectors. For more complex biological samples, more selective detectors are used: Fluorescence detector, Coluometric detector and the most sensitive and selective chromatographic detector, the triple quadruple mass spectrometer.

Figure 14: LC-MS/MS chromatogram of 41 analytes quantified in a single run from a tissue permeability sample (Žakelj et al. *Curr Drug Metab* 2013; 14(1):21-7).



Key words:
HPLC, sample preparation,
LC-MS/MS

Capacities:

- Determination of various analytes in complex biological samples: blood, serum, plasma, urine.-bioanalytical method development in accordance with FDA guidelines

BIORELEVANT DISSOLUTION TESTING

Dissolution/release testing is a powerful tool for prediction of *in vivo* dosage forms and active pharmaceutical ingredients performance after dosage form administration. To increase the probability of correct *in vivo* dissolution prediction the conditions of dissolution testing have to be as close as possible to those in human body. Thus different biorelevant conditions are introduced to simulate situation in gastrointestinal tract environment after oral administration: composition of media, physical contact and pressures on the dosage forms, transit through the stomach and intestine, volumes, flows etc. The main goal is the estimation of good *in vitro in vivo* correlation for chosen solid oral dosage form.

The areas of our research on biorelevant dissolution testing are as follows:

1. Development of novel dissolution system simulating physiological flows, volumes, media, physical contact with mucosa and physiological movement (shown in Figure 1)
2. Evaluation, simulation and modelling of data on gastric emptying of pellets in fasted and fed state. Use of developed models in prediction of *in vivo* dissolution and estimation of *in vitro in vivo* correlation
3. Evaluation of data on tablet transit through small intestine to be subsequently introduced in dissolution testing
4. Influence of variability of physiological tract conditions on overall variability of plasma profiles

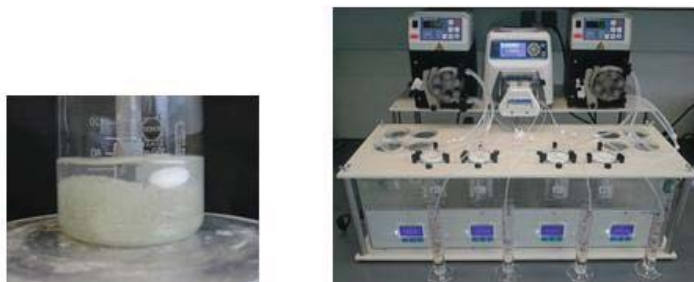


Figure 15: Novel flow-through dissolution testing system developed on FFA UL: basic unit (left) and complete system with four working units (right).

Key words: biorelevant dissolution testing, biorelevant release testing, gastric emptying, small intestine transit, tablets, pellets

Capacities:

- Dissolution/release testing under compendial and biorelevant conditions
- Prediction of *in vivo* dissolution profiles of solid dosage forms after oral administration
- Establishment of *in vitro in vivo* correlation of dissolution / release results with *in vivo* data

LADME – ABSORPTION/PERMEABILITY, DRUG TRANSPORTERS

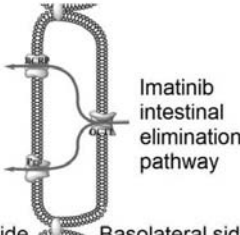
The absorption of the drug from gastrointestinal tract (GIT) after peroral application is very important for its bioavailability (activity). The extent and the rate of absorption can differ in different segments of GIT, depending on the epithelial properties, transporters...

In our laboratory, we study the permeability of different substances *in vitro* through the isolated segment of animal GIT or through the cell culture monolayer in the Sweetana-Grass side-by-side diffusion cells. During the experiment, the solutions are gassed with carbogen (95% O₂ and 5%CO₂) and kept at 37°C to maintain the rat intestinal tissue viability. Additionally, tissue integrity is validated by measuring the permeability of a fluorescent transport marker fluorescein, across the isolated rat intestinal tissue. The electrophysiologic parameters (potential difference PD, short circuit current, I_{sc} and transepithelial electrical resistance, TEER) are monitored to estimate tissue viability too.

