

## **Description of the main research directions investigated by the institute**

The research conducted in 10 scientific departments of the ICPF can be divided into five main research topics: Chemical and Environmental Engineering, Chemical Sciences, New Materials and Biotechnologies.

### **1. Chemical Engineering**

The ICPF represents a unique chemical engineering workplace within the Czech Republic and has a long history of research in this field. This involves top-tier fundamental research with a tradition reaching back practically to the founding of the institute. Our wide-ranging knowledge finds applications in the industrial sphere, where we maintain an array of collaboration at the national and international level. Chemical engineering research at ICPF includes the classical topics, such as Separation Processes, Catalysis and Reaction Engineering, Multiphase Systems, as well as Microreactor Engineering, which has emerged as a result of recent development at the Institute.

- **Separation Processes**

Research in the field of separation processes at the ICPF has in recent years been concentrated in the Department of Membrane Separation Processes. Membrane separation is utilized here especially for the separation of gas mixtures (cleaning raw biogas, separation of volatile organic compounds from air, flue gas cleaning). Various polymer membranes, ionic liquid membranes, polymers of intrinsic microporosity, and composite membranes are utilized for these purposes. Pertraction is used for the separation of liquid mixtures. Another long-term focus is separation of individual enantiomers and elimination of drugs and endocrine disruptors from water. Pervaporation is utilized for the separation of components of azeotropic mixtures. Research includes modelling of transport properties of membranes such as permeability, activation energy of permeation, and selectivity of the separation process. Data is evaluated with the aid of models based on activity coefficients, equations of state, and occasionally by modelling of molecular dynamics.

- **Catalysis and Reaction Engineering**

Research in the field of catalysis and reaction engineering at the ICPF takes place in the Department of Catalysis and Reaction Engineering. This field covers a wide range of scientific activity, from the preparation of catalysts and the study of mass transport in catalysts and their microstructure, to their use in process engineering. Clarifying the relationships between the structure, composition, activity, and selectivity of oxidation catalysts allows for more efficient use of biofuels in the automotive industry, for example, or the development of new types of photocatalysts for environmental technologies. An integral part of our research is the precise characterization of the textures and microstructures of materials, including the elucidation of transport in the pores of a catalyst. For this, we have developed our own software. This know-how is used for describing catalytic processes, designing optimal pore structures of heterogeneous catalysts, and ultimately preparing our own catalysts.

- **Multiphase Systems**

Research in the field of multiphase systems at the ICPF takes place in the Department of Multiphase Reactors. The research stems from a long tradition of study of transport and reaction processes in liquid-gas systems such as bubble columns and other aerated systems. This has expanded to include fluid-solid systems for the study of flow in granular media and liquid-gas-solid systems, as in three-phase apparatuses, bioreactors, and flotation. Research on multiphase phenomena is carried out on three levels according to the natural hierarchy of the structure of physical reality—that is, on the level of one to two particles (micro), the level of particle clusters (meso), and the level of the vessel (continuum—macro). In addition to experiments, theoretical models and numerical simulations are used in the study of all systems. Experiments are based on advanced diagnostic methods such as time-resolved PIV, microPIV, electrodiffusion diagnostics, and high-speed imaging. Our theoretical and numerical methods make use of the most up-to-date software such as ANSYS Fluent, COMSOL, MATLAB, etc. An integral part of our research on multiphase systems is characterization of phases and study of steady-state and dynamic processes at the phase interface. For this, rheological and tensiometric methods are utilized.

- **Microreactor Engineering**

Research in the field of microreactor engineering at the ICPF is undertaken by the Microreactor Technologies Group in the Department of Multiphase Reactors. The main focus is the application of flow microreactors to the intensification and optimization of existing chemical technologies and the design of new ones. This includes multiphase processes that are non-catalytic, as well as those that are homogeneously or heterogeneously catalysed. Examples have included the study of sulfonation and sulfation reactions for the surfactant industry, absorption of gases in liquids, selective catalytic oxidation and hydrogenation in the gas phase, homogeneous and heterogeneous catalytic hydrogenation in the liquid phase, and radical polymerization. Processes are studied from the perspective of hydrodynamics, heat and mass transfer, and chemical kinetics with the objective of designing flow technologies for the efficient development of specific products. Our laboratory is equipped with several types of flow microreactors of various scales, and emphasis is placed on online analysis as well. Research activity also includes equipment design, 3D print prototyping, and manufacturing of microreactors for specific applications.

## **2. Environmental Engineering**

In environmental engineering we apply our years of experience in the fields of chemical sciences and chemical engineering to the development of processes for the protection of the environment. We combine fundamental and applied research in the attempt to understand the essence of phenomena and then apply this to the current requirements of our partners in industry.

- **Energetics**

Research in the field of energetics at the ICPF takes place in the Department of Environmental Engineering. This primarily involves the development of new procedures for increasing the efficiency of thermochemical conversion of fuels with the

use of new and alternative fuels and further reducing the impact on the environment. These activities are oriented in two main directions. The first includes development of new and more effective processes of flue gas treatment, effective and clean utilization of new waste-based fuels, and the search for suitable methods of processing and using solid waste from energy production. The second is the development of technologies to advance decentralized systems for the use of alternative fuels and biomass by gasification and pyrolysis and their transformation to synthesis gas of a quality suitable for the production of chemical commodities, primarily transport fuels and hydrogen.

- Waste-to-Energy

Research in the field of waste-to-energy at the ICPF is concentrated in the Department of Environmental Engineering. The department is occupied with, among other things, the energy and material recovery of waste and the recycling of waste. Efforts in energy recovery involve the safe use of various atypical wastes, the search for new methods of flue gas treatment, and the development of methods for treatment of solid residues from waste incineration such as fly ash and bottom ash for the purpose of obtaining valuable components. In the field of waste treatment and recycling, we develop methods for processing municipal waste and industrial waste such as electronic waste, plastic waste, biological waste such as food waste and sewage sludge, etc. We consider waste as a source of raw materials or energy and approach it accordingly.

- Aerosols

Research in the field aerosols has a long tradition at the ICPF and takes place in the Department of Aerosols Chemistry and Physics. The department is occupied with the study of the chemical and physical properties of atmospheric aerosols and determining the share of various sources.

Further topics include the behaviour of aerosols in indoor environments, kinetics of nucleation and growth, emission sampling of aerosol particles, as well as preparation of nanoparticles for inhalation experiments. The department coordinates aerosol research at the National Atmospheric Observatory Košetice and is part of the large international research structure ACTRIS (ESFRI), where we measure number concentrations and aerosol particle size distribution and determine the precise chemical composition of aerosols.

- Water

Research in the field of water purification at the ICPF is predominantly concentrated in the Department of Catalysis and Reaction Engineering. The research is focused on removal of pollutants from wastewater and industrial wastewater. This includes substances such as endocrine disruptors, organic compounds, heavy metals, and pharmaceuticals. Various processes for removing such substances are tested, including adsorption, electrocoagulation, and photocatalysis. Research also focuses on combinations of these methods for comprehensive treatment of polluted water, with regard to the economics of the overall process.

### 3. Chemical Sciences

Chemical sciences have been cultivated at the ICPF since its founding and have a long tradition here. We are one of the six institutes of the Section of Chemical Sciences at the Czech Academy of Sciences and we collaborate in chemical sciences with a number of universities, where we participate in teaching of undergraduate students. In addition, we function as a training workplace for doctoral students and postdocs. We also work with the industrial sector.

- Analytical Chemistry

Research in the field of analytical chemistry at the ICPF takes place in the Department of Analytical Chemistry, with a concentration on various aspects of nuclear magnetic resonance (NMR) spectroscopy. The primary focus is on so-called NMR-omics, which, based on concentrations of substances and with the help of multidimensional statistical methods, can distinguish samples originating from patients from those of healthy individuals (metabolomics). We are the only ones in the world using the same principle for analysis of organic substances in aerosols (aerosolomics). Another research interest is the use of receptors for anion complexation and chiral recognition. The department also provides support to other departments in the institute when their own analytical equipment does not suffice. For analysis of inorganic substances, the laboratory is equipped with MP-AES and ICP-OES atomic spectrometers. For analysis of organic compounds, high-resolution mass spectrometry (HMRS), UV-Vis spectroscopy, or high-performance liquid chromatography (HPLC) can be utilized, in addition to NMR. For qualitative and quantitative analysis of more complicated mixtures of organic substances, it is possible to utilize combined techniques like LC-NMR, LC-HRMS, or GC-MS.

- Organic Chemistry

Research in the field of organic chemistry at the ICPF takes place in two scientific departments. The Department of Advanced Materials and Organic Synthesis focuses primarily on photochemical synthesis of polycyclic aromatic hydrocarbons, primarily helicenes and phenacenes. The parent compounds are then derivatized with the objective of improving their optical and electronic properties, which are subsequently tested for various applications in wide-ranging branches of research. The Department of Bioorganic Compounds and Nanocomposites concentrates on, among other things, the preparation of fluorinated carbohydrates, glycosides, and their multivalent analogues, which find applications in glycobiology as enzyme inhibitors, model molecules to study the interaction of carbohydrates with proteins, and as substances that modulate the cellular glycome. The department's other key topic is the design and preparation of new dendrimers, dendritic and hyperbranched molecules, and molecular formations with precisely defined structures. These macromolecules are further studied as drug delivery vehicles, non-viral vectors for therapeutic siRNA/DNA delivery, or as components of advanced composite materials.

- Physical Chemistry

Research in the field of physical chemistry at the ICPF is concentrated in two scientific departments. In the Department of Molecular and Mesoscopic Modelling, complex fluid systems in the volume phase and in nanospace under equilibrium and non-equilibrium conditions are studied. Computer modelling at the molecular and mesoscopic level serves here as a complement to realistic experiments and finds applications in chemical and material engineering, as it clarifies the relation between the microstructure of systems and their macroscopic behaviour. The second group dedicated to physical chemistry is the Thermodynamics of Specific Materials group, situated in the Department of Aerosols Chemistry and Physics. The main topic of their research is the properties of phase change substances and heat transfer fluids that can be used in heat accumulators. Areas of interest include both experimental determination of thermophysical properties of substances important for energy storage and mathematical modelling of heat and mass transfer in porous materials with thermochemical transformation.

#### **4. New Materials**

Materials engineering is a relatively new field at the ICPF, where we study materials originating primarily from our fundamental research in chemical sciences. Our materials find applications in fields such as organic electronics, photovoltaics, and biomedicine. We work with a number of universities and industry partners in these fields.

- Inorganic Materials

Research in the field of inorganic materials at the ICPF takes place almost exclusively in the Department of Laser Chemistry. The department focuses on preparation of thin films and nanostructured materials by an array of deposition techniques and is well equipped with vacuum equipment for deposition of new materials, but also with instrumental technology for characterization of the prepared materials. Through laser ablation, radiofrequency deposition, or chemical deposition from the gas phase, we prepare nanostructured materials based on silicon and germanium for photovoltaic applications and oxides, suboxides, oxycarbides, and oxynitrides for photocatalytic and photoelectrochemical applications. Characterization of materials is done by a plethora of spectroscopic techniques such as IR, UV-Vis, Raman, photoelectronic, and EDX spectroscopy or with scanning electron microscopy. Crystalline samples are analysed by powder diffractometry, while gaseous and liquid samples are analysed by gas chromatography and mass spectrometry.

- Organic Materials

Research in the field of new organic materials at the ICPF takes place in three scientific departments. Materials based on carbosilane structures such as dendrimers, dendrons, and amphiphiles are prepared for use in biomedical applications in the Department of Bioorganic Compounds and Nanocomposites. Phosphonium dendrimers are capable of forming complexes with therapeutic sequences of nucleic acids and demonstrate both lower toxicity and more effective transfection into cells.

Polycyclic aromatic compounds suitable for construction of organic electronic components are synthesized in the Department of Advanced Materials and Organic Synthesis. Compounds prepared on the basis of helicenes and phenacenes, derived from the structure of 2D graphene, demonstrate semiconducting characteristics. Thin films prepared from such materials can then be used for the construction of OFET or OLED devices or, with electropolymerization, for preparation of a permselective sensor layer directly on the electrode.

Within the Department of Aerosols Chemistry and Physics, the Thermodynamics of Specific Materials Group studies the use of ionic liquids for energy storage. As heat transfer fluids offering greater efficiency in energy use from renewable resources, these materials represent an interesting alternative to materials currently used in heat accumulators.

- **Composite Materials**

Research in the field of composite materials at the ICPF takes place in the Department of Bioorganic Compounds and Nanocomposites. Prepared materials are designed primarily for capturing gases such as CO<sub>2</sub> or for effective gas separation. Adsorbents derived from layered silicates and porous polymers are tested for these purposes. These matrices are then modified by hyperbranched synthetic macromolecules – dendrimers. These molecules have significant internal volume and a large number of functional groups on the surface. These properties increase the adsorption capacity and selectivity of the new material.

## **5. Biotechnologies**

Biotechnologies are a relatively newly developing field for the ICPF. Here we apply years of experience in the sphere of chemical engineering to an entirely new, dynamically developing area. Research is usually conducted alongside specialists from biological fields.

- **Biorefining Processes**

Research in the area of biorefining at the ICPF is carried out in the Department of Catalysis and Reaction Engineering. Biorefining and biotechnology these days are producing new and desired products. The Department of Catalysis and Reaction Engineering is the leading entity at the BIORAF Centre of Competence and the BIOCIRTECH National Centre of Competence—projects undertaken with academic and industry partners to solve problems related, for example, to the creation of new products such as joint supplements from poultry cartilage or cosmetic and anti-inflammatory dermatological agents from plants, and to isolation of essential unsaturated fatty acids from microalgae. We are currently working with the medical community to solve issues related to the adhesion of animal cells to various biopolymer meshes and membranes produced by an original method of electrospinning.

- **Microbial Biotechnologies**

Research in the field of microbial biotechnologies at the ICPF is undertaken by the group within the Department of Multiphase Reactors. The core of this research lies in the utilization of microorganisms in environmental and food technologies. This

involves primarily unicellular eukaryotic algae and cyanobacteria, but also bacteria and yeasts. Important activities include optimization and scale-up of cultivation processes, including development of photobioreactors, optimization of separation processes, and innovation of down-stream processes, drawing significantly on findings gained from fundamental research of multiphase hydrodynamics carried out within the department.

- Biocatalysis

Research in the field of biocatalysis at the ICPF is undertaken by the Supercritical Technologies Group within the Department of Advanced Materials and Organic Synthesis. The biocatalysis is carried out in supercritical carbon dioxide for the purpose of enriching vegetable oils with essential fatty acids through hydrolysis. Here, vegetable oil reacts with water or ethanol in  $\text{scCO}_2$  medium, catalysed by a regiospecific enzyme. The profile of fatty acids in the reaction product then depends on the various reaction rates of individual components of the oil. When the reaction mixture is enriched with essential fatty acids the reaction can then be carried out on the basis of different  $\text{scCO}_2$  solubility or different affinity for the sorbent.

## **Research activity and characterisation of the main scientific results**

The team members from Department of Membrane Separation Processes were the principal investigator of 6 standard (1 of them as coinvestigator) and 2 junior projects supported by Czech Science Foundation and 2 projects (1 of them as coinvestigator) supported the Ministry of Education, Youth and Sports of the Czech Republic and as 1 coinvestigator for the project supported by Technology Agency of the Czech Republic. Furthermore, the team also cooperated on Contract work with the total sum 1 269 000 CZK with the industrial partners (2015: Česká Hlava – Czech Head, 2016: Membrain, Veolia and ASIO, 2018: INTERPHARMA and DHCWOD, 2019: Atrea). The contracts were based on the constructing biogas purification unit (JINPO), ideas how to bring the process from a laboratory to industry (Czech head), research and development in membrane technologies (Membrain), water purification (Veolia), producing new chemical products (ASIO and Intherfarma for pharmacy), flue gas purification (DHCWOD), and development of recuperation units (Atrea).

The details of all researcher projects are given below.

### **Czech Science Foundation, Standard projects**

**Pavel Izák, Membrane separation – the more effective separation of a pure enantiomer from a racemic mixture, 01.01.2012 - 31.12.2015, GAP106/12/0569 (6 389 000 CZK)**

Drug stereochemistry has become an issue for the pharmaceutical industry and the regulatory authorities, because each of the enantiomers frequently shows different impacts to living organisms. The goal of the proposal is to develop new membrane separation techniques for successful resolution of racemic mixtures allowing optimization of the therapeutic value of enantiomeric drugs (pharmacological and toxicological) and avoiding their adverse effects. The key objective is to separate enantiomers by a new membrane separation method, based on the proposed supported chiral room temperature ionic liquid membrane, that has never been studied yet. In comparison with classical methods employed earlier, it should show higher efficiency and cost-effectiveness in the processes of enantiomer separation. To greatly reduce the amount of experimental work, particle-based modelling will be employed. After gathering all transport characteristics, it will be possible to model the separation process and to estimate permeability and selectivity of the separation.

**Pavel Izák, Separation of polar and non-polar gasses by membrane processes, 01.01.2014 - 31.12.2016, GA14-12695S (8 712 000 CZK)**

The use of upgraded biogas is considered as one of the most efficient means of utilizing renewable and sustainable energy. Replacing of conventional methods by low-costs, membrane separations is therefore of a great interest. The aim of this project is to develop innovative highly selective membranes with improved performance for efficient biogas upgrading. Two parallel approaches of unique membrane preparation will be used: (i) the supported ionic liquid membranes and (ii) the water-swollen thin film composite membranes. A complementary and multidisciplinary approach to laboratory experiments for gas separation can be provided by modelling, which can pre-select the best ionic liquid and save a lot of experimental work. The aspect connected with designing, preparation, characterization and evaluation of membranes



for efficient biogas upgrading will be addressed to achieve the most successful membrane. The major issues are achieving high selectivity towards the target gas (methane) and testing this new separation method also for other polar gasses.

**Ivan Wichterle, Thermodynamics of polymer solutions: experiments meet theory and vice versa, 01.01.2015 - 31.12.2017, 15-19542S (7 113 000 CZK)**

The project aims at (1) obtaining accurate vapour-liquid equilibrium data for industrially important systems containing polymer (mostly biodegradable) + solvent, (2) obtaining results on the conformational behaviour of polymers in solutions at the given thermodynamic conditions and their impact on the phase equilibria, (3) developing a truly molecular-based computer-aided theory as an alternative to empirical and semi-empirical models as e.g., SAFT. The experimental part will focus on improving methodology of getting accurate data on phase equilibria over a wide range of thermodynamic conditions. New simple short-range models will be developed to be used in a perturbation expansion for the Helmholtz free energy. Correctness and accuracy of the theory will be verified by molecular simulations and experimental data. The results acquired can be immediately applied in the design of efficient separation units in chemical and petroleum industry.

**Pavel Izák, Membrane separation – the more effective separation of a pure enantiomer from a racemic mixture, 01.01.2017 – 31.12. 2019, 17-00089S (3 381 000 CZK)**

The preparation of enantiomerically pure compounds remains is a challenging task despite the quick progress in asymmetric synthesis and separation techniques. The aim of our work is to obtain complete data sets for membrane transport of individual enantiomers and separations of enantiomers. The molecular simulations are used to significantly reduce the amount of experimental work. Therefore, the knowledge of the interactions between individual enantiomers and membranes is essential for further improvement the quality of predictions and understand the achieved results of separation processes.

**Coinvestigator: Pavel Izák, New effective membrane for efficiency separation H<sub>2</sub>/CO<sub>2</sub> (HySME), 01.01.2017 – 31.12. 2019, 17-05421S (4 131 000 CZK)**

The aim of the project is the development of new separation membranes for the effective separation of hydrogen from carbon dioxide. The preparation of H<sub>2</sub> and CO<sub>2</sub> selective membranes combine two parallel approaches based on different transport mechanisms, in particular the sieve effect mechanism and solubility-diffusivity transport mechanism. In the first case, stable and highly permeable membranes are prepared as graphene and graphene oxide foils with targeted surface modification. Secondly, highly selective membranes are prepared from a specific ionic liquid that offers enormously high CO<sub>2</sub> absorption.

**Pavel Izák, Flue gas purification by membrane separation processes, 01.01.2018 – 31.12.2020, 18-05484S (6 801 000 CZK)**

The project is focused on the search for new type of membranes that will be comparable with commercially available membranes or better them. Representative membranes will be studied, i.e. polymers with intrinsic microporosity, ionic liquid membranes and water-swollen composite membranes. Various gaseous pollutants will

be removed from the flue gas as well as fine particles. The separation of real flue gas will be tested with the membrane that will show the best transport and separation properties during the laboratory tests.

### **Czech Science Foundation, Junior projects**

#### **Zuzana Petrusová, Membrane separation of gasoline vapours from air and its optimisation, 01.01.2017 – 31.12. 2019, 17-03367Y (4 809 000 CZK)**

The junior project deals with a process of membrane separation of gasoline vapours from air, which is desirable for both ecologic and economic reasons. The main goal of the project is a systematic study of input parameters (temperature, rate and concentration of membrane feed, pressure above and below the membrane, counter-current/co-current flow arrangement in the permeation cell and the type of membrane) on the permeate flux and selectivity of organic vapours separation from air.

#### **Jana Gaálová, Elimination of pharmaceutical drugs and endocrine disruptors from water by membrane processes, 01.01.2019 – 31.12.2021, 19-08153Y (6 894 000 CZK)**

Endocrine disrupting chemicals are the focus of current environmental issues, as they can cause adverse health effects to animals and human, subsequent to endocrine function. Together with pharmaceutical chemicals, they present dangerous threats as water pollutants. Furthermore, the conventional methods of water and sewage treatment are not completely effective in their removal. The target of the project is to investigate the elimination of pharmaceutical chemicals and endocrine disruptors, emphasising on water environment, by membrane processes.

### **Ministry of Education, Youth and Sports of the Czech Republic**

#### **Pavel Izák, Supported ionic liquid membrane for separation of volatile organic compounds and pollutants from flue gases, 01.04.2014 - 30.06.2017, LD14094, COST action (2 290 000 CZK)**

The aim of proposed project is the development of ionic-liquid containing membranes for the separation of volatile organic compounds and pollutants from flue gases. Also development of the model, which allows testing hypotheses about non-constancy of membrane characteristics along its surface and its possible dependency on various parameters, is part of the project. By comparison with experimental data the model will also be validated. The validated model will be then used for the supported room temperature ionic liquid membrane optimisation of its operating parameters and geometry.

#### **Coinvestigator: Pavel Izák, Enrichment of raw biogas by methane, 01.04.2014 - 31.12.2016, LH14006, LH - KONTAKT II (995 000 CZK)**

The principal aim of this project is preparation, characterization and testing of advanced separation polymeric membranes with enhanced performance containing anchored ionic liquids in a suitable non-porous polymeric membrane using (i) Van der

Waals forces in coated (composite) porous asymmetric polymeric membrane or (ii) a covalent bond or (iii) high ionic liquid content cross-linked polymer gels. Preparation of such novel separation membranes is targeted for an efficient methane separation from biogas. It will also be developed a model enabling prediction the transport and separation characteristics of these membranes intended for separation of gas mixtures.

### **Technology Agency of the Czech Republic**

**Coinvestigator: Pavel Izák: Membrane separation of carbon dioxide from flue gas and its subsequent use, 01.07.2019 – 30.06.2024, TK02030155 (main investigator: MemBrain s.r.o.) (15 444 000 CZK)**

The greenhouse gas emissions are still increasing although all efforts with "green energy sources", especially CO<sub>2</sub> emissions from fossil fuels. The aim of this project is to construct and verify the mobile pilot plant with the membrane module for flue gas separation available to connect to additional pre-treatments. The capacity of the technology will be determined from our previous studies and simulations. The pilot plant will be independent and it will be possible to connect it as new technology for CO<sub>2</sub> capture.

### **The main scientific results achieved by the team within these projects are:**

59 impacted international publications

1 EU patent

## **Research activity and characterisation of the main scientific results**

The research activity of DACP during the evaluated period 2015 – 2019 is possible to divide into 9 topics focused on different aspects. Additionally, the main scientific results achieved within the individual research activity are listed below each topic.

### **Involvement in a comprehensive characterization of physical and chemical properties of atmospheric aerosol at pan-European scale**

The main focus of DACP concentrates on characterization of atmospheric aerosol in various environments. The key infrastructure for atmospheric aerosol observation is National Atmospheric Observatory Kosetice (NAOK) and suburban Suchdol observatory in the ICPF campus. DACP covers measurements of aerosol in-situ variables (physical properties including size distribution, number concentration, optical properties and chemical composition). All these activities are inline with participation of DACP in pan-European research infrastructure ACTRIS aiming at the observation of Aerosol, Clouds and Trace Gases. ACTRIS is composed of observing stations, exploratory platforms, instrument calibration centres, and a data centre. ACTRIS serves a vast community of users working on atmospheric research, climate and Earth system and air quality models, satellite retrievals, weather analysis and forecast systems by offering high quality data and research infrastructure services for atmospheric aerosols, clouds, and trace gases. ACTRIS aims to contribute in the resolving of such challenges by providing a platform for researchers to combine their efforts more effectively, and by providing observational data of aerosols, clouds and trace gases openly to anyone who might want to use them.

### **Topic 1: New particle formation and transformation**

In the 2015-2019 period, one of the main topics studied at the DACP, aerosol formation and transformation, continued to be studied intensively. The formation and transformation have been studied both in laboratory conditions and using field data. The laboratory works focus on the theoretical assumptions behind the new aerosol formation, also called nucleation, as the full understanding of the process is still missing, although its climate relevance is enormous. Nucleation of aerosol particles from gaseous precursors is an important stage in the formation of atmospheric secondary aerosols or in industrial applications, particularly coal burning boilers.

#### Description of results and share of the team

In the work of Trávníčková et al. (2018), called “Laboratory study of H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O nucleation using a new technique—a laminar co-flow tube” a novel laboratory device, laminar co-flow tube, was used for studying binary or ternary nucleation. The sulphuric acid, one of the main agents in the nucleation, has been described also in flow tube measurements of the effective sulfuric acid diffusion coefficients published by Brus et al (2017) in the paper called “Temperature-Dependent Diffusion of H<sub>2</sub>SO<sub>4</sub> in Air at Atmospherically Relevant Conditions: Laboratory Measurements Using Laminar Flow Technique”. The measurements and simulations done suggested that in the atmosphere the attachment of sulfuric acid molecules with base molecules can lead to a lower than expected effective sulfuric acid diffusion coefficient with a higher than expected temperature dependence. The laboratory studies with laminar flow technique were done in cooperation with a Finnish team, with L. Škrabalová from ICPF designing

and performing the experiments and contributed to synthesis of data and the article writing (share of the DACP team 30%).

The works based on the field data analyse new particle formation (NPF) that significantly influences the number concentrations and size distributions of an atmospheric aerosol and is often followed by the rapid growth of the newly formed particles. The process seems to have a large geographical extent, so multi-station datasets and approaches are typically needed or an influence of various air masses needs to be taken into account. In the work of Nemeth et al. (2018), simultaneous particle number size distribution measurements were performed in the urban environment of Budapest, Vienna, and Prague for two years and NPF events were classified using an adapted classification scheme for urban sites. The main difference between stations was related to source and sink terms of gas-phase sulphuric acid. For all stations, however, the new particle formation is a significant source of ultrafine (UF) particles, and the amount of them is comparable to the directly emitted UF particles. For Vienna, the impact of air mass origin and season on aerosol chemical composition and new particle formation and growth events was also investigated using impactor samples from short-term campaigns and two long-term number size distribution datasets (Wonaschütz et al. 2015). The results suggest that air mass origin is most important for bulk PM concentrations, chemical composition of the coarse fraction ( $>1.5\ \mu\text{m}$ ) and the mass size distribution, and less important for chemical composition of the fine fraction ( $<1.5\ \mu\text{m}$ ). Air mass origin was a secondary influence on NPF, largely through its association with meteorological conditions. Neither a strong dependence on the PM<sub>2.5</sub> loading of the air masses, nor indications of a source area for NPF precursors outside the city were found. Both works were done in cooperation with international team; ICPF scientist provided part of the data, analysed them and also participated on the writing, the share of the DACP team on the topic was 30 %.

During the data analyses, also a reversal process to formation, particle shrinkage, was found to occur under specific atmospheric conditions, which are unfavourable for the growth of nucleated particles. In the study by Škrabalová et al. (2015), an analysis of particle shrinkage events following prior NPF at an urban background station in Prague, Czech Republic, was done. During the most intensive events, the grown particles shrank back to the smallest measurable size of 10 nm. The particle shrinkage was attributed to the evaporation of previously condensed volatile and semi-volatile species from the particulate phase to the gas phase. When analysing the dependence on meteorological conditions, the particle shrinkage was found to occur under peak global radiance intensity, which was gradually decreasing, or under a sharp drop of global radiance intensity. This work was done 100 % by DACP team members.

#### Supporting projects and Students involved

MEYS MOBILITY grant 7AMB12AT021, Czech Science Foundation grants P503/12/G147 and P209/11/1342, European Union's Horizon 2020 research and innovation programme ACTRIS-2 (grant agreement No. 654109), ACTRIS - CZ (num. LM2015037), and ACTRIS – CZ RI (CZ.02.1.01/0.0/0.0/16\_013/0001315). One PhD student from DACP (L. Škrabalová) worked on the topic and successfully defended her thesis.

#### References:

Trávníčková, T. et al (2018). Tellus B: Chemical and Physical Meteorology, 70(1), 1-11. Brus, D. et al. (2017). Atmosphere, 8(7), 132. Németh, Z. et al. (2018). Atmospheric

Environment, 178, 191-197. Wonaschütz, A. (2015). Atmospheric Environment, 118, 118-126. Skrabalova, L. et al. (2015). Aerosol Air Qual. Res, 15, 1313-1324.

## **Topic 2: Chemical composition of aerosols**

Study of aerosol chemical composition serve as a base for many other related topics but also for determination of processes related to aerosol formation and transformation, related effects in aerosol transport and mainly relations between particle size and its chemical composition. We have covered three basic topics in aerosol chemical composition during recent years.

Detailed offline chemical characterisation of suspended particulate matter of defined size fraction (main focus on fine particles) was used for basic description of aerosol chemical composition in Central Europe and their seasonal variations and transport. Another topic has been related to characterization of chemical composition with high time resolution where diurnal variations in chemical composition served as indicators of aerosol origin and transport ways to a receptor site. Third main topic is connected to chemical characterisation of indoor aerosols, e.g. one study was performed within model kitchen to study effects of various sources on indoor aerosols. A new topic related to P. Vodička postdoctoral stay in Japan was introduced after his arrival connected with isotopic composition of aerosols.

### Description of results and share of the team

The results on ambient aerosols were evaluated especially with regard to aerosol origin – differentiation between aerosols of local, regional or long range transported origin and to different air masses. EC/OC analysis with 2 hour time resolution was used for characterisation of differences between urban and rural aerosols. Strong effect of urban environment on presence of more volatile OC fractions in winter and their diurnal pattern clearly showed their origin in anthropogenic emissions in a city. Double site study in Ostrava air pollution hot spot lead to elucidation of transboundary pollution transport in this area. Chemical analysis of non-refractory PM<sub>1</sub> aerosol in high time resolution using aerosol mass spectrometry in Prague confirmed local origin of organic aerosol in winter, however, regional or long range transport of sulphates was proved based on downmixing of sulphates during periods with high vertical mixing. The most interesting result from chemical point of view was related to usage a kitchen degreaser containing monoethanol amine. The single usage of the degreaser changed substantially the indoor aerosol composition by replacing ammonium salts with monoethanol amine salt for more than 24 hours. In the same time indoor nitrate concentration were increased substantially due to higher stability of monoethanol amine nitrate in comparison with ammonium nitrate. Possible health effects were suggested. The size resolved chemical analysis of a workshop aerosols were performed as a part of exposure studies. Characterisation of possible aerosol processes at NAOK using isotopic approach was performed in collaboration with Japan. The part of the studies were carried out by DACP only but the others also within a national and international cooperation and fractional count of the DACP authors differ from study to study from 0.25 to 1.

### Supporting projects and Students involved

National research infrastructures ACTRIS CZ, ACTRIS CZ RI and ACTRIS CZ RI2, and MEYES INTER-COST (LTC18068), and Czech Science Foundation

(P503/12/G147 - CENATOX , P209/19/06110Y, P209/20/08304J). Three PhD students have been working on this topic.

#### References:

Kubelová, L., et al. (2015) *Atmos. Environ.* 118, 45-57. doi:10.1016/j.atmosenv.2015.07.030. Pokorná, P., et al. (2018) *Environ. Pollut.* 241, 841–851. doi:10.1016/j.envpol.2018.06.015. Schwarz, J., et al. (2016) *Atmos. Res.* 176–177, 108–120. doi:10.1016/j.atmosres.2016.02.017. Schwarz, J., et al. (2017). *Environ. Sci. Technol.* 51. doi:10.1021/acs.est.6b06050. Schwarz, J., et al. (2019). *Sci. Total Environ.* 664, 1107–1116. doi:https://doi.org/10.1016/j.scitotenv.2019.01.426. Kozáková, J., et al. (2019), *Environ. Sci. Pollution Res.* 26, 1675-1692. Vodička, P., et al., (2019) *Atmos. Chem. Phys.* 19, 3463-3479, doi:10.5194/acp-2018-604. Vodička, P., et al. (2015). *Sci. Total Environ.* 518–519, 424–433. doi:10.1016/j.scitotenv.2015.03.029.

### **Topic 3: Source apportionment of atmospheric aerosol based on receptor modelling**

In order to analyse the spatial and temporal changes in airborne PM, the application of receptor model is highly effective. Receptor models analyse data matrices of aerosol chemical and physical characteristics of samples collected at a given locality, the receptor, to apportion their sources. There are several receptor model approaches of which factor analysis (FA) is the most frequently used to estimate the number and chemical profiles of the sources and their contributions to the receptor concentrations. Recent air quality studies use the advanced variant of the FA, Positive Matrix Factorization (PMF). PMF is a powerful tool for solving receptor models with highly time resolved aerosol composition data as well as particle size distribution data. The advanced receptor model currently used most often for highly time and size resolved chemical composition obtained using a aerosol mass spectrometer (AMS) is positive matrix factorization coupled with multilinear engine procedure (PMF-ME2). However, the bilinear multivariate models are not directly applicable to data with different sampling integration times (on-line offline measurements). To solve this expanded receptor modelling problem multi-time factor analysis model was developed using the ME algorithm that can utilize data collected at different time scales. Combining off-line and on-line PM measurements enables us to improve source apportionment of atmospheric aerosol.

#### Description of results and share of the team

The published source apportionment studies present results of receptor model applications on size-resolved aerosol composition and particle size distribution data collected in different environments (urban, suburban, urban with industry, rural background) with different integration times (24-h, 2-h, 5-min) during the intensive campaigns as well as for long-term period (Leoni et al., 2018; Pokorná et al., 2018; Kozáková et al., 2019; Schwarz et al., 2019; Lhotka et al., 2019). The studies arised from a national and international cooperation and fractional count of the Department of Aerosol Chemistry and Physics authors differ from study to study from 0.1 to 1.

#### Supporting projects and Students involved

National research infrastructures ACTRIS CZ, ACTRIS CZ RI and ACTRIS CZ RI2, and MEYES INTER-COST (LTC18068) and INTER-ACTION (LTAUSA1906), and Czech Science Foundation (P209/19/06110Y, P209/20/08304J). Two PhD students are working currently on this topic.

#### References:

Leoni, C., et al. (2018) *Environ. Pollution* 234, 145-154. Pokorná, P., et al. (2018) *Environ. Pollution* 241, 841-851. Kozáková, J., (2019), *Environ. Sci. Pollution Res.* 26, 1675-1692. Schwarz, J., et al. (2019), *Sci. Total Environ.* 664, 1107-1116. Lhotka, R., et al. (2019), *Atmosphere* 10, 687, 2-16.

#### **Topic 4: Aerosol-cloud-interactions**

In 2015, a new research topic focused on interaction between atmospheric aerosol and hydrometeors (product of condensation or deposition of atmospheric water vapor, for example clouds, fog, rain etc.) was started. Mainly, studies on connection between submicron aerosol and its influence on cloud physics were done, as the coupling between clouds and aerosol is one of the largest sources of uncertainties in the current climate models.

#### Description of results and share of the team

First, the influence of hydrometeors on submicron aerosol was studied at National Atmospheric Observatory Kosetice (NAOK), a background station in the Czech Republic, using long-term data series measured at the station. In the paper by Zíková and Ždímal (2016) called "Precipitation Scavenging of Aerosol Particles at a Rural Site in the Czech Republic" the in- and below- scavenging of aerosol was described on mist, fog, rain, and snow. It was found that both obscurities (fog and mist) and precipitation have a strong influence on the aerosol concentrations, both on the total number concentrations and on the particle number size distributions (PNSDs). The presence of phenomena even changes the number of modes on the PNSDs. The PNSD main mode is shifted to the larger particles, and the concentrations of particles smaller than 50 nm in diameter are considerably lower. The results were based on the interstitial aerosol only, i.e. aerosol not activated into the hydrometeors. Thus, in 2018 a project incorporating also measurements of activated aerosol was started under the Czech Science Foundation, called "Cloud scavenging of atmospheric aerosol". The project aims to examine the cloud processing of atmospheric aerosol using ground based data taken during four intensive measurement campaigns at Milešovka station, where fog/low clouds are present for most of the time of the year (225 out of 365 days). In 2018 and 2019, three out of the four campaigns were taken, and the results have been sent to Atmospheric environment in the manuscript called "Activation of atmospheric aerosol in fog and low clouds". The results have been also presented at several national and international conferences: European Aerosol Conference (2019), International Fog and Dew Association (IFDA) conference, Annual conference of the Czech Aerosol Society in 2018 and in 2019, and the conference Air in 2015 and in 2019. The share of the DACP authors on the results varies from 0.9 to 1.

#### Supporting projects and Students involved

Czech Science Foundation (P209/18/15065Y, P209/20/08304J). One PhD student is expected to work on this topic.



References:

Zíková, N., Ždímal, V. (2016) Tellus Series B – Chemical and Physical Meteorology 68, 27343.

**Topic 5: Indoor and workplace aerosols**

People in developed countries spend the majority of their time (approximately 90 %) in indoor environments, and as consequence they may be exposed to a range of pollutants of an indoor origin, particularly ultrafine particles which may cause cardiovascular, respiratory, and neurological hazards to human health. The indoor environments include homes, but also working environments including special cases, where people are exposed to various nanoparticles (either being directly the final product or being a by-product of other manufacturing procedures). DACP was dealing with different studies including indoor/outdoor relationship, changes of aerosol particles when traversing from outdoor to indoor environment, comparison of transport of gases and particles in indoor environment and also emissions of different indoor sources, and characterization of indoor workplace environments including exposure studies.

Description of results and share of the team

One laboratory study was aimed to better understanding of the relationship between the transport behaviour of gas and particles. Such knowledge is important for the realistic prediction of aerosol distribution in ventilated rooms when using tracer gas techniques. The data can be used to validate CFD models for the evaluation of the distribution of pollutant concentrations and airflow patterns in rooms with overhead mixing ventilation. The study investigated the separate and combined effects of ventilation rate, free convection flow produced by a thermal manikin, and the presence of objects on the distribution of tracer gas and particles in indoor air (Bivolarova et al., 2017). Share of the DACP team was 50%.

Another study related to indoor/outdoor relationship was dealing with near real-time dynamic aerosol behaviour along a migration path from an outdoor to indoor environment. This paper describes the use of a unique valve switching system that allowed for high temporal resolution indoor and outdoor data to be collected concurrently from online and offline aerosol spectrometers covering physical and chemical composition of indoor/outdoor aerosol with high time resolution (Talbot et al., 2016). ). Share of the DACP team was 100%.

Following study on changes of physical and chemical properties of aerosol when the aerosol particles were migrating from indoor to outdoor environment was conducted using the same measurement design as the previous study. In this study we concentrated specifically on changes in size distribution and chemical changes connected to nitrate particles. During the study, no known indoor sources were present and the sampled room was unoccupied throughout the whole measurement (Talbot et al., 2017). Share of the DACP team was 100%.

Various studies on nanoparticle emissions and the following exposure were conducted during this period as well. The aim of this study was to evaluate the nanoparticle emissions from a laser printer and to characterize the processes that lead to increased nanoparticle concentrations, as well as to estimate the human particle dose of the printers' users (Serfozo et al., 2018, share of DACP was 25%).

There is little information about potential risks of inhalational exposure of stomatologists and their patients to nanoparticles released from dental composites during their finishing in dental cabinets. The aim of this work was to map the

dimensions of aerosol particles released during grinding typical dental composite materials using a few rotational instruments under conditions simulating work in the dental cabinet. We found that under specific conditions release of particles in the nanorange cannot be excluded and that aerosol nanoparticles may originate from the thermal decomposition of the composite polymeric matrix due to friction heat rather than from filler nanoparticles. (Bradna et al, 2017, share of DACP was 65%).

Aerosol exposure of workers and the related health effects have been studied in a series of papers, where the role of DACP was to determine the aerosol exposure, using a combination of online and offline methods, while the medical doctors from Charles University were taking samples of body liquids of workers in nanomaterial production, another team of analytical chemists and statisticians analysed the samples for biomarkers and their correlation with exposure (Pelclova, et al. (2015); Pelclova, et al. (2016). Pelclova, et al. (2017). Share of DACP team was 30%).

#### Supporting projects and Students involved

EU project HEXACOMM (FP7-PEOPLE-2012-ITN - Marie-Curie Action: "Initial Training Networks"). Czech Science foundation grant No. P503/12/G147. - Centre of Excellence CeNaTox. Three PhD students worked on this topic.

#### References:

Bivolarova, M., et al. (2017) *Indoor Air*. 2017, 27(6), 1201-1212. Talbot, N., et al. *Atmospheric Environment*. 2016, 131, 171-184. Talbot, N., et al. (2017) *Aerosol and Air Quality Research*. 17(3), 653-665. Serfozo, N., et al. (2018). *Environmental Science and Pollution Research*. 2018, 25(13), 13103-13117. Bradna, P., et al. (2017). *Monatsh Chem* 148, 531–537. Pelclova, D., et al. (2015). *Journal of breath research*, 9(3), 036008. Pelclova, D., et al. (2016). *Journal of breath research*, 10(1), 016004. Pelclova, D., et al. (2017). *Nanotoxicology*, 11(1), 52-63.

#### **Topic 6: Indoor aerosols and cultural heritage**

A complex study was performed in libraries, archives, museums, churches, and depositories located in areas with different outdoor air quality. It included long-term investigation of chemical composition of indoor particulate matter (PM), behaviour of gaseous pollutants, modelling of indoor/outdoor relationship, deposition on indoor surfaces, influence of visitors, and adverse effects of dust deposits. The results revealed that the most important source of the indoor pollution is penetration from the outdoor air. The chemical composition of PM indicated that all the particles found indoors can be considered to be potentially threatening to the stored materials. Thus, the higher level of pollution and therefore the higher degree of degradation is expected in depositories located in highly polluted regions. The results can be used for a selection of strategies of the indoor air quality control management.

#### Description of results and share of the team

The published studies present characterisation of indoor PM (Mašková et al., 2015), gaseous pollutants (Vichi et al., 2016, Mašková et al., 2017), indoor/outdoor relationship (Chatoutsidou et al., 2015, Mašková et al., 2020), influence of visitors (Mašková et al., 2016), and effects of PM on cellulose (Bartl et al., 2016). The studies arised from a national and international cooperation and fractional count the Department of Aerosol Chemistry and Physics authors differ from study to study from 0.5 to 1.

#### Supporting projects and Students involved

Ministry of Culture NAKI (DF11P01OVV020) and NAKI II (DG18P02OVV048), EU 7th framework program HEXACOMM (315760), and AVCR/CNR Bilateral Agreement Exchange Programme.

#### References:

Chatoutsidou, S.E., et al. (2015) *Build. Environ.* 89, 253-263. Mašková, L., et al. (2015) *Atmosp. Environ.* 107, 217-224. Bartl, B., et al. (2016) *Stud. Conser.* 61(4), 203-208. Mašková, L., et al. (2016) *Aerosol Air Qual. Res.* 16(7), 1713-1721. Vichi, F., et al. (2016) *Heritage Sci.* 4(4), 1-8. Mašková, L., et al. (2017) *Build. Environ.* 120, 77-84. Mašková, L., et al. (2020) *Build. Environ.* 180, 107024.

#### **Topic 7: Nanoparticle synthesis for inhalation studies**

For inhalation exposure studies of toxicity of NPs, there is a requirement for continual generation source of NPs for a duration of weeks and sufficiently high number concentration, with appropriate emission rates, size distribution and well-defined composition. For these studies, the ICPF research team tested the methods of NPs generation of various species, with the task to find suitable experimental conditions of NPs generation of each species for follow-up long term inhalation exposure experiments with laboratory animals. During the project we tested the generation of Pb NPs by evaporation/condensation method, generation of titania NPs by pyrolysis and oxidation of liquid metalorganic precursor titanium tetra-iso-propoxide, generation of ZnO NPs by evaporation of metallic zinc into stream of carrier gas and oxidation of Zn vapour by a stream of diluting air, generation of Cu/Cu<sub>2</sub>O/CuO NPs by pyrolysis and oxidation of solid metalorganic precursor copper(II) acetylacetonate and generation of FeOx NPs by pyrolysis and oxidation of solid metalorganic precursor iron(III) acetylacetonate. All NPs generation experiments were carried out in an externally heated tube flow reactor. Particle production (number, surface and mass concentration, mean particle size and particle size distribution) of all species was monitored using scanning mobility particle sizer. Samples of NPs were deposited on TEM grids for TEM (morphology), EDS (composition) and SAED (crystallinity) analysis and on various kinds of filters for ICP-OES, EC/OC, EDS, XPS (composition) and XRD (crystallinity) analyses. NP production of all species were investigated in wide range of experimental conditions and optimal/suitable experimental conditions for generation of all species were found and recommended for inhalation exposure experiments. We also participated on Inhalation exposure experiments itself by providing the aerosol generating system and know-how of NPs generation to the Institute of Analytical Chemistry (IAC), where the inhalation experiment with laboratory animals were carried out. The toxicity of the NPs itself was studied in the Veterinary Research Institute (VRI), Institute of Animal Physiology and Genetics (IAPG) and Institute of Experimental Medicine (IEM).

#### Description of results and share of the team

The results of Pb NPs generation study were published in Moravec et al., 2015, TiO<sub>2</sub> NPs production in Moravec et al., 2016 and ZnO NPs synthesis in Moravec et al., 2019. In all of these papers the fractional count of DACP was 0.8. Some aspects of toxicity of ZnO NPs were presented in Rossner et al., 2019 (share of DACP was 0,05) and Vysloužil et al., 2020 (share of DACP was 0.1).

#### Supporting projects

Czech Science foundation grant No. P503/12/G147. - Centre of Excellence CeNaTox.

#### References:

Moravec, P., et al. (2015) Aerosol. Sci. Technol. 49, 655-666. Moravec, P., et al. (2016) Aerosol. Sci. Technol. 50, 1068-1076. Moravec, P., et al. (2019) Aerosol. Sci. Technol. 53, 172-183. Rossner et al. (2019) Toxicol. Sci. 168, 190-200. Vysloužil et al. (2020) J. Trace Elements Medicine Biology 61, 126511.

#### **Topic 8. Size resolved testing of filters and personal protective equipment**

Various industrial processes produce a wide range of aerosol particles of different composition and size. Thus, workers in these workplaces may be exposed to a broad range of aerosol particles possibly causing adverse health effects. Many organizations worldwide recommend protective respiratory devices as a preventative form of protecting workers from exposure to hazardous aerosol particles. Filtering facepiece respirators (FFR) are widely used against harmful aerosol particles and protect the user if worn properly. Throughout the years, the performance of the FFRs has been the topic of extensive applied studies, resulting mainly in the characterization of aerosol penetration through filters using aerosols that are thought to be similar to those encountered in workplaces. Our approach included measurements of size resolved filtration efficiency of a set of selected respirators having different protection level (FFP1, FFP2, FFP3) from different manufacturers. Two studies arise from this topic: one dealing just with the material itself and the second one dealing with the whole respirator.

#### Description of results and share of the team

In the first study, the penetrations and the most penetrating particle size (MPPS) of samples cut out from different CE-marked respirator half masks were evaluated (Serfozo et al., 2017a, share of DACP was 80%).

Following study was dealing with the performance of the whole FFRs. The research was conducted using manikin-based method to determine the penetrations through various CE-marked filtering facepiece respirator models. The study also compares the two approaches – measurement of the penetration through only the material of the FFRs and the penetration through the whole FFR fitted to the manikin face. This research also reveals the disadvantages and shortcomings of the currently valid European Norm (EN 149:2001) for testing the filtering facepiece respirators. As a final remark it has to be taken into consideration that any of the personal protective equipment (PPE, especially respirators and face masks) the most important parameter is the tightness of the PPE on the face. Any leak around the PPE can significantly lower the filtration efficiency (sometimes even by tens of percent). Therefore, the CE-marked respirators examined in this study may not be efficient in providing the expected level of protection for workers exposed to nanoparticles (Serfozo et al., 2017b, share of DACP was 80%).

#### Supporting projects and Students involved

EU project HEXACOMM (FP7-PEOPLE-2012-ITN - Marie-Curie Action: "Initial Training Networks"). Two PhD students who were also involved in this topic finished their PhD within HEXACOMM project.

#### References:

Serfozo, N., et al., (2017) *Aerosol and Air Quality Research*. **17**(5), 1305-1315.  
Serfozo, N., et al. *Journal of Occupational and Environmental Hygiene*. 2017b, **14**(12), 965-974.

#### **Topic 9: Thermodynamics of Task-Specific Materials (TTSM)**

Two groups of compounds were studied in this topic. Ionic liquids have been investigated both from the fundamental point of view, in terms of their structure-property relationships, and with regard to their possible use as thermal storage media. Two series of imidazolium-based ionic liquids with a bis(trifluoromethylsulfonyl)imide (bistriflimide) anion were synthesized. The influence of the fine tuning of the isomeric structure on the IL properties was thus studied as well as the role of the second substituent structure. The application potential of ILs in thermal energy storage was then assessed for two 1-hexadecyl-3-methylimidazolium ILs with a chloride and a saccharinate anion. Industrially relevant data were obtained for the temperatures and enthalpies of melting. Vapour pressures, surface tension, and thermal properties of selected terpenes were then studied to contribute to a better understanding of nucleation in the atmosphere. All the experimental data were analyzed using an advanced data analysis based on mathematical gnostics to validate measurements of thermophysical properties of ionic liquids and terpenes. In both projects, marginal analysis was used.

#### Description of results and share of the team

The published data on ionic liquids brought novel and industrially relevant information on their thermophysical and thermodynamic properties. The studied ILs were found to show some application potential for thermal storage, either by their high energy density or a suitable latent heat of melting (Bendova et al. 2019, Čanji et al. 2020). Moreover, interesting structure-property relationships were found in isomeric and quasi-isomeric ionic liquids, showing the possibility of fine-tuning the properties of ILs by subtle structural changes (Rotrekl2017). Novel data were also measured for vapour pressures and heat capacities of terpenes (Wagner2019) and the correlation of vapour pressures by means of the Clarke-Glew equation was discussed. Rigorous mathematical tools were used to calculate the sensitivities of its parameters and hence assess how many parameters can in principle be estimated from the experimental data. The advanced data analysis based on mathematical gnostics then enabled us to critically assess the experimental data and to use robust regression methods on their description.

#### Supporting projects and Students involved

Czech Science Foundation projects 17-08218S and 17-19798S, Ministry of Education, Sports and Youth Project LD14090. Three PhD students were involved in these projects.

#### References:

Bendová et al. (2019) *J. Solut. Chem.* **48**(7), 949-961; Čanji et al. *J. Mol. Liq.* **292**, Article 111222; Rotrekl et al. (2017) *Fluid Phase Equilib.* **443**, 32-43.; Wagner et al. (2019) *J. Solut. Chem.* **48**(7), 1147-1166.

## **Research activity and characterisation of the main scientific results**

Recently, the Department has pursued its scientific orientation to fulfil the new concept of chemical engineering, which during its existence goes through the orientation towards unit operations, transport phenomena and product engineering to the current focus on sustainable chemical engineering forced by the expected energy demand, constraining water supplies and climate changes. Scientific activities of the Department correspond to the progressive directions of chemical engineering aimed at ensuring a scientifically based environmentally healthy life and economic use of renewable resources. Therefore, concerning the period under the review, scientific research conducted in the Department was interdisciplinary. The main topics mentioned above Catalysis for 21st century, Novel materials for modern technologies, Reaction engineering for environment sustainability and Biorefining and biotechnology cover the priority areas of catalysis including all major aspects of homogenous and heterogeneous catalysis, mathematical modelling, reaction engineering, surface science, materials' science with special focus on texture and morphology studies, transport phenomena and materials engineering, chemical and environmental technologies, together with process development, environmental processes, biotechnology and other related fields. It is evident that the research activities were very diverse and broad.

Department activities clearly reflected in the numbers and variety of projects which have been solved within the last five years; EU projects, NATO project, collaborative international projects supported by Ministry of Education, grants supported by Grant agency of the Czech republic, another technology related projects supported by Ministry of Industry and Ministry of the Interior and projects supported by Technology Agency of the Czech Republic including Centre of Competence focused on Biotechnology and National Centre of Competence focused on bio-refining in intention of the circular economy.

The Department's strategy seems to focus on many various topics; nevertheless, these topics have been carefully selected to take advantage of detailed basic research knowledge for applied research that focuses on the real problems of contemporary society. Applied research supported by various grant projects involved collaboration with companies and other research institutions. In two cases, the implementation phase was achieved, in cooperation with a number of companies, to verify the scale-up of the proposed technology for practice. Obviously, it was not a simple task to characterize briefly the main research achievements at the Department during the inspected period 2015-2019. However, to summarize, 114 scientific achievements could be tracked in the form of research papers, books and book chapters, patents, industrial approved technologies, and best available technologies. Apparently, only some of them may be introduced below.

### **Catalysis for 21st century**

Besides the large number of research areas mentioned above, the substantial Department's activity is usually associated in some way with catalysis and/or reaction engineering, catalysts/materials research, and the design, development and construction of various types of reactors. Obviously, the catalyst's and the reactor's design have always been intimately linked. No doubts catalysis is a key principle for selective transformations in the industrial-scale manufacture and this part was one of the major focuses in the department catalytic research during the inspected period.

The research was focused on understanding of hydrotreating reactions such as hydrodesulfurization and hydrodeoxygenation, which are crucial for effective environmental protection and sustainable development. The subject of research covers synergism of catalytically active transition metal components, advanced catalyst syntheses including our original method of solvent-assisted spreading, and the role of support. Our catalyst research emphasizes the increase of catalytic activity as well as the aspects of price and lifespan. A new method of preparation of CoMo and NiMo catalysts has been successfully developed. It is called slurry impregnation method (SIM) or solvent-assisted spreading (SAS). The SAS method uses precursors of low solubility in water such as  $\text{MoO}_3$ ,  $\text{CoCO}_3$  or  $\text{NiCO}_3 \cdot 2\text{Ni(OH)}_2$  to prepare solid catalysts instead of high solubility precursors (solubility higher than 40 g per 100 ml of  $\text{H}_2\text{O}$ ) such as  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ ,  $\text{Co(NO}_3)_2$  or  $\text{Ni(NO}_3)_2$  typical for other methods of preparation. Low solubility (mostly below 0.13 g per 100 ml of  $\text{H}_2\text{O}$ ) was found to be sufficient to gradually dissolve the compound and to simultaneously absorb the compound onto support surface. The SAS method is suitable in the deposition of  $\text{MoO}_3$  onto supports such as  $\text{Al}_2\text{O}_3$ ,  $\text{AlO(OH)}$ ,  $\text{ZrO}_2$ ,  $\text{ZrO(OH)}_2$ ,  $\text{TiO}_2$ , C,  $\text{MgO}$ , and  $\text{SiO}_2\text{-Al}_2\text{O}_3$ . Co and Ni can be deposited over Mo catalysts containing a saturated adsorption monolayer of  $\text{MoO}_3$  or sulfidic layers of  $\text{MoS}_2$  by SAS, i.e. from aqueous slurries of low solubility carbonates or hydroxycarbonates. The SAS method does not make it possible to exceed the dispersion capacity of the support surface or to form three dimensional (bulky), and therefore catalytically inactive, phase on the support. This method is a clean and simple method, which does not introduce any auxiliary ions like  $\text{NH}_4^+$  or  $\text{NO}_3^-$ . Calcination is not needed. No environmentally harmful  $\text{NO}_x$ es are, therefore, formed during calcination. Only water is produced on drying. Water, thus, represents the only side products of the SAS. For these reasons, the SAS method represents a practical implication of concepts of green chemistry. The dried form of catalysts can be sulfided without calcination. The prepared catalysts in their sulfidic forms exhibit superior activity in model hydrotreating reactions important for sustainable refining of both fossil and renewable feeds, e.g. results 1, 3, 5.

Furthermore, the issues related to the total oxidation of volatile organic compounds (VOC) was intimately studied regarding the fact that VOC emissions are detrimental both to the environment (several times more efficient greenhouse gases than  $\text{CO}_2$ ) and to human health (the formation of ground-level ozone, which is a major component of photochemical smog). A significant success in this field has been achieved by publishing a series of articles describing the effect of gold nanoparticle deposition on the catalytic properties of the mixed cerium oxide and zirconium. The studies were created in cooperation with top European laboratories: Prof. E. Gaigneaux at the Université catholique de Louvain (Belgium), Prof. R. Keiski at the University of Oulu (Finland) and Prof. J. Barbier Jr. at the University of Poitiers (France), and they were published in highly impacted international journals (e. g., Applied Catalysis B, IF = 14.2). The first and corresponding authors are members of the ICPF, where the idea and major part of the research (catalyst preparation and test design) came from. For the first time, attention was drawn to a rather surprising fact that even nanoparticle agglomerates of considerable size (of the order of 100 nm) can be catalytically active in the oxidation of certain types of VOCs (until then, such large particles had been considered catalytically inactive). The studies describing the powder catalysts were subsequently extended to the preparation of monolithic catalysts, which, due to their difficulty, have been relatively little studied, even though they are crucial for the application of catalysts in industrial practice.

Research was also focused on the possibility of using plant residues as biocatalysts for VOC oxidation. Carbonaceous materials (catalyst supports) were prepared from a broad range of waste agricultural biomass involving tropical wastes (e.g. cocoa pod husk, corn cobs, coffee husk or red mombin seeds in collaboration with National University of Tumbes) or waste biomass coming from the local agriculture industry such as, e.g., vascular plants and algae after appropriate biotechnology processes. These materials were then used as a support for palladium or platinum nanoparticles introduced by wet impregnation. Before applying them as VOC catalysts, all prepared catalysts were thoroughly characterized to obtain their texture and structure properties. Their activity and selectivity as catalysts for VOC oxidation was, subsequently, correlated with their texture and physicochemical properties.

Simultaneously, the research was focused on transition metal oxides catalysts in various forms: particles, pellets, monoliths, meshes. These results could be obtained due to long-term cooperation of the team from ICPF with Prof. L. Obalová and Prof. F. Kovanda and their respective teams. The team of Ostrava focused mainly on examination of catalytic activity in N<sub>2</sub>O or NO decomposition and some physicochemical properties (FTIR, SR-TAD), team of Prof. Kovanda concentrated on the preparation of precipitated catalysts or hydrothermal technique and examination of some properties (XRD, SEM). The team of ICPF tested catalysts in VOC oxidation, the preparation of structured catalysts (over monoliths or Al meshes) and examination of catalysts properties by TPR and TPD techniques. The portion of the team contribution can be estimated to one third for each team.

The investigation showed that particles of transition metal oxides emerging after calcination of layered double hydroxides serve as very active catalysts in the abatement of organic compounds of air. Using various combinations of transition metal M-II and M-III cations on oxidation activity revealed that ternary mixed oxides containing Mn were more active than the binary Cu-Mn, Co-Mn, and Ni-Mn ones, as well as the ternary Al-containing catalysts; the Cu-Ni-Mn mixed oxide showed the highest activity in ethanol oxidation. The catalytic activity increased with an increasing amount of easily reducible components and amount of oxygen desorbed from catalysts surface at lower temperatures (up to 500°C). Deposition of mixed transition metal oxides on monoliths or wire meshes was studied as they show a low-pressure drop of the catalytic layer and absence of internal diffusion of reactants. Optimum conditions were found for the preparation of sufficiently adherent catalytic layers to the support in the form of monoliths. Concerning wire meshes, Al meshes were treated under hydrothermal conditions in the solution of Co and Mn nitrates, and transformed into Co-Mn-Al oxides after heating. Co<sub>3</sub>O<sub>4</sub> deposited from Co(OH)<sub>2</sub> suspension provided a more active catalyst than Co-Mn-Al oxides. The deposition of thin films of mixed oxide enabled its high utilization in the catalytic reaction due to low influence of internal diffusion of reactants and products in comparison with the pelleted catalyst. Magnetron sputtering method was also applied, and various combinations of magnetron methods and hydrothermal introduction or electrochemical deposition of transition metal oxides over meshes were tested. The best mechanical stability of the deposited Co oxide layer was achieved on the thin Co oxide layer deposited by magnetron sputtering on stainless steel meshes first, before the electrochemical deposition was applied; e.g. results 4, 8, 10, 11, 15, 18.

Attention was also paid to the amperometric analysis of Ru  $\pi$ -conjugated polymers, prospective supports for enantioselective Ru catalysts. The output has brought a completely new point of view on a family of catalytic supports for complicated organometallic complexes with an inherent stereoselectivity. Their potential utilization



lies in the stereoselective synthesis, namely the asymmetric hydrogenations of unsaturated ketoesters. The data on the electronic structure of the new class of support in respect of these special reactions were published in detail; e.g. the result 12.

An integral part of the research concerning catalysis was also an accurate characterization of the texture and microstructure of materials. It is obvious, that fully understanding the functional relationship between the inner pore structure and texture and transport properties of porous solids is quite essential for optimization in terms of their introduction to real applications. Mass transport measurements were carried out both in dynamic and steady-state regime involving an inverse gas chromatography method, Wicke-Kallenbach diffusion cell, Graham's diffusion cell and both quasi-stationary and dynamic permeation cells. Subsequently, fitting of the primary data to the Mean Transport-Pore Model provides corresponding transport characteristics. Within the textural studies, special attention was paid to the detailed analysis of micropores using advanced texture methods based on the Density Function Theory. The obtained transport characteristics were put into the context with the evaluated textural properties. Tested materials involved not only catalysts, but also biochar, activated carbon and chitosan.

Our own unique software was used to clarify the relation between a material structure and mass transport in pores. This knowledge was applied to explain catalytic processes and to make reliable predictions on the optimal pore structure of tested materials. The optimal pore structure of heterogeneous catalysts and other applied porous materials (adsorbents and membranes, etc.), and, consequently, optimization of the catalyst preparation was possible to propose based on the knowledge of individual process steps. This unique combination of catalyst preparation, process design and validation, characterization, modelling and optimization was used to address today's challenges in sustainable chemistry.

#### Novel materials for modern technologies

The research was focused on preparation of nanomaterials including highly crosslink polymers and thin layers of materials with a wide range of structures and properties based on oxides of transition metals which can be modified according to the demand. In the framework of our long time orientation towards research of polymer-based functional materials in the past years, we focused our effort on investigation of a novel type of porous highly poly-divinylbenzene. Our original method for evaluation of morphology of polymer materials in their swollen state help us discover really unique and novel morphology of these materials. The research was focused on detailed investigation of the influence of preparation conditions on the resulting polymer morphology. It was revealed that the foam-like morphology of the novel porous polymers is created by microsineretic mechanism of phase separation, so far considered possible in very low-crosslinked materials only. It was found that for its initiation, the "solvothermal" conditions are not necessary, and it was proved that the mesoporous foam-like morphology formation is important especially for high dilution of monomers with a "good" solvent, which is a solvent with high affinity to the polymer chains. The method of the novel mesoporous polymer preparation was patented (Czech pat. 307994). In cooperation with colleagues from the University of Maribor, Slovenia, it was proved that creation of the novel mesoporous morphology is not limited to poly-divinylbenzenes but can also be initiated in polymerization of other monomers. (ICPF idea and test description, University of Maribor scale-up of polymerization (M. Paljevac, P. Krajnc, L. Hankova, L. Holub, B. Le Droumaguet, D. Grande, K. Jerabek, Mater. Today Commun. 7 (2016) 16-21.)) The novel mesoporous poly-divinylbenzenes

exhibit unique properties differing from currently available commercial materials. They offer a very high surface area in relatively wide pores and morphology independent of the nature of surrounding solvent. Searching for applications in which such properties could be advantageous, we found, in cooperation with University of Padua, Italy, that, in solid state, peptide syntheses supports based on our mesoporous polymers can, in some aspects, be significantly better than existing commercial materials. This use of our novel polymer is a subject of European patent application nr. EP18164049, and industrial scale tests of these novel supports are under negotiations; more details also in the result 2.

The activities were directed towards preparation, modification and testing of nanomaterials related with thin layers based on titania, ceria, zirconia with various dopants as copper, platinum, iron and graphitized carbon nitride to clarify relationships between the properties of modified photocatalysts depending on a dopant type and its amount. The research was also focused on the photocatalytic activity and selectivity and photosensitivity of prepared materials. Based on the obtained results, two application projects oriented on photo-sensitive sensors for protection of old books and another archive documents were solved. The technical solution relates to the surface element for the visual indication of the exposure dose of UV radiation by a calibrated colour change. Thin layers, which exhibit a time-dependent calibratable response as a colour change, are an extremely effective tool for simple visual evaluation of a time variable. It is the memory element which converts a continuous timeline with almost arbitrary precision into a cumulative form, which can be used for various applications, e.g. protection of archive materials from light. This unique new solution, which can be used to evaluate the dose of a particular type of radiation, safe exposure time, expiration time, or light resistance as well durability limits, was tested in the National Library of the Czech Republic. Thus, we obtained an effective and economically advantageous time monitoring tool with an option of directly assessing time-dependent variables; the result 29.

The main objective of the second application project was the development of invisible sensors on selected types of archival materials to protect them in cases of alienation, which is a serious problem affecting the whole Central European region. The element cannot prevent theft itself, but is able to unambiguously provide information about its origin upon the capture of the stolen documents. This type of information is regarded as a key security expert clue. We tested the developed thin layer elements, which are nearly invisible. It should be emphasized that the element is really small with the dimension order of  $\text{mm}^2$  and a thickness of 300 to 600 nanometers; moreover, it is transparent; thus, it is visible only when using an electron microscope and knowledge of the exact position on the document. Our technical solution relates to a surface element for the visual indication of the exposure dose of UV radiation by a calibrated colour change. We prepared and tested thin layers, which exhibit a time-dependent calibratable colour change. It is, basically, a memory element which converts a continuous timeline with almost arbitrary precision into a cumulative form, which was tested for protection of artefact for Ministry of Interior.

During the inspected period, we significantly deepened the research focused on preparation of nanofibers by an electrospinning method, which can be objectively modified regarding their use. They were tested as specific catalysts and live cell carriers. Research activities related to the utilization of electrospinning technique in heterogeneous catalysis were focused on assessment of the specific properties of the novel polymeric electrospun materials in applications as catalyst supports and reaction carriers. Especially, optimization of their porosity, attachment of the catalytically active

centres or catalyst precursors, tests of various possible applications, evaluation of importance of working-state pore structure of the tested catalysts for overall kinetics of the catalytic reaction and investigation of possible effect of differences between nature of the polymer surface of nanofibers and conventional supports on the catalytic properties (activity and selectivity) were the subject of the complex investigation.

Another research area was specifically addressed to preparation of electrospun supports for living cells immobilization. Living cells are deposited directly on the electrospun nanofibrous carriers to prepare the cell based scaffolds. Additionally, this research was specifically addressed to investigation of the effect of both morphology and microstructural properties (texture and transport characteristics) of nanofibrous carriers on the cell attachment and growth; e.g. the result 17.

#### Reaction engineering for environment sustainability

Attention was paid to those processes, which could guarantee environmental protection by elimination of undesirable substances from water, gas and soil; our long-term theme. The research was devoted to thorough investigation of a series of specially designed methods like electrocoagulation, photolysis, photocatalysis, adsorption, but also zerovalent iron and nanoremediation for decontamination of polluted industrial and waste waters. These activities were supported by a number of grants as well as application projects; thus, efficiency and economic feasibility of laboratory designed and experimentally verified methods of decontamination were tested on pilot plant units. The research was focused on the optimal combination of methods with regard to the presence of individual types of contaminants to guarantee both efficiency and process economics. Subsequently, the proposed methods of decontamination of industrial waste water were verified with regard to the treatment and management of contaminated water also in brownfields. Within the verified technology, a technological scheme for the removal of pollutants from industrial wastewater, including individual case studies, was published. Moreover, the research brought new details on the design, optimization and utilization of the electrocoagulation method under conditions of the real industrial effluent treatment. The work was also focused on several different toxic ions, including ions of lead, zinc, chromium, copper and aluminum. Various types of process conditions were inspected with the intention to describe the optimal operational mode regarding various types of effluents. The process was designed in such a way that would also allow the scaling up calculations based on critically assessed process data. Furthermore, special attention was paid to water treatment containing toxic metals. The verified industrial technology pattern was realized in cooperation with Dekonta,a.s. (ICPF – idea, design and tests, Dekonta – scale up covering application under conditions of the real industrial effluent treatment; results e.g. 19, 21, 22.)

Besides the design, optimization and utilization of the methods for the real industrial effluents treatment, an important part of our effort in this field had been spent on photocatalytic and photolytic methods for an endocrine disruptor elimination and clarification of the photocatalyst effect. It has been verified that although endocrine disruptors (EDs) are often considered as one group of pollutants, their elimination from water needs to be approached individually, depending on the type of disruptor. Direct photolytic decomposition brought significantly increasing values of toxicity during the reaction for various types of substances (e.g. Ethynyl estradiol, Bisphenol A and 4-Nonylphenol). It was found that the presence of a photocatalyst significantly affected the resulting toxicity of the solution; the toxicity decreased slightly in parallel with the

decomposition of individual EDs; e.g. *Journal of Sol-Gel Science and Technology* (2018) 88:22–32 <https://doi.org/10.1007/s10971-018-4654-6>.

It is also necessary to mention a new direction of our research which is aimed at the solar driven photo-electrolysis of water. Our original electrochemical method using solar radiation allows generation of hydrogen at cathodes and, under the catalytic effect of co-producing nanoparticles (eg Pd, Zn, Fe, etc.), allows PCB dechlorination to harmless biphenyl. Similarly, it is possible to degrade very difficult-to-decompose chlorinated pesticides (e.g. aldrin, dieldrin), which are also present in 12 POPs. This original method brings new scientific knowledge but at the same time has a perspective of a practical use; e.g. *Silicon Nanowires' Based Photoanode for Efficient Hydrogen Evolution*. *International Journal of Hydrogen Energy*. 2018, 43(39), 18136-18141. ISSN 0360-3199 doi: 10.1016/j.ijhydene.2018.04.244

Combined thermal desorption and catalytic oxidation for soil decontamination supported by several grants, which was studied not only at laboratory but also at the pilot plant scale, must also be emphasized. Decontamination methods of soil polluted by a broad range of contaminants, such as petroleum substances, polyaromatic hydrocarbons, and heavy metals, were verified. Moreover, the influence of strong oxidant addition on consequent catalytic oxidation or alternatively applied biodegradation of condensates was successfully tested. Numerous unique species attributable to 2-methylnaphthalene oxidation together with products of motor oil oxidation and benzoyl peroxide destruction were identified in condensed vapors, whereas oxidation products of xylene were not detected. The application of benzoyl peroxide significantly accelerated biodegradation of condensates by *Pseudomonas* sp. Moreover, it was confirmed that the presence of partial oxidation products in the condensates did not increase their eco-toxicity. Efficiency and economic feasibility of laboratory experiments were proven on pilot-plant units. Based on our results, the new inventions were reported. This inventions report a new solution for remediation of soils from brownfields contaminated with organic pollutants and heavy metals. During the thermal desorption, toxic substances as mercury, cadmium and arsenic may be released into the atmosphere. According to the inventions, capturing of these metals is performed via the adsorber. Adsorbent is prepared by impregnation with water solution of calcium polysulphides and alkali metal polysulphides, while the support can be silicates, aluminosilicates, activated carbon or biochar. Heavy metal vapors (in particular mercury, cadmium, or arsenic) adsorb on the adsorbent surface, and react with the sulfur and/or alkali components of the adsorbent to form the compounds which are insoluble in water and firmly anchored on the adsorbent and, thus, removed from the vapor phase; results 9, 23, 24, 27.

Another topic supported by an EU project had been focused on investigation of the formation and release of contaminants, as well as evaluation of microstructure and sorption properties of experimental post-gasification solid residues to specify the impact of the Underground Coal Gasification (UCG) process to the environment. Within this project the solid post-gasification residues (char, slag and rock strata) in terms of fluids migration, heat dissipation, mechanical strength, catalytic activity, as well as CO<sub>2</sub> storage capacity were obtained, studied and physically characterized.

One of the most important theme related to the development of a new technology for regeneration of electronic grade silicon and germanium from waste photovoltaic cells and discarded optical lenses, which is possible to apply on any other material. This method is based on the reaction of grounded waste materials including silicon and/or germanium with waste magnesium to form magnesium silicide and/or germanide with subsequent hydrolysis by phosphoric acid. Our research was focused on process

optimization including the optimal design of the reactor. As a result, silanes, germanes or a mixture of silanes and germanes can be obtained. Consequently, there are many possibilities for their use. The mixture of silanes and germanes can serve as a source of individual products with high purity due to high differences of their boiling points. Thus, it may be a source of highly desired and shortage silanes and germanes, e.g.  $\text{Si}_3\text{H}_8$ ,  $\text{Ge}_4\text{H}_{10}$ . Isolated products have various applications in metallurgy, chemistry, electronics and energetics. Moreover, silanes and germanes could be thermally or radiofrequently decomposed to ultrapure elements (Ge, Si) applicable either for electronic or optical lenses, and hydrogen applicable for chemistry or energetics. All these reaction steps are fast and exothermic, the whole technology is energy neutral or rather positive with the total yield of ultrapure silicon and/ or germanium with above 97 w%. The research resulted in 2 patents. The scale - up of the technological process has currently been under way, and an industrial partner has been found who is interested in bringing the technology into operation. Results 14, 16, 25, 28.

#### Biorefining and biotechnology

A systematic solution of utilization of renewable material and energy provided by natural ecosystems based on the application of chemical engineering principles launched in the past period has become an important topic. This research area respects the rational concept of sustainable development and besides new scientific knowledge contributes to the acquisition of alternative products with high added value based on the so-called Green chemistry. This new scientific field found its platform in the project BIORAF – a competence centre and in the project BIOCIRTECH – a national competence centre, both supported by Technology Agency of CR, and, in both, ICPF is being the coordinator. The projects cover the entire issue of microbial, plant, animal and waste biomass processing, including municipal origin and sewage sludge, using chemical, biochemical and physical processes to obtain valuable, high value-added products for the use in food, agriculture, manufacturing and energy industries. The aim is new products from renewable sources processed by environmentally friendly technologies in accordance with the circular economy addressing current social issues (food sustainability, use of natural resources, sustainable energy). The Department as a leader of both projects participated in many new technologies including proteins hydrolysis of chicken cartilage and feathers carried out at increased temperature and pressure in the presence of malic acid, which needs to be emphasized. The resulting aqueous solution of amino acids contained the same representation as peptides forming collagen and keratin of raw material. This product, which was successfully scaled-up to industrial scale, batch  $8\text{m}^3$ , is really perspective for various applications: first, for dampening the composted agricultural waste; second, it could be useful as nutritional additives to livestock feed, similarly as a nutrient supporting plant growing in aeroponic or hydroponic systems for biotechnology applications; and finally, as a source of special proteins suitable for cartilage regeneration. Tests on human chondrocytes and chondroblasts are currently being prepared with an industrial partner and CEITEC Research Centre. Results e.g. 26, 30.

## Research activity and characterisation of the main scientific results

The topical pattern of our past and present research activities can be described as the main trunk of the fundamental multiphase investigation into the gas-liquid-solid systems (I), here labelled as: bubbles (I-a) and granulars (I-b), from which two new branches side-off: microreactors (II) and bioreactors (III), complemented with a number of smaller often multi-disciplinary studies providing the amusing variedness to our daily work (IV). The Reference Numbers [1-79] refer to the list of our publications.

### I. Multiphase flows

#### I-a) Bubbles

The gas-liquid and gas-liquid-solid systems represent the pivotal subject of the investigation into the fundamental aspects of the multiphase flows. Our activity in this key area show the application of the multiscale approach and the combination of different research tools to reach the goals we made.

##### \* Desaturation

On the small-scale of the individual bubbles formation we experimentally studied the desaturation process where the bubble nucleation occurred on the macromolecules of hydrophobins, which are the protein substances exerting the surface activity. The molecular clusters of the dissolved gas ( $\text{CO}_2$ ) formed at the locations on the molecular surface where specific polar/nonpolar interactions occurred. This study was motivated by the practical problem of beer overfoaming (gushing) when the hydrophobins presence in the malt exceeds certain limits. We designed a new testing apparatus and performed the study. Based on this, a simple physical model was suggested. References [7, 26].

##### \* Bubble chains

When the bubbles are continuously issued from a point source, bubble chains are generated. It is the example where the hydrodynamic bubble-bubble interactions dominate in the vertical directions and due to its relative simplicity the problem can be studied in some depth. It is the model case of one-dimensional bubble cluster. We numerically studied the dynamics of bubble chains with the particular respect to their mixing capacity since the rising bubbles induce the liquid circulation in the chain vicinity. Based on this a simple physical model was suggested. This flow situation is relevant to the pneumatic mixing of glass melts, where other ways of stirring (e.g. mechanical) are difficult to apply. Ref. [1]

##### \* Added mass

When the bubbles move in real chemical-engineering equipment, they typically behave in a strongly unsteady way where their acceleration is far from being the zero-vector. Here, the added mass effects come in play and produce substantial inertial forces, namely because the mass of the gaseous particles (bubbles in liquids) is negligible. These effects then govern the bubbles dynamics, starting from the single bubble trajectory, through the hydrodynamic interactions including the coalescence and breakup, up to the gross properties like the gas holdup and mass transfer. We numerically studied the added mass phenomenon with bubbles in both the bubble columns and fluidized beds. In particular, the cap-shaped bubbles were brought into

focus since they are typically observed in experiments. This problem is difficult to solve analytically due to the lack of the front-rear symmetry of the cap body. We obtained several important results and correlation formulas for the added mass coefficient. [9, 30, 70]

#### \* Coalescence

When the bubbles move within and through the bubble beds they undergo many types of hydrodynamic interactions, and the coalescence is one of the most important. It affects the bubble size distribution within the ensemble, whence the mean bubble rise speed (whence holdup) and also the interfacial area critical for the transport processes (mass, heat). The coalescence phenomenon has been studied for a long time but still many problems are left open. For instance, the effect of the bubble approach velocity on the coalescence efficiency and time is off the mainstream research due to the difficulty to control it. Therefore, we developed an apparatus where this is possible and started a detailed and systematic investigation along this line. Valuable results were obtained indicating the importance of this bothersome control parameter (approach velocity), which, however, can dominate the coalescence dynamics. [8, 46]

#### \* Breakup

The inverse process to the bubble merger (coalescence) is the bubble breakup which is an equally important process affecting the final bubble size distribution. In bubble beds, the breakup process is controlled by the energy of the flow in many cases with turbulent conditions. The study of these turbulent systems comes with many difficulties and limitations, leading to the need to simplify and to control better the conditions for investigation of the breakup process. In our laboratory, we developed two apparatuses to study this problem: (i) cell with well-defined turbulence and (ii) a cell for controlled interaction between a single bubble and a single well-defined vortex structure in order to implement a deterministic approach of the bubble breakup. Both devices proved to provide highly valuable data required in numerical methods predicting the behaviour of multiphase systems. [48, 67]

#### \* Bubble columns

On the large-scale of the whole bubbly layers, with a large number of bubbles, typically laterally confined in a container (bubble column), the noticeable feature is the particular flow regime prevailing inside the equipment. Here, we can apply the continuum approach and the Euler/Euler CFD simulation strategy. Due to the strong two-way coupling between the gas and liquid phases this approach can give unrealistic results. Therefore our main tool is the systematic experimental work aimed at the flow regime identification and characterization. As the next step we study their stability and transition. Based on the measured data, simple physical models can be suggested, working with the mean quantities. This has been done and both two-phase and three-phases systems were studied. Important results were obtained by coupling the information gathered on small-scales (e.g. bubble size distribution) and large-scale (e.g. gross holdup), together with the impact of the boundary condition at the gas input. The presence of the solid phase, as the perturbation factor to the gas-liquid flow, shows the gradual process of the bubble bed slurrification as the solid load increases. It shows how far we can apply the two-phase approximation to the slurry systems. [24, 53]

#### \* Effect of surfactants

The presence of surfactants in multiphase systems is inevitable, and it can result from additives, used for promoting a specific process (e.g. in flotation processes) or removing unwanted foam, or from naturally produced species during an industrial process (e.g. change of the liquid phase composition during fermentation). Surfactants have affinity with the gas-liquid interphase and their presence has consequences on the bubble dynamics. We study experimentally the effect of surfactants on bubble behaviour in various situations: on the bubble free rise velocity and on the bubble-bubble (coalescence) or bubble-particle interactions (bubble interaction with horizontal solid surfaces or solid particle). As support for the study of multiphase systems, the detailed characterisation of gas-liquid interphase and liquid phase physico-chemical properties are also carried in our group. The research on this topic is done with close collaboration with UCT, Prague (Dr.P.Basařová), sharing the experimental facilities and solving joint projects. [46, 58, 72, 78]. Effect of Surfactants on Bubble-Particle Interactions. Basařová, P., Zedníková, Mária. In: Dutta, A.K., ed. Surfactants and Detergents. London: IntechOpen, 2019, s. 29-41. ISBN 978-1-78984-660-7. doi: 10.5772/intechopen.85436

#### \* Taylor bubbles

The objective of this work is to improve understanding of bubble rise in inclined rectangular channels within the inertial flow regime. The experiments based on bubble observation by means of a high-speed camera are carried-out in a versatile channel with easily adaptable geometry over a wide range of inclination angles. Our experiments on bubble shapes and velocities provided original and comprehensive data suitable for subsequent velocity scaling and modelling. Based on results analysis, we present a simple model [71] for the prediction of the rise velocity of large bubbles in inclined channels. Although based just on two principal parameters (the translation velocity in horizontal channels and the rise velocity in vertical channels; both these velocities are easily predictable), the model is able to fit very well experimental data measured in inclined channels of various cross-sections and aspect ratios. The subsequent experiments employ two-segment electrodiffusion sensors to measure wall shear rate profiles along the passing bubbles and thus provide information on reverse flow in a liquid film separating the bubble from the wall, capillary waves appearing at the bubble tail, and near-wall flow fluctuations in the bubble wake. These results will be published this year.

### **I-b) Granulars**

In the area of the granular media, our activity were focussed on few particular aspects, namely the rheology and flow properties of dry and wet materials, static and dynamic properties of the anisometric grains, behaviour of multicomponent mixtures, the numerical modelling of the confined granular flows with the accent on mixing.

#### \* Anisometric grains

The majority of granular studies in the literature concerns various kinds of materials called 'common powders' due to their widespread occurrence and technological importance. Much less attention is paid to the specific case of coarse anisometric grains that are not so often encountered. However they can be highly relevant to



certain branches of industry, for instance where crystallization and crystal materials are produced and handled (e.g. chemicals, pharma). We performed few basic experimental studies on the model and real samples of anisometric particles, where the relevant quantities describing their static and dynamic behaviour were evaluated. The data were also fitted with the granular media models, primarily designed for common powders. The similarities between these two categories of materials were highlighted. [47, 60]

#### \* Granular mixtures

We also studied the behaviour of granular mixtures, which is an important issue with far reaching practical consequences (e.g. pharma). The main research challenge here is to discover how the properties of the resulting mixture relate to the properties of its individual constituent components. The inspiration came from the pharmaceutical industry and a partial success was reached. [34, 62]

#### \* Granular modelling

Numerical simulations of the granular mixing process was investigated, for the vertical rotational blades mixer that causes the rotary motion of the grains, the primary flow. Unlike the fluid mixing, where the secondary flow results from the centrifugal forces, this kind of flow, its existence and features, is still an open problem for mixing the granular media. The secondary flows are often superimposed on the complex 3D structures of the primary flows and their presence is not always apparent. Their detection is also complicated by the fact that a large number of mutual interactions among the particles makes the granular material transport complex. Therefore, we proposed a methodology for the detection of secondary flows [21] based on the elimination of the influence of the dominant primary flows from the raw simulation data. We described the effect of blades rotational speed on the formation of secondary flow [20, 21] and showed the influence of bed depth on the formation of these secondary flows [20]. Our experimental measurements confirmed the presence of these secondary flows during the mixing process [61]. Two competing mechanisms of the secondary flow formation we found: (i) the different contact time of the particles with the blade; (ii) the different magnitude of inertial forces acting on the particles around the stirrer blade [61]. We also opened the problem of granular segregation [55], to which we want to pay more attention in the future.

#### \* Dispersion rheology

Rheology of dispersions is a typical multiphase phenomena which can be investigated in different levels: (i) characterization of individual phases exhibiting simple rheology behaviour, (ii) characterization of multiphase dispersions (suspensions, emulsions, foams etc.) as continuum exhibiting complex rheology behaviour, and (iii) description of interphase behaviour, i.e. anomaly flow properties of multiphase dispersions in proximity of a wall, e.g. apparent wall slip or stagnant layer formation.

Aqueous kaolin suspensions were used as model materials for investigation of dispersion rheology. They represent very interesting multiphase dispersions of platelet clay particles of specific molecular structure which lead to strong electrochemical interactions and hydrogen bonding with water and ionic materials. An addition of electrolytes into aqueous kaolin suspensions leads to increasing their flowability due to electrical double layer surrounding clay particles which leads to electrochemical repulsions between clay particles and therefore their deflocculation. This is widely used in ceramic industry for transport facilitation. Effect of various electrolytes on flowability

and stability of resulting dispersions was systematically studied [6]. The study of complex rheology behaviour is not a trivial task because the traditional rotational rheometry considers principally Newtonian behaviour and therefore a further data treatment can lead to some misinterpretation. The end effects in Couette flow increase torque about 10% for Newtonian fluids while this increasing can be up to 30% for non-Newtonian fluids. In [22] the torque increasing due to end effects is calculated numerically for pseudoplastic fluids under higher Reynolds numbers. A simple empirical formula for end effects correction is evaluated there for using in precision data treatment in rheometry. This correction is necessary for data treatment in the very sensitive rheometry (apparent wall slip rheometry) which enable to detect interphase phenomena ad (iii). The anomalous flow behaviour of concentrated aqueous kaolin suspensions in proximity of a wall was systematically studied in the dependence on the concentration [31, 42], temperature [31] and material of the wall [42]. In the dependence of the wall material, i.e. nonexisting or existing electrochemical interaction between suspension and the wall, either apparent wall slip [31, 42] or stagnant layer formation [42] was detected.

## **II. Microreactors**

The group of Microreactors is focused on the fundamental research of multiphase phenomena in microreactors and catalysis with the particular accent on the application potential: e.g. intrinsic reaction kinetics, interphase mass and heat transfer, phase and reaction equilibria, to design a continuous process that provide optimal performance in terms of yield, selectivity and economy. The microreactor applications include e.g. gas phase catalytic reactions, gas-liquid heterogeneously catalysed reactions, homogeneous catalysis for flow chemistry and photochemistry. We cooperate on the international basis with academic and industrial partners from the area of bulk chemicals, fine chemicals or pharmaceuticals.

### **\* Gas-solid catalytic reactions**

The motivation was driven by a task to investigate intrinsic kinetics of gas phase catalytic hydrogenation of isobutene, a model component of C4 fraction, which is a valuable intermediate stream in process of crude oil refining. The application of a cassette microreactor prototype surprisingly revealed the regular oscillation behaviour of this reaction with specific characteristics, which was not yet reported. Rigorous analysis of this observation from transport phenomena point of view was published in a paper [17]. The obtained experiences were exploited to other type of reactions such as VOC oxidations and selective oxidations. In this field there was a long-term cooperative research established in 2013 with industrial partner Synthomer a. s., Sokolov, CZE. As a result of this collaboration, the two microreactor prototypes were designed, fabricated by ICPF workshop and experimentally validated. Some of the experimental results are already being implemented by the partner at the industrial scale. Results are summarized in about 10 confidential research reports. Within the field of gaseous phase reactions, the cooperation with group of Dr. Murzin at Åbo Akademi University, Turku, Finland was established on topics of catalyst development and kinetic study of ethene oxychlorination performed in various type of microreactors [51, 56, 57]. The experimental work has been performed by our team member at Åbo Akademi, Turku.

\* Gas-liquid-solid reactions

Investigation of gas-liquid-solid catalytic reaction includes mainly hydrogenation reactions that became one of the team expertise. In 2015 the team successfully applied for the EC funded collaborative project PrintCR3DIT (2015-2018), that focused on exploiting of the additive manufacturing for catalyst technology (<https://www.sintef.no/projectweb/printcr3dit>). The consortium consisted of 13 partners from EU, the task ascribed to ICPF consisted of kinetics and reaction mechanism study of two case study hydrogenation reactions from field of fine chemicals and cosmetics. The work consisted of experimental study of hydrogenations in conventional batch and continuous mili-trickle bed reactor. As a result, the mathematical reaction model was formulated, that enabled 3D catalyst design and modelling of an industrial pilot plant. Finally, the successful application of 3D printed catalyst to continuous squalene hydrogenation has been reported [76]. The achieved results with both hydrogenation reactions are also summarized into two book chapters currently under peer review and will be published in a book with working title "Industrial Green Chemistry" by De Gruyter in autumn 2020. Following the educational activities of the PrintCR3DIT project, the experts in additive manufacturing and its application in chemical sciences were invited to the "First European school for additive manufacturing applied to chemical industries", that was organized in Prague with attendance of about 20 Master or PhD students from EU.

\* Two-phase flow through complex structures

For the industrial trickle bed reactors the two-phase flow hydrodynamics is one of the key parameter influencing the pressure drop, catalyst efficiency utilization and safe operation, therefore, this topic also receive our particular attention. Investigation of two-phase flow has a long history in the team that has well established expertise. In combination of techniques of additive manufacturing newly introduced to the team by PrintCR3DIT project, the team aims at 3D optimization of catalyst support for two-phase flow in the collaboration with University Porto, which has been already established. The activities included experimental investigation of hydrodynamics and two-phase CFD modelling in 3D complex domain. Some initial results were already published in previously mentioned paper [76]. An initial hydrodynamic study with metal foams was performed in collaboration with Technical University Dresden [27, 44] (the experimental work was performed in TU Dresden).

\* Flow chemistry for fine chemical and pharmaceutical industry

This topic represents application of team multidisciplinary approach to preparation of fine chemicals. The remarkable achievement to mention is the development of unique temperature-driven reversible-biphasic solvent system for stereoselective hydrogenations. The reversible-biphasic system has been systematically developed with use of microfluidic chip reactors starting from hydrodynamic measurements in microfluidic chip reactor, phase equilibrium calculations of multicomponent mixtures and hydrogenation reaction experiments. The solvent system comprised of methanol, low content of water and a particular ionic liquid that served as immobilization agent for Ru-BINAP chiral catalyst. The system was homogeneous under reaction conditions, while it became biphasic when cooling down, that facilitated product separation and reuse of Ru-BINAP catalyst. The solvent system was validated with selective hydrogenation of methyl-acetoacetate [35, 37] and preparation of 4-chloro-3-hydroxybutyrate [69]. A membrane method for product separation from reaction

mixture was also successfully approached in cooperation with ICPF's department no. 1, which was responsible for pervaporation experiments [64]. The research of stereoselective hydrogenations was performed under co-supervision by Dr. Petr Kluson, member of ICPF's Department no. 3.

The microreactor feature of excellent control of reaction conditions together with possibility of well-defined and uniform light irradiation of the reaction volume was exploited for photochemical reaction studies. In particular, the study of PBDE [4] and 4-chlorophenol [15] photodegradation in a photo microreactor with a thin liquid layer have shown that such a devices are very suitable for detailed mechanism and kinetic studies of photochemical reactions.

#### \* Flow diagnostics

The electrodiffusion technique has been mostly used for the near-wall flow diagnostics on large scales. A novel technique for fabrication of plastic microfluidic systems with integrated metal microelectrodes (called technique of sacrificed substrate) enables us to produce microfluidic devices with precisely shaped sensors for wall shear stress measurements. These gold sensors are built-in a plastic substrate and therefore exhibit good mechanical resistance and smoothness. Our results obtained for separating/reattaching flow behind a backward-facing step and for gas-liquid Taylor flow in microchannels then demonstrate its applicability for the detection of near-wall flow reversal, the delimitation of flow -recirculation zones, and the determination of wall shear stress response to moving bubbles. Other applications of these sensors in microfluidics (e.g. characterization of liquid films, capillary waves, bubbles or drops) can be also envisaged. Tihon, J.; Penkavova, V.; Stanovsky, P.; Vejrazka J. (2015) Electrodiffusion Method of Near-Wall Flow Diagnostics in Microfluidic Systems, EPJ Web of Conferences 92: 02098.

### **III. Bioreactors**

The group of Bioreactors was established in 2017. The main focus is to bring together fundamental knowledge and expertise in multiphase systems, physical chemistry, microbiology and biotechnology and create a synergistic multidisciplinary cooperation. One of the main areas of our involvement is the microalgae biotechnology – photobioreactor design and downstream processing of microalgae biomass. Both prokaryotic (cyanobacteria) and eukaryotic microalgae related technological operation are studied in the way to describe, understand and model the basic processes to be subsequently able to predict the behaviour and to improve (develop) the performance of unit operations according to technological requirements (optimization).

#### \* Biosurfaces in dispersions

Surface properties of the microbial cells and composed bioparticles (clusters, flocks) are studied in relation to their hydrodynamic behaviour in dispersion and their interfacial interactions with other object as surfaces and colloids. This area profits greatly from the diverse expertize of the Department members and also from the cooperation with the external partners (UCT Prague, Institute of Hydrodynamics CAS, etc.). In last years, we were mainly focussed on the flocculation (coagulation) of microalgae, which has a great application potential in lowering energy consumption during harvesting the biotechnologically produced microalgal biomass. [49, 65, 77, 79]

\* Enviro and food bioprocesses

This domain includes our practice-oriented research and application of biotechnology in environmental protection and food production. We were focused on reducing the environmental impact of mining (via biotechnological accumulation of alkali metals from mining waste waters), malting process (via application of bio-fungicide) and large scale microalgae production (energy consumption reduction via electrocoagulation introduction). [45, 52, 59]. Utility Model No. CZ 33022 (2019-36298): Electrocoagulation equipment for algae biomass separation.

**IV. Miscellanea**

Besides the activities within our main multiphase arena, occasionally some studies are performed reaching beyond our nominal scope. Here are few examples.

Within the collaboration with another team of our Institute (Department of Aerosols Chemistry and Physics), we contributed with our skill of building simple theoretical models capturing the physical essence of the problem under study. Their published data on the permanent internal rain inside the water well was described and explained with help of such a model. [43]

Further we helped with the numerical simulations of various aerosol processes, e.g. nucleation, transport, settling, deposition, supported with measurements. Together with the Finnish Meteorological Institute in Helsinki, we worked on the study of the aerosols involving sulphuric acid, e.g. determining the diffusion coefficient, effect of temperature, presence of impurities, nucleation kinetics. [40, 50]

Besides, we also worked on the topic of indoor aerosols: penetration, settling, and deposition of dust particles in book depositories. The study also included the influence of the visitors as a local aerosol source. Within this cooperation, a methodology for modelling these processes in an enclosed space was developed, and the importance of a source term representing visitors for the realistic modelling this type of system was shown [32].

Within the collaboration with another team of our Institute (Department of Membrane Separation Processes), we contributed with our CFD simulations to understanding the flow in the experimental apparatus for the membrane separation processes, to better interpret the measured data and to make an attempt at scale-up. Further we cooperated on the experimental measurement and interpretation of results for vapour permeation of organic compounds and membrane assisted removal of CO<sub>2</sub> and SO<sub>2</sub> from a flue gas. We contributed to elucidation of the membrane regeneration mechanism and to correct calculation of the transport of the reactive gases in humid conditions. [29, 63, 66, 74]

Within the mutual project with another institute of ASCR (Institute of Hydrobiology), we developed a device for controlled bubble production in the field studies. We cooperated on the measurement of the acoustic response of bubbles of a desired size in the natural lake for different sonar directions. A practical protocol and straightforward relation between size and response were created in order to quantify ebullitive methane emissions and to distinguish bubbles from fishes. [2]

Within the collaboration with another institute of AVCR (Institute of Hydrodynamics), we contributed with our CFD simulations to describe and understand the experiments on the dynamics of reaction fronts, where the chemical reaction was strongly coupled with the hydrodynamics via the density changes. [41]

## Research activity and characterization of the main scientific results

As mentioned above, the original research activities of the department are mainly connected with NMR spectroscopy. The traditional focus on  $^{29}\text{Si}$  NMR and its various aspects like  $^{29}\text{Si}$ - $^{29}\text{Si}$  and  $^{29}\text{Si}$ - $^{13}\text{C}$  coupling constants were gradually replaced by more current topics as the utilization of  $^{29}\text{Si}$  NMR diminished with the decline of organosilicon chemistry at the Institute. It is common worldwide that the NMR laboratory follows and reflects research topics of other departments of the home institute.

Therefore, the gained knowledge of coupling constants determination in organosilicon compounds was transformed into investigation of  $^{13}\text{C}$ - $^{13}\text{C}$  coupling constants in polyaromatic compounds synthesized within the Department of Advanced Materials Organic Synthesis. Indirect spin-spin coupling constants represent a unique source of information about the electron distribution between involved nuclei. These couplings can be correlated to bond-related structural parameters like bond distance, bond angle, the fraction of  $\sigma$  character in the bond and so on. Generally speaking, couplings reflect the bonding environment. Using standard experiments like INADEQUATE and the more recent ADEQUATE, we faced the limitations of both experiments when determining couplings between nuclei of similar chemical shift or even symmetrically equivalent nuclei. Coupling constants between such atoms are difficult to measure due to the collapse of the NMR signal by a symmetry-equivalent pair into a single line. For this purpose, we developed two new pulse sequences. Both methods utilize one-bond hydrogen-carbon coupling for creation of effective chemical shift difference. The first method, SYMONA (SYmmetrical MOlecules Natural Abundance double-quantum experiment), uses indirect detection via hydrogen and possesses good sensitivity [SYMONA]. It is mainly suitable for determination of large single-bond carbon-carbon couplings in symmetrical molecules. On the other hand, the STRONG INADEQUATE experiment uses direct carbon detection and is suitable for measurement of small couplings between equivalent carbon atoms over several bonds [STRONG INADEQUATE]. STRONG INADEQUATE works superbly for  $^nJ_{\text{CC}}$ , where  $n \geq 3$ . In that case, the resulting pattern in F1 dimension is reduced to a simple antiphase doublet with  $^nJ_{\text{CC}}$  separation. This pattern is preserved even when the symmetrical  $\text{HC}\cdots\text{C}'\text{H}'$  system is coupled to other hydrogen atoms. This is the main advantage of the STRONG INADEQUATE experiment compared to other experiments for determination of coupling constants between equivalent carbon atoms. The latter technique can prove the connectivity between carbon atoms of similar chemical shift as demonstrated in structure determination of ascorbigen A [Ascorbigen A]. The development of analytical formulas took a certain time. All three works were published recently in journals dedicated to NMR spectroscopy. Currently, we are working on combination of pure-shift NMR decoupling schemes and developed pulse sequences. A successful combination could provide a much simpler signal pattern without the need of pattern simulation which is necessary for complicated spin systems.

- SYMONA Pulse Sequence. *Journal of Magnetic Resonance* **2019**, 298, 107-114.
- STRONG INADEQUATE. *Magnetic Resonance in Chemistry* **2019**, 57(12), 1107-1120.
- Ascorbigen A. *Magnetic Resonance in Chemistry* **2019**, 57(12), 1084-1096.

The second research topic is associated with supramolecular chemistry. The design and synthesis of artificial receptors for anion and enantiomer recognition is the main aim of our research. Here, NMR spectroscopy represents a basic and irreplaceable tool for determination of the properties of complexes formed. The strength of individual complexes is reflected in association constants providing the ratio between complex formation and its dissociation. The most common way of determining association constants is NMR titration. In this technique, the aliquots of substrate are gradually added into solution of receptor, and the shifts of NMR signals induced by complexation are observed. The observed signals then represent average signals of the uncomplexed and complexed species. The desired information of the complex stability is then derived by specialized computing programs. In our group, a computing program, ESTAC, was developed in the past, providing advantageous features over commercially available ones. This program works with tuneable uncertainties in both variables, taking into account the errors in accurate determination of concentrations of both substrate and receptor solutions. These can be significant especially in low concentrations. Using this tool and long-term experience in NMR titration techniques, the selectivity of various receptors was studied and many structural features were described. In collaboration with the University of Chemistry and Technology in Prague (UCT), calixarene-based receptors and their derivatives were studied in chiral recognition of amino acids [Mačková et al.], and complexation abilities towards anions. Beside the expected selectivity towards carboxylates over halides, a significant shape-based chiral discrimination of one enantiomer was found in several cases of amino- and hydroxyacids. In a subsequent work, it was proved that calixarenes can serve as a suitable molecular scaffold for modelling of a complexation site into a desired shape; e.g. two hydrogen-donating groups can be bridged and can subsequently cooperate, yielding an enhancement of the receptor selectivity [Řezanková et al.]. Comparing the bridged receptors to the unbridged ones, the bridged receptors were found to be almost 20-times more efficient than their unbridged analogues in the complexation of acetate. It was also found that for too strong complexes, the two possible complexation sites do not necessarily have to cooperate, which results in the formation of complexes with higher stoichiometry [Klejšch et al.]. Furthermore, sulphonic esters were used to enhance the effectivity of hydrogen bonding in the receptors. Unexpectedly, this structural feature enhanced the complexation properties of the receptors to the extent of leading to preferential complexation of two anions over cooperation upon complexation of one anion.

The use of NMR in determination of the stability of supramolecular complexes is not limited only to hydrogen-bound species. Even the formation of charge transfer complexes causes detectable changes in electronic properties of molecules that can be studied by NMR. In addition, a complexation process based on electrostatic and hydrophobic interactions can be monitored similarly [Stejskal et al.]. Such complexes can be of high importance for practical use. Based on our findings, the electrodes incorporating calixarene-based receptor were prepared for forensic analysis of MDMA derivatives. Specifically, the detection and quantification of buphedrone among other new psychoactive substances including those belonging to the cathinone family was studied [Shishkanova et al.].

In our recent research, we accent the practical use of the designed and prepared receptors. In cooperation with UCT Prague, we prepared and successfully tested receptors working as NMR solvating agents [Cuřínová et al. 2018, Cuřínová et al. 2019]. The prepared binaphthyl-based compounds form diastereomeric complexes with chiral drugs enabling observation of their ee by NMR techniques. These



compounds selectively interact with chiral alcohols and acids. Among the targeted use, these compounds were also employed for determination of product ee in stereoselective synthesis performed in the Department of Multiphase Reactors [Klusoň et al.]. In current studies, we focus on the anchorage of effective receptors to more elaborated structures suitable for separation techniques like nanofiltration and others. These activities taking place in collaboration with UCT Prague and the Department of Bioorganic Compounds and Nanocomposites have been supported by the Czech Science Foundation recently.

- Mačková et al. *New Journal of Chemistry* **2015**, 39(2), 1382-1389.
- Řezanková et al. *Tetrahedron* **2017**, 73(6), 742-749.
- Klejch et al. *New Journal of Chemistry* **2016**, 40(9), 7935-7942.
- Stejskal et al. *Tetrahedron* **2016**, 72(5), 760-766.
- Shishkanova et al. *Forensic Toxicology* **2020**, 38, 70-78.
- Cuřínová et al. *Chirality* **2018**, 30(6), 798-806.
- Cuřínová et al. *Chirality* **2019**, 31(5), 410-417.
- Klusoň et al. *Journal of Flow Chemistry* **2019**, 9(4), 221-230.

The third main research topic is focused on omic sciences exploiting  $^1\text{H}$  NMR data. In the past, the NMR lab was recognized at least at the national level for the ability to analyse mixtures of organic compounds using NMR techniques; namely LC-NMR hyphenation. Once we were asked by our colleagues from the Department of Aerosols Chemistry and Physics to analyse a sample of aerosol and, using a  $^1\text{H}$  NMR spectrum, employ so-called Functional Group Analysis, which means to integrate several parts of the spectrum. The  $^1\text{H}$  NMR experiment is usually the only measurement that can be done on such diluted samples as aerosols in reasonable time. The resulting spectrum looks overcrowded on first sight but most of the chemical individuals possess at least one isolated signal that can be used for compound identification. Therefore, we have decided to apply a “metabolomic” approach, which uses a comprehensive library of compounds that can occur in a given environment. The  $^1\text{H}$  NMR spectra can then be analysed via special refining programs which allow direct comparison of the real spectrum with the spectra of individual compounds in the library. The matching compounds can be easily identified, and the original spectrum is simplified by the subtraction of individual compound spectra. Using this approach, we are currently able to identify and quantify about 60 compounds in the  $^1\text{H}$  NMR spectrum of a real aerosol sample. The concentration data are then subjected to multivariate statistical analysis, which can reveal the main features of the sample. Currently, we manage a database of approximately 150 organic compounds associated with aerosols. The database consists of a metabolomic database of ChenomX software (ChenomX, Canada) and its extension towards aerosol compounds. The supplement to the original database can be downloaded from a webpage of the department. This method, called NMR aerosolomics, has been introduced in the pilot publication describing its principles and the results of statistical analysis performed on series of winter and summer aerosols. The method works beautifully. In agreement with the literature, it revealed the main differences between winter and summer aerosols, indicated different composition according to particle size, confirmed presence of some compounds that had been suggested by theory and also identified a few new compounds. However, the publication is in the revision process for six months now, facing a lack of will to accept a new method from the reviewers.

In the future, we will use this method to investigate the chemical composition of size-resolved aerosol or for aerosol source apportionment. These activities have been supported by the Technology Agency of the Czech Republic.

In 2016, the decision was made that our experience with metabolomic software should be utilized in real metabolomic studies. Having no contacts with the medical sector, we started a collaboration with the Department of Analytic Chemistry of UCT and stepped into an already established collaboration with the Military University Hospital Prague. In 2018, the first diploma thesis dedicated to diagnosis of pancreatic cancer via  $^1\text{H}$  NMR metabolomics of blood plasma was defended. The pilot study performed on a limited cohort was published subsequently [Michálková et al.]. Currently, we are finishing a study focused on association of pancreatic cancer with diabetes mellitus type 2. This work was done on larger cohorts and includes a prediction model for recently diagnosed diabetes mellitus patients.

Recently, we were invited to analyze the metabolomic profile of workers exposed to nanoparticles. This project is done in collaboration with the Department of Aerosols Chemistry and Physics and General University Hospital in Prague. Here, we analyse samples of blood plasma and exhaled breath condensate of pre- and after-shift workers and identify changes induced by processing of nanocomposite materials.

Another study started in collaboration with Motol University Hospital. The aim of the study is to identify a source of facial nerve paralysis via  $^1\text{H}$  NMR metabolomics of blood serum and cerebrospinal fluid samples.

Nowadays, NMR metabolomics is a well-established method abroad. However, our position is still as a kind of supportive lab. The samples we usually obtain are “leftovers” from other analyses, and we have to prove that the method works. So far, we are working on our reputation, as we would like to become a respectable partner in regular projects. There are several studies we would like to start in the near future.

- Michálková et al. *Analyst*, 2018, 143, 5974.

The Department of Analytical Chemistry in cooperation with the Department of Inorganic Chemistry of the Faculty of Science, Charles University (CU) also provides single crystal X-ray analysis. Suitable crystals are transferred to CU and measured. The final data refinement, the analysis of crystal structure and intermolecular interactions in the crystal lattice are performed at the ICPF. A number of publications arose in the monitored period, especially in collaboration with the Department of Advanced Materials and Organic Synthesis focusing on the relation between molecular structure and compound properties. Some of these activities were supported by the Czech Science Foundation (Project no. 15-12719S, 2015-2018).

The arrangement of molecules in the crystal lattice was investigated for helicene-based imidazolium salt, which was prepared and utilized in an organic electronic device [Storch et al.] or for helicene-silver complex [Makrlík et al.].

The isolation of individual enantiomers of racemic helicene afforded their utilization as selective anodic [Vacek et al.] or plasmonic layers [Kalachyova et al.] for enantiomer discrimination. Chiral HPLC played a crucial role also in utilization of 2-bromo[6]helicene as a key intermediate for [6]helicene functionalization [Jakubec et al.].

Thorough analysis of NMR and X-ray data supported by calculations of molecular dynamics provided a description of conformation behaviour of helicene molecules and its limits [Dračínský et al.].

The formation of CH...F intermolecular interactions in fluorohelicene derivatives were found to be responsible for the lower solubility of these compounds when compared to unsubstituted helicenes [Církva et al.].

Long-standing experience in the analysis of intermolecular interactions was utilized in the procedure for construction of a crystal lattice using only intermolecular interactions that are accessible by solid state NMR techniques [Hejtmánek et al.]. This procedure, as an alternative to common approaches, was proposed as part of a special issue dedicated to NMR crystallography.

It is worth noting that the above-described activities can form a substantial part of the publication, which is then reflected in shared correspondence or even exclusive correspondence of a given publication.

- Storch et al. *Chemistry - A European Journal* **2015**, 21(6), 2343-2347.
- Makrlík et al. *Chemical Physics Letters* **2015**, 633, 105-108.
- Vacek et al. *ChemElectroChem* **2018**, 5(15), 2080-2088.
- Kalachyova et al. *ACS Applied Materials and Interfaces* **2019**, 11(1), 1555-1562.
- Jakubec et al. *The Journal of Organic Chemistry* **2018**, 83(7), 3607-3616.
- Dračinský et al. *Physical Chemistry Chemical Physics* **2017**, 19(4), 2900-2907.
- Církva et al. *The Journal of Organic Chemistry* **2019**, 84(4), 1980-1993.
- Hejtmánek et al. *Crystals* **2019**, 9(3), 159.

Naturally, the department provides “service” measurements for the majority of research departments of the Institute. The contribution to these publications is rather small; therefore, the scope, form and impact of the publication cannot be affected significantly. However, in many cases it is necessary to propose the optimal settings of an experiment and the sampling in order to provide samples suitable for a convenient analytical method.

#### 1. Department of Membrane Separation Processes

The analysis of mixtures in the course of membrane separation process are performed to monitor the separation. The separation of volatile components is usually monitored by NMR.

- Gaálová, Jana, Hejda, Stanislav, Stavárek, Petr, **Sýkora**, Jan, Fajgar, Radek, Klusoň, Petr, Izák, Pavel. Pervaporation of (R)/(S)-Methyl 3-Hydroxybutyrate ( $\Sigma$ MHB) from a Mixture Containing an Ionic Liquid, Methanol and Ru Catalyst. *Separation and Purification Technology* **2019**, 222, 45-52.
- Gaálová, Jana, Vojtek, Lukáš, Lasnier, Sara, Tadic, Toma, **Sýkora**, Jan, Izák, Pavel. Separation of Trimethyl Borate from a Liquid Mixture by Pervaporation. *Chemical Engineering & Technology* **2019**, 42(4), 769-773.

#### 2. Department of Chemistry and Physics of Aerosols

As mentioned above, there is a close collaboration with this department resulting in up-to-date research topics. One publication is currently in the revision process, the other will follow.

3. Department of Catalysis and Reaction Engineering

Analysis of noble metals in the catalyst prepared is usually requested. However, the collaboration is slowly decreasing.

- Kaluža, Luděk, Larsen, M.J., Zdražil, Miroslav, Gulková, Daniela, Vít, Zdeněk, Šolcová, Olga, Soukup, Karel, Koštejn, Martin, Bonde, J.L., **Maixnerová**, Lucie, Odgaard, M. Highly Loaded Carbon Black Supported Pt Catalysts for Fuel Cells. *Catalysis Today* **2015**, 256, 375-383.

4. Department of Multiphase Reactors

Analytical services are required only from the Group of Microreactors. Analyses of crude reaction mixtures by NMR or GC/MS are most common.

5. Department of Analytical Chemistry

6. Department of Environmental Engineering

Analyses of heavy metals in fly and bottom ash are required. For this purpose atomic spectroscopy is used.

- Rumayor, Marta, Svoboda, Karel, **Švehla**, Jaroslav, Pohořelý, Michael, Šyc, Michal. Mitigation of gaseous Mercury Emissions from Waste-to-Energy Facilities: Homogeneous and Heterogeneous Hg-Oxidation Pathways in Presence of Fly Ash. *Journal of Environmental Management* **2018**, 206, 276-283.
- **Švehla**, Jaroslav, Židek, R., Ružovič, Tomáš, Svoboda, Karel, Kratzer, J. Simple Approaches to on-Line and off-Line Speciation Analysis of Mercury in Flue Gases with Detection by Atomic Absorption Spectrometry: A Pilot Study. *Spectrochimica Acta Part B: Atomic Spectroscopy* **2019**, 156, 51-58.
- Rumayor, Marta, Svoboda, Karel, **Švehla**, Jaroslav, Pohořelý, Michael, Šyc, Michal. Mercury Removal from MSW Incineration Flue Gas by Mineral-based Sorbents. *Waste Management* **2018**, 73, 265-270.

7. Department of Molecular and Mesoscopic Modelling

There are no requests from this department.

8. Department of Laser Chemistry

Commonly, the performance of prepared catalyst is tested in degradation of a target group of molecules; e.g. antibiotics in waste water. The reaction mixture is analysed to monitor the course of degradation process. LC/MS is widely used.

- Jandová, Věra, Pokorná, Dana, Kupčík, Jaroslav, Dytrych, Pavel, **Cuřínová**, Petra, Fajgar, Radek, Pola, Josef. Infrared Laser Radiation-Produced TiO-doped Si/SiO<sub>x</sub>/SiO<sub>2</sub> Nanocomposite – Entry to TiO-containing Materials. *Journal of Photochemistry and Photobiology A-Chemistry* **2017**, 332, 376-383.
- Křenek, T., Tesař, J., Kupčík, Jaroslav, Netrvalová, M., Pola, M., Jandová, Věra, Pokorná, Dana, **Cuřínová**, Petra, Bezdička, Petr, Pola, Josef. CW-Laser-Induced Solid-State Reactions in Mixed Micron-Sized Particles of Silicon Monoxide and Titanium Monoxide: Nano-Structured Composite with Visible

Light Absorption. *Journal of Inorganic and Organometallic Polymers and Materials* **2017**, 27(6), 1640-1648.

- Jandová, Věra, Pokorná, Dana, Kupčík, Jaroslav, Bezdička, P., Křenek, T., Netřvalová, M., **Cuřínová**, Petra, Pola, Josef. Thermal Reactions in Mixtures of Micron-sized Silicon Monoxide and Titanium Monoxide - Redox Paths Overcoming Passivation Shells. *Research on Chemical Intermediates* **2018**, 44(1), 503-516.
- Pokorná, Dana, **Cuřínová**, Petra, Pola, Josef. Calcium Hydroxide Effect in Degradation of Aqueous Naphthalene: Nucleophilic Substitution of Hydrogen at the C(sp<sup>2</sup>)–H Bond. *Polycyclic Aromatic Compounds* 2019(xx)(xx), 1-10. doi: 10.1080/10406638.2019.1624975.

#### 9. Department of Advanced Materials and Organic Synthesis

Besides the above-described activities, routine NMR and MS analysis of prepared organic compounds is requested. The Group of Supercritical Technologies requests analysis of natural extracts. For this purpose, GC/MS and LC/MS techniques are employed.

- Papoušková, B., Bernard, Martin, Ottenschlager, Jakub, Karban, Jindřich, Velíšek, Petr, Hrbáč, J., **Sýkora**, Jan, Storch, Jan, Vacek, J. A Comprehensive LC/MS Analysis of Novel Cyclopentenedione Library. *Journal of Pharmaceutical and Biomedical Analysis* **2016**, 128, 342-351.

#### 10. Department of Bioorganic Compounds and Nanocomposites

HRMS and LC/MS are often required for characterization of carbohydrates and dendritic structures prepared in this department.

- **Cuřínová**, Petra, Krupková, Alena, Červenková Šťastná, Lucie, Müllerová, Monika, Čermák, Jan, Strašák, Tomáš. ESI-TOF Mass Spectrometry of Cationic Carbosilane Dendrimers: a Potent Tool for Characterization of Structural Defects. *Journal of Mass Spectrometry* **2018**, 53(10), 986-996.
- **Horník**, Štěpán, Červenková Šťastná, Lucie, **Cuřínová**, Petra, **Sýkora**, Jan, Káňová, K., Hrstka, R., Císařová, I., Dračínský, Martin, Karban, Jindřich. Synthesis and in Vitro Cytotoxicity of Acetylated 3-Fluoro,4-Fluoro and 3,4-Difluoro Analogs of D-glucosamine and D-galactosamine *Beilstein Journal of Organic Chemistry* **2016**, 12, 750-759.
- Strašák, Tomáš, Malý, J., Wróbel, D., Malý, M., Herma, R., Čermák, Jan, Müllerová, Monika, Červenková Šťastná, Lucie, **Cuřínová**, Petra. Phosphonium Carbosilane Dendrimers for Biomedical Applications – Synthesis, Characterization and Cytotoxicity Evaluation. *RSC Advances* **2017**, 7(30), 18724-18744.
- Hodík, Tomáš, Lamač, Martin, Červenková Šťastná, Lucie, **Cuřínová**, Petra, Karban, Jindřich, Skoupilová, H., Hrstka, R., Císařová, I., Gyepes, Robert, Pinkas, Jiří. Improving cytotoxic properties of ferrocenes by incorporation of saturated N-heterocycles. *Journal of Organometallic Chemistry* **2017**, 846, 141-151.
- Liegertová, M., Wróbel, D., Herma, R., Müllerová, Monika, Červenková Šťastná, Lucie, **Cuřínová**, Petra, Strašák, Tomáš, Malý, M., Čermák, Jan, Smejkal, J., Štofík, M., Malý, J. Evaluation of Toxicological and Teratogenic Effects of

Carbosilane Glucose Glycodendrimers in Zebrafish Embryos and Model Rodent Cell Lines. *Nanotoxicology* 2018, 12(8), 797-818.

- Strašák, Tomáš, Červenková Šťastná, Lucie, Bílková, Veronika, Skoupá, Veronika, Karban, Jindřich, **Cuřínová**, Petra, Čermák, Jan K. Synthesis and Fluorophilicity of Compounds with Tris(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)silyl Substituent. *Journal of Fluorine Chemistry* 2015, 178, 23-29.
- Kurfiřt, Martin, Červenková Šťastná, Lucie, Dračínský, Martin, Müllerová, Monika, Hamala, Vojtěch, **Cuřínová**, Petra, Karban, Jindřich. Stereoselectivity in Glycosylation with Deoxofluorinated Glucosazide and Galactosazide Thiodonors. *The Journal of Organic Chemistry* 2019, 84(10), 6405-6431.

The department provides “service” activities also for external subjects. There is extensive collaboration with the University of Pardubice and the J. Heyrovsky Institute of Physical Chemistry of the CAS in the field of organometallic chemistry. Usually, HRMS and heteronuclear NMR measurements are demanded.

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## Research activity and characterisation of the main scientific results

Within the last period, the research team focused attention on following topics with high social and economic impact and relevance. Generally, the topics lead towards minimization of environmental impacts of human activities as described above. Names of topics have slightly changed since the last evaluation report. We have presented and commented on the most important and compact scientific results, the full list of results related to these topics and other results achieved within the evaluation period is part of another evaluation phase.

### Topic 1 Flue gas treatment for the achievement of low concentrations of pollutants

Flue gas treatment research focused originally on the development of an integrated one-step flue gas cleaning system for small-scale waste-to-energy plants. This approach involved simultaneous removal of particulate matter, acid gases, nitrogen oxides, organic pollutants, and heavy metals including mercury. Catalytic filtration (temperature about 200-220 °C) combined with various solid sorbents injected for the removal of acid gases and mercury is an original idea which was experimentally verified. Based on these conditions, sodium bicarbonate is the most suitable sorbent for the removal of acid gases, however, we focused also on other sorbents on theoretical levels (1). Within the period, a pilot-scale unit for studying of sorbent reaction with acid gases within the pseudo-real flue gas environment was constructed. The core of the study focused on the evaluation of reaction kinetics of concurrent reactions of HCl and SO<sub>2</sub> with the sorbent. Based on the experimental data, a regression model for sorbent consumption with respect to HCl and SO<sub>2</sub> concentrations was proposed. The paper describing it is under preparation nowadays. To our best knowledge, it is the first paper based on real data. The data will be used in cooperation with our industrial partners within the Waste-to-energy competence centre. i.e. engineering company EVECO Brno and flue gas cleaning system supplier ZVVZ Enven Engineering.

Moreover, no commercial sorbent for effective Hg removal at temperature over 200 °C was available on the market, therefore, to find and develop such sorbent was another task for the progress in integrated flue gas cleaning unit. It was a part of a broad study on mercury behaviour in flue gases and technologies for its abatement. Mercury is one of the most significant pollutants in terms of emissions from coal combustion, besides, it is present also in flue gases from other combustion processes, such as waste incineration. For an effective capture of mercury, it is necessary to have a knowledge of its speciation in the flue gases. Therefore, an extensive experimental activity focused on the elucidation of mercury behaviour during combustion and the key factors influencing its speciation was performed. Moreover, possible mercury capture methods were experimentally verified as well. It can be concluded from our research that there are three important factors for the effective sorption of mercury (esp. in the form of the vapour of elementary mercury Hg<sup>0</sup>):

- specific sorbent surface (should be, if possible, more than 200 m<sup>2</sup>/g),
- sorption temperature (suitable to avoid temperatures above 150 °C),
- and impregnation (oxidizing compounds with chlorides and bromides, and agents with sulfur, in elementary form as well as polysulfides).

During the sorption of  $\text{Hg}^0$  and mercury compounds on fly ashes presented in flue gases, the decisive factors are the content of unburned carbon, chloride content, specific surface area and sorption temperature. The content of water vapour usually slightly hinders the sorption of mercury on sorbents. At higher ratios of  $\text{HCl}/\text{SO}_2$  concentrations in the flue gas and oxygen content above approx. 4 vol. %,  $\text{Hg}^0$  is oxidized in the gas to  $\text{HgCl}_2$ . The oxidation depends on the temperature, catalytically active surface, and reaction time. Organometallic mercury compounds are transformed in the combustion processes to a mixture of  $\text{Hg}^0$  vapour and inorganic compounds (esp.  $\text{HgCl}_2$ ). In flue gas with a very low content of VOC, almost no organometallic compounds are formed. When mercury is to be removed by wet methods together with  $\text{SO}_2$ , it is necessary to pay attention to so-called re-emission of mercury caused by the reduction of  $\text{Hg}^{2+}$  to  $\text{Hg}^0$  with subsequent volatilization into flue gas. These findings and assessments were published in a number of original works and a review article (2-5) and serve as a base for the development of a capture technology for mercury in flue gas. For dry method of Hg removal, sulphur-impregnated mineral sorbents based on soda, zeolite, bentonite or diatomaceous earth were prepared, experimentally verified and protected (6). The sorbent efficiency can be comparable with activated carbon, however, they are applicable at higher temperatures appropriate for integrated flue gas cleaning unit, without danger of explosion. The team participated also in a project that monitored mercury behaviour in real-scale coal firing power plants. Our role, as chemistry experts, was the mercury behaviour and speciation analysis. As mentioned before, mercury re-emission from wet-scrubbing systems (that are used for large scale incineration units such as coal power plants) because of mercury reduction is a crucial factor causing an increase in Hg emissions often leading to exceeding of emission limits. A method for Hg removal from re-circulated scrubbing water by an electrolytic process was invented, patented (7) and verified on lab-scale basis. Nowadays, negotiations with industrial partner for pilot scale verification of this method are ongoing.

## Topic 2 Thermochemical conversion of fuels

In the field of solid fuels gasification and pyrolysis we have a prominent role on the national level. Originally, we focused predominantly on fluidized bed gasification and on proposals of primary and secondary measures for syngas cleaning and pollutants minimization. The influence of individual parameters on biomass gasification in a bubbling fluidized bed was the subject of a large experimental campaign. One of the investigated problematics was the influence of catalytically active calcium oxide and inert quartz sand in the fluidized bed of the gasifier during autothermal gasification of biomass with the use of a mixture of  $\text{CO}_2 + \text{O}_2$  as a gasification agent. The influence of calcium oxide in the fluidized bed on the gasification of biomass with this gasification agent was compared with a commonly used gasification agent  $\text{H}_2\text{O} + \text{O}_2$ . While sufficient concentration of catalytic calcium oxide in the fluidized bed was only 50 % when  $\text{H}_2\text{O} + \text{O}_2$  was used, in the case of  $\text{CO}_2 + \text{O}_2$ , the optimal results were achieved with pure  $\text{CaO}$  in the fluidized bed. The catalytic effect of  $\text{CaO}$  in the fluidized bed resulted, in this case, in an 8.7 times lower tar yield in the producer gas and a decrease of the tar dew-point by 124 °C to 71 °C (8-10).

Secondary measures research focused mainly on deep removal of chlorine- and sulfur-containing pollutants from syn-gas at medium to high temperatures (200-900 °C). Solid sorbents based on rare earth elements oxides have been considered as promising sorbents. We performed thermodynamic analyses on the determination of theoretical



equilibrium concentrations of  $\text{H}_2\text{S}$ ,  $\text{HCl}$  and  $\text{HF}$  for  $\text{La}_2\text{O}_3$ ,  $\text{La}_2\text{O}_2\text{CO}_3$  and  $\text{Ce}_x\text{O}_y$ -based sulphur compounds sorbents, on possible interferences of hydrogen halides in  $\text{H}_2\text{S}$  removal in the temperature range 600–1100 K and on upstream selective deep removal of  $\text{HCl}$  and  $\text{HF}$  in producer gas. The lowest  $\text{H}_2\text{S}$  concentrations ( $<0.1$  ppm-v) were achieved by  $\text{Ce}_2\text{O}_3$ -based sorbents at temperatures below 800 K. Oxidation of the  $\text{Ce}_2\text{O}_3$ -based sorbents to less efficient higher Ce-oxides (with limit of  $\text{CeO}_2$ ) is thermodynamically preferred in real producer gas. The presence of  $\text{CO}_2$  in gas phase leads to formation of lanthanum oxy-carbonate ( $\text{La}_2\text{O}_2\text{CO}_3$ ) with lower efficiency in  $\text{H}_2\text{S}$  removal than  $\text{La}_2\text{O}_3$ . The thermodynamic analyses were also focused on suitable ways and conditions for regeneration of spent desulfurization sorbents ( $\text{Ce}_2\text{O}_2\text{S}$  and  $\text{La}_2\text{O}_2\text{S}$ ) to the corresponding oxides or oxy-carbonates at temperatures 600 K–1100 K. The  $\text{Ce}_2\text{O}_2\text{S}$ -based spent cerium sorbents were advantageously regenerated by reaction with  $\text{SO}_2$  at temperatures over 700 K. In this way  $\text{CeO}_2$ , elemental sulfur and small concentrations of  $\text{S}_2\text{O}$  can be theoretically produced. If the cerium halides are formed, their decomposition and full Ce-sorbent regeneration is thermodynamically possible rather by reaction with steam. The La-based spent sulfur sorbents are restored by reaction with  $\text{CO}_2$  and  $\text{H}_2\text{O}$  vapor leading to formation of  $\text{La}_2\text{O}_2\text{CO}_3$  and  $\text{H}_2\text{S}$ . The suitable operating conditions involve temperatures between 700 and 800 K and higher operating pressures. Potential small contents of La-oxy-halides ( $\text{LaOF}$  and  $\text{LaOCl}$ ) in the spent sorbent are converted into  $\text{La}_2\text{O}_2\text{CO}_3$  in such a regeneration process. The thermodynamic analysis showed why the efficiencies found in practical applications are lower than expected, which is crucial for deeper understanding of this phenomenon and utilization of the Ce- and La-based sorbents in practise. The development of primary and secondary measures is a part of a long-term task focused on syn-gas cleaning on a certain level allowing its utilization in solid oxide fuels cells (11, 12).

Apart from fluidized bed gasification, we participated in development of downdraft gasifier technology for biomass gasification in real scale. The unit is multi-staged and used for wood biomass gasification. The allothermal torrefaction and autothermal pyrolysis of the wooden biomass take place in the upper part of the twin-fire generator, i.e. first stage, at temperatures of approximately 500–600°C. Subsequently, both partial oxidation of the primary producer gas (temperature exceeding 1200°C) and auto-activation of the biochar by steam and carbon dioxide (cooling reactions, 950 → 750°C) take place in the second stage of a lower container. The overall concept and design led to high efficiency of the process. The pilot unit is used for combined heat and power production along with biochar production by company Energo Zlatá Olešnice, with nominal power output 200 kW<sub>e</sub>. The technology concept is covered by patent (13) and offered on the market by cooperating company Tarpo.

The last sub-topic related to biomass conversion is the research on biochar production and its utilization as soil amendment agent and modified biochar for contaminated water purification. We cooperate with agriculture sector, particularly with University of Life Sciences, Prague. Our role is the study of optimal pyrolysis conditions and the evaluation of influence of input biomass on produced biochar properties and modification of its properties based on requests from end users. It is an emerging topic within our team and some of the results have been already published (14-16).

### **Topic 3 New technologies for the treatment or recycling of wastes and waste materials**

Our original research on incineration solid residue treatment focused on its valorisation. It is possible to recover valuable components, such as ferrous and non-ferrous metals, and to use treated metal-depleted bottom ash mineral matrix in various application of construction industry. We started with thorough characterization of bottom ash from three Czech WtE plants and determination of the potential for metals recovery. The collecting areas of each plant and incinerated waste were different (type of housing, share of commercial waste, separate collection system efficiency, etc.), hence, in cooperation with Brno Technical University, we tried to predict the bottom ash composition based on the data concerning municipal solid waste composition and character of the collecting area. Bottom ash material analysis is based on hand picking (i.e. very laborious), so its prediction can be valuable for precisising the bottom ash recovery potential. The results were published in Waste Management Journal (17). Subsequently, we obtained a project of the Ministry of Industry and Trade in cooperation with Prague waste-to-energy plant for development and experimental verification of high effective technology for ferrous and non-ferrous metals recovery from bottom ash particles over 4 mm. Within the project, the pilot plant unit with the capacity of up to 2 tonnes per hour was constructed in Prague WtE plant and verified with determined efficiency of over 85 % for these particles (18). The data from the pilot plant were used by Prague WtE plant and, nowadays, the feasibility study and real scale plant are under consideration. The acquired knowledge and experience about the principles and efficiency of the separation process were utilized and disseminated in an invited lecture given by Dr. Šyc at a prestigious bottom ash workshop within IRRC Waste-to-energy conference (19) on behalf of international team focused on this topic. Subsequently, a comprehensive review paper about state-of-the art was prepared and published in highly-impacted Journal of Hazardous Materials in 2020 ([doi.org/10.1016/j.jhazmat.2020.122433](https://doi.org/10.1016/j.jhazmat.2020.122433)). We focused also on the utilization of mineral matrix of bottom ash after metal recovery, as it can be used in construction industry. Due to legal restriction, the utilization of the mineral matrix has not been possible in the Czech Republic. Hence, we focused on a thorough analysis of bottom ash parameters, environmental criteria, and possible ways of utilization, which was again presented at prestigious IRRC Waste-to-energy conference a year later (20). Later, a comprehensive paper about legal framework of bottom ash utilization across nearly all the European countries was prepared and published with our team participation in 2020 ([doi.org/10.1016/j.wasman.2019.11.031](https://doi.org/10.1016/j.wasman.2019.11.031)). Simultaneously, we cooperated with Danish Waste Solution company on the development of suitable criteria for bottom ash utilization in the Czech Republic and prepared series of studies for Czech Ministry of the Environment and on behalf of plant operators have negotiated with the Ministry since 2017. Finally, the proposed criteria (environmental and technical) will be included in legislation change proceeding in 2020 in the Czech Republic and will allow safe utilization of nearly 200 ths. tons of bottom ash annually, saving the same amount of primary materials.

Sewage sludge treatment and phosphorous recovery is an important part of our activities. Our original research on sewage sludge incineration, associated emissions etc. had been realized before this evaluation period. Within the evaluation period, we focused on the evaluation of sewage sludge treatment in oxygen-enriched atmosphere that had not been investigated before. An increase in combustion temperature typical for oxygen enriched atmosphere resulted in an increase in NO<sub>x</sub> and SO<sub>2</sub> emissions and in decrease in N<sub>2</sub>O emissions. An increase in inlet oxygen concentration led to a decrease in NO<sub>x</sub> and N<sub>2</sub>O emissions. N<sub>2</sub>O and SO<sub>2</sub> emissions were higher in CO<sub>2</sub> rich atmosphere. The presence of water vapor in the inlet combustion medium resulted

mainly in the reduction of NO<sub>x</sub> emissions (21). The results can serve the purpose of the proposal of real-scale process leading to lower greenhouse gas emission in the future.

We consider sewage sludge thermal treatment as a first step for phosphorous recovery. Within the evaluation period, we thoroughly studied or have started to study various thermochemical methods for sewage sludge treatment, such as hydrothermal treatment, incineration, gasification, and pyrolysis. Papers describing results of the last two methods are under evaluation nowadays. For all these methods, the evaluation of agriculture potential and bioavailability of phosphorus from P-bearing product is an important factor. Hence, we investigated the potential of ashes from sewage sludge incineration as P-bearing materials for agriculture. In our study, we found an inadequacy of current tests, based mainly on the solubility of P and toxic metals in neutral ammonium citrate (NAC) or 2% citric acid (2%CA) solution. The key factors are the bioavailability and the degree of sewage sludge ash contamination by heavy metals. The results showed an apparent influence of Ca and Al content, pointing out the necessity to understand the behaviour of these materials under various soil conditions. Therefore, a simple testing apparatus using standardized soil tests (Olsen, Mehlich3, or soil sorption test) was designed. This apparatus produces results with significantly higher data validity regarding the application of sewage sludge ash in agriculture than the currently used tests, while having comparable demands in terms of economy and time. The tests currently used for sewage sludge ash significantly overestimate the bioavailability of P in comparison with the bioavailability determined by the soil solubility test apparatus. The results were published in prestigious journal (22) and we continue to develop ideas and methods for accurate evaluation of nutrient potential of secondary materials, such as sewage sludge.

Composite packaging materials are a part of everyday life, as they are mainly used for food and feed packaging. Perhaps the best-known composite material is the Tetra Pak packaging, which is composed of paper, foil and aluminium, where every layer has its own functional property. So far, these materials have been non-recyclable and are dominantly landfilled or incinerated. It is a significant waste flow, as composite materials comprise around 20 % of plastic waste in Europe. We developed a new unique technology that deals with the material processing of several types of composite wastes like Tetra Pak. The technology is based on the principle of their separation by means of a leaching mixture into individual components, which are then separated from each other into single-species materials. This is a unique solution to the recycling of these so far non-recyclable wastes. A pilot plant with the capacity of 100 kg/hour was created for patent validation and acquiring reliable data. Based on these data, a full-scale plant with the capacity of 10,000 t/year is currently being built and will be commissioned in 2020/2021. The technology is patented (23) and a licence was sold to the company Plastigram a.s. The institute has a share from the revenues during the patent validity.

Another example of a successful technology developed in our team is the technology for biowastes hydrolysis. This technology is based on a high-temperature high-pressure hydrolysis in the presence of carbon dioxide that acts as a catalyst and allows the acquisition of hydrolysates without inorganic salts. The input raw materials for the developed original technology are biowastes with high nutrient content that are difficult to process, such as feathers, blood, confiscates, canteen residues, etc. The product of the technology is a hydrolysate, which is nowadays certified as fertilizing agent under the commercial name Organico and contains 19 % (wt., dry) nitrogen. The whole

procedure is patented (24), and a licence was sold to the company FERGIA, a.s. that currently constructs a pilot plant.

Neodymium–Iron–Boron permanent magnets (NIB) are used in a variety of applications in amounts from milligrams in small electronics, kilograms in electric vehicles, to 1000–2000 kilograms in wind turbine generators. Accordingly, the life cycle of NIB magnets ranges 2–3 years in consumer electronics to 20–30 years in wind turbines. NIB permanent magnets contain about 31–32 wt. % of rare earth elements (REEs). Their recycling from spent NIB permanent magnets is expected to play an important role in the supply of REEs in the future. A complete process of recovery of rare earth elements from neodymium-iron-boron permanent magnets scrap by acid dissolution and liquid-liquid extraction has been proposed. A hydrometallurgical process containing acid leaching with subsequent two-step solvent extraction on a counter-current multistage extraction column is employed to separate iron from lanthanides, re-extract lanthanides in the form of sulphates, precipitate as oxalates, and finally calcinate to a pure state as didymium oxide (praseodymium + neodymium) and dysprosium oxide that can be sold on the market. This technique is unique and allows the preparation of individual REE that can be used again in primary production. The method was patented (25) and verified on a pilot scale level (26). Nowadays, negotiations to license the patent are ongoing in cooperation with technological scout world widely. The main obstacle for the utilization is the long life-time for the products containing high amount of REEs, i.e. the lack of input material and its complicated collection at the moment. However, we expect a change in the near future and application of these unique results based on our deep knowledge of hydrometallurgical processes in real practise.

The most important and promising results with respect to industrial application at the moment are described above, however, several other re-use, recovery and recycling methods for various wastes were studied and developed within the evaluation period. Within the topic we have establish cooperation with the most relevant industrial subjects in the Czech Republic such as all four waste-to-energy plant operators, large international waste management companies like SUEZ or SME with feeling for innovative solutions like Plastigram etc.

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## Research activity and characterisation of the main scientific results

The first research activity dealt with nanoconfined fluids. The physics of fluids in nanoconfinement such as micropores or at interfaces with solid surfaces such as graphene differs significantly from the physics in the bulk. The significant and often counterintuitive changes in fluid behaviour in nanospace are caused by dominant effects of interactions between the fluid molecules and surface atoms. Results of strong fluid-solid interactions are inhomogeneities in fluid density, appearance of specific fluid phases, slow-down of fluid diffusion and transport, e.g. aqueous solutions in hydrophilic mineral micropores, or speed-up of fluid diffusion and transport, e.g. water in carbon nanotubes. We used molecular dynamics and Monte Carlo simulations to understand, describe and quantify at the molecular level effects of nanospace and fluid-solid interactions on the phase, structural, diffusion and transport behaviour of nanoconfined fluids. A molecular-level understanding of nanoconfined fluid behaviour plays a critical role in rational design of porous materials or in development of graphene-based technologies. This research has been supported by two grants: the Czech-Science-Foundation project no. 16-12291S and HORIZON2020 project [ShaleXenvironment](#) no. 640979, and resulted in 13 papers in international journals since 2015.

The second research activity considered nanoscopic heat transport. Heat transport in nanometric dimensions plays an important role in many technologies, ranging from microelectronics, to propagation of reactive fronts, to nanolubrication. The temperature gradients associated with nanoscopic heat transport may lie in the range of  $10^6$ - $10^8$  K/m and depend on the coupling between the different non-equilibrium processes. Molecular-level description and quantification of these non-equilibrium processes becomes critical for understanding e.g. the effects of surface roughness on heat transport or thermally initiated decompositions in reactive energetic materials. We focused on the development of a general and flexible methodology based on mesoscopic models and dissipative particle dynamics which enables us to model the time evolution of the microstructure, local thermodynamics and composition in systems with temperature gradients. This research has been supported by the US ARL-ICPF collaborative project no. W911NF-10-2-0039 and resulted in 5 papers in international journals since 2015.

The third research activity focused on a modification of solid surfaces which may have a dramatic impact on their wetting, thermodynamic or optical properties; a proper fundamental understanding of these effects is still missing. Our effort was to describe and interpret in terms of microscopic, intermolecular interactions the impact of the geometric and chemical heterogeneity of solid surfaces on the adsorption behaviour of simple and complex fluids. Our main focus was on surfaces whose heterogeneity is characterized by a nanoscopically small length-scale, which is a research area still in its infancy. Here, the recent microscopic studies revealed not only strong quantitative but often also a qualitative disagreement with the well-established meso- and macroscopic approaches. Apart from the theoretical importance the research has also tight connections with modern technologies for fabrication of functional materials or for developing of tiny devices such as “lab-on-a-chip”. This research has been supported by several grants within Czech-Science-Foundation projects nos. 13-09914S, 16-12291S, 17-25100S and newly also Czech-Science-Foundation project no. 20-14547S, and resulted in 19 papers in international journals since 2015.

The last research activity involved study of mechanics of granular and multi-phase media that controls deformation and evolution of rocks. This research was started in

2019, when SP was awarded a Czech-Science-Foundation project No. 19-21114Y, while it led to a publication only in 2020. The work under the grant focuses on mechanics of fluid-saturated granular media with respect to stability of geological faults and landslides. The long-term goal is to understand how pore fluid in fault zones participate in bringing the fault up to its mechanical failure during seismic shaking. This phenomenon, known as dynamic triggering, may generate new earthquakes as the mechanical failure is associated with fault slip and increased seismicity.

## Research activity and characterisation of the main scientific results

### Research activities of DLC

1. Growing nanostructured materials by CVD technique or combination of conventional CVD with laser ablation. Nanowires and nanoparticles of different nature were grown at specific conditions and they were characterized by available techniques in the DLC. Crystal structure was studied completely by experts from Institute of Physics CAS, photoelectrochemical activity (Si nanowires as a photoanode) was studied together with Dept. of Catalysis and Reaction Engineering.

Dřínek V. et al.: *Synthesis and characterization of nanostructured molybdenum oxynitride films fabricated by sub-atmospheric chemical vapor deposition*  
JOURNAL OF ALLOYS AND COMPOUNDS 808, Article Number: UNSP 151470, (2019)

Dřínek V. et al.: *Ytterbium silicide nanostructures prepared by pulsed laser ablation in oven: Structural and electrical characterization*  
MATERIALS LETTERS 246, 17-19 (2019)

Dytrych P. et al.: *Silicon nanowires' based photoanode for hydrogen evolution*  
INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 43 (39), 18136-18141 (2018)

Dřínek V. et al.: *Silicon nanowires grown on metal substrates via self-catalyst mechanism* MATERIALS LETTERS 160, 109-112 (2015)

Correa C. A. et al.: *Crystal structure redetermination of e-Ni<sub>3</sub>Si<sub>2</sub> from a single nanowire by dynamical refinement of precession electron diffraction data*  
JOURNAL OF ALLOYS AND COMPOUNDS 672 505-509 (2016)

2. Deposition of materials prepared by laser ablation and reactive laser ablation. As targets, e.g. manganese, titanium, titanium dioxide etc. were used and based on deposition conditions, layers, nanoparticles or encapsulated nanoparticles were prepared. The material was characterized by microscopic, spectroscopic and diffraction techniques in the DLC. HRTEM and magnetic properties were studied in partner institutes (IIC, UCT). Ferromagnetic properties and application potential as catalysts and photocatalysts was demonstrated.

Jandova V. et al. *Reactive laser-induced ablation as approach to titanium oxycarbide films*, THIN SOLID FILMS, 590, 270-275 (2015)

Koštejn, M., et al. Characterization of thin MnSi and MnGe layers prepared by reactive UV pulsed laser deposition. THIN SOLID FILMS, 619, 73-80 (2016)

Jandová V. et al., *Infrared laser radiation-produced TiO-doped Si/SiO<sub>x</sub>/SiO<sub>2</sub> nanocomposite - Entry to TiO-containing materials*, JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY A: CHEMISTRY, 332, 376-383 (2017)

Vála, L. et al.: *Laser-Induced Reactive Deposition on Nanostructured CoS<sub>2</sub>- and Co<sub>2</sub>CuS<sub>4</sub>-Based Films with Fenton Catalytic Properties*. EUROPEAN JOURNAL OF INORGANIC CHEMISTRY, 9, 1220-1227 (2019)

3. Study of solid state reactions induced by laser irradiation of highly homogeneous mixtures. Novel Ti/Si/O nanocomposite films with red-shifted UV-Vis absorption when compared to various TiO<sub>2</sub> or TiO<sub>2</sub>-SiO<sub>2</sub> composite materials were prepared, which suggests its use as a visible light photocatalyst. Thermal processes



were used to prepare catalysts and catalyst carriers for Fenton catalysis tested as a means of degradation of waste water pollutants.

The work was supported by TACR grant project TA04020860. Functional samples and a patent application were also results of the research.

Jandova V. et al.: *Thermal reactions in mixtures of micron-sized silicon monoxide and titanium monoxide: redox paths overcoming passivation shells*, RESEARCH ON CHEMICAL INTERMEDIATES, 44, 503-516 (2018)

Kreněk T. et al.: *CW-Laser-Induced Solid-State Reactions in Mixed Micron-Sized Particles of Silicon Monoxide and Titanium Monoxide: Nano-Structured Composite with Visible Light Absorption*, JOURNAL OF INORGANIC AND ORGANOMETALLIC POLYMERS AND MATERIALS, 27, 1640-1648(2017)

A functional sample of the macro-meso porous glass carrier for nano-catalysis has been prepared by the sol-gel foam procedure using tetraethoxysilane and calcium nitrate hydrate co-hydrolysis in the presence of surfactants and optimized concentration of HF:

Urbanová, M. et al.: *A functional Sample of the Macro-Meso Porous Glass Carrier for Nano-Catalysis*. 0488542 - ÚCHP 2018 RIV CZ cze, <http://hdl.handle.net/11104/0283122>

Functional samples of porous catalysts to be used in Fenton degradation of antibiotics in waste water have been prepared by TEA CO<sub>2</sub> laser ablative deposition of magnetite onto porous alumina substrates. Comparison with Nd:YAG laser deposition (in West Bohemian University) was performed. The samples were characterized by mercury porosimetry and by means of SEM and BET analyses. Due to their mechanical stability they were found to be suitable for the use in flow reactors:

**Křenek, T. et al:** Functional Samples of Porous Catalysts., 0452865 - ÚCHP 2016 RIV CZ cze, <http://hdl.handle.net/11104/0253786>

Functional samples of composite catalysts for Fenton degradation of antibiotics in waste water were prepared by laser ablative deposition of magnetite on porous geopolymer, and on extruded high-density polyethylene. The samples were characterized by EDX-SEM electron microscopy and their efficiency for degradation of antibiotics was assessed by means of LC-MS spectrometry.:

Pokorná D. **et al.:** Functional Samples of Porous Catalysts. 0469182 - ÚCHP 2017 RIV CZ cze, 2016, <http://hdl.handle.net/11104/0267036>

The patented solution affords method of preparation of Al<sub>2</sub>O<sub>3</sub> catalyst carrier covered with Fe(II) spinel nanoparticles for Fenton degradation of antibiotics and trace concentration of drugs from waste water:

J. Pola et al.: Preparation of material based on aluminium oxide with iron (II) spinels  
CZ Patent No. 307971, 18.09.2019

TEA CO<sub>2</sub> and Nd:YAG laser ablation depositions of mixture of TiO and SiO were performed in ICPF and West Bohemian University. The results show that ablative deposition of an equimolar mixture of TiO and SiO results in the formation of films rich in SiO with good adhesion. The research was supported by TACR project TA04010169:

Jandová, Věra - Pola, Josef - Křenek, T. - Pola, M. - Němeček, S. - Kašparová, A. Titanium-silicate Laser Deposition Coatings on Titanium Porous Surfaces - Creating Si/Ti-Porous Surfaces from Powder Materials. 0470092 - ÚCHP 2017 RIV CZ cze, 2016, <http://hdl.handle.net/11104/0267834>

4. Up to 2018, DLC was an integral part of Department of Catalysis and Reaction Engineering. At that period members of DLC were involved in research activities of this Department and papers published. Members of DLC participated mainly in SEM/EDS, Raman, XPS, FTIR and TGA characterizations of catalysts and thermal treatment at specific conditions. The techniques enabled description of deposited catalyst layers – surface concentration and homogeneity, oxidation state and chemical states of elements. The interpretation of data by DLC helped to fully describe materials and understand of the impact of changes on catalytic processes. Comparison of SEM/EDX technique with XPS depth profiling, conducted by DLC members explained penetration dynamics of the catalyst elements Co and/or Ni into the carrier. DLC members contributed to 7 joint papers.

Dvořáková M. et al.: *Preparation of cobalt oxide catalysts on stainless steel wire mesh by combination of magnetron sputtering and electrochemical deposition.*

CATALYSIS TODAY, 334, 13-23 (2019)

Jirátová K. et al.: *Cobalt Oxide Catalysts in the Form of Thin Films Prepared by Magnetron Sputtering on Stainless-Steel Meshes: Performance in Ethanol Oxidation.*

CATALYSTS, 9 (10), 806 (2019)

Jirátová. K. et al.: *Precipitated K-Promoted Co–Mn–Al Mixed Oxides for Direct NO Decomposition: Preparation and Properties.* CATALYSTS, 9(7), 592 (2019)

Pacultová K. et al.: *Co-Mn-Al mixed oxides promoted by K for direct NO decomposition: Effect of preparation parameters.* CATALYSTS, 9 (7), 593 (2019)

Kaluža L. et al.: *Deposition of Co onto Supported MoS<sub>2</sub> by Water-Assisted Spreading: Increased Activity Promotion in Hydrodesulphurization of 1-Benzothiophene.* CATALYSTS, 9 (12), 987 (2019)

Kaluža, L. et al.: *Hydrodesulfurization NiMo catalysts over gamma-alumina prepared mechanochemically.* REACTION KINETICS, MECHANISMS AND CATALYSIS, 125 (1), 319-337 (2018)

Kaluža L. et al.: *Highly loaded carbon black supported Pt catalysts for fuel cells.* CATALYSIS TODAY, 256, 375-383 (2015)

5. Our experience with Surface Enhanced Raman Spectroscopy (SERS) was exploited to study SERS activity of samples prepared at UCT. Different silver structures, deposited on polymer films with various morphology were characterized by microscopic and spectroscopic techniques in DLC. SERS response was tested using a wavelength 473 nm and optimized response was demonstrated using a model compound of Rhodamine 6G:

Kalachyova Y. et al.: *Plasmooptoelectronic tuning of optical properties and SERS response of ordered silver grating by free carrier generation.* RSC ADVANCES, 5, (113), 92869-92877 (2015)

Kalachyova Y. et al.: *Silver nanostructures: From individual dots to coupled strips for the tailoring of SERS excitation wavelength from near-UV to near-IR.* ELECTRONIC MATERIALS LETTERS, 11 (2), 288-294 (2015)

Kalachyova Y. et al.: *Surface plasmon polaritons on silver gratings for optimal SERS response.* JOURNAL OF PHYSICAL CHEMISTRY C, 119 (17) 9506-9512 (2015)

#### Service activities

As a part time service unit, DLC was carrying out characterization of materials, prepared by other ICPF departments. Activities were focused on characterization of received materials, optimization of analyses, processing of samples to afford the best results. Contribution in characterization and qualified evaluation of outcomes of investigation resulted in many articles participation.

#### *Department of Membrane Separation Processes*

Evaluation of separation processes in membranes. Cutting of membranes was developed and the distribution of elements in membranes was measured by SEM/EDX. XPS and FTIR were applied to explain separation processes.

3 joint publications

#### *Department of Aerosols Chemistry and Physics*

DLC contribution was focused on analyses of nanoparticles of selected size deposited in impactors under various condition Elemental mapping by SEM/EDS and chemical state analyses by XPS were conducted. Elemental analyses of ionic liquids and their thermal behaviour by thermogravimetry was studied in the DLC. 9 joint publications

#### *Department of Bioorganic Compounds and Nanocomposites*

Elemental composition and spectroscopic characterization of dendrimers were studied. Thermal stability and decomposition products by TGA-FTIR were used to characterize organosilane dendrimers. FTIR and EDX were applied to describe composition of dendrimers, prepared in the ICPF. 2 joint publications

## Research activity and characterization of the main scientific results

During the last ten years, the group has emerged and brought an ambitious topic of novel materials suitable for molecular electronics. The target molecules were polycyclic aromatic hydrocarbons (PAHs), particularly  $[n]$ helicenes,  $[n]$ phenacenes, and their derivatives. The original synthetic approach developed in the group was based on metal-catalyzed cyclotrimerization (Storch, 2010). However, this approach could not be applied generally, e.g. some helicenes can be hardly produced by this procedure. Also, another synthetic approach leading to heterohelicenes, based on intramolecular cascade hydroarylation/ cycloisomerization was soon abandoned (Storch, 2013) due to general drawbacks. Simultaneously, the former photocyclization procedure was revisited in our group and new methods leading to the preparation of various substituted helicenes in the multigram scale as well as various other types of PAHs were introduced (Storch, 2012). The invention dealing with the development of methodology and apparatus for the production of various  $[n]$ helicenes in the multigram scale has been patented in cooperation with an industrial partner (Lach-Ner Company). Lach-Ner launched the production of racemic helicenes in 2014 and its distribution to customers in the specialty chemicals market (<https://www.lach-ner.cz/helicenyn>). Owing to these efforts, helicenes, and phenacenes can be integrated into applied research more intensively (Žádný, 2013).

As a result of the successful synthetic handling of photocyclization reactions, we have been able to prepare a number of different helicene and phenacene derivatives over the past five years (**Topic 1**, WP1-1 to WP1-3). For further study, helicene derivatives must be separated into individual enantiomers. Thus, it was necessary to find suitable methods of separation and subsequently to characterize the enantiomers (by measuring the CD spectra, optical rotation and racemization barriers, **Topic 2**, WP2-1 to WP2-2). The prepared helicene and phenacene derivatives had to be characterized by many different physico-chemical methods (X-ray, NMR, UV-vis, fluorescence, quantum yield, CV, etc., **Topic 3**, WP3-1 to WP3-3). DFT quantum chemical calculations were also performed allowing correlation with the measured data (WP3-4). Initial toxicological measurements have also started recently (WP3-5), as this knowledge is required when trying to use these materials in molecular electronics or sensing of biomolecules. Finally, the first efforts to applications were also started, i.e. preparation of helicene-based layers (by spin-coating or electropolymerization) and their characterization (**Topic 4**, WP4-1 to WP4-2). Also, the use of helicenes and phenacenes as active layers in humidity sensors is interesting, as well as their utilization in chiral plasmonics or graphene exfoliation (**Topic 5**, WP5-1 to WP5-3).

Storch J., *et al.* *J. Org. Chem.* **2010**, 75(9), 3137. Storch J. *et al.* CZ Pat. No. 303997 (**2012**) / PV 2012-245. Storch J. *et al.* *Eur. J. Org. Chem.* **2013**, 2013(2), 260. Žádný J. *et al.* *Tetrahedron* **2013**, 69(30), 6213.

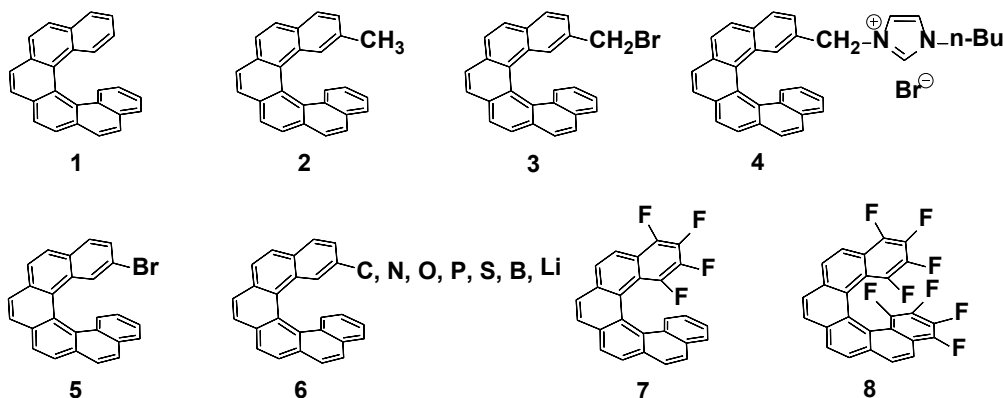
### Topic 1: Synthesis of helicene and phenacene derivatives

The preparation of helicenes and phenacenes can be divided into three groups (see WP1-1 to WP1-3) according to the length (number of benzene units) and type of the side-chain.

#### WP1-1: [6]Helicene derivatives

The simplest member of this group, [6]helicene (**1**), was prepared according to our improved procedure (Storch, 2012) by photocyclization of 2,7-distyrylnaphthalene. The

required helicene-based imidazolium salt **4** was prepared (Storch, 2015) starting from racemic 2-methyl[6]helicene (**2**), which was subjected to radical bromination to yield 2-(bromomethyl)[6]helicene (**3**). Subsequent treatment with 1-butylimidazole furnished the corresponding 1-butyl-3-(2-methyl[6]helicenyl)-imidazolium bromide (**4**).



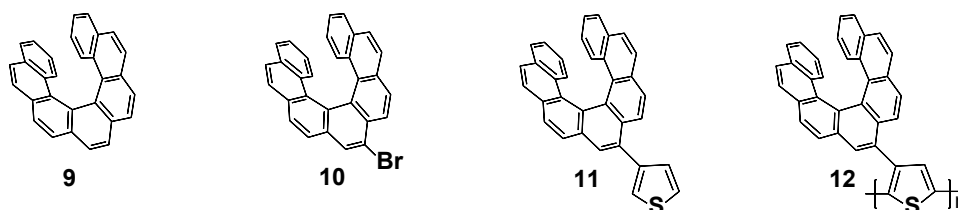
The parallel activity was focused on 2-bromo[6]helicene (**5**), which was shown to be a suitable starting material for the synthesis of functional molecules based on a [6]helicene skeleton (Jakubec, 2018). The photochemical method for obtaining of 2-bromo[6]helicene (**5**) was improved, bringing the overall yield up to 51% over 5 steps. Its reactivity was investigated and a library of 17 different carbon, nitrogen, oxygen, phosphorus, sulfur, boron, and lithium substituted derivatives **6** bearing a [6]helicene-2-yl moiety was synthesized including 13 previously unknown [6]helicene derivatives. All of the mentioned reactions were optimized for racemic starting material **5**, which can be easily separated into individual 2-bromo[6]helicene enantiomers (**5**). Upon measuring of racemization barrier of nonracemic **5**, the three most energy-demanding reactions (phosphorylation conducted at 160 °C, Buchwald-Hartwig amination at 170 °C, and Rosemund-von Braun cyanation at 210 °C) were tested using enantiomerically pure **5** to confirm the predicted drop-off of enantiomeric excess of the products. These finding opening further applications of these molecules in various fields of chemistry and material science.

Ongoing work (Církva, 2019) was focused on the preparation of partially fluorinated [6]helicenes to understand the impact of fluorine substitution on the properties of the newly synthesized helicenes. The introduction of fluorine substituents into helicene skeletons has been a prospective strategy for tuning the properties required for application in organic electronic devices. Fluorine substitution can affect C-H/ $\pi$  interactions, which prevail in a molecular assembly in the solid-state, or can improve the solubility. Therefore, the first racemization-stable helicene derivatives fluorinated at terminal rings, i.e. 1,2,3,4-tetrafluoro[6]helicene (**7**) and 1,2,3,4,13,14,15,16-octafluoro[6]helicene (**8**), have been successfully synthesized via the Mizoroki-Heck cross-coupling reaction and Wittig reaction, followed by eliminative or oxidative Mallory photocyclization with an overall yield of 41% of **7** and 76% of **8**. It was found that the photocyclodehydrofluorination reaction followed by HF elimination is not suitable for this purpose (2% yield of **7** and 0% yield of **8**) due to the formation of dimeric structures.

#### WP1-2: [7]Helicene derivatives

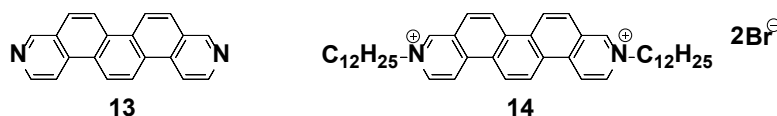
The simplest members of this group, [7]helicene (**9**) and 9-bromo[7]helicene (**10**), were also prepared according to our improved photochemical procedure (Žádný, 2013) by photocyclization of brominated 1,4-bis[2-(2-naphthyl)vinyl]benzene. The required

monomeric unit, 3-([7]helicen-9-yl)thiophene (**11**), was synthesized using the methodology developed in our group (Hrbáč, 2014), starting from 9-bromo[7]helicene (**10**). **10** was subjected to Suzuki coupling with thienylboronic acid catalyzed by a platinum compound with 80% yield. Subsequently, monomer **11** was electropolymerized to form a polymer, poly[3-([7]helicen-9-yl)thiophene] (**12**) (Hrbáč, 2017). The potential-driven on/off switch concept was used to prepare two types of helicene-derived polymer. This concept is based on cyclic anodization of the electrode to +1.5 or +2.5 V, leading to compact conductive or non-conductive polymeric thin layers. The above results will serve as the basis for further applications for the preparation of helicene-based materials. The next step will be the synthesis of hybrid thiophene monomers from optically pure forms of helicene (P and M enantiomers).



### WP1-3: [5]Phenacene derivatives

The parent 3,10-diazapicene (**13**), a member of the hitherto rather underrepresented family of hetero[*n*]phenacenes, was accessible through microwave-assisted photocyclization of the corresponding diazastilbenes (i.e. 1,2-di(5-isoquinoliny)ethene). To enhance the solubility of **13** and to introduce electron-accepting properties, *n*-dodecyl alkyl chains were introduced at the pyridyl nitrogens (Roth, 2017). The reaction of **13** with 1-bromododecane in DMF furnished the *N,N'*-didodecyl decorated 3,10-diazapicenium dibromide salt (**14**) in 89% yield.



Storch J. *et al.* CZ Pat. No. 303997 (**2012**)/PV 2012-245. Žádný J. *et al.* Tetrahedron **2013**, 69(30), 6213. Hrbáč *et al.* RSC Adv. **2014**, 4, 46102. Storch J. *et al.* Chem. Eur. J. **2015**, 21(6), 2343. Roth A. *et al.* Chem. Sci. **2017**, 8(5), 3494. Hrbáč J. *et al.* ChemElectroChem **2017**, 4(12), 3047. Jakubec M. *et al.* J. Org. Chem. **2018**, 83(7), 3607. Církva V. *et al.* J. Org. Chem. **2019**, 84(4), 1980.

## Topic 2: Separation of helicenes into single enantiomers

The development and utilization of effective synthetic protocols and enantioselective separation approaches are in general one of the most important steps in the technological applicability of helicenes. For practically all applications, especially in the field of precise optoelectronics, the main prerequisite is the multigram synthesis of helicenes with high chemical purity including enantiopurity.

### WP2-1: Separation study

To the best of our knowledge, there is a limited range of experimental setups and robust methodologies for the chiral resolution of helicenes.

Our first report (Storch, 2016) is focused on a chiral resolution study of capillary electrophoresis (CE), high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) of [6]helicene (**1**) and the 1-butyl-3-(2-methyl-

[6]helicenyl)imidazolium bromide (**4**). Both non-ionic and cationic helicenes exist as *P* and *M* enantiomers. The results obtained in HPLC and SFC clearly shown that both methods were suitable for the enantioseparation of compounds **1** and **4**. However, SFC enabled the highly selective resolution of enantiopure helicenes of **1** and **4** in a single run within 5 min. CE exhibited a significantly lower potential for enantioseparations of the studied helicene derivatives. Only partial enantioseparation of compound **4** was observed, employing  $\gamma$ -CD as the chiral selector. The **1** was resolved to optical antipodes in the preparative scale using HPLC with a chiral stationary phase for chiral hybrid plasmonic nanostructures preparation (Kalachyovy, 2019).

In the other study, the enantiomers 2-bromo[6]helicene (*M*)-**5** and (*P*)-**5** were isolated using a chiral HPLC (Kromasil Cellucoat column) and *n*-heptane/2-propanol as a mobile phase (Jakubec, 2018). The enantiomerically pure isomer **5** was tested on three energy-demanding model reactions (see WP1-1).

In the next study, the enantiomeric resolution of 1,2,3,4-tetrafluoro[6]helicene (**7**) was also accomplished by preparative HPLC using a Kromasil Cellucoat column (Církva, 2019). The chiral separation was not attempted in the case of octafluorinated helicene **8** due to its low solubility (e.g., only 0.5 mg/mL in chloroform).

## WP2-2: CD, optical rotation and racemization barrier

With pure enantiomers in hand, we were able to study the circular dichroism (CD) spectra, optical rotation, and racemization barriers.

First, the CD spectra of [6]helicene enantiomers (**1**) were measured and determined. Then we focused on the characterization of chiral [6]helicene *P*- and *M* layers prepared by anodic electrodeposition (Vacek, 2018). It was typical that the positive (or negative) CD-active band could be clearly seen about 310 nm for the *P*-layer (or *M*-layer). This band exhibiting strong ellipticity was assigned to the typical  $\pi$ - $\pi^*$  transitions found at 325 nm in [6]helicene monomer. In addition, new CD-active bands appeared between 380 and 580 nm, and were assigned to the deposited layers. It was demonstrated that the *P*- and *M*- enantiomers of [6]helicene can be immobilized electrochemically to form chiral (enantiopure) thin layers on ITO substrate, and that these layers exhibit new CD signatures compared to the parent [6]helicene.

The formation of chiral response by optically active hybrid plasmonic structures was also determined by electronic CD spectroscopy upon deposition of [6]helicene on the Au grating surface. This arrangement allows the direct enantiomers SERS discrimination (see WP5-3) (Kalachyova, 2019).

In the next study, 1,2,3,4-tetrafluoro[6]helicene (**7**) enantiomers exhibited clear mirror images in the CD spectra similar to that of [6]helicene (Církva, 2019). Lower specific rotation of (**7**) compared to parent [6]helicene was also discussed.

First, the specific optical rotation of [6]helicene were measured in chloroform and these values were obtained, (+)-(*P*)-**1**  $[\alpha]_{\text{D}}^{20} = +3645 \pm 4^\circ$  and (-)-(*M*)-**1**  $[\alpha]_{\text{D}}^{20} = -3669 \pm 2^\circ$ , respectively (Kalachyova, 2019).

Enantiomers of 1,2,3,4-tetrafluoro[6]helicene (**7**) exhibited also high specific optical rotation. According to the X-ray structure analysis, the dextrorotatory enantiomer was attributed to the *P* form and levorotatory to the *M* form. The obtained specific rotation values in chloroform (+)-(*P*)-**7**  $[\alpha]_{\text{D}}^{20} = +2882 \pm 4^\circ$  and (-)-(*M*)-**7**  $[\alpha]_{\text{D}}^{20} = -2849 \pm 4^\circ$  were lower than values published for unsubstituted [6]helicene **1**. This observation can be attributed to a lower electron density and also a shorter distance between terminal rings found for **7** in comparison with [6]helicene (**1**) (Církva, 2019).

It is well documented that helicenes can racemize. Therefore, the Gibbs free energy ( $\Delta G^\ddagger$ ), the rate constant of racemization ( $k_{rac}$ ) and the rate constant of enantiomerization ( $k_e$ ,  $k_{rac} = 2k_e$ ) values for the helicenes **5** and **7** were determined. Three different approaches were used and mutually compared to obtain the best fit to the experimental data and reliable half-time of the racemization ( $t_{1/2}$ ).

In the case of the model compound, [6]helicene, the conformational interconversion takes place at elevated temperature and is characterized by the Gibbs free energy barrier  $\Delta G^\ddagger$  (503 K) = 36.5 kcal mol<sup>-1</sup> (152.8 kJ mol<sup>-1</sup>) at 230 °C. When 2-bromo[6]helicene (**5**) was measured, the racemization process was monitored and the determined Gibbs free energy of **5** at 465 K (36.5 kcal/mol) was close to that reported for unsubstituted [6]helicene (Jakubec, 2018).

On the other hand, the racemization barrier of 1,2,3,4-tetrafluoro[6]helicene (**7**) can be significantly affected by the introduction of bulkier fluorine substituents into the interior of the helix. Therefore, it can be reasonably expected that enantiomers of **7** should possess a higher racemization barrier and provide better thermal stability than unsubstituted [6]helicene. The thermal racemization process was monitored at 233 °C following the decay of the ee over time ( $t$ ). The plot of  $\ln(ee_t/ee_0)$  against  $t$  allowed the determination of  $\Delta G^\ddagger$  (506 K) = 38.9 kcal mol<sup>-1</sup> (162.9 kJ mol<sup>-1</sup>) for **7** (Církva, 2019).

Storch J. *et al.* *J. Chromatogr. A* **2016**, 1476(DEC 9), 130. Jakubec M. *et al.* *J. Org. Chem.* **2018**, 83(7), 3607. Vacek J. *et al.* *ChemElectroChem* **2018**, 5(15), 2080. Kalachyova Y. *et al.* *ACS Appl. Mater. Interfaces* **2019**, 11(1), 1555. Církva V. *et al.* *J. Org. Chem.* **2019**, 84(4), 1980.

### Topic 3: Physico-chemical properties of helicene and phenacene derivatives, toxicology

The newly prepared helicene and phenacene derivatives were characterized by various physico-chemical methods (i.e. X-ray, NMR, UV-vis, fluorescence, or CV) (WP3-1 to WP3-3), which were supported by DFT quantum chemical calculations (WP3-4). Moreover, the initial toxicological measurements (WP3-5) were also started.

#### WP3-1: X-ray and NMR study

The main focus was the study of the internal dynamics in helicene molecules and variable-temperature X-ray diffraction study of [6]helicene (**1**) in the temperature range of 100-300 K. The analysis has shown that the higher temperature increases only the oscillations of individual atoms manifested by larger thermal ellipsoids without any significant effect on molecular conformation that is almost temperature-independent. On the other hand, variable-temperature NMR experiments performed in a 200-400 K range have revealed significant temperature dependence of the nuclear shielding of peripheral atoms, which are the most strongly influenced by through-space ring-current effects. Chemical shifts were always higher at elevated temperatures, which could be explained by a larger distance between the peripheral rings (Dračínský, 2017).

In another study, the work was focused on 1-butyl-3-(2-methyl-[6]helicenyl)imidazolium bromide (**4**). Two remarkable kinds of interaction within helicene domains have been considered: 1)  $\pi$ - $\pi$  stacking of two adjacent molecules when *P*- and *M*-type helicenes alternated in this direction with interactions between the electron-rich and -poor parts of the helicene subunits. 2) A strong CH- $\pi$  interaction was found (Storch, 2015).



All the structures of partially fluorinated [6]helicenes **7** and **8** utilized a similar motif in the crystal packing (known from the structures of unsubstituted helicenes), i.e. a pair of two mutually interlocked helicene molecules. It was also found that increasing fluorination also significantly affected the intermolecular interactions in the crystal lattice. The  $\pi$ - $\pi$  and CH- $\pi$  interactions were gradually substituted by CH-F interactions which constituted 1D-molecular chains in the structures of both fluorinated helicenes. The fluorination of both peripheral rings in **8** enabled the formation of very strong intermolecular interactions within the 1D-chain, resulting in an elevated melting point and significantly decreased solubility of the compound **8** (Církva, 2019).

The experimental and theoretical investigation performed within all this study describes the unexpectedly large conformation flexibility and dynamic behavior of [6]helicene molecules. These properties should be taken into account in the design of helicene-like structures for future applications.

### WP3-2: UV-vis and fluorescence study

The ongoing research was focused on the study of the optical properties of helicene and phenacene derivatives.

The UV-vis absorption spectra of prepared compounds **1**, **4**, **7**, **8**, **9**, **11**, **12**, **13**, and **14** were measured (Storch, 2015; Roth, 2017; Hrbáč, 2017; Vacek, 2018; Církva, 2019). The presence of the [6]helicene moiety influenced the absorption spectra considerably. There were two peaks, the major one located at 266 nm and the lower one with an apparent doublet at  $\lambda = 318$  and 328 nm. These values were in line with previously published data for [6]helicenes and are rather typical for  $\pi$ - $\pi^*$  transitions.

Steady-state excitation and emission fluorescence spectra, the fluorescence quantum yields and fluorescence decays of **1**, **4**, **7**, **8**, **11**, **12**, **13**, and **14** were also measured (Storch, 2015; Roth, 2017; Hrbáč, 2017; Církva, 2019). The fluorescence spectra of the helicene derivatives did not differ fundamentally from the spectra of basic helicene. A wavelength close to the excitation maximum (325 nm) was subsequently used for fluorescence emission spectra measurement. The fluorescence originated from the  $^1L_b$  state, which was not observed in the absorption spectra. The band contained two maxima coming from the  $S_0$  symmetrical vibrational mode and a weaker unresolved shoulder at lower energies.

### WP3-3: Cyclic voltammetry (CV) study

The electrochemical properties of [5]helicene, [6]helicene (**1**), and [7]helicene (**9**) were studied by CV at a glassy carbon electrode using acetonitrile supported by tetrabutylammonium perchlorate (TBAP) as an electrolyte (Vacek, 2018). Although the helicenes are composed only of nonsubstituted ortho-condensed benzene rings, their electrochemical properties were significantly different. The helicenes were studied as racemic equimolar mixtures of *P* and *M* enantiomers. Based on oxidation and reduction  $E_p$  values, it was evident that [7]helicene was oxidized more easily than [5]- or [6]helicene.

The 3-([7]helicene-9-yl)-thiophene (**11**) hybrid monomer was electrooxidized (Hrbáč, 2017) in acetonitrile by using CV with anodic potential limits of +1.5 or +2.5 V, resulting in a conductive or non-conductive novel polymer, poly[3-([7]helicene-9-yl)-thiophene] (**12**). The potential-driven on/off switch concept was thus used to prepare two types of helicene-derived polymer.

Redox properties of the studied fluorinated [6]helicenes **7** and **8** were also investigated by CV at a glassy carbon electrode (Církva, 2019). The cathodic scan revealed a multistep reduction process, manifested as three rather broad reduction CV peaks.

The CV pattern was shifted toward positive potentials with increasing degree of fluorination, indicating that the reduction was facilitated after the functionalization. On the other hand, in anodic CV scans two overlapped peaks merging with the anodic limit occurred. The anodic features are shifted toward positive potentials with increasing fluorination.

In the case of the *N,N'*-didodecyl decorated 3,10-diaza-picenium dibromide salt (**14**), CV measurements were carried out in a conventional three-electrode cell using Pt button working electrodes, a platinum wire counter electrode, and Ag/AgCl as a reference electrode (Roth, 2017). The one-electron reduction of **14** to the corresponding radical cation was observed.

#### WP3-4: DFT study

DFT calculations were performed with the quantum computational density functional B3LYP or B3PW91 methods and 6-311++G\*\*, 6-31G(d,p) and 6-311++G(d,p) basis set using the Gaussian 09 software package.

It was found that in [5], [6], and [7]helicene order with the increasing number of helicene rings the HOMO-LUMO gap was decreased while HOMO energy increased and LUMO energy decreased (Vacek, 2018). Also, DFT calculations confirmed high flexibility of [6]helicene conformation and the trend of increasing chemical shifts of peripheral atoms in conformations with a larger opening of the helicene molecule (Dračínský, 2017).

In another investigation, the study was focused on 1-butyl-3-(2-methyl[6]helicenyl)-imidazolium bromide (**4**) (Storch, 2015). When comparing the HOMO orbitals of **4** to the 2-methyl-[6]helicene (**3**), the electronic density slightly decreased along with the aromatic system toward the imidazolium ring. This trend followed the distribution of the  $\pi$ -surface of the helicenes with decreasing HOMO and LUMO levels.

It was observed in another study, that the effect of fluorine substitution was well illustrated in the comparison of electrostatic potentials mapped onto the electron density surface calculated for fluorinated helicenes **7**, **8** and parent [6]helicene (**1**) (Církva, 2019). The calculated HOMO and LUMO representations of the studied compounds showed that the electron density distribution was pronounced in the nonfluorinated rings in each case. While the HOMO-LUMO gap was almost independent of the substitution, the HOMO and LUMO energy levels decreased within the degree of fluorination.

The experimental and theoretical investigation performed within these works describes the unexpectedly large conformation flexibility and dynamic behavior of [6]helicene molecules. These properties should be taken into account in the design of helicene-like structures for future applications (Dračínský, 2017).

#### WP3-5: Toxicology

This initial study investigated ecotoxic properties of [6]helicene (**1**) to aquatic (*Daphnia magna*, *Desmodesmus subspicatus*, *Vibrio fischeri*) and soil (*Folsomia candida*, *Enchytraeus crypticus*) organisms. In the case of the aquatic species, no significant effect was observed even at the highest nominal concentration tested of 0.63 mg/L. In the case of the soil species, the higher of the two tested concentrations of 1.7 mg/g showed an inhibition of reproduction of 51% and 39% of *Enchytraeus crypticus* and *Folsomia candida*, respectively. The soil toxicity of [6]helicene was higher than expected from its lipophilicity, which might suggest a specific toxicity mechanism (Sovová, 2015).

This ongoing study examined the effect of [6]helicene (**1**) and 1-butyl-3-(2-methyl-[6]helicenyl)-imidazolium bromide (**4**) on the activity of the aryl hydrocarbon receptor (AhR) and expression of cytochrome P450 1A1 (CYP1A1) in human hepatoma HepG2 cells. An MTT viability assay showed that both **1** and **4** were cytotoxic to HepG2 cells after 24 h of exposure, with IC<sub>50</sub> values of 0.9 and 8.4  $\mu$ M, respectively. Using a gene reporter assay performed in transiently transfected HepG2 cells, we found that 1  $\mu$ M [6]helicene, unlike **4**, significantly increased the activity of AhR to 2.1-fold compared to the control after 24 h of exposure. Moreover, [6]helicene induced a small but significant increase in the level of CYP1A1 mRNA. On the other hand, neither the protein level nor activity of CYP1A1 was affected by [6]helicene in HepG2 cells. The effect of [6]helicene on the AhR pathway was thus much lower than that of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin, a potent AhR activator. It was possible to say that [6]helicene was a poor activator of the AhR pathway in HepG2 cells, and that the possible activation of the AhR pathway in vivo remains to be investigated (Vrba, 2019).

Storch J. *et al. Chem. Eur. J.* **2015**, 21(6), 2343. Sovová T. *et al. Pol. J. Environ. Stud.* **2015**, 24(5), 2329. Roth A. *et al. Chem. Sci.* **2017**, 8(5), 3494. Dračinský M. *et al. Phys.Chem.Chem.Phys.* **2017**, 19(4), 2900. Hrbáč J. *et al. ChemElectroChem* **2017**, 4(12), 3047. Vacek J. *et al. ChemElectroChem* **2018**, 5(15), 2080. Církva V. *et al. J. Org. Chem.* **2019**, 84(4), 1980. Vrba J. *et al. Toxicol. in Vitro* **2019**, 57(June 2019), 105.

#### **Topic 4: Preparation of helicene-based layers and their characterization**

The first applications were started, i.e. preparation of helicene-based layers (by spin-coating or electropolymerization) and their characterization (WP4-1 to WP4-2).

##### **WP4-1: Spin-coating**

The 1-butyl-3-(2-methyl-[6]helicenyl)-imidazolium bromide (**4**) salt was immobilized by spin-coating onto a SiO<sub>2</sub> substrate as a crystalline or amorphous deposit. Concerning the fact that annealing at 190 °C improved the conductivity of the film, both annealed samples (at 100 and 190 °C) using Bragg diffraction and optical microscopy were investigated for possible structural modifications of the spin-coated **4** film. From the macroscopic point of view, the annealed sample at 100 °C appeared homogeneous, nevertheless, it contained domains with the size of a few microns. On the other hand, the annealed sample at 190 °C was mostly composed of crystals with a size of several hundred microns, which overlapped each other, and thus they created a connected path across the sample between the two gold electrodes. Since the crystalline phase enhances the conductivity of the studied material, it was crucial to know the onset of crystallization. The deposited layers were used for a fully reversible humidity sensor (Storch, 2015) (see WP2-1). The technique of spin-coating was used also for the preparation of unique optically active plasmonic structures based on SPP-supported Au grating combined with helically chiral and extremely optically active molecules of [6]helicene (Kalachyova, 2019), which can be used for detection of enantiomers in picomolar concentration by SERS but also can recognize and quantify them simultaneously and directly in their mixtures, without their previous chiral separation (see WP5-3).

##### **WP4-2: Electropolymerization**

The new material, bearing thiophene and helicene moieties, was prepared by using a potential-driven on/off switch strategy on the surface of glassy carbon and ITO

substrates. Specifically, 3-([7]helicen-9-yl)thiophene (**12**) hybrid monomer was electro-oxidized in acetonitrile by using CV with anodic potential limits of +1.5 or +2.5 V, resulting in a conductive or non-conductive polymer. The electrodeposited polymers could be used for the further development of materials applicable in organic electronics, optoelectronics, and sensing technologies (Hrbáč, 2017).

The multicomponent anodic process of helicenes was observable at potentials from +1.5 to +2.5 V, leading to the formation of deposited structures (layers) on the electrode surface. The anodic deposition of *P* and *M* enantiomers of [6]helicene (**1**) was also performed using ITO substrates, resulting in the formation of enantiopure layers of nanometer thicknesses, as was confirmed by CD spectroscopy and AFM. This discovered electrosynthetic procedure opened up a new possibility for the immobilization of chiral helicene layers onto solid supports (Vacek, 2018).

Storch J. *et al. Chem. Eur. J.* **2015**, 21(6), 2343. Kalachyova Y. *et al. ACS Appl. Mater. Interfaces* **2019**, 11(1), 1555. Hrbáč J. *et al. ChemElectroChem* **2017**, 4(12), 3047. Vacek J. *et al. ChemElectroChem* **2018**, 5(15), 2080.

### Topic 5: Application of helicenes and phenacenes

The use of helicenes and phenacenes as a humidity sensor, or in plasmonic structures and graphene exfoliation were studied (WP5-1 to WP5-3).

#### WP5-1: Humidity sensor

The deposited layers of 1-butyl-3-(2-methyl-[6]helicenyl)-imidazolium bromide (**4**) onto SiO<sub>2</sub> were used for the development of organic molecular semiconductor devices and the construction of a fully reversible humidity sensor. The crystalline (dry) imidazolium itself had a strong affinity to being dissolved in the moisture in the air. Similarly, the imidazolium component in the spin-coated **4** could be affected by the humidity in the air and thus it could change the material's conductivity (Storch, 2015).

#### WP5-2: Exfoliation of graphene

The *N,N'*-didodecyl 3,10-diazapicenium dibromide salt (**14**) was successfully applied to exfoliate graphite and to stabilize the graphene flakes. It was corroborated that the ground state of these hybrids was dominated by a shift of charge density from the basal plane of graphene to the positively charged 3,10-diazapicenium - the result of this shift was p-doped graphene. Unambiguously, the presence of 5-8 layers of turbostratic graphene flakes with modified electronic properties was demonstrated (Roth, 2017).

#### WP5-3: Plasmonic structures

This initial study concerned with the fabrication of chiral plasmon substrates based on surface plasmon-polariton-supported structure coupled with optically active helicene enantiomers (see WP2-1). Such an approach allowed us to excite chiral plasmon waves and to design optically active substrates for surface-enhanced Raman spectroscopy (SERS). They allowed for enantio-selective detection/recognition of optical enantiomers with detection limits below those of standard spectral techniques. The chiral optical response of the new plasmonic system was observed and controlled by the optical rotation of helicenes. Without the necessity of previous chiral separation or implementation of sophisticated experimental equipment, we were able to estimate the concentration of enantiomers in their mixtures by using left- or right-handed chiral plasmon substrates (Kalachyova, 2019).

Storch J. *et al.* *Chem. Eur. J.* **2015**, 21(6), 2343. Roth A. *et al.* *Chem. Sci.* **2017**, 8(5), 3494. Kalachyova Y. *et al.* *ACS Appl. Mater. Interfaces* **2019**, 11(1), 1555.

## Research activity and characterisation of the main scientific results

The team's activities were traditionally focused on extracting health-promoting substances from plants, including mathematical description of the process. To increase the added value of extracts and their utility, we also studied various applications of scCO<sub>2</sub> such as extract micronization, its fractionation on sorbents, and modification using enzymatic reactions. In addition to the extractions, we introduced new applications of supercritical fluids in the preparation of nanostructured metal oxides, and we studied the process of polymer foaming by rapid CO<sub>2</sub> expansion.

### Topic 1: Extraction of biologically active components from plants

Plant material is the source of many healthy ingredients, which can be obtained from plants using various extraction methods. SCTG uses its rich experience in the extraction from plants especially in application projects and in research for companies. In the field of basic research, we focus on mathematical modelling of supercritical extraction from plants.

#### 1.1. High-pressure extraction of plant adaptogens

The Group is involved, together with the company ASIPO s.r.o. and the University of Chemistry and Technology in Prague (UCT), in the project MPO FV30300 (Isolation and application of plant products having effects against stress and other neurodegenerative diseases) solved in the period from 2018 to 2021. The objective of the project are plant products with effects on neurodegenerative and other stress diseases, capable of augmenting the resistance of organism against stress diseases and augmenting the resistance of organism to resist to changed environment. Many plants, often called medicinal plants, produce such substances. The target of the project is both growth of the plants and use of their products. This is the reason for setting the project as collaborative one between two research institutions and a small company. The research institutions are focused on developing ways of isolation and investigation of active components in the studied plants, and the small company is engaged in the growing of target plants and utilization of the results of the collaborative investigation. The above mentioned plant products are isolated by modern technologies, e.g. by supercritical carbon dioxide extraction and pressurised liquid extraction, which are compared with conventional extraction methods, and, finally, incorporated into the novel products.

The aim of the work in the SCTG was to develop an efficient procedure for the extraction of selected plants to obtain quality extracts with high yields and concentrations of monitored substances. The extraction efficiency was assessed in terms of (a) the method for obtaining biologically active substances, (b) the type of solvent (ethanol, water, scCO<sub>2</sub>), (c) the process conditions, (d) the consumption of solvents and time, and (e) the origin and quality of the raw material. The composition of extracts was analysed by HPLC-MS and UV spectroscopy.

The bark of birch (*Betula pendula*), seeds of *Laserpitium archangelica*, and leaves and branches of sea buckthorn (*Hippophae rhamnoides*) and *Rhodiola rosea* were used to obtain extracts, and the plant material was processed at the SCTG by high-pressure liquid extraction, supercritical extraction, maceration, Soxhlet extraction and leaching. Attention was mainly paid to obtaining extracts from the leaves and branches of sea buckthorn and *Rhodiola rosea*, which samples were prepared by various extraction

methods and solvents in order to assess their suitability for a given material in terms of extract yields, composition and biological effects.

The individual extraction methods were performed under different conditions and subsequently compared with each other in terms of the yield of the individual biologically active components of plants. When optimizing the methodology of extraction, the optimization criteria were the yield and concentration of monitored substances, it is triterpene components from birch bark and sesquiterpene lactones from *Laserpitium archangelica*.

The content of tyrosol, salidroside and rosavin was monitored in the extracts from *Rhodiola rosea*, while the content of serotonin, caffeic acid and ferulic acid was determined in the extracts from sea buckthorn. The total content of phenolic substances and flavonoids was also determined in both plant materials. At the same time, samples of extracts from target plants with different compositions were prepared and submitted to the UCT to test their biological activity.

Several students took part in the research and wrote their bachelor's theses on the given topic as part of their studies. The results concerning the extraction of *Laserpitium archangelica* were presented at an international conference and the results from sea buckthorn and *Rhodiola* at a national conference.

## 1.2. Conventional extraction of grape by-products

The Czech Republic is a country with a highly developed winegrowing and winemaking industry. During the wine production, quite a high amount of plant wastes is generated. This waste, such as grape cane, grape pomace, or grape seeds, contains a significant amount of biologically active substances (resveratrol, viniferines, omega-3 fatty acids, and others) which can be used as active ingredients in human dietary products. Unfortunately, almost all mentioned wastes are composted or combusted without obtaining any other benefit.

This topic which has been solved between 2018 and 2019 dealt with the complex treatment of grape wastes into semi-products with added value. The research was supported by the project no. TJ01000249 "Development of multipurpose extraction unit for the processing of waste from wine industry into products with substantial added value" from the Technology Agency of the Czech Republic, and solved in cooperation with an industrial partner MikroChem LKT.

The project was focused on the extraction of grape cane and grape pomace using traditional extraction techniques (maceration, Soxhlet extraction, ultrasound-assisted extraction) and their combination. The chemical composition of isolates was determined via HPLC/MS. The main bioactive compounds of extracts were *trans*-resveratrol and viniferines, compounds from the group of stilbenes. Extracted material from the South Moravian region contained 0.2 – 9.6 wt. % of *trans*-resveratrol and 0.1 – 9.5 wt. % of *trans*- $\epsilon$ -viniferine. The chemical composition of isolates varied based on the extraction method and process conditions. The experiments conducted at lower temperatures were more selective for the extraction of *trans*- $\epsilon$ -viniferine, while using higher temperatures led predominantly to the *trans*-resveratrol isolation. Adopting ultrasound had a significant effect on the decrease of extraction times, from days in the process of maceration to minutes when using ultrasound.

Extracts obtained by different extraction methods were exposed to variable stress conditions (light, temperature, and time) to evaluate the stability of each target compound and easiness of its release from the plant matrix. *Trans*-resveratrol, which often remains bound to other molecular structures even in extracts, was found to be relatively thermally stable, but it gradually undergoes dimerization when exposed to

light. Extracts containing high amounts of this stilbene can therefore be obtained using extraction in dark at elevated temperatures for periods no longer than one day. For extraction methods at ambient temperature, longer periods are possible, such as 14 days. The main routes of formation of *cis*- $\epsilon$ -viniferine are light-induced dimerization of resveratrol and photoisomerisation of *trans*- $\epsilon$ -viniferine. According to this, the exposure to light during extraction leads to extracts rich in *cis*- $\epsilon$ -viniferine. Elevated temperatures as well as longer extraction times should be avoided due to the low thermal stability of this stilbene. To obtain extracts containing a high amount of *trans*- $\epsilon$ -viniferine, also the protection of material from light during extraction is crucial. Use of elevated temperatures during extraction is in the case of *trans*- $\epsilon$ -viniferine less favorable due to lower thermal stability of this stilbene.

Results obtained from laboratory experiments served for the construction of semi-production multipurpose extraction unit (function sample) which allows combining the mentioned extraction methods in one operational unit. This unit was used to propose the best combination of process conditions for stilbene extraction (proven technology). Primary outcomes of this project were a functional sample (pilot plant), proven technology, article in an impacted journal (Kosovic, 2020) and nine communications at prestigious international scientific conferences.

### 1.3. Extraction of botanical pesticides

*Pelargonium graveolens* is a valuable source of biologically active compounds and a promising botanical pesticide. In this study, supercritical fluid extraction (SFE) of *Pelargonium* leaves under different operational conditions (pressure 9–30 MPa, temperature 40 and 50 °C) was optimized to improve the extraction yields, selectivity and pesticidal activity. The composition and pesticidal activity of scCO<sub>2</sub> extracts were compared with the isolates obtained by maceration and hydrodistillation. GC–MS and GC–FID were used to determine the chemical composition of isolates. The insecticidal activity (acute and chronic toxicity, and antifeedancy) of the isolates was measured on larvae of *Spodoptera littoralis*. The antifungal activity was evaluated as the inhibition effect on the growth of model pathogenic and toxinogenic fungi (*Fusarium oxysporum*, *Penicillium expansum*, *Aspergillus fumigatus*). The highest yield of CO<sub>2</sub> extract was 44.5 mg g plant<sup>-1</sup> at 30 MPa, 40 °C, and the maximum concentration of volatiles in extract was 56 % w/w at optimal conditions 9 MPa and 50 °C. The insecticidal activity of isolates differed according to the type of test: CO<sub>2</sub> extracts obtained at 9 MPa, 50 °C and at 12 MPa, 40 °C exhibited the highest chronic toxicity (LD<sub>50</sub> = 6 µg) and antifeedancy (ED<sub>50</sub> = 95 µg cm<sup>-2</sup>), respectively. The essential oil obtained by hydrodistillation with the yield of 8.5 mg g plant<sup>-1</sup> showed the strongest acute toxicity (LD<sub>50</sub> = 28 µg). The antifungal activity of isolates increased with increasing concentration of volatiles in isolates. The results suggest that CO<sub>2</sub> extracts of *P. graveolens* can be suitable substitutes to the essential oil in their use as botanical pesticides (Machalová, 2015).

Supercritical fluid extraction combined with two fractionation methods, sorption and use of a series of separators, was applied on *Eucalyptus globulus* leaves in order to maximize the insecticidal activity of extracts against *Leptinotarasa decemlineata* larvae (Topiar, 2015). The yields and composition of eucalyptus extracts and essential oil were compared. The major compounds in the essential oil isolated with the yield 26.7 mg g<sup>-1</sup> were 1,8-cineole (42.6 wt%), alpha-pinene (10.7 wt%) and globulol (5.9 wt%). The concentration of total volatiles in the extracts varied from 2.8 to 59.5 wt%, depending on the extraction and fractionation conditions. The extract obtained at 50



°C and 9 MPa exhibited the highest acute toxicity with  $LD_{50} = 38 \mu\text{g}$ .  $\text{ScCO}_2$  extracts exhibited several times stronger insecticidal activity in terms of chronic toxicity, antifeedant activity and growth inhibition than essential oil. Increase in the concentration of volatiles in isolates resulted into increase of chronic toxicity and antifeedant activity while growth inhibition decreased.

This research was funded in 2011-2014 within the framework of the project TA01010578, which was focused on the development of botanical pesticides.

#### **1.4. Mathematical modeling of supercritical fluid extraction from plants and microalgae**

Mathematical models for extraction kinetics were used for better understanding the process of supercritical fluid extraction of different natural products. Thus, the generally observed decreased proportion of most volatile terpenes in  $\text{CO}_2$  extracts compared to essential oils from steam distillation or hydrodistillation was explained by escape of their vapours in the stream of gaseous  $\text{CO}_2$ , and the phenomenon was described quantitatively (Sovová, 2015a). Another phenomenon, an early decrease of initially intensive action of polar co-solvent, which was added to  $\text{CO}_2$  in order to dissolve polar substances and thus enhance their extraction rate, was explained as a switch from one state of solvent,  $\text{CO}_2$ -expanded liquid, to another one, supercritical fluid. Initially, the extract was dissolved in liquid co-solvent expanded with supercritical  $\text{CO}_2$ . When the co-solvent completely evaporated into supercritical  $\text{CO}_2$ , the solvating power of the solvent was rapidly reduced (Zachová, 2018). Changing between the  $\text{CO}_2$ -expanded liquid and modified supercritical  $\text{CO}_2$  opens space for new possibilities of natural products extraction.

A series of studies on mathematical modelling of changes of extract composition in the course of supercritical fluid extraction was carried out together with dr. R.P. Stateva from the Bulgarian Academy of Sciences, Institute of Chemical Engineering, who is a specialist in thermodynamic modelling of high pressure equilibria. The procedure was as follows. Experimental data on extract composition development was pre-processed with a simple model for extraction kinetics to obtain approximate equilibrium composition at several stages of extraction. The equilibrium composition served to adjust parameters of thermodynamic model, and the results of thermodynamic modelling were by return implemented into the model for extraction kinetics which could then yield a complete simulation of extract composition development in dependence on extraction conditions. The modelling was first tested on the data on extraction of cuticular waxes (Sovová, 2015b) and then applied to the extraction of bioactive furanocoumarins from *Ruta graveolens* (Sovová, 2017a), where essential oil and cuticular waxes are co-extracted. Next, modelling of supercritical fluid extraction of carotenoids from microalgae enabled us to prove that their solubility in the solvent and thus the extraction rate and economy of this process depend on the proportion of carotenoids to co-extracted oil in microalgae, and on the adsorption capacity of microalgae matrix (Sovová, 2019). The effect of oil adsorption on the rate of its extraction from microalgae was examined previously in cooperation with colleagues from the Lisbon University who carried out extensive experimental studies on supercritical fluid extraction from microalgae (Sovová, 2016a).

As a model for supercritical fluid extraction published by us already in 1994 is frequently used by other researchers, mainly because its approximate expression for time dependence of extraction yield enables them to avoid solving differential

equations, it was felt necessary to warn against its misuse and show what are its limitations (Sovová, 2017b).

A chapter on modelling supercritical fluid extraction was written for a monography (Sovová, 2016b), and the results of modelling were presented at international conferences (5) and national conferences (1).

### 1.5. Practically oriented research for companies

The GSCT collaborated with industry to perform custom extractions and optimize extraction conditions for various plant materials. For company Kanebos, s.r.o./xMed21 s.r.o., the conditions for supercritical extraction of cannabinoids from technical hemp were optimized. The result of this collaboration is a technology that is summarized in a confidential research report. For the company Algamo s.r.o., algae extract was fractionated to concentrate astaxanthin. The GSCT has optimized the extraction conditions for pink pepper, coral mushroom, and sea buckthorn berries with supercritical carbon dioxide for company Trumf Internationals.r.o., which owns a supercritical extraction industrial plant.

Results: 10 papers, 18 conference presentations, 2 bachelor's thesis, 1 chapter in book, 1 proven technology, 1 function sample.

*Kosovic E. et al., Sci Rep, 10 (1), 5564 (2020, J<sub>imp</sub>). Machalová Z. et al., Ind. Crops and Prod., 67, 310-317 (2015, J<sub>imp</sub>). Topiar, M. et al., J. Supercrit. Fluids, 97, 202-210 (2015). Sovová H. et al., J. Supercrit. Fluids, 105, 29-34 (2015a, J<sub>imp</sub>). Zachová Z. et al. J. Supercrit. Fluids. 142, 38-44 (2018, J<sub>imp</sub>). Sovová H. et al., Ind. and Eng Chem Res., 54(17), 4861-4870 (2015b, J<sub>imp</sub>). Sovová H. et al., J. Supercrit. Fluids, 120 (Part 1), 102-112 (2017a, J<sub>imp</sub>). Sovová H. et al., J. Supercrit. Fluids, 148(JUN 15), 93-103 (2019, J<sub>imp</sub>). Sovová H. et al., Materials., 9(6), 423-441 (2016a, J<sub>imp</sub>). Sovová H. et al., J. Supercrit. Fluids., 129(SI), 3-8 (2017b, J<sub>imp</sub>). Sovová H.: Mathematical Modeling of Supercritical Fluid Extraction. In: Shi, J. ed., Functional Food Ingredients and Nutraceuticals: Processing Technologies. 2nd ed. Boca Raton: Taylor & Francis Group, pp. 105-125. Functional Foods and Nutraceuticals Series. ISBN 978-1-4822-4064-1 (2016b, chapter in a book). Topiar, M. et al., J. Verified technology for the preparation of plant isolates rich in stilbenes with using of multi-functional extraction unit enabling processing of plant wastes from the wine industry (2019, proven technology). Topiar, M. et al., Multipurpose Extraction Unit for the Processing of Waste from Wine Industry (2018, function sample).*

## Topic 2: Extract processing

### 2.1. Supercritical preparation chromatography

Extraction techniques used for obtaining plant isolates are mostly not selective and thus the isolates contain a complex mixture of many chemical compounds. Supercritical preparative chromatography (SFS), a high-pressure chromatographic method where the sorbent forms the stationary phase and the supercritical carbon dioxide (scCO<sub>2</sub>) forms the mobile phase, was used to fractionate extracts in order to increase the concentration of valuable components or to remove undesirable components from the extract. Besides tailoring the properties of scCO<sub>2</sub> by pressure and temperature, the advantage of this mobile phase is that the fractions obtained by the SFS contain no traces of organic solvents. This technology has been applied for the fractionation of turmerons from turmeric extracts and cannabinoids from hemp extract.

### **Fractionation of turmerones from turmeric rhizomes**

The use of supercritical fractionation on sorbents was investigated as a method for the fractionation of turmerones from turmeric extract in doctoral thesis of M. Topiar (Topiar, 2012-2019). Turmerones are in the interest of many types of research lately, because of their possible positive effects in the treatment of neurodegenerative diseases. For the fractionation experiments, SFE extract obtained after the optimization of extraction conditions was used. The influence of sorbent type (silica gel, aluminum oxide, activated carbon), extract-to-sorbent ratio (1:6.25 – 1:50 v/w), temperature (40 – 80 °C), and pressure (9 – 66 MPa) on the fractionation efficiency evaluated in terms of concentration of active compounds in the fractions, total eluate yield, or duration of the fractionation process was monitored. The target compounds were predominantly terpenes, namely turmerone, ar-turmerone and curlone. After the optimization of SFS conditions for the SFE isolate, a combination of SFE and SFS into one technological step was successfully executed. The chemical composition of the fractions was analyzed by GC/MS and GC/FID. The obtained experimental data were evaluated using a mathematical model valid for SFE, SFS and their combination.

In contrast to alumina and activated carbon, the use of silica gel as stationary phase in the SFS resulted in the elution of almost all deposited substances (recovery up to 98 wt. %). Moreover, it was possible to use the silica gel column repeatedly without lowering its separation ability. Increasing the pressure, temperature, and amount of injected extract had a positive effect on the rate of elution of target compounds. The concentration of turmerones in the SFS fraction was up to 40% higher than in the extract. Moreover, the concentrations of ar-curcumen, 2-epi- $\alpha$ -funebrene, and  $\beta$ -sesquiphellandrene increased up to seventeen times in another SFS fraction. The results of biological tests of extracts have shown that the fractions obtained by SFS often have a higher activity than the original SFE isolates.

Our research proved the supercritical fractionation on sorbents is a suitable technique for turmerones fractionation when their concentration in obtained fractions increased several times in comparison with the initial feed. The quality of research was proved by publication of the article in a high-ranked scientific journal from the chemical engineering category (Topiar, 2019), presentation of 5 communications on scientific conferences and defense of M. Topiař thesis.

### **Fractionation of hemp extract**

SCTG dealt with the supercritical chromatography of hemp extract within the partial application project GAMA Nr.840206 (Separation of cannabinoids from industrial cannabis extract by supercritical adsorption) financed by the TACR in the years 2017-2018. The technical hemp extract obtained by liquid extraction and purified by distillation was subjected to fractionation using sorbents in a supercritical CO<sub>2</sub> environment. The experiments took place in laboratory equipment, which was adapted to the needs of the project. To achieve maximum fractionation efficiency, it was necessary to examine in detail the effect of pressure, temperature, type of sorbent used and the ratio of crude extract to sorbent amount on the composition and yield of fractions obtained by supercritical fractionation on sorbents. The efficiency of the fractionation was assessed in terms of recovery (recovery = yield, is the ratio of the amount of component obtained by fractionation to its amount in the original extract) and the concentration of CBD and THC in the obtained fractions and the amount of solvent consumed (indicates the duration of the whole process). A part of the research was also scaling up, in which the identified optimal conditions were tested. Based on

the developed procedure for the separation of cannabinoids from hemp extract, a special laboratory apparatus working with a gram amount was assembled for these purposes, which directly allows the processing of smaller volumes of hemp extracts after connection to a high-pressure pump. A supercritical adsorption device for the separation of cannabinoids has been reported as a utility model (Sajfrtova, 2018). The supercritical adsorption device according to the present invention provides an unconventional way of fractionating cannabinoids from a mixture of substances in cannabis extract using sorbents in combination with supercritical carbon dioxide, which serves instead of solvents. An unquestionable advantage over conventional chromatographic techniques is that the CO<sub>2</sub> is transferred from supercritical to gaseous state at the end of experiment. The result is a concentrated fraction of cannabinoids without any traces of solvents that would be necessary to evaporate. The device can be used to separate different mixtures of substances using supercritical CO<sub>2</sub>.

The efficiency of cannabinoid fractionation is shown in the Table, which compares yields and concentrations of CBD and THC in the original extract and in the fractions obtained. Fractions containing less than 80% CBD were viscous, while crystalline at higher concentrations.

Table: Composition of raw extract and individual fractions collected during supercritical fractionation on sorbents under optimal conditions and their recovery

Extract	Concentration of the component in the extract, wt. %		Component yield (based on original extract), wt. %	
	CBD	THC	CBD	THC
<b>Original extract</b>	<b>42.7</b>	<b>1.1</b>	<b>100</b>	<b>100</b>
1. fraction	11	0.25	1.6	1.4
<b>2. fraction</b>	<b>84</b>	<b>0.08</b>	<b>77</b>	<b>4</b>
3. fraction	64	0.35	20.4	6

## 2.2. Particle design by supercritical antisolvent micronization

Extracts obtained from natural materials are mostly pasty consistency or are liquid. For better market use, it is necessary to dry them in powder form, thereby reducing the storage cost, increasing the concentration and stability of the active ingredients, and the solubility and applicability of the extracts. For this reason, we focused on the microparticle preparation from extracts containing bioactive components (especially polyphenols and adaptogens) using the so-called supercritical anti-solvent process (SAS). This issue is financially supported by the Ministry of Industry and Trade in the framework of the application project FV40252 (Development of device and procedure for formation of microparticles by supercritical antisolvent) solved since 2019 (01/2019 - 12/2022) in cooperation with a small company ASIPO s.r.o. The use of this micronization method prevents the degradation of the active components, their contamination with organic solvents, and the undesired production of excessive particles, which occur when conventional drying methods (spray drying, lyophilization, dry grinding, etc.) are applied.

The sub-objectives of the project are to (a) design and assemble a laboratory micronization apparatus for SAS, (b) evaluate the effect of operating parameters

(pressure, temperature, concentration of dissolved substance, type of injection needle, etc.) on the properties of the particles formed from selected plant extracts, (c) develop effective procedures for the preparation of high quality input extracts for micronization using combination of highly efficient extraction and fractionation techniques, (d) design a pilot-plant device for SAS and (e) apply the most promising micronized extract to the cosmetic product.

In the first year of the project, a primary screening (systematic detection) of source materials and basic technological procedures applied in high-pressure micronization using the supercritical antisolvent (SAS) method was performed with help of available literature data. Based on this screening, a design of the micronization apparatus was compiled, assembled and tested with the help of Mechanical Workshops of ICPF. The developed equipment enables production of high quality and stable powder extracts, which are non-toxic thanks to the green solvent used and therefore safe and harmless to consumers. In the context of the Czech economy, the implementation of the project represents a significant step in the production of natural products of new quality. It provides the production of input raw materials based on purely natural extracts for the cosmetic, food and pharmaceutical industries, through so-called clean technology, which is generally the basis for the development of new technologies worldwide.

### **2.3. Biocatalysis of vegetable oils**

The aim of the project 19-19245S (A study on biocatalysis using supercritical carbon dioxide: enrichment of vegetable oils with essential fatty acids, 2019-2021) supported by the Czech Science Foundation is to develop a new, environmentally benign procedure for the fractionation of oils rich in essential fatty acids, which is based on the application of supercritical CO<sub>2</sub>, regiospecific enzyme, and sorbent. Two main steps of the procedure are: (i) reaction of oil, either hydrolysis or ethanolysis, and (ii) separation of reaction products from reaction mixture by adsorptive fractionation. As the regiospecific enzyme catalyses reaction at the primary *sn*-positions of oil triacylglycerols, the composition of released fatty acids will correspond to their composition at primary positions and the fatty acid composition of partial glycerides will be enriched by fatty acids at the secondary *sn*-position. Two fractions suitable for dietary applications should be obtained, one richer in omega-3 fatty acids and one richer in omega-6 fatty acids.

Hydrolysis of hemp oil and blackcurrant seed oil was carried out in the first year of the project. The evaluation of experimental results depends on the accuracy of chemical analysis, which was made using HPLC with MS detector. As the accuracy and reproducibility of analytical results was not satisfactory, much effort was put into developing a software that enables a better data mining from raw chromatograms. This will be a part of Master's thesis of O. Pleskač. Another research direction within the project was focused on the possibility to predict stereochemical distribution of fatty acids in vegetable oils, which would simplify the choice of oils suitable for regiospecific reactions leading to desired products.

### **2.2. Impregnation**

The SCTG became a member of international COST project CA18224 "Green Chemical Engineering Network towards upscaling sustainable processes" since 2019. As part of this event, the group is working on two projects with titles "Supercritical CO<sub>2</sub> - based upgrading of natural products and agricultural wastes" and "Supercritical extraction and fractionation of bioactive substances from plant and fungal materials and their applications in food and medicine" in collaboration with the University of



Chemistry and Technology Prague. Within this project, the group focuses on the development of ecologically friendly procedures using renewable resources and substituting the conventionally applied organic solvents with those harmless to health and nature. The objective of this project is efficient processing of medicinal plants, fungi and wastes from industrial processing of agricultural crops rich in health-promoting substances to products with high added value and utility using green technologies. Valuable substances with various properties will be obtained from each natural material using supercritical CO<sub>2</sub> extraction, pressurized liquid extraction, microwave-assisted extraction and/or ultrasonic extraction. The combination of various techniques and solvents allows complete and gentle isolation of valuable substances with high yields. The extracts will be subsequently subjected to fractionation, micronization and impregnation to obtain products with high concentration of health-promoting substances that can be widely used in food supplements and cosmetics or applied to bakery products. Extracts with high content of high molecular weight substances will be used for isolation of polysaccharide matrix and development of novel functional polysaccharide films that can increase the added value of conventional wound dressing materials. These films will be impregnated by extracts rich in health-promoting substances using supercritical CO<sub>2</sub> and non-thermal plasma. A broad spectrum of methods and devices will be applied to characterize the properties of obtained products. This project allows to create research teams that join together young and experienced scientists with understanding for science on a green chemistry base. Developed “green” procedures will enable companies producing health and food products to efficiently utilize agro-industrial waste, reduce production impact on the environment and improve product quality.

Results: 1 paper, 9 conference presentations, 1 doctoral thesis, 1 utility model.

*Topiar, M. et al. J. Ind. Eng. Chem. (Amsterdam, Neth.) 77, 223-229 (2019, J<sub>imp</sub>). Sajfrtová M.: Zařízení pro superkritickou adsorpci. (Czech) A Device for Supercritical Adsorption. Pat. No. UV 32450/PUV 2018-35668. Applied: 18.11.28, Patented: 18.12.18 (2018, utility model).*

### **Topic 3: Supercritical crystallization of metal oxides**

Preparation of nanostructured metal oxides by using extraction techniques belongs to unconventional preparation routes. Using assets of supercritical and pressurized fluids the metal oxides possessing significantly improved textural and structural properties can be synthesized than using conventional calcination.

This topic has been solved since 2013, and in 2014-2016 it was supported by the project no. 14-23274S “Unconventional preparation of nanostructured metal oxides by using pressurized and supercritical fluids” from the Czech Science Foundation solved in cooperation with the Technical University of Ostrava (Nanotechnology Centre CNT-coordinator, VSB-TUO) and the Faculty of Mathematics and Physics from Charles University (X-ray group).

The nanostructured metal oxides based on TiO<sub>2</sub> in various macroscopic forms (thin films and blocks/monoliths) were prepared by combination of sol-gel processes (templated, chelating agent-assisted and acid-catalyzed), thermal hydrolysis using titanyl sulphate as Ti source and several unique processing techniques utilizing pressurized (subcritical) and/or supercritical fluids. Besides the processing by pressurized water and/or pressurized /supercritical methanol, also a novel processing

based on injection of modifier(s) (water, methanol, ethanol, acetone and ethanol:water mixture) into supercritical carbon dioxide was investigated and optimized. This newly developed processing by water- and organic solvent modified supercritical carbon dioxide was optimized especially for preparation of thin films and blocks. All investigated processing approaches were optimized in a broad range of temperatures, pressures, volumes of fluids and fluid flow rates in such way to reach crystallization of synthesized metal oxides/mixed metal oxides in all their macroscopic forms (thin films, blocks/monoliths) and removal of used organic precursors (template and solvent(s) used for sol-gel synthesis). The influence of individual types of extraction approaches and experimental conditions on microstructure was examined. Extracted metal oxides were compared with calcined analogues. Their photocatalytic activity and toxicity were assessed.

The research related to preparation of powdery form of metal oxides/mixed metal oxides by processing by pressurized water and/or pressurized /supercritical methanol/ethanol, preparation of calcined thin films for specific XRD studies, ab-initio calculations, most of characterizations, photocatalytic and toxicity testing was realized in laboratories at VSB-TUO. The development and optimization of processing by water- and organic solvent-modified supercritical carbon dioxide and processing by subcritical water for preparation of crystalline TiO<sub>2</sub> thin films and blocks was a research responsibility of the laboratory at ICPF ASCR. Last, but not least the important and inseparable part of the project was the precise characterization of all macroscopic forms of developed nanostructured metal oxides/mixed metal oxides with the special focus on their structure (quantitative phase composition), microstructure, optical, electronic, electrochemical, surface and textural properties. The knowledge of all these properties helped to reveal for most of developed materials the key facts about the relationship 'preparation-property-performance'.

Results: 4 papers, 19 conference presentations, 1 doctoral work - planned defence in 2020.

*Sajfrtová M. et al. J. Supercrit. Fluids 117, 289-296 (2016, J<sub>imp</sub>). Sajfrtová M. et al. J. Supercrit. Fluids 133, 211-217 (2018, J<sub>imp</sub>). Cerhová M. et al. J. Supercrit. Fluids 137, 93-100 (2018, J<sub>imp</sub>). Šubrt, J. et al. Pure and Applied Chem. 89(4), 501-509 (2017, J<sub>imp</sub>).*

#### **Topic 4: Polymer foams**

Within a project led by Dr. J. Kosek from the University of Chemistry and Technology Prague (grant No. GA14-18938S, Czech Science Foundation: Morphology evolution of nano- and micro-cellular polymeric foams, 2014-2016), the research was focused on preparing microcellular polymer foams by rapid depressurization of polymer saturated with supercritical carbon dioxide. This method has been examined in several laboratories over the world with the aim to substitute in the future present technology which uses different environmentally hazardous chemicals. Presently, however, the porosity of CO<sub>2</sub>-foamed polymers is not yet high enough to compare with insulating properties of products on the market.

In this research, cell diameters and bulk porosities were determined from SEM images. Two series of experiments with foaming a hanging polystyrene film were conducted. In the first series, effects of film width, saturation pressure and temperature, and depressurization rate on polymer porosity and cell size were studied. In the interpretation of experimental data, the porosity profile in the film was related to the

cooling by Joule-Thomson effect of expanding gas to polymer glass transition temperature. Using heat balance equations, the moment was determined when the local temperature dropped to the glass transition temperature, polymer was vitrified and bubbles of CO<sub>2</sub> stopped growing (Sovová, 2017).

In the second series, the polymer was impregnated with co-blowing agent prior to its saturation with CO<sub>2</sub>. Two hydrocarbons used as co-blowing agents acted mainly as plasticizers, *n*-pentane and cyclopentane. Both porosity and cell diameters increased with concentration of co-blowing agents, however, after crossing a particular concentration, the cell size remained almost constant. Saturation conditions and concentration of hydrocarbons were optimized to maximize the increase of porosity, keeping the cell size sufficiently small. Impregnation of neat PS with high-pressure liquid CO<sub>2</sub> near room temperature enabled to prepare foams with cell diameters below 10 µm and porosities about 80 %, which has not been reported before. Hence, organic co-blowing agents could be used to increase the porosity of CO<sub>2</sub> blown foams (Nistor, 2017).

Results: 2 papers, 6 conference presentations.



## Research activity and characterisation of the main scientific results

### Functionalised carbosilane dendrimers (CS-DDMs) and branched macromolecules

Our pivotal theme is a rational design of **carbosilane dendrimers and complex macromolecular and supramolecular systems** based on dendritic compounds. The products of our research are finding applications in a number of areas, e.g. in gene therapy, targeted transport of drugs or catalysis.

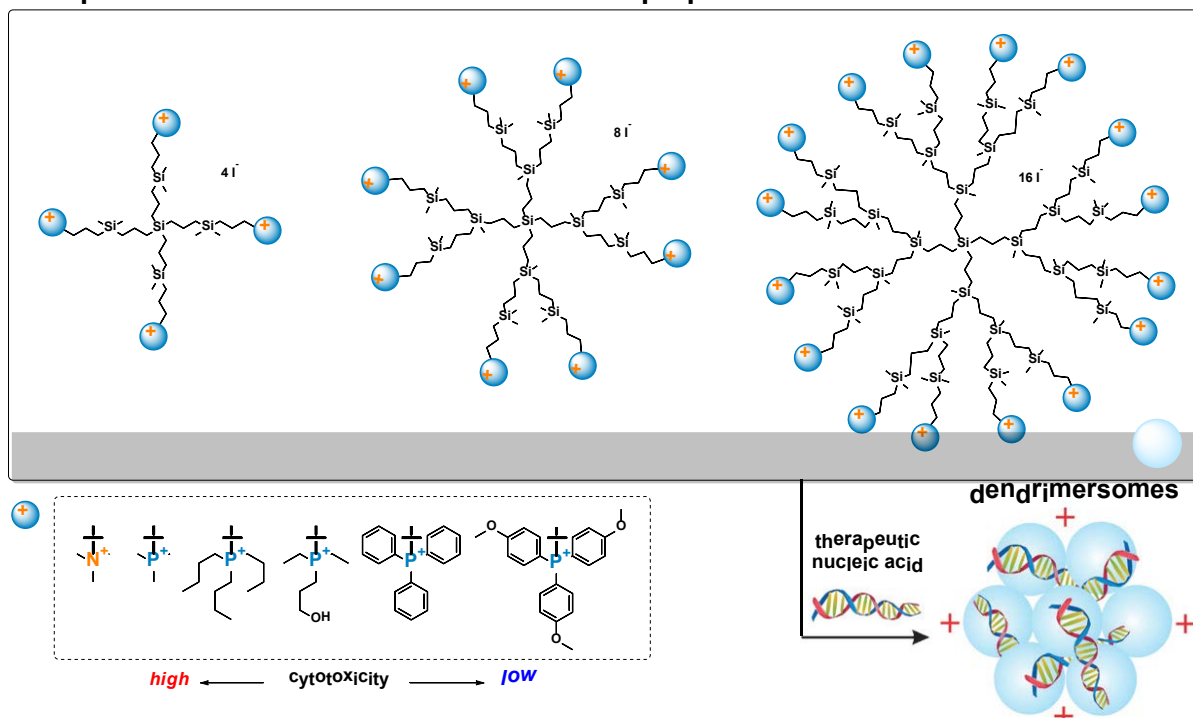
Chemistry of CS-DDMs started to be pursued around 2002 after we had a collaboration offer from Portuguese colleagues (Prof. J. Rodrigues, Chair of the latest IDS Conference and an eminent expert in dendrimer chemistry). We realized that since there had been a long and important tradition of our Institute in organosilicon chemistry, this would allow us to concentrate on so far only marginally investigated class of commercially unavailable carbosilane dendrimers. First, sufficient synthetic know-how had to be obtained (A. Krupková, PhD Thesis – 2009), then we became engaged in development of catalysts based on dendrimers decorated with transition metal complexes (T. Strašák, PhD Thesis - 2012). The work was supported from the Ministry of Education program Centers of Fundamental Research (LC06070). Later we shifted our attention towards organocatalysis and bioapplications. In 2015 we received an unspecified request by our colleague dr. Malý from Jan Evangelista Purkyně University in Ústí nad Labem (UJEP) to prepare some type of cationic nanoparticles for their testing as non-viral vectors for gene therapy targeted research. These rapidly developing concepts bring great expectations in potential treatment of several fatal genetic-based diseases as are cystic fibrosis, haemophilia, or various types of neurodegenerative diseases. Cationic character of the vector is desirable for its efficient interaction with partially anionic nucleic acids. Up to now, most of dendrimer-based gene delivery systems have been surface-modified by nitrogen-containing cationic groups including ammonium cations but toxicity of positively charged amine-based groups is the main obstacle for *in vivo* use of such cationic vectors as gene delivery vehicles. Several different approaches were presented, which address this problem by surface engineering of dendrimers with low-toxic outer shell.

In our group, we proposed a design of completely new class of **generation 1–3 CS-DDMs surface- modified with various types of phosphonium groups**<sup>1</sup>. Optimized three-step synthetic protocol starts from allylic CS-DDMs synthesized according to our well-mastered methodology, and the protocol can be used to prepare ammonium and azolium terminated CS-DDM. Briefly, in the first step, carbosilane dendrimers with 3-chloropropyl terminal groups were synthesized via hydrosilylation of allyl terminated CS-DDMs by (3-chloropropyl)dimethylsilane. The reactions were performed in Schlenk tubes using pentane as solvent and the Karstedt catalyst. After optimization, no side products resulting from undesired  $\alpha$ -hydrosilylation leading to branched alkyl spacers in the structure were detected. The chloropropyl functionality was subsequently transformed to iodopropyl by Finkelstein reaction. The last step of the synthesis consists in the formation of onium salts on the periphery of dendrimers. The quaternization reaction with 3-iodopropyl-terminated dendrimers was carried out in acetonitrile with slight excess of appropriate amine or phosphine. By this way, we prepared phosphonium dendrimers with both aliphatic (-PMe<sub>3</sub>, -PBu<sub>3</sub>, -PEt<sub>2</sub>(CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OH) and aromatic (-P(Ph)<sub>3</sub>, -P(PhOMe)<sub>3</sub>) substituents, and -NMe<sub>3</sub> terminated ammonium CS-DDMs for comparison. After extensive analysis and characterization, the products were subjected to series of biophysical, biological and

medical tests performed mostly by our colleagues at UJEP. Firstly, our assumption of low cytotoxicity of phosphonium dendrimers was confirmed. Significant improvement in comparison with ammonium as well as with other phosphonium dendrimers was observed with  $-P(\text{PhOMe})_3$  type and to lesser extent also with  $-P(\text{Ph})_3$  type dendrimers.  $-P(\text{PhOMe})_3$  dendrimers exhibit very low cytotoxicity across all cell lines or assay methods used and they can be even compared to non-ionic sugar or PEG coated dendrimers (cytotoxicity studies – UJEP).

To understand more deeply the interaction of CS-DDMs with model cell membrane, the interactions of all derivatives of carbosilane dendrimers with dimyristoylphosphatidylcholine (DMPC) or dimyristoylphosphatidylcholine/dimyristoylphosphatidylglycerol (DMPC/DMPG) liposomes were studied. Generally, weak interactions as well as destabilization of the lipid membranes at low concentrations, regardless of liposome type, were observed in the case of  $-N\text{Me}_3$ ,  $-P\text{Me}_3$ ,  $-P\text{Bu}_3$ , and  $-P\text{Et}_2(\text{CH}_2\text{CH}_2\text{CH}_2\text{OH})$  functionalized dendrimers. Dendrimers with  $-P(\text{Ph})_3$  and  $-P(\text{PhOMe})_3$  peripheral functional groups interacted much more strongly and increased the rigidity of liposomes. We suggest that, among other types of dendrimers, the dendrimer bearing  $-P(\text{PhOMe})_3$  groups is a highly promising candidate for the design of a drug delivery system (DDS) due to its positive charge, efficient interaction with lipidic membranes and low cytotoxicity<sup>2</sup>.

#### Examples of cationic carbosilane dendrimers prepared in our team



The following studies showed that the 3rd generation carbosilane dendrimer bearing  $-P\text{Me}_3$  groups on its periphery was able to effectively deliver functional siRNA into cells (B14, *Cricetulus griseus*), release it into the cytosol and finally to achieve up to 40% gene silencing of a targeted gene (glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) with the comparable or, in some cases, even better effectivity than its ammonium analogue ( $-N\text{Me}_3$ ). Moreover, this cationic dendrimer showed relatively low in vivo toxicity compared to its ammonium analogue when analyzed by standard fish embryo test (FET) on *Danio rerio* model, with  $\text{LD}_{50} = 6.26$

$\mu\text{M}$  after 48 h of incubation. This is more than 10-fold improvement compared to published values for various other types of cationic dendrimers<sup>3</sup>.

These studies and feedback from our colleague-biologists (UJEP) gave us valuable impulses for further design and improvement of phosphonium dendrimers as DDSs and currently we continue engineering the outer layer of CS-DDMs. Our synthetic protocol enables the construction of various cationic CS-DDMs where cationic species results from electrophilic attack of alkyl iodide on relevant neutral Lewis bases. For example, we already prepared analogous series of imidazolium and thiazolium CS-DDMs by this way.

As a special type of branched compounds we are interested in, we should mention polyfluorinated tags, designed and developed in our group for the purpose of easy fluorophilization of various compounds. As well as with carbosilane dendrimers, silicon atom is used as a branching point, substituted with three perfluorinated chains and a spacer ending with a reactive group for the attachment to a substrate. Obtained tagged fluorophilic compounds are then tested in applications utilizing fluorous phase, which is orthogonal to both aqueous and organic phases (fluorous biphasic extraction or catalysis). Considering the well-known affinity of aliphatic fluorinated compounds to carbon dioxide, we also test selected fluorinated molecules as components of composite materials for sorption and separation of  $\text{CO}_2$ .

### **Mono- and multivalent (dendrimer supported) carbohydrate compounds**

1. Multivalency is a common principle to increase the affinity and specificity of ligand–receptor interactions in nature and results in a cooperative, over-additive enhancement of binding affinity. Synthetic multivalent carbohydrates, especially well-defined glycodendrimers, represent efficient tool for study of glycobiological processes in living systems, which are important in various processes. Currently, we are the only groups developing **carbosilane glycodendrimers (glyco-CS-DDMs)** as a class of well-defined functional bionanomaterials. We have synthesized the first series of glyco-CS-DDMs containing 4, 8, and 16  $\beta$ -D-glucopyranosyl units at the periphery, by methodology using copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC). As the starting compounds we utilized CS-DDMs with 3-iodopropyl terminal groups. These substrates were developed for synthesis of cationic DDMs (see above). In the first step, halogens were converted to azide functional groups by substitution with sodium azide. The glucose moieties were attached to azide-functionalized DDMs using a CuAAC click reaction ( $\text{CuSO}_4$ , sodium ascorbate)<sup>4</sup>.

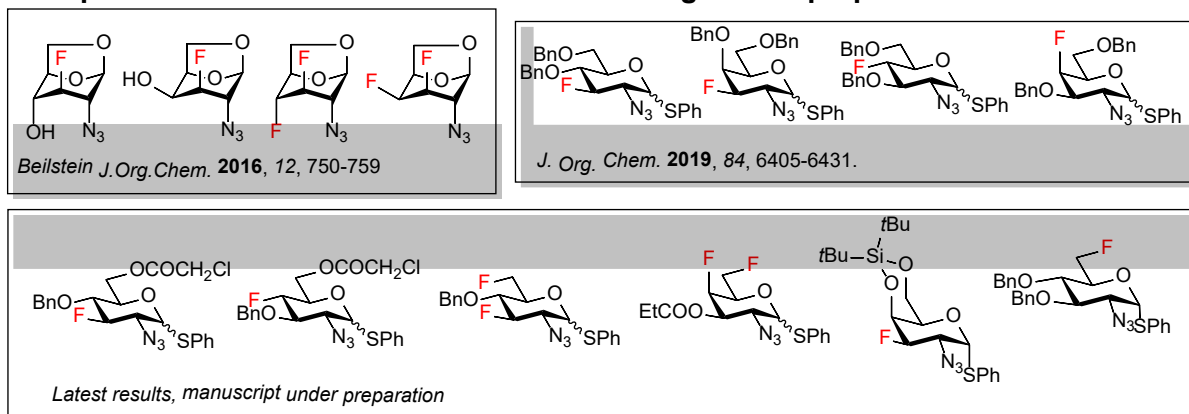
Subsequently, *in vitro* and also *in vivo* cytotoxicity of glycodendrimers<sup>4</sup> were tested and then we tested their interactions with two models of biological structures: lipid membranes (liposomes) and serum protein (human serum albumin, HSA)<sup>5</sup>. These studies were performed primarily by the team of dr. Jan Malý at UJEP and partially by prof. Klajnert-Maculewicz from University of Lodz, Lodz in Poland.

Apart from design and application of glycodendrimers, we also concentrate on two topics concerning design of carbohydrate mimetic and conjugates: fluorinated carbohydrates, and antitumor conjugates of carbohydrates and organometallics.

2. **Fluorinated carbohydrates** are indispensable as research tools, for example in the investigation of carbohydrate-binding proteins, glycosylation stereoselectivity, hydrogen bonding, and carbohydrate antigens. We are particularly interested in development of synthetic procedures for the preparation of glycostructures containing deoxofluorinated glucosamine and galactosamine. We have already developed synthesis of several key monofluorinated monosaccharide building blocks<sup>6</sup>. This synthesis exploits versatile 1,6-anhydrofuranose chemistry. At present, we extend this

methodology to the synthesis of orthogonally protected mono- and difluorinated hexosazide glycosyl donors. We are investigating their use in assembly of fluorinated analogues of naturally-occurring glycans such as T<sub>N</sub> antigen, *N*-acetylglucosamine, *N,N'*-diacetylglucosamine, *N,N'*-diacetylchitobiose, chitooligosaccharides, glycosaminoglycan segments, and their multivalent constructs. This involves detailed study of anomeric stereoselectivity in glycosylations with fluorinated glycosyl donors including mechanistic aspects and stereoselectivity tuning. We have already mapped the stereoselectivity in glycosylation with fluorinated glucosazide and galactosazide donors under preactivation conditions<sup>7</sup>. Using orthogonal acyl and silyl protecting groups, we are currently focusing on the development of  $\beta$ - as well as  $\alpha$ -selective glycosylations. The envisaged synthetic targets will find use as degradation-resistant glycomaterials, probes for <sup>19</sup>F NMR studies of carbohydrate-protein interactions, enzyme and lectin inhibitors, tailor-made immunogens and antigens with finely-tuned properties, as well as model compounds for the study of hydrogen bonding in polysaccharides.

#### Examples of fluorinated monosaccharide building blocks prepared in our team



3. As for **antitumor organometallic-carbohydrates conjugates**, we first concentrated on the synthesis of cytotoxic titanocenes decorated by protected monosaccharide moiety because titanocenes were the first non-platinum organometallic anticancer agents<sup>8</sup>. We have then extended our research effort to carbohydrate- and iminosugar-modified ferrocenes and prepared a series of ferrocene conjugates with cytotoxicities comparable to or better than cisplatin *in vitro*<sup>9</sup>. Ensuing effort focused on the elucidation of biological mechanism of action of our ferrocene- and titanocene-substituted carbohydrates and research in this direction continues in collaboration with research division (RECAMO, Regional Centre of Applied Molecular Oncology) of Masaryk Memorial Cancer Institute in Brno<sup>10</sup>. Electrochemical properties of ferrocenes inspired us to develop a simple and non-expensive electrochemical method for determination of the uptake and distribution of ferrocene anticancer agents<sup>11</sup>.

Our original rationale for conjugation of an organometallic compound to carbohydrates was enhancement of selectivity exploiting highly increased uptake of glucose, which is characteristic for many types of cancer. While we were developing ruthenium arene-glucose conjugates, we discovered that some of these glucose-substituted complexes are potent anti-invasive agents comparable to well-established antimetastatic drug candidates RAPTA T and RAPTA C<sup>12</sup>. This prompted us to start work on the preparation of a series of galectin inhibitors by linking ruthenium arene or ferrocene

complex to known small-molecule galectin inhibitors. Resulting conjugates should feature dual mode of action through combination of antimetastatic activity of galectin inhibitors and anti-invasive or cytotoxic activity of the organometallic complex. Their cell distribution and protein interaction will be conveniently followed by electrochemistry. In addition, we have also embarked on development of ferrocene-platinum-sugar conjugates.

### **Nanocomposites and advanced hybrid materials**

The preparation of **hybrid composite materials** constitutes another area of our research. We are working on several strategies and applications:

1. Besides tailored dendritic compounds we use **layered clays** as constituents of organic/inorganic composites. **We have developed an effective protocol for anchoring of polyionic dendrimers to the layered structure of the silicate. By means of ion exchange, positively charged dendritic macromolecules are immobilized within the matrix due to multiple electrostatic interaction with negatively charged inorganic layers.** This connection facilitates fine optimization of the interface between the inorganic matrix and the organic filler and preparation of unique architectures. We have shown that cationic dendrimers intercalate into the layers of montmorillonite and form stable nanocomposites<sup>13</sup>. As inorganic cations of the natural silicate are substituted by macromolecular organic cations, the layers of the silicate open up. No leaching of the organic component from the composite material was observed both under ambient conditions and at elevated temperature. We focus on the use of these advanced materials for catalysis and gas, especially CO<sub>2</sub>, capture. We performed a comparative studies to evaluate the influence of cationic moieties (ammonium or phosphonium) and dendrimer generation on the characteristics of resulting materials. The target nanocomposites were thoroughly characterized (X-ray, TGA, SEM, EDX, IR). To illustrate the catalytic activity of the prepared materials, selected samples were tested as catalysts in the cycloaddition of CO<sub>2</sub> to epoxides<sup>14</sup>. Ensuing effort summarized in recently submitted paper focused primarily on catalysis (scope of substrates, reaction conditions etc.) and different modes of catalyst recycling. While homogeneous dendritic catalysts could be recycled by means of nanofiltration, composite materials prepared by intercalation of the most active ammonium dendrimers into natural montmorillonite were easily separable by simple centrifugation, while retaining reasonable level of activity. This research was done completely in our department except for characterization of heterogeneous materials which were realized either by other departments of ICPF (IR, TGA, EDX, SEM) or externally (X-ray spectroscopy - UJEP; MAS-NMR - University of Pardubice).

2. In cooperation with colleagues from Chung Medical University in Taiwan, we are engaged in the preparation and study of gas transport through **carbon molecular sieve (CMS)** membranes. CMS membranes are studied as promising and energy-efficient membranes for gas separation due to their high permeability and selectivity, as well as their high thermal and chemical stability under adverse and harsh conditions, especially for separating gas mixtures with quite similar molecular kinetic diameter. In general, CMS membranes are fabricated via controlling carbonization of a polymer precursor, when the operating conditions greatly affects the CMS membranes structure and thus separation performances. In case of supported CMS membranes consisting of a thin and dense top selective carbon layer and a porous supporting layer plays a key role also a support preparation/modification because the properties of selective layer strongly depend on support structure.

In this study a new C/TiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> molecular sieving membrane was proposed for the first time and its separation performance for binary mixtures of gases (H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>, O<sub>2</sub>) was tested. Prepared composite membranes consist of a carrier, a porous  $\alpha$  alumina substrate, modified with TiO<sub>2</sub> interlayer via a sol–gel spin coating method and a very thin carbon selective layer created by a carbonization of polyetherimide precursor. After calcination, the titanium gel provided an interconnected nanonetwork intermediate layer for casting dope to penetrate the support and form an interlocking matrix. By adjusting the hydrolysis-condensation rate using acid catalysts during sol-gel preparation, the intermediate layer structure can be modified, which is beneficial for supporting CMS membrane with high adhesion, but not with an interlocking depth too high to increase mass-transfer resistance. The effect of the modified support properties, such as surface roughness, porosity, and pore size distribution (depended on the sol–gel preparation conditions) on interfacial adhesion between the support and carbon layer and on the gas separation performance of the CMS membrane was investigated. Our results indicated that the TiO<sub>2</sub> intermediate layer controls the interlocking pattern between the carbon layer and porous Al<sub>2</sub>O<sub>3</sub> support. An adequate/slight pore penetration with strong mechanical interlocking can enhance adhesion without sacrificing high permeance. The gas permeabilities in new CMS membranes followed the sequence of  $P_{H_2}$  (2.8 Å) >  $P_{CO_2}$  (3.3 Å) >  $P_{O_2}$  (3.46 Å) >  $P_{N_2}$  (3.64 Å) >  $P_{CH_4}$  (3.8 Å) (the number in parentheses is the kinetic diameter of the penetrating gas), indicating that the CMS selective layer prepared in this study had a pore size distribution close to the kinetic diameter of the smaller gases, and the gas transport phenomenon was dominated by a molecular sieving mechanism. New C/TiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> membranes exhibited an increased hydrogen permeability and a superior permselectivity for H<sub>2</sub>/CH<sub>4</sub> and H<sub>2</sub>/CO<sub>2</sub> (hydrogen permeability up to 601 Barrer, H<sub>2</sub>/CH<sub>4</sub> and H<sub>2</sub>/CO<sub>2</sub> separation factors 726 and 8.6, respectively) compared to the C/Al<sub>2</sub>O<sub>3</sub> membranes (hydrogen permeability 538 Barrer, H<sub>2</sub>/CH<sub>4</sub> and H<sub>2</sub>/CO<sub>2</sub> separation factors 198 and 3.3, respectively). In the Robeson plot often used to membrane performance assessment (the trade-off permeability vs. selectivity) the new C/TiO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> molecular sieving membranes surpassed the upper-bound line for the separation of H<sub>2</sub>/CH<sub>4</sub> and H<sub>2</sub>/CO<sub>2</sub>. Our team contributed about 50 % in this study. The preparation and structural characterization of the membranes was carried out in a laboratory of Taiwanese colleagues who possess the equipment for this purpose. We performed all permeation experiments for studied gases (H<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub> and O<sub>2</sub>) to determination of separation performance of prepared CMS membranes and provide feedback to colleagues for adjustments of the membranes preparation process. The permeation apparatus used in this study was designed and constructed in our laboratory in cooperation with mechanical workshop of institute.

The results of this study we reported in two published papers<sup>15,16</sup>, and one manuscript is in preparation.

### 3. Composite materials for toluene/*n*-heptane separation

The goal of this work was to obtain insight into the relationship between pervaporation performance and a structure of composite membranes with a poly( $\gamma$ -benzyl-L-glutamate) (PBG) separation layer as a perspective material for C<sub>7</sub>-aromatic/aliphatic hydrocarbon separations.

New composite membrane with PBG separation layer on a micro-porous poly(amide-imide) support was prepared and tested as a pervaporation membrane for selective separation of toluene/*n*-heptane mixtures. Composite membrane was formed by coating a solution of PBG in chloroform on the top surface of micro-porous PAI-SO<sub>2</sub>

support. PBG layer fulfilled indispensable requirement for the preferential adsorption of the most penetrating component (toluene) of a separated mixture. The structure of the membrane was studied in detail. X-ray diffraction, electron microscopy and IR spectroscopy measurements were performed. Crystalline regions formed by backbones of macromolecules were low permeable barriers to penetrants, but also limiters for swelling of high permeable polymer phase. The knowledge of the material structure in dependence of conditions of its preparation enabled to adapt the process of the membrane formation to reach the efficiency of separating aromatic/aliphatic compounds. The separation ability (diffusion coefficients, fluxes and separation factors) was performed by pervaporation measurements for different toluene concentrations in binary mixtures toluene/*n*-heptane. The composite membrane was substantially more selective (~3 times) than homogeneous PBG film. Its permeability exceeds significantly the permeability of PBG homogeneous film as a result of reducing the thickness of the diffusion layer. The effect of increasing selectivity while increasing the permeability of the transition from nonporous self-supporting film (diffusion membrane) to a composite membrane with the same polymer in the diffusion layer could be explain by a formation of a thin diffusion layers oriented supramolecular structure of the polymer. Further it was shown that conformation of  $\alpha$ -helix PBG molecules in the coating layer of the composite membrane have not changed after the pervaporation. The membrane retains its integrity and can be used in subsequent cycles of a process. Pervaporation study through new PBG/PAI membranes for model system toluene/*n*-heptane showed the PBG layer of a supramolecular structure with crystalline regions is very efficient material for C7- aromatic/aliphatic hydrocarbon separations due to its high selectivity and stability.

Our team contributed to about 50 % of this work. Our share is to conduct all pervaporation experiments with new composite membranes for separating toluene/*n*-heptane mixtures of different compositions using two measurement techniques (vacuum and sweeping gas arrangements) and evaluation of membranes' performance (mass fluxes, diffusion coefficients, separation factors). Also the pervaporation apparatus was designed and manufactured in our institute<sup>17</sup>.

### **Associated research and development for purification and characterization of products**

1. The main difference between dendrimers and hyperbranched polymers, which are prepared much more easily, is well defined structure of the former in contrast with random layout of the latter. To fully exploit this advantage of dendrimers, precise analytical tools are needed to confirm the accordance between the targeted and actually obtained structure. Within the class of polyionic dendrimers prepared in our group for both biomedical and catalytic applications **high-resolution mass spectrometry with electrospray ionization (ESI MS)** proved in our hands to be a very valuable tool for structure verification. By thorough analysis of ESI MS spectra we can assess the dendritic purity and disclose the amount and type of defects arising not only from the last synthetic step, but from the whole synthetic pathway, which is very helpful for its optimization. Thus, we are able to prepare high purity materials yielding reproducible results in subsequent applications<sup>18</sup>.

In order to further increase the purity of prepared dendrimers we employed **nanofiltration and developed purification protocols utilizing commercially available membranes and equipment**. In contrast to dialysis, which has already received some attention from dendrimer community but (as a diffusion-driven process) it is very time and solvent consuming, nanofiltration allows for highly effective

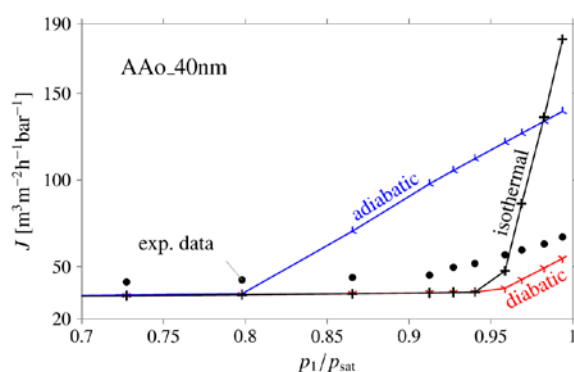


separation of target dendrimers and low MW impurities. Thus, we can employ large excess of reagents to drive all synthetic steps to completion, which also contributes to generating products with low amount of structural defects. We also tested the applicability of nanofiltration for the recycling of dendrimer-based organocatalysts. The results are very promising in terms of purity and quantity of both the dendritic catalyst and the target product.

2. We are also concerned with the **effect of condensation conditions in porous media**. Gas capillary condensation in small pores can significantly affect the efficiency of gas separation through a porous membrane. It occurs under pressure conditions close to the partial pressure of the condensable gas, usually before completion of the multilayer adsorption. However, this mechanism is not fully understood and described. We have dealt with condensation of gaseous substances when passing through a multilayer porous medium theoretically and experimentally for more than twenty years. This process is interesting not only from the theoretical point of view, but it can also be of practical use. The direction of gas transport through the multilayer material can significantly influence its size. We first published this effect in *Journal of Membrane Science* in 1994. We cooperate on this topic with Dr. Thomas Loimer from Technical University Vienna. During this research, we designed, and in cooperation with mechanical workshops constructed a number of measuring devices.

In recent work<sup>19</sup>, the transport of isobutane vapors in anodic membranes of uniform size of pore was studied. Experimental results were compared with the predicted data of three different models. On the basis of supposed various simplifying conditions of transport in the area of condensation there were designed three models. These were (i) a well-known isothermal description and two non-isothermal descriptions for which the energy balance is taken into account and (ii) an adiabatic or (iii) a diabatic boundary condition was applied at the downstream front of the membrane. A comparison with experimental data indicated that a description with heat flux towards the downstream front of the membrane could agree best with data. However, the conclusion was that a determination of the radius of curvature of the meniscus within the membrane from permeance data alone is uncertain. Accurate temperature measurements at the upstream and downstream fronts of the membrane would increase the reliability of the determination of the radius of curvature of the meniscus within the membrane. Although the data comparison showed that none of models can accurately describe the experiments, the obtained model results are very beneficial because they relatively well predict the pressure conditions at which the isobutane flow through the membrane decreases or increases.

#### Permeation fluxes of isobutane vapor through anodic multilayer membrane obtained experimentally and from used models





Our team contributed to about 60 % of this research. All experimental work, included the construction and testing of apparatus, all experiments, evaluation, was carried out in laboratory of our team at Institute of Chemical Process Fundamental. Obtained experimental data were then described using the transport mechanism models proposed by our colleague Dr. Loimer from Technical University in Vienna.

Our results dealing with investigation of capillary condensation in porous media obtained during evaluated period we published in two works<sup>19,20</sup>.

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