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DETAILED RESEARCH REPORT

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Contents

Statutory Research

Catalytic Materials and Processes for Sustainable Development

The Pd-Ir catalysts of different Pd/Ir proportions; influence of Ir content on the hydrogenation of furfural

(prof. Alicja Drelinkiewicz, Robert Kosydar PhD, Erwin. Lalik PhD, Aleksandra Pacuła DSc)

The aim of studies was to determine activity of bimetallic PdIr/C catalysts of different Pd/Ir proportion for the hydrogenation of bio-reagent, furfural (FU).

The research included the preparation of catalysts by a reverse water-in-oil microemulsion (w/o) method (Triton X 114, cycloheksane, $NaBH_4$ reducing agent) supported on the carbon black Vulcan XC72 (228 m^2/g, 1.77 cm^3/g). The catalysts consisting of the 2wt%Pd and growing Ir content in range 5 - 50 mol% Ir ($Pd_{95}Ir_5$ - $Pd_{50}Ir_{50}$) using 0.02 M aqueous solution of $PdCl_2$ i $IrCl_3$. The monometallic 2%Pd/C and 2%Ir/C catalysts were also prepared by microemulsion method. The catalysts were characterized by BET, XRD, XPS, SEM, HRTEM techniques. The surface fraction of Ir and Pd in the PdIr particles were evaluated by CV (0.5 M H_2SO_4) measurements.

Results. The nearly monodisperse metallic particles of size within narrow range 3-6 nm (SEM, TEM) and similar average size ca. 4 nm (XRD, TEM) were observed regardless of the bulk Ir content. The average size of the PdIr particles was close to that of metallic Ir (3.8 nm) but below that of Pd (6.2 nm). The preservation of fcc crystalline structure assisted by a small shift of the XRD reflexes to higher angles proved the Pd-Ir alloyed structures. The EDS and STEM techniques showed the particles nominal compositions, being in accordance with the intended ones. At a moderate Ir content 15 - 35 mol%, the surface Pd/Ir atomic ratio determined by XPS and CV was higher than the corresponding bulk value (Table) indicating segregation of Pd and Ir resulting in the Pd-enriched surface. With growing bulk Ir content, the surface Ir fraction slowly increased. The surface of $Pd_{95}Ir_5$ and $Pd_{91}Ir_9$ with the lowest Ir content was enriched in Ir. An electron modification of Pd in the PdIr was observed by XPS (Pd binding energy shift) and weaker strength of hydrogen binding observed by CV (lowering of the hydrogen desorption potential) and lower heat of sorption (Q kJ/mol H_2) (Table).

In the hydrogenation of bio-reagent, furfural (2-propanol, 35^{0}C, 6 bar) the activity of PdIr was determined by the Ir content. The PdIr/C catalysts with Pd-enriched surface of the metal particles were more active than Pd and the most active (2-times more active) was PdIr-0.26/C with the surface Pd/Ir = 5.7 and the weakest heat of hydrogen sorption. Over all the PdIr/C catalysts the selectivity to furfuryl alcohol (FAL) was superior to that on Pd, the highest (81 %) was displayed by the PdIr-0.26/C. The hydrogenation of the ring in the FAL yielding THFAL proceeded competitively with the reaction of furfural with 2-propanol generating isopropyl acetal (AC), the additive to diesel fuels. The selectivity to AC correlated with the contribution of oxidized Ir species (XPS) in the PdIr surface, which was especially high in the 2%Ir/C and PdIr-0.26/C, PdIr-0.5/C prepared using precursor solution of different concentrations 0.04 and 0.2M, respectively.

The obtained results could be utilized to design the PdIr catalysts of high activity and selectivity to unsaturated alcohol and furfural diisopropyl acetal, both technologically important reagents.

Table Furfural conversion (C) and selectivity (S) to FAL, THFAL i AC (%)

Catalyst	Q kJ/mol H_2	Pd/Ir bulk.	Pd/Ir$_S$ surface.	C (FU)	S (FAL)	S (THFA)	S (AC)
Pd/C	23.5			47.4	45.5	5.7	25.8
PdIr-0.05		19	6.7	40.8	77.2	6.4	11.8
PdIr-0.09	15.9	10	6.7	35.6	76.4	6.5	14
PdIr-0.15	13.4	5.7	8.1	68	80.9	8.9	5.2
PdIr-0.26	6.9	2.9	5.7	89	81.8	17.9	1.5
PdIr-0.35	9.4	1.9	5.3	52.6	71.5	9.7	12
Ir/C				18			100
PdIr-0.26 0.04		2.9	4.9	23	38.7	2.2	50
PdIr-0.5 0.2		1.0	4.5	21	62.7	3.9	38.7

Q - the heat of hydrogen sorption determined by calorimetric measurements

New peroxo and polyoxo compounds of Mo (VI), W (VI) and V (V). Synthesis, structural studies and applications in oxidation processes.

(prof. Wiesław. Łasocha, Łukasz Kuterasiński PhD, Adrianna Sławińska MSc)

The conducted research concerned on the synthesis and structural studies of new polyoxomolybdates; reaction products of molybdic acid and o-phenylenediamine derivatives. The most important results of the conducted research can be presented as:

1) A number of syntheses were carried out using methyl-o-phenylenediamine, 4-halogeno-o-phenylenediamine and dimethyl-o-phenylenediamine. Syntheses under reflux condenser and using autoclaves were performed. Tests were carried out for solutions acidified with hydrochloric or acetic acid. The obtained solid phases were tested using powder diffraction and elemental analysis methods.

2) It was found that typical hydrothermal syntheses result in poorly crystalline mixtures of phases. Reaction conditions require refinement and optimization.

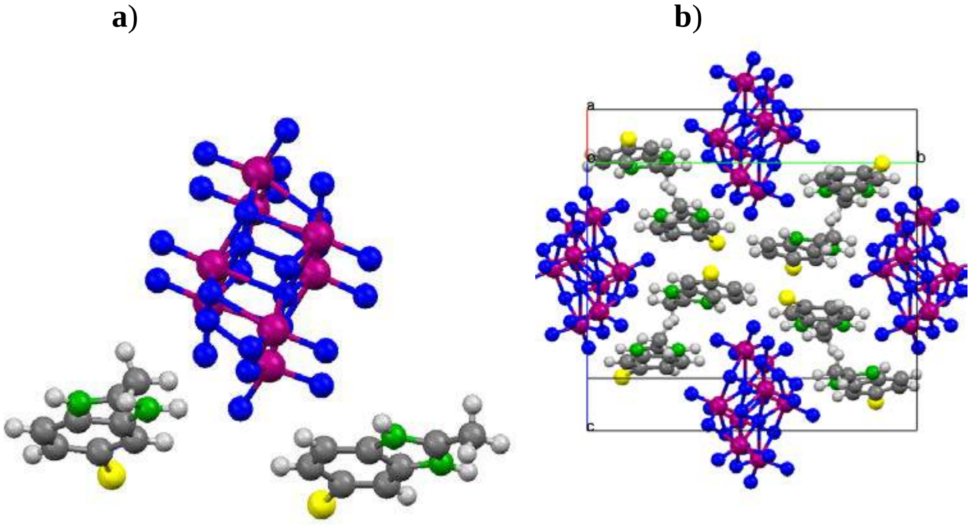
3) Short synthesis (under reflux) with HCl acidification, resulting in diaminoaniline octamolybdates. Prolonged syntheses using acetic acid leads to the formation of imidiazole polyoxomolybdates (reaction products of o-phenylenediamine derivatives and acetic acid).

4) The crystal structure of 6-chloro-2-methyl-benzimidazolium octamolybdate was refined (**1**). The crystallochemical data and drawings are presented below. Studies indicate that a similar compound was obtained by reaction with 4-bromo-o-phenylenediamine (**2**).

5) A number of attempts have been made to synthesize new polymolybdates of aniline and its derivatives. The solids obtained are at the stage of powder diffraction and physicochemical tests.

6) For selected compounds, tests of catalytic oxidation of cyclic hydrocarbons (IKiFP PAN) and epoxidation of cyclooctene were conducted (University of Aveiro, Portugal). Selected preparations are tested in IF PAS as part of pharmacological investigations.

The figure below shows the asymmetric unit (a), and a projection of the structure (b) of monoclinic β-octamolybdate (VI) tetra(6-chloro-2-methyl-benzimidazolium) (1), Spheres with colors: magenta, gray, blue, yellow and green means Mo, C, O, Cl and N, small light gray balls mean H.



Crystallochemical data:

(1) Chemical formula C32 H32 Cl4 Mo8 N8 O26. SG $P2_1/c$, λ= 0.71073 , a,b,c,β, 10.6378(2), 17.2379(2) , 13.9937(2) Å, 104.192(2), V = 2487.75 Å^3, Z=2

(**2**) Chemical formula C32 H32 Br4 Mo8 N8 O26. SG $P2_1/c$, λ= 1.54178, a,b,c,β, 10.788(2), 17.355(3), 14.098(5), 104.91(2) . V=2550.9, Å^3, Z=2

Studies of structural effects and influence of suport on the catalytic activity of copper-based systems

(assoc prof. Dorota Rutkowska-Żbik, prof. Małgorzata Witko, prof. Mirosław Derewiński, assoc prof. Renata Tokarz-Sobieraj, assoc prof. Anna Micek-Ilnicka, Michał Śliwa PhD, Katarzyna Samson PhD, Małgorzata Ruggiero-Mikołajczyk PhD, Łukasz Kuterasiński PhD, Mariusz Gackowski PhD, Urszula Filek PhD, Małgorzata Zimowska PhD, Wojciech Rojek MSc, Jerzy Podobiński MSc, Natalia Ogrodowicz MSc, Joanna Kaim MSc, Małgorzata Smoliło MSc)

The aim of our research task is to define how the structure of copper-based systems modulates their catalytic activity in model gas-phase reactions. As the first step, one had to establish the methodology to quantitatively measure the amount and type of different forms of copper (Cu^+, Cu^{2+}) exposed to reagents: oxide-type copper, Cu_{oxide} (in which Cu ions are neutralized by oxygen anions) or as so called "exchanged" ions in zeolites, $Cu_{exch.}$ (which neutralize AlO_4^- tetrahedra).

The studies were done for the systems in which 1, 2, and 5 wt.% of Cu were introduced by impregnation into FAU zeolite in its protonic (HFAU) and sodium (NaFAU) forms. The physico-chemical properties of the samples were characterized with the following techniques: XRD, low temperature nitrogen sorption, H_2-TPR, NMR, IR, SEM. IR spectroscopy with CO (for the detection of Cu^+) and NO (for the detection of Cu^+ and Cu^{2+}) as probe molecules was chosen as the main tool to investigate the status and properties of Cu at room temperature and at -100°C. It was found that in the prepared zeolite samples there are four types of copper centres: $Cu^+_{exch.}$ as exchanged ions, Cu^+_{oxide} in the oxide form, $Cu^{2+}_{exch.}$ and Cu^{2+}_{oxide}. The proportion between various forms of Cu depends on both the impregnation procedure and the amount of Cu introduced into the zeolite.