We competently and comprehensively evaluate the permeability mechanism of substances using different transporters by this model. In the absorption process absorptive and secretory transporters pertaining to different groups, mostly ABC (ATP-binding cassette) proteins and SLC (solute carrier) proteins can be involved:

1. we examined Rhodamine 123 transport as an actively secreted Pgp (P-glycoprotein, ABCB1) marker substrate, while verapamil or PSC833 inhibits its transport;
2. the transport of monocarboxylic acid type drugs (ketoprofen, ibuprofen) which depends on inwardly directed proton gradient across the apical membrane of the enterocytes was researched; the role of absorptive monocarboxylate transporter, SLC16A was investigated;
3. we established that imatinib is eliminated through the intestinal mucosa by synergistic activity of efflux transporters Pgp and BCRP (breast cancer resistance protein, ABCG2) on the apical side and uptake transporter OCT1 (organic cation transporter, SLC22A) on the basolateral side of the intestinal cells.

Inhibited transport protein	Efflux ratio (ER) (S-M)/(M-S) Papp
None	2.8
Pgp and BCRP	1.0
Pgp	1.6
BCRP	1.4
OCT1 (OCT3)	1.2
OCT3	2.2
OCT2 (OCT1)	1.7



Imatinib intestinal elimination pathway

Apical side Basolateral side

Figure 16: Characteristics of imatinib efflux transport through the rat intestine. (Int. J. Pharm., 2014, 460, 144-149).

Key words: permeability, absorption, transporters, active transport, ABC proteins, SLC transporters

Capacities:

- Determination of 'in vitro' permeability (in absorptive and secretory direction) across isolated segment of rat intestinal tissue and Caco-2 monolayer
- Involvement of different transporters (like Pgp, MRP, BCRP, OCT and others) in the absorption process of tested substance
- Study of metabolism in GIT (enzymes in intestinal tissue)

PHARMACOKINETIC/PHARMACODYNAMIC EVALUATION

Pharmacometric methods can be applied to all stages of drug development as well as to personalized pharmacotherapy. We develop pharmacokinetic (PK), pharmacodynamic (PD), PK/PD and disease progression models to support drug development and optimise therapeutic strategies, thereby increasing the safety and efficacy of the pharmaceuticals in clinical settings. We predict PK/PD of novel drug delivery systems and collaborate with hospital services in therapeutic drug monitoring for drugs with a narrow therapeutic range and in dosage regimen design in children and elderly. We have state-of-the-art instrumentation and automation equipment for sample analyses including 2 LC/MS/MS, several HPLCs with DAD, FD, RI and ECD, and automated SPE stations. We investigate the influence of genetic polymorphisms of drug-metabolising enzymes, drug transporters, and drug targets. In our recent studies, we have applied population pharmacokinetic analyses to optimize pharmacotherapy of patients with epilepsy. We have also studied the association of genetic polymorphism in the folate metabolic pathway with methotrexate pharmacokinetics and toxicity in childhood acute lymphoblastic leukaemia and malignant lymphoma and the role of CYP2D6 genotype in risperidone metabolism and incidence of extrapyramidal symptoms in patients with schizophrenia.

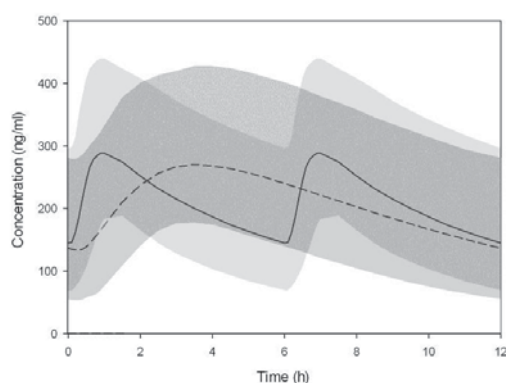


Figure 17: Simulated steady-state plasma concentration of tramadol in a population of patients. Typical profiles of the immediate release formulation 50 mg q6h (solid line) and 100 mg q12h as sustained release formulation (dashed line) with 95% prediction intervals (shaded area). This simulation reveals that tramadol exposure with sustained release formulation is comparable with that of immediate release formulation and supports switching patients to twice-daily dosing of sustained release formulation.

Key words: pharmacokinetic/pharmacodynamic modelling, pharmacometrics, bioavailability

Capacities:

- Bioavailability/bioequivalence studies and PK/PD studies
- Development of selective and sensitive assays for determination of drugs, metabolites, degradation products and endogenous compounds in various matrices.
- PK and PD data analyses, including population analyses using nonlinear mixed effects modelling, target mediated drug disposition modelling, clinical trial simulation, and optimisation of study design
- Development of methods for covariate modelling in population analyses and mixture modelling for identification of subpopulations of patients with various phenotypes to guide the discovery of new pharmacogenetic markers

CLINICAL PHARMACY RESEARCH AND SERVICES

We perform two types of clinical research together with the translation of the results to routine clinical services: Medication Reconciliation and Therapeutic Drug Monitoring. The main goal is to achieve optimal clinical outcomes in patients on drug therapy.

Research in the field of Medication Reconciliation is implemented at the hospital wards, during admission, transfer and discharge of the patient, as well as at the general practitioners, nursing homes and home care. We put the results of this research into practice as a three-stage process in which we:

1. Get patient's medication history,
2. Evaluate intended and unintended discrepancies in medication, and
3. Document intended and eliminate unintended discrepancies.

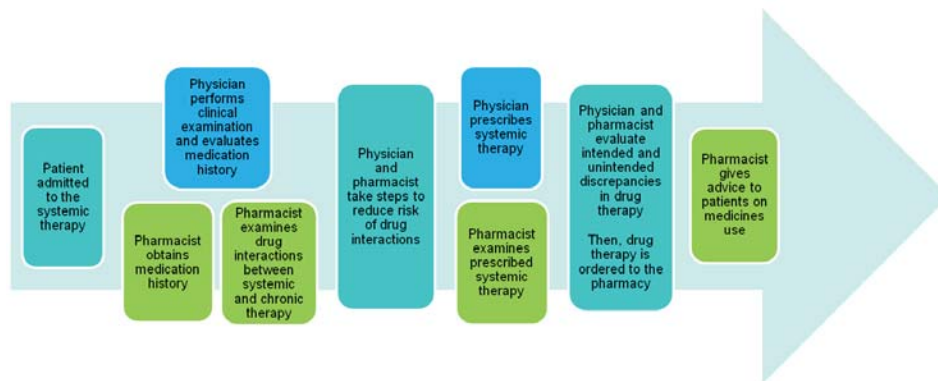


Figure 18: Medication Reconciliation - how to optimize systemic therapy of cancer patients

Research in the area of Therapeutic Drug Monitoring is relevant for optimization of therapy with narrow therapeutic index drugs. On the basis of pharmacokinetic data and therapeutic range we:

1. Define optimal time(s) for withdrawal of plasma sample(s),
2. Measure drug and/or metabolite(s) levels by suitable bioanalytical method, and
3. Calculate optimal drug dosage regimen.

Key words: clinical pharmacy, medication history, medication discrepancies, drug counselling, narrow therapeutic index drugs, monitoring drug therapy

Capacities:

- Medication Reconciliation at tertiary, secondary and primary level of health-care system
- Therapeutic Drug Monitoring in clinics and hospitals

PERSONALISED MEDICINE: COMPLEX DISORDERS UNDER THE MAGNIFYING GLASS OF GENOMICS, PHARMACOGENOMICS, EPIGENETICS AND FUNCTIONAL GENOMICS

Chronic diseases as complex genetic disorders represent one of the most attractive research fields in regard to identification of new diagnostic and therapeutic targets. Over the last 15 years our research group has been studying the genetic backgrounds and pharmacogenetics of complex diseases like osteoporosis, osteoarthritis, insulin resistance, obesity, and also heavy metal toxicity. With genotype-phenotype association studies, genome-wide analyses and gene expression profiling our researchers want to identify important molecules (biomarkers) and/or pathways in those diseases. As part of the pharmacogenetic studies the group is studying the genetic variations in drug metabolic enzymes and drug transporters of selected antiosteoporotic, anticoagulant, anticancer and immunosuppressive drugs. During the last years our group is focusing on research of micro RNA and other epigenetic mechanisms of cell regulation with implication to personalized diagnostics and treatment of complex diseases.

Objectives:

1. Searching and validation of genetic factors in complex diseases
2. Designing of cell models for in vitro gene function testing
3. Cell-material interactions (joint arthroplasty, nanofibres)
4. Genetic and epigenetic cell responses to different factors (hypoxia, oxidative stress, heavy metals)
5. Pharmacogenomics
6. Nutrigenomics

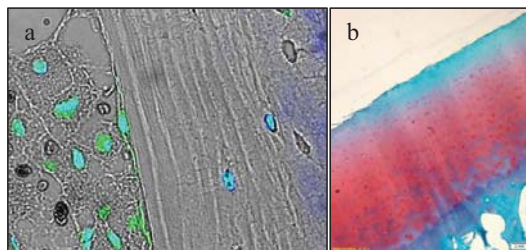


Figure 19: (a) Immunolocalization of alkaline phosphatase in human bone tissue using indirect immunofluorescence; (b) Safranin O staining for cartilage assessment

Key words: personalized medicine, osteoporosis, osteoarthritis, bone cancer, genetics, epigenetics, pharmacogenomics

Capacities:

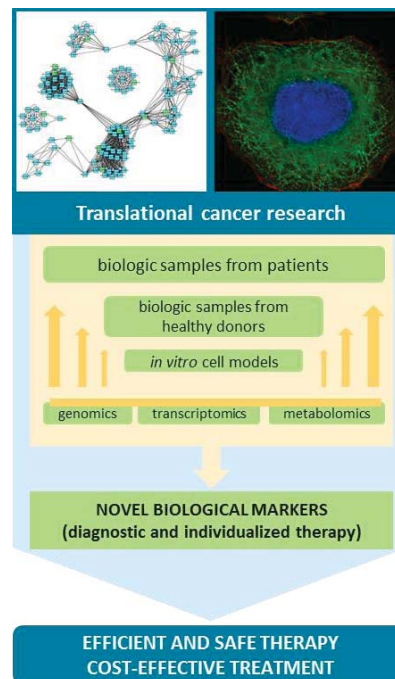
- Molecular diagnostics
- Pharmacogenetic testing
- Epigenetic testing and miRNA analysis
- Molecular cloning
- Site-directed mutagenesis
- Indirect immunofluorescence
- Immunohistochemistry
- Evaluation of tissue structure and quality (cartilage histochemistry and OARSI evaluation, bone histomorphometry using von Kossa, paragon and TRAP staining)

TRANSLATIONAL CANCER RESEARCH: PHARMACOGENOMICS AND NEW THERAPEUTIC TARGETS FOR TREATMENT OF LEUKAEMIA

The main efforts of our research group are aimed to identify and validate novel biomarkers predicting safety and efficacy of existing treatments as well as identifying new therapeutic targets for treatment of leukaemia, focusing on acute lymphoblastic leukaemia (ALL) and chronic lymphoblastic leukaemia (CLL) patients. In the experimental design of our studies, we apply the contemporary methods using horizontal 'omic' technologies, such as genomics, transcriptomics, proteomics and metabolomics on vertical models of growing complexity; from *in vitro* biochemical tests and cell line models, to biological samples of healthy volunteers and patients on treatment.

Objectives:

1. Identification of genetic and metabolomic biomarkers for individualization of ALL treatment
2. Development of dosing algorithms for individualized thiopurine treatment using innovative bioinformatics approaches
3. Identification of markers of resistance to therapy through studies on lymphoblastoid cell lines models
4. Studying the proteasome and immunoproteasome systems as targets for cancer treatment
5. Evaluation of novel therapeutic targets in *in vitro* studies



Key words: pharmacogenomics, biomarkers, bioinformatics, translational cancer research, genomics, transcriptomics, proteomics, metabolomics, apoptosis

Capacities:

- Biomarkers discovery and validation
- Clinical trials
- Functional analysis of therapeutic targets in pre-clinical *in vitro* studies
- Evaluation of new potential therapeutic agents in pre-clinical *in vitro* studies

CELL AND TISSUE ENGINEERING FOR ADVANCED THERAPIES AND DEVELOPMENT OF *IN VITRO* TESTING MODELS

Advanced therapies are encompassing the use of gene, somatic cell and tissue-engineered therapeutic products. They are becoming important in the treatment of numerous diseases, especially cancer and autoimmune disorders and represent a basis for regenerative medicine. Additionally, cell and tissue engineering techniques are enabling us to plan and develop various rather simple or highly complex *in vitro* models for testing drugs as well as other substances on specific human tissue- or organotypic cultures. For this purpose different types of cells can be used, such as: stem cells (SCs), induced pluripotent stem cells (iPSCs) and tissue-specific adult somatic cells.

Our expertise:

1. cellular immunology: antigen-specific priming and expansion of immune cells, *in vitro* production of monocyte-derived dendritic cells (DCs), immune cell phenotyping and functional analyses, immunomodulation, alloreactivity;
2. cell and tissue engineering: preparation and evaluation of anti-cancer cellular vaccines, tissue and organotypic cultures for clinical and experimental use (*in vitro* testing models);
3. designing expertise-related clinical studies

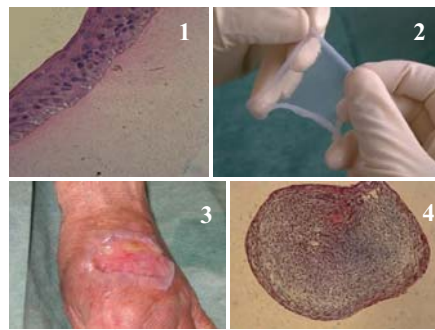
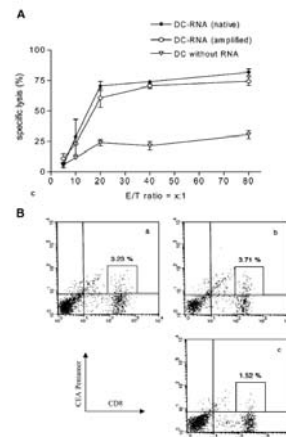


Figure 20: 1-histology of epidermal fibrin based substitute; 2 - fibrin based skin substitute before application (International Journal of Pharmaceutics 291 (2005) 31-37); 3 - the same, applied on a chronic wound; 4 - histology of a dense microculture of bone-marrow derived mesenchymal stem cells in a chondrogenic medium; A- CEA-specific cytotoxic immune responses generated *in vitro* with native and amplified total tumor RNA lipofected autologous DCs; B - flow cytometry quantification of CEA-specific cytotoxic T lymphocytes with HLA pentameres (Immunobiology 211 (2006) 179-189)



Key words: cell and tissue engineering, tissue and organotypic cultures, advanced therapies, immunotherapy of cancer, immunomodulation

Capacities:

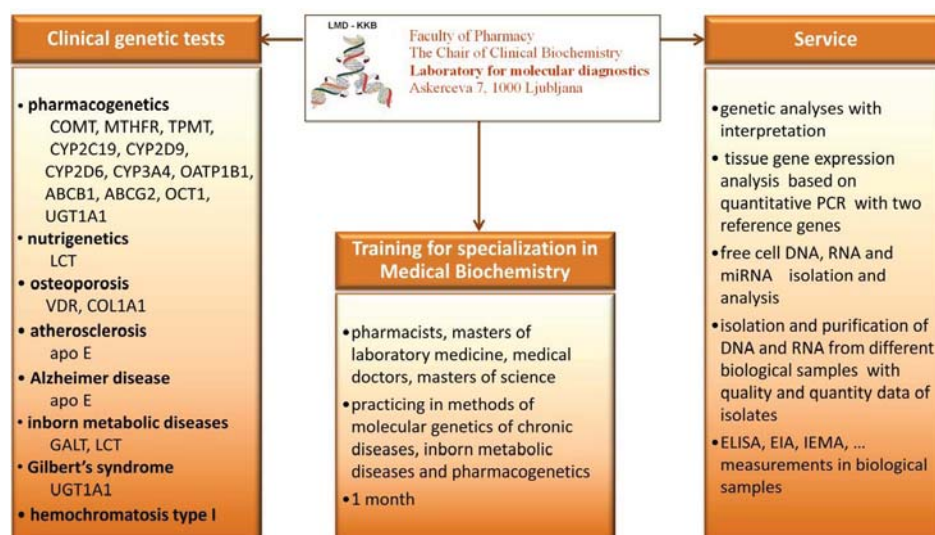
- Human cell isolation, phenotyping, morphology assessment, quantification and cultivation;
- Cell and molecular biology-based *in vitro* functional cell testing;
- Simple and complex tissue and organotypic production (i.e. epidermal/dermal skin substitutes);
- Dendritic cell – based vaccines preparation and evaluation;
- Total tumor RNA isolation, amplification and dendritic cell transfection;
- Ongoing clinical study - immunotherapy of prostate cancer (EudraCT: 2012-005498-29)

TRANSLATIONAL LABORATORY MEDICINE: FROM RESEARCH TO CLINICAL PRACTICE

In the medicine and context of patient care, more than 75 % of clinical decisions are based solely on the results of laboratory medicine. Laboratory for molecular diagnostic (LMD) at The Chair of Clinical Biochemistry was established in 2009. As a professional, scientific and pedagogical integration promoting laboratory it has a pleasure to serve physicians, patients and research community in Slovenia. A panel of 19 genetic clinical tests is available for differential diagnosis and personalized medicine in selected chronic and inborn metabolic diseases as well as pharmacogenetics and nutrigenetics. LMD is a Ministry of Health work permit holder, based on fulfillment of all legal requirements and regulations in the field of laboratory medicine in Slovenia. Laboratory participates in the proficiency test programs for external quality assessment (RfB, INSTAND). The LMD staff includes 3 professors, registered as specialists at European Federation of Laboratory Medicine, 1 assistant professor, 2 post-doctoral fellows all with expertise in genetics or protein analysis and 2 experienced technicians.

More information is available at:

<http://www.ffa.uni-lj.si/raziskave/laboratorij-za-molekularno-diagnostiko/>



Key words: molecular diagnostics; licenced medical laboratory; translational laboratory medicine;

Capabilities:

- Genetic analyses
- Gene expression analysis
- Cell-free nucleic acid analyses
- Protein analyses
- Clinical interpretation of results

PHARMACOTHERAPY AND PHARMACEUTICAL CARE

Health related outcomes are primarily objective parameters defined as clinical outcomes observed by health care providers. On the other hand, humanistic outcomes take into account the patients' perspective and include the evaluation of quality of life and patient satisfaction. Drug related problems form the base of pharmacist cognitive services usually described by the terms pharmaceutical care and clinical pharmacy. The aim of such activities is to optimise and rationalise medicine treatment in order to achieve definite outcomes. Several risk factors lead to drug related problems including drug-drug interactions, poor adherence, inappropriate prescribing and dispensing. Pharmacists are experts in pharmacotherapy and are responsible for ensuring the safe, appropriate, and economical use of medicines. The integration of pharmacist services into the health care system and the cooperation of pharmacist with other health care personnel and patients are of utmost importance.

Our research:

1. Health outcomes of pharmacotherapy
2. Drug related problems and their risk factors
3. Context, structure, processes, outcomes and impact of health care services
4. Public health research

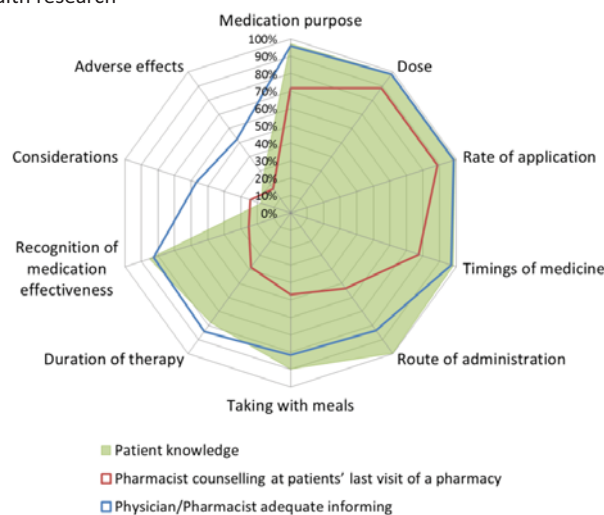


Figure 21: Patient knowledge of different counselling elements, Pharmacist counselling at patients' last visit of a pharmacy and Physician/Pharmacist adequate informing. [In submission].

Keywords: patient reported outcomes, health related quality of life, patient satisfaction, drug related problems, medication reviews, medication counselling, pharmaceutical care, clinical pharmacy, competency framework

Capacities:

- Design and evaluation of pharmacy services (i.e. medication review, pharmaceutical care and clinical pharmacy programs)
- Design and/or validation of instruments (e.g. quality of life and patient satisfaction questionnaires)
- Design of clinical trials and biostatistical data analysis

PHARMACOEPIDEMOLOGY AND PHARMACOECONOMICS

Research focus is the study of medicine effectiveness and safety after marketing authorization (i.e. while getting into the hands of patients). Research includes the evaluation of benefit-risk profiles of drugs, comparative effectiveness and cost-effectiveness and is predominantly evaluated in the area of oncology, cardiovascular diseases and vaccination. Market uptake and the accessibility of patients to new treatment strategies are of particular interest. The research area can be embedded into health technology assessment that forms an integral part of health care policy:

1. Pharmacoepidemiology
2. Comparative effectiveness and safety
3. Medicine pricing and reimbursement policy
4. Pharmacoeconomics and health economics
5. Health care policy research

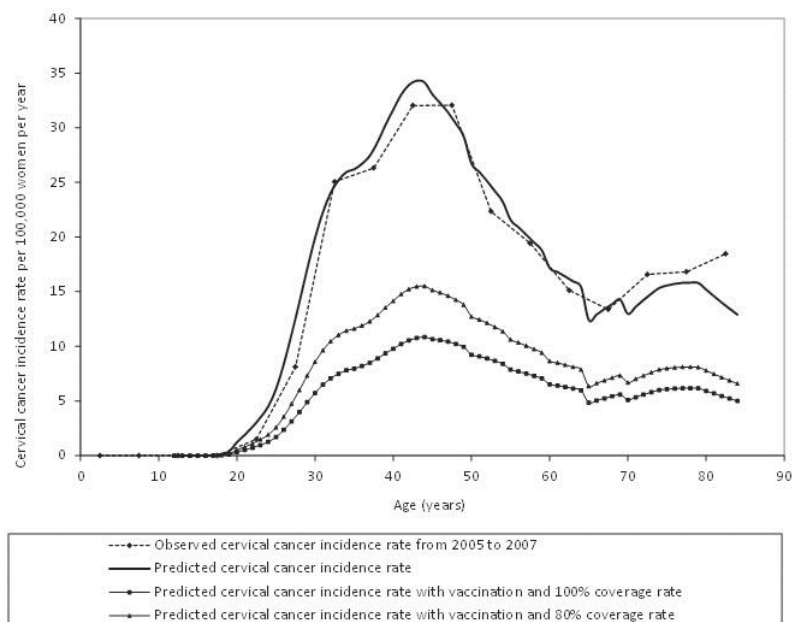


Figure 22: Predicted and observed age-specific cervical cancer incidence rate from 2005 to 2007, and predicted reduction of cervical cancer incidence rate by HPV vaccination, assuming 80 and 100% vaccination coverage rate. [European journal of public health 2010]

Keywords: pharmacoepidemiology, pharmacovigilance, comparative effectiveness, systematic reviews, meta-analysis, pharmacometrics, pharmacoeconomics, health economics, cost-effectiveness; budget impact, burden of disease, medicine reimbursement, cost containment, health care policy

Capacities:

- Data mining of databases
- Model development and simulation
- Health technology assessment
- Medicine market consultancy

Research at the Faculty of Pharmacy University of Ljubljana

About the University of Ljubljana
About the Faculty of Pharmacy

CHAIR OF PHARMACEUTICAL BIOLOGY

- Isolation and identification of plant secondary metabolites
- Pharmaceutical biotechnology
- Peptidase signalling in cancer, neurodegenerative and immune disorders

CHAIR OF PHARMACEUTICAL CHEMISTRY

- Custom synthesis of building blocks, biologically active small molecules, fluorescent/spin labels and probes
- Computer-aided discovery and design of novel biologically active compound
- Structure-based design of novel enzyme inhibitors and receptor modulators
- Mechanistic toxicology and the role of metabolism in toxicity
- Pharmaceutical analysis

CHAIR OF PHARMACEUTICAL TECHNOLOGY

- Preformulation studies: Tailoring and engineering of drug ingredients
- Conventional and advanced drug delivery systems
- Lipid based formulations and microemulsions
- Nanotechnology and nanodelivery systems
- Safe and effective cosmetic products

CHAIR OF BIOPHARMACEUTICS AND PHARMACOKINETICS

- Bioanalytics
- Biorelevant dissolution testing
- LADME – Absorption/Permeability, drug transporters
- Pharmacokinetic/Pharmacodynamic Evaluation
- Clinical Pharmacy

CHAIR OF CLINICAL BIOCHEMISTRY

- Personalised medicine: Complex disorders under the magnifying glass of genomics, pharmacogenomics, epigenetics and functional genomics
- Translational cancer research: Pharmacogenomics and new therapeutic targets for treatment of leukaemia
- Cell and tissue engineering for advanced therapies and development of *in vitro* testing models
- Translational laboratory medicine: from research to clinical practice

CHAIR OF SOCIAL PHARMACY

- Pharmacotherapy and pharmaceutical care
- Pharmacoepidemiology and pharmacoconomics

Research
Faculty of Pharmacy, University of Ljubljana, Slovenia

Publisher:
Faculty of Pharmacy, University of Ljubljana
Aškerčeva 7
Ljubljana, Slovenia

Editorial Board:
Julijana Kristl
Borut Božič
Samo Kreft
Stanislav Gobec
Mirjana Gašperlin
Albin Kristl
Darko Černe
Mitja Kos

Design & Layout
Irena Prodan Žitnik

Issued: 2014

Print: Ofsa d.o.o.

Edition: 500 copies



<http://www.ffa.uni-lj.si/>