In the CuHFAU sample containing 1 wt.% of Cu, all of the Cu species were in the form of $Cu^+_{exch.}$. The quantitative IR studies of CO sorption evidenced that half of $Cu^+_{exch.}$ was inside supercages which were accessible to reactant molecules, and half inside cuboctahedra and hexagonal prisms of the FAU structure. The ions located in the latter positions are inaccessible to adsorbates. The increase of Cu content up to 2 and 5 wt%. caused an increase of the $Cu^+_{exch.}$ amount (peak at 2158 cm^{-1}) and an appearance of oxide type forms Cu^+_{oxide} (peak at 2130 cm^{-1}) as well as Cu^{2+}_{oxide} (peak Cu^{2+}_{oxide}-NO 1860 cm^{-1}). Furthermore, in the samples having higher amounts of copper, the $Cu^{2+}_{exch.}$ was also observed in the „square planar" form, as coordinated by the lattice oxygen atoms. The amount of Cu^{2+} increases in line with the amount of copper introduced into the samples. Two kinds of Cu^+_{oxide} are present with various electrodonor properties (peaks at 2120 and 2130 cm^{-1}). The Cu_xNaFAU series demonstrated much lower concentration of $Cu^+_{exch.}$ ions than is found in the Cu_xHFAU series at the same copper loading. The concentration of Cu^+_{oxide} and Cu^{2+} (both as $Cu^+_{exch.}$ and CuO) is higher, what should probably be related to the lower dispersion of copper phase. In the next step, an attempt was made to check which of the present Cu forms are responsible for the catalytic activity of the samples the model reaction, modelled as their reduction in the flow of hydrogen. To study the process, the examined systems were reduced with hydrogen at different temperatures (starting from room temperature up to 500°C) while the copper status was monitored with IR spectroscopy with NO and CO as probe molecules. The reduction of the copper catalysts leads to the generation of additional acid centres, what was further evidenced with IR with NH_3 as a probe molecule. The kinetic studies of reduction of accessible copper centres showed that they are prone to the reduction in the following order: $Cu^+_{oxide} > Cu^{2+}_{exch.} > Cu^{2+}_{oxide} > Cu^+_{exch.}$.

New catalytic materials for „green chemistry" processes

(prof. Ewa Serwicka-Bahranowska, Roman Dula PhD, Dorota Duraczyńska PhD, Robert Karcz PhD, Joanna Kryściak-Czerwenka PhD, Alicja Michalik PhD, Bogna D. Napruszewska MSc, Katarzyna Pamin DSc, Anna Walczyk MSc)

Novel catalytic materials for reactions catalyzed by basic sites were derived from the natural clay mineral sepiolite, by means of alkaline activation. The other type of studied catalysts consisted of metalloporphyrin-based mixed system for liquid phase oxidation of hydrocarbons.

The search for means of efficient valorization of biomass or CO_2 entrapment, contributed to the rapidly growing interest in development of new solid base catalysts and adsorbents. We have chosen sepiolite, a cheap, nontoxic, natural magnesium silicate mineral with ribbon structure and high microporosity, as basis for the catalyst design. A series of solids were obtained by treatment of sepiolite with NaOH at different temperatures and for various periods of time. The materials were characterized with XRD, SEM/TEM, N_2 adsorption/desorption at -196^{o}C, XRF, AAS, XPS, FTIR (MIR, NIR), and ^{29}Si MAS NMR. Surface basicity was determined by titration with benzoic acid, and the solids were tested as catalysts in aldol self condensation of acetone and as CO_2 sorbents. The study led to a major reinterpretation of the existing knowledge on the nature of alkali treated sepiolite. We have shown that activation with NaOH involves a significant desilication of sepiolite, while the loss of Mg from the solid is prevented due to the intracrystalline preciptation of amorphous $Mg(OH)_2$, which dramatically affects the solid composition and texture, and enhances the product basicity. For the first time the Si sites at the centre of structural ribbons have been identified as part of the silicate framework most susceptible to leaching upon alkaline treatment. Although the NaOH treated product assumes, as previously established, the structure of sodium form of sepiolite (loughlinite), it is in fact a composite of loughlinite, whose tetrahedral layers are depleted of central Si sites, and magnesium hydroxide.

Liquid phase oxidation of hydrocarbons with molecular oxygen is a process frequently employed in industry. For this reason new solutions enabling improvement of productivity and/or adherence to the ever stricter environmental regulations are sought after. The present study concerned modification of the catalytic system based on cobalt porphyrin (CoTPP) for oxidation of cyclooctane with molecular oxygen. Alteration consisted in the use of cheap and nontoxic compound, N-hydroxyfthalimid (NHPI), as a co-catalyst. Apart of NHPI, a series of its derivatives, such as 3-F-N-hydroxyfthalimid (3F-NHPI), 4-F-N-hydroxyfthalimid (4F-NHPI), tetra-F-N-hydroxyfthalimid (TF-NHPI) i N-N'-dihydroxypyromellitimid (NNDNHPI), were examined as well. It has been found that the joint use of metalloporphyrin and a NHPI type compound leads to the more efficient transformation of cyclooctane to the desired products (cyclooctanol and cyclooctanone) than in the case of pure metalloporphyrin catalyst.

New cathode materials for high-temperature fuel cells with reduced operating temperatures

(Michał Mosiałek DSc, Małgorzata Zimowska PhD, Dmitry Kharytonau PhD, Prof. Paweł Nowak, Grzegorz Mordarski PhD)

The slow oxygen reduction reaction on a cathode is the main source of energy losses in solid oxide fuel cells (SOFCs). Significant reduction of energy losses may be attained by using materials of mixed (ionic and electronic) conductivity for the cathode construction. Those materials enables preparation of the electrodes with highly developed surface (3D electrodes). An example of such a material is $La_{1-x}Sr_xMnO_{3-\delta}$ (LSM), remaining nowadays the most frequently used cathode material for SOFC. Recently LSM has been also studied as an electrode material for the cells operating reversibly as a fuel cell or as an electrolysis cell, which are promising for the storage of energy from solar and wind energy sources. The main drawback of LSM is a decrease in cathode efficiency at lower operating temperatures. $La_{1-x}Sr_xCo_yFe_{1-y}O_{3-\delta}$ (LSCF) is the most promising cathode material for the 500−800 °C temperature range. Therefore, we attempted to improve the above-mentioned cathode materials developing composite cathode materials containing $YFe_{0.5}Co_{0.5}O_3$ (YFC) as the second phase.

$YFe_{0.5}Co_{0.5}O_3$ was synthesized by the citric acid method. The solution of iron, yttrium, and cobalt nitrates as well as citric acid was continuously stirred under heating until the gel was obtained. The gel was dried and sintered at 950 and 1100 °C. X-ray diffraction patterns of tested composites revealed the presence of pure perovskite phases in samples sintered at 950 °C and the presence of $Sr_4Fe_4O_{11}$, $YMnO_3$ and $La_{0.775}Sr_{0.225}MnO_{3.047}$ in samples sintered at 1100 °C. Composite cathodes containing YFC and LSM in 5:95 and 50:50 weight ratios (5YFC-95LSM and 50YFC-50LSM), as well as YFC and LSCF in 50:50 weight ratio, were prepared applying the suspension of the composite in the form of thin films on the surface of $Ce_{0.8}Sm_{0.2}O_{1.9}$ disks. The properties of such obtained new materials were compared with the properties of LSM and LSCF, respectively.

Electrochemical impedance spectroscopy measurements revealed the polarization resistance (R_p) of 5YFC-95LSM cathode to be significantly lower than R_p of LSM cathode. The difference is larger and larger with a decrease in the oxygen concentration and with a decrease in the temperature. 50YFC-50LSM cathode after activation by 24 h of polarization at −0.5 V achieved the lowest R_p among all tested cathodes in the 750−800 °C temperature range. No improvement of the working parameters of the LSCF cathodes was achieved. Composite cathodes containing YFC and LSCF exhibited R_p similar to R_p of LSCF cathodes at 800 °C whereas its R_p was much greater at lower temperatures.

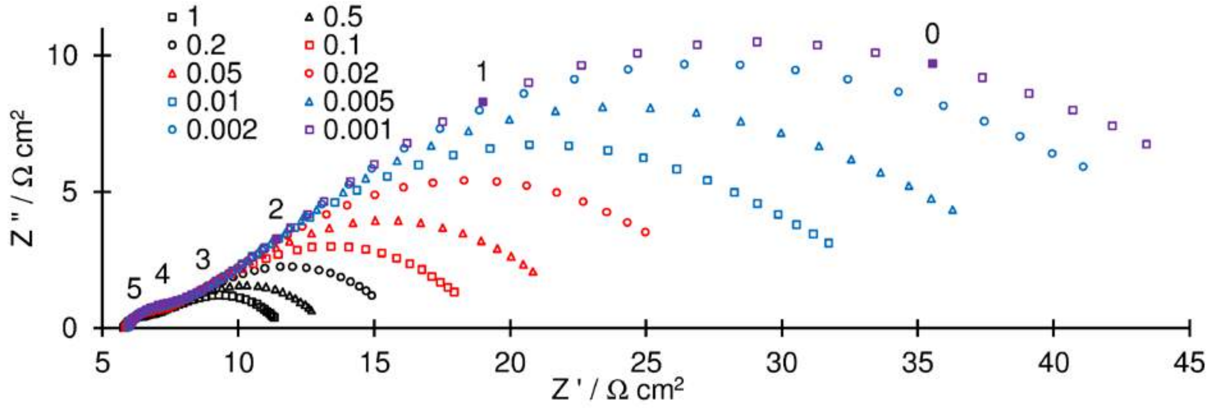


Fig. 1. Examples of EIS spectra in the Nyquist representation of (a) 50YFC-50 LSM electrode at 800 °C in oxygen and mixtures of argon and oxygen before activation by polarization, $P(O_2)/P_{atm}$ is given in the legend. The numbers placed above filled experimental points denote the logarithm of the frequency

Enzymatic processes – basic and applied research

(prof. Tomasz Borowski, prof. Maciej Szaleniec, Maciej Guzik PhD, Agnieszka Wojtkiewicz PhD, Mateusz Tataruch PhD)

a) *biotechnological modification of enzymes with the His_6 tag*
Oligonucleotides were designed to clone the R-PEDH gene into a bacterial vector capable of expressing that gene in the Escherichia coli system. Work is underway to optimize the expression of a protein modified with a histidine tag.

b) *studies of compounds based on polyhydroxyalkanoate monomers ((R)-3-hydroxy acids or their derivatives) as potential substrates for these enzymes*
A number of enzymatic tests were carried out to optimize the process of changing the absolute configuration of PHA monomers from R to S. Biocatalysts in the reactions were two dehydrogenases: S-PEDH and R-PEDH. The reaction for obtaining pure levorotatory enantiomers took place in two stages. The first of these was NAD^+ dependent oxidation of R-3-hydroxy acid methyl esters to 3-oxo acid esters using S-PEDH. The oxidation reaction of PHA monomers took place in TRIS buffer (pH 8) with the addition of NAD^+ and S-PEDH (Batch I). Substrate monomers were dissolved in acetone, which was also necessary for reconstitution of the NAD^+ cofactor. In the next step, the oxo-compounds were reduced using the R-PEDH enzyme to their S-enantiomers. The reduction of the 3-oxo acids obtained was carried out in K_2HPO_4 / KH_2PO_4 buffer (pH 5.8) with the addition of NADH and R-PEDH. The reactions were initiated by the addition of substrates in isopropanol, which was used to restore NADH (Batch II). The course of the reaction was monitored using a UV-Vis spectrometer.

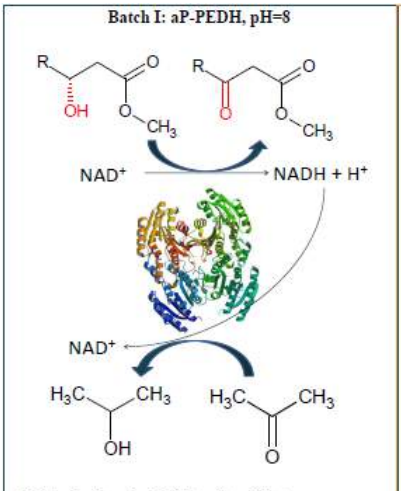


Fig 3. A schematic synthesis of 3-keto-acids methyl esters.

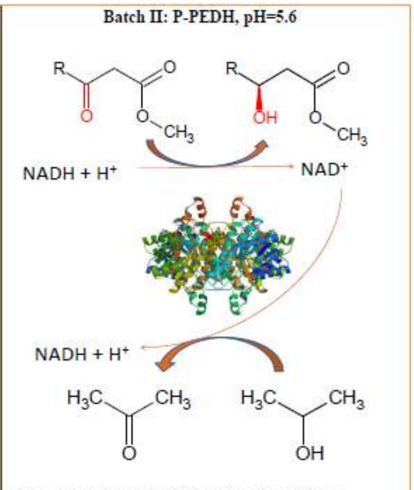


Fig 4. A schematic synthesis of (3S)-3-hydroxyacids methyl esters

c) computational studies - molecular docking of potential substrates to the PEDH enzyme. Docking into the active form of the enzyme was carried out using the AutoDock4.2 program using a rigid enzyme model and flexible ligand.

Physics and Chemistry of Surfaces and Nanostructures – Experiment and Theory

Structural, electronic and dynamics properties of surface and nanostructures studied with microscopic and spectroscopic techniques under ultra-high vacuum conditions.

(prof. Józef Korecki, assoc. prof. Nika Spiridis, Jacek Gurgul MSc, Kinga Freindl PhD, Ewa Madej PhD, Ewa Młyńczak PhD, Piotr Mazalski PhD, Robert Socha PhD, Dorota Wilgocka-Ślęzak PhD, Joanna Wojas MSc, Natalia Kwiatek MSc)

Research carried out in the framework of the statutory activity by the "Surface Nanostructures" group concerned metallic and oxide nanostructures, in particular grown by molecular beam epitaxy, with potential spintronic and catalytic applications.

Studies of multilayer magnetic systems containing epitaxial layers the FeRh alloy have been continued , with emphasis on the antiferromagnet (AFM) / ferromagnet (FM) phase transformation that occurs in the FeRh alloy near the room temperature. For Au/FeRh/MgO(001) systems, it was found that a small amount of ferromagnetic phase survives to low temperatures. This residual FM phase, located at the interfacer with MgO, is responsible for the perpendicular magnetization component. On the other hand, for the thinnest Fe layers, a small fraction of the AFM phase is observed above the transition temperature, which is explained by epitaxial strains induced generated in the FeRh / Au interface.

The studies of the magnetic anisotropy in Fe(110)-based systems were also continued and extended to systems exchange-coupled through a non-magnetic layer. In Au/Fe(110) and Co/Au/Fe(110) epitaxial double and triple layers obtained in the process of epitaxial growth on a monocrystalline W (110) substrate, the oscillatory character of the surface magnetic anisotropy as a function of the Au-layer thickness was found, with an additional enhancement of the surface magnetic anisotropy induced by the indirect exchange coupling between the ferromagnetic layers.

The global structure of the interface layer structure plays an important role in shaping the chemical, electron and magnetic properties in metal-oxide epitaxial systems. In this respect, systems based on iron oxides were studied: FeO (wustite), Fe_3O_4 (magnetite) and Fe_2O3 (hematite). Analysis of the chemical structure of the Fe/MgO /FeO trilayers showed the effect of the presence of the MgO layer on the stoichiometry of the FeO layer in comparison to the Fe/FeO system, in which iron is reduced in the interfacial area.

In the field of the model catalysis, the adsorption of CO on the Fe_3O_4(111) magnetite surface with different, precisely controlled terminations was studied under ultra high vacuum conditions, using a comprehensive methodology involving, among others, scanning tunneling microscopy and temperature-programmed desorption.

The second group of tasks related to materials prepared *ex situ* concerned the analysis of the elemental surface composition and electronic states of in various types of catalytic, photovoltaic, and electrochemical systems.

Single crystalline layers of magnetite of different orientations obtained in our laboratory using the technique of reactive molecular beam epitaxy were used to study the interaction between biomolecules and inorganic surfaces.

A significant amount of group efforts was devoted to measurements, calibration and operation of the PEEM/ XAS beam line at the National Synchrotron Radiation Center "Solaris", and above all to making the end-stations available to external users, both as a result of the open calls for the beamtime and as part of the CERIC/ERIC consortium. In 2019, in the framework of three consequtive calls for proposals, fourteen experiments of external users were carried out at the PEEM end station that is fully supported by ICSC PAS and a number of experiments at the XAS end-station, to operation of which ICSC PAS gives a considerable contribution.

Molecules with biological importance in reference to their interaction with a surface and refining their theoretical description

(prof. Tomasz Pańczyk, Wojciech Płaziński DSc, Agnieszka Brzyska PhD)

Molecules with biological importance can be formally divided into two groups, that is molecules which form living organisms or play important role in cells or molecules which come from outside but interact in specific way with living cells. The former group is comprised, among the others, of proteins and saccharides, while the latter of drugs. Compounds belonging to both these groups were accounted for in our investigations.

One of the raised issues was the preliminary research that explored the possibility of applying the machine learning (ML) methods for description of conformational properties of small molecules (consisting up to 25 atoms). The standard procedure of using the ML approach to study the molecular conformation relies on generating a certain amount of data that link molecular structures with the corresponding energies calculated at a high level of accuracy. Usually, for this purpose, a trajectory is generated by using molecular dynamics simulations carried out at the *ab initio* level of accuracy. Due to the low computational efficiency of such simulations, the generated data cover only a scarce part of the total conformational space, which may affect the accuracy of ML potential in poorly sampled areas (e.g. energy barriers). As part of the research, we proposed an alternative procedure, involving the use of data generated at a lower level of accuracy (classical molecular dynamics, carried out at the level of molecular mechanics force fields). In the subsequent stage, the energy of the generated structures is recalculated at a higher level of accuracy (*ab initio*). Such two-step procedure allows for: (i) better sampling of high-energy regions of conformational space or those separated by energy barriers; (ii) the possibility of using the enhanced-sampling methods in order to focus on selected degrees of freedom. Accounting for both these factors may significantly increase the accuracy of ML potential. This stage of the study relied on the model compounds belonging the group of monosaccharides.

Another studied systems were bisazo dyes molecules interacting with doxorubicin - the well known anticancer drug which is however highly cardiotoxic. There are premises that supramolecular structures formed by bisazo dyes can selectively interact with immune complexes and do not interact with free antibodies. This creates the possibility of targeted delivering of drugs associated with the ribbon like supramolecular structures, selectively bound only to antibodies involved in the complex with the antigen. The studies were carried out using molecular dynamics which allows us to analyze the mechanisms of the relevant processes occurring at the molecular level. It was shown that doxorubicin binds to all the studied dyes that is Congo Red, Titan Yellow and Evan Blue leading to formation of supramolecular structures. However, the biding energy depends on the dye molecule though in all cases it is so large that the supramolecular structures are stable and can interact with immune complexes. In the case of Congo Red it was also found that biding energy depends strongly on pH - it can be utilized as a factor facilitating release of doxorubicin in acidic microenvironment of tumor tissue.

Nanostructures of Soft Matter

Topology of protein monolayers at the solid/electrolyte interfaces and mechanisms of their interactions with ionic and macromolecular ligands. Influence of sensor topography on the kinetics of albumin adsorption.

(prof. Zbigniew Adamczyk, Jakub Barbasz DSc, Aneta Michna DSc, Maria Morga PhD, Małgorzata Nattich-Rak DSc, Magdalena Oćwieja DSc, Agata Pomorska PhD, Marta Sadowska PhD, Monika Wasilewska PhD)

Human serum albumin (HSA) binds many endogenic and egzogenic ligands, controls their concentration and facilitates their efficient transport to various tissues. It is also used for medical device coating (membranes and peacemakers, orthopedic titanium implants, catheters) which prevents adhesion of other proteins, platelets and bacteria. Given its essential significance, adsorption kinetics of HSA on silica substrates of different surface topography was studied in this work using the quartz microbalance (QCM), optical light mode spectroscopy (OWLS), atomic force microscopy (AFM) and some electrokinetic methods. Measurements were performed for various flow rates, pHs and ionic strengths. A comparison of the QCM experimental data with the OWLS measurements acquired for lower pH range is presented in Fig. 1. It is seen that the mass coverage of albumin determined by QCM significantly exceeds the coverage determined by OWLS at the same convective transport conditions, which was attributed to the dynamic solvation of HSA layers due to the sensor oscillatory motion. The experimental data were quantitatively interpreted in terms of a new hydrodynamic model. It was shown that the amount of solvation depends on the geometrical heterogeneity of sensors, characterized in terms of the root mean square parameter *rms*. This theoretical model was also applied for the calculation of the dynamic solvation of sensor produced by a controlled deposition of gold nanoparticle mono- and bilayers. Measurements performed at larger pHs confirmed that the HSA adsorption kinetics is considerably slower than at pH 3.5. This effect was attributed to a slow aggregation of HSA solutions and interpreted in terms of a theoretical model combining the Smoluchowski theory with the convective diffusion mass transfer theory.

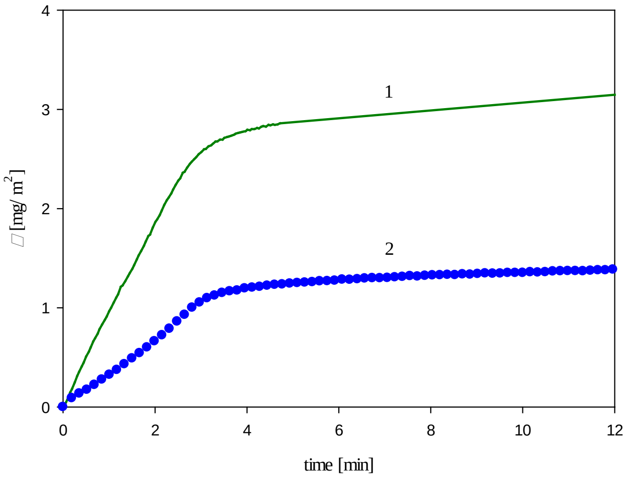


Fig. 1. The kinetics of HSA adsorption on the silica sensor determined by QCM (green curve number 1, adsorption kinetic constants $k_Q = 3.1\times10^{-4}$ cm s^{-1}, $k_c = 1.2\times10^{-4}$ cm s^{-1} and OWLS (blue solid points, $k_c = 1.2\times10^{-4}$ cm s^{-1}), pH 3.5, NaCl concentration 0.15 M, bulk HSA solution concentration 5 mg L^{-1}.

Determination of mechanisms of synthesis, structure, and transport parameters of supported colloidal particle multilayers

(assoc. prof. Paweł Weroński, Karolina Pałka MSc)

A thin layer of colloidal particles adsorbed on the solid surface significantly determines the macroscopic properties of this boundary area. Modification of the structure of the deposited layer causes significant changes in optical, mechanical, magnetic, hydrophobic-hydrophilic, transport, or antibacterial properties. Commonly used characteristics describing the structure of thin layers include the correlation function of adsorbed particle pairs and power spectral density function of height of this layer. The latter allows a multi-scale, quantitative description of the roughness of the adsorbed layer. Both functions are closely related. Unlike the correlation function, the analytical description of power spectral density has been limited to the simplest monodisperse systems of pillars and disks so far.

The aim of our research was to generalize the previously developed theoretical model of the roughness of the pillar layer for monodisperse spherical particle systems and the numerical and experimental verification of the obtained analytical results. The first stage of this task consisted in deriving analytical equations for the Fourier transform and the spectral power density of the monolayer of spherical particles. Then, we developed numerical algorithms and computer programs for numerical studies. They allowed the generation of monolayers of spherical particles with a given surface coverage, calculation of the height function of these layers, calculation of the Fourier transform of this function and its spectral power density. We then compared the numerical results obtained from computer simulations with analytical equations and found their good agreement.

The final stage of the task was experimental research. That included the adsorption of monodisperse polystyrene latex particles with diameters from 200 to 500 nm on the surface of mica and scanning the surface of the obtained layers using an AFM microscope. The particle layer height functions obtained in this way allowed the calculation of the power spectral density of these functions. The obtained experimental results allowed to state that the measurements of the height function are strongly dependent on the geometrical dimensions of the AFM probe used. In order to take this effect into account, we developed a generalized analytical model describing the height function for the system of spherical colloidal particle and conical AFM probe with a given conic angle and radius of curvature of the tip. We also derived the Fourier transform equations of this function. The results of experimental studies also showed that the use of the AFM microscope for measuring the height function is limited to layers of particles with not very high surface coverage, even after taking into account corrections for the dimensions of the AMF probe. This limitation results from the fact that as the surface coverage increases, the number of pairs of particles located at a distance smaller than the width of the probe tip, where the correct measurement of the layer height is not possible, increases. Considering this effect would require the use of a much more complicated mathematical model, which significantly exceeds the scope of planned research. It also seems that the correct measurement of the height function of layers with high coverage could be done using other experimental methods with higher measurement accuracy, such as SEM microscopy. To this end, however, it is necessary to develop a theoretical model linking the brightness of the image with the height of the surface, which also does not seem trivial.

Influence of initial adsorption coverage at liquid/gas interface on stability of foam and wetting films under dynamic conditions

(assoc prof. Jan Zawała, Dominik Kosior PhD, Agata Wiertel-Pochopień MSc, Dorota Gaweł MSc, Mariusz Borkowski Msc)

The aim of this research task was to investigate the stability of foam films forming the real foam, i.e. the dispersed system comprising gas bubbles dispersed into the aqueous phase of proper physicochemical properties. In the reporting period the influence of concentration of cationic surface-active substances (SAS), namely n-alkyl-trimethyl-ammonium bromides (C_nTAB, where n = 8, 12, 16, and 18), on solutions foamability and stability of formed foams, was investigated. The experiments were focused on comparison between above-mentioned parameters measured for one-component cationic SAS solutions and mixtures of cationic/non-ionic SAS, where cationic SAS was the main component. The research was carried out using KRÜSS DSA100 apparatus. The experiments were performed in cooperation with the research group at Norwegian University of Science and Technology.

During experiments, the adsorption coverage at the liquid/gas interface was changed in a traditional way, i.e. by changing the bulk SAS concentration. Higher was the bulk concentration, higher was the adsorption coverage, and, as could be expected, the foamability of solution was better (measured height of the formed foam column was higher). Nevertheless, comparison between foamability of one-component and mixed solutions revealed that addition of non-ionic SAS of small concentration can improve the solution foamability remarkably. The example of results obtained for C_8TAB (OTAB) is presented in Fig. 1. The foam height measured after 20 s of foaming in one-component solutions of different concentrations and in mixed solutions, where 5×10^{-4} M of n-octanol was added, is presented there. It was found that even small amount of non-ionic SAS causes synergistic effect, i.e. at identical main-component (cationic SAS) concentration, the foam height is much higher in the non-ionic SAS presence. However, this conclusion is true only for specific cationic SAS concentration regime. As can be seen in Fig. 1 above some specific concentration the synergistic effect disappears and the foamability in no longer enhanced. Similar results were obtained for other C_nTABs in homologous series and for different kinds of non-ionic SAS additives. It was shown that small amount of non-ionic SAS addition can remarkably reduce the concentration of main solution component (cationic SAS) needed to obtain identical foamability properties without change in the foam stability. Analysis of the obtained results, based on surface elasticity (E) – the parameter determining stability of foam films in so-called wet foams, allowed describing the mechanism and role of non-ionic SAS in the synergistic effect existence. Moreover, a simple method of determination of threshold concentration, above which the synergistic effect disappears (called critical synergistic concentration), was proposed. This method is based on determination of changes of the solution surface tension of given concentration ($d\sigma$), which is proportional to the E parameter.

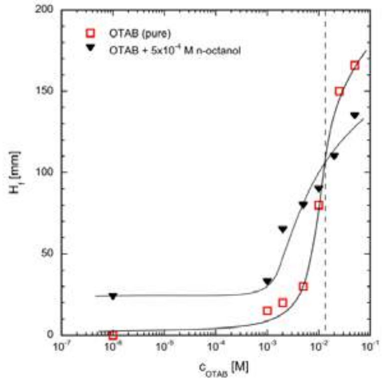


Fig. 1. Foam height as a function of C_8TAB solution concentration with and without n-octanol

Functional multilayer polyelectrolyte films

(prof. Piotr Warszyński, Marta Kolasińska-Sojka DSc, Ewelina Jarek PhD, Lilianna Szyk-Warszyńska PhD, Dzmitry Kharytonau PhD)

As a continuation of the last year's task, we have focused on various aspects of the characteristics of nanocomposite materials containing inorganic nanoparticles and polyelectrolyte films. Nanoparticles (NPs) can be incorporated into supported lipid bilayers (SLBs) to form NP-SLB hybrids, which are promising candidates for bioanalytical applications, *e.g.* tracking of vesicle fusion on surfaces *via* fluorescence visualization of the NPs in the resulting lipid bilayers. Considering its crucial importance to the design and use of nanomaterials in biomedical applications, there is an urgent need for a better fundamental understanding of NPs' roles in the lipid reorganization and the membrane morphology of NP-SLBs.

The main aim was to investigate structural changes of negatively charged -palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) / 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine (POPE) supported lipid bilayers intercalated with quantum dots on a positively charged poly(ethyleneimine) (PEI) film as a function of incubation time. X-ray reflectivity (XRR) was applied to monitor the temporal structural changes of mixed SLBs of (POPC)/(POPE) intercalated with 4.9 nm hydrophobic cadmium sulphide quantum dots (CdS QDs). The QD-embedded SLBs were formed *via* rupture of the liposomes on a PEI monolayer. Atomic force microscopy (AFM) imaging provided complementary characterisation of the bilayer morphology.

Our results show time-dependent perturbations in the SLB due to the interaction upon QD incorporation. Compared to the SLB without QDs, at 3 h incubation time, there was a measurable decrease in the bilayer thickness and a concurrent increase in the scattering length density (SLD) of the QD-SLB. It then became progressively thicker with increasing incubation time (d = 4,9 nm at 14 h incubation and d = 5,3 nm at 24 h incubation). The fitted scattering length density (SLD) profiles suggest that the QDs were expelled from the inner leaflet to the outer leaflet of the bilayer. Such structural reorganisation was related to the insertion of hydrophobic QDs in the hydrocarbon tails region of the SLBs which enhanced disorder in the conformation of the lipid chains in the POPC/POPE bilayer. Initially (at 3 h incubation), this reduced membrane thickness was due to lipid tails tilting to accommodate the hydrophobic QDs. Subsequent QD expulsion from the inner membrane led to a more ordered packing of the lipid molecules in the inner leaflet, whilst the outer membrane leaflet in contact with water, less constrained and more flexible, more readily accommodated the QDs.

Our results give unprecedented mechanistic insights into the structural evolution of QD-SLBs on a polyelectrolyte cushion, important to their potential biomedical and biosensing applications.

We also analysed the antibacterial properties of Chitosan-AgNPs coatings containing copper. They were deposited onto AISI 316L stainless steel. First, a 20-nm-thick layer of copper was deposited from a standard copper plating sulphate electrolyte. Then, chitosan-AgNPs coating was deposited by electrochemical anodic technique at the current density of 2 A/dm^2. The deposition bath contained 5 g/L of chitosan, 50 mM/L of acetic acid, and varying amount (0; 50; 100; 150, and 200 mL/L) of AgNPs suspension, synthesized by a citric method. Antibacterial properties of the coatings formed were examined towards ATCC 35984/RP62A *Staphylococcus epidermidis* bacteria. The tests showed that introduction of copper ions and AgNPs improved antibacterial properties of chitosan. After 1 h of tests, the fraction of dead bacteria increased from 84% to 98%, while surface coverage decreased from 14 to 11% for pure chitosan and chitosan–150 mL/L AgNPs, respectively.

Nanoparticles as neuroprotective substance carriers

(assoc. prof. Krzysztof Szczepanowicz, Marta Szczęch MSc, prof. Piotr Warszyński, Anna Pajor-Świerzy PhD)

Despite many chemicals show a protective effect on neuronal cells, they are less effective in clinical trials, which may be related to their poor absorption and distribution as well as adverse reactions of the immune system after systemic applications. Nanotechnology creates new perspectives because formulations in nano-form can possess unique pharmacokinetic and pharmacodynamic properties, that can contribute to their high effectiveness in therapeutic action while minimizing undesirable side effects. The scientific aim of this task is to develop a methodology of synthesis of biodegradable and biocompatible nanocarriers for neuroprotective substances. Final results will broaden knowledge in the field of neuropharmacology, particularly concerning the design and synthesis of novel, nano-scale drug delivery systems for neuroprotective agents.

In 2019, we were focused on the optimization of the preparation procedure of multilayered nanocarriers for neuroprotective substances, nanocarriers with controllable physicochemical properties and proper functionality. The method of the preparation of biocompatible and biodegradable polylactic acid (PLA) nanoparticles was developed (Fig. 1). Selected neuroprotective substance, Edaravone, was successfully encapsulated in developed PLA nanoparticles. Furthermore, drug-loaded PLA nanoparticles were functionalized by the formation of a multilayered shell. The polyelectrolyte multilayer shell was constructed by sequential adsorption of polyelectrolytes (layer-by-layer) method. Polyelectrolyte multilayer shells were constructed with biocompatible and biodegradable polyelectrolytes: poly-L-lysine as polycation and poly-L-glutamic acid as polyanion. The average size of drug-loaded multilayered nanocarriers was 120nm (Fig. 2). Synthesized multilayered nanocarriers will be further tested for their cytotoxicity and activity of the encapsulated drug.

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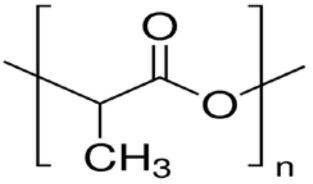

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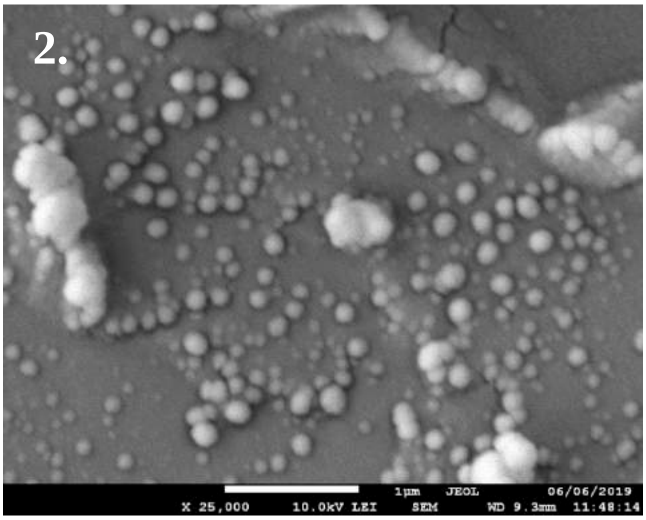


Figure 1. Polylactic acid (PLA).
Figure 2. Cryo-SEM picture of PLA nanoparticles.

Physicochemical properties of functional nanocarriers based on dendrimer and protein systems

(assoc. prof. Barbara Jachimska, Paulina Komorek MSc, Magdalena Szota MSc)

Applications of bovine β-lactoglobulin (LGB) as a biomimetic transport system. The specific structure of this protein allows the drug to be immobilized both on the surface and in the binding pocket located inside the protein structure. The effectiveness of the formation of protein-drug complexes was studied by the effectiveness of immobilization in the LGB structure of tetracaine hydrochloride (TET) and determining the effects of Tanford transition on the formation of the active complex.

The formation of LGB-TET complexes was analyzed for various molar ratios under conditions when access to the β-barrel is closed or open (pH 5.5 and pH 7.5). Optimal conditions for complex formation were obtained for a 1:50 molar ratio at pH 7.5. The formation of the LGB-TET complex was verified on many levels using, among others, the UV-Vis method, QCM-D and zeta potential measurement. The zeta potential of LGB-A-TET and LGB-AB-TET complexes is lower than for the native form of the protein, which indicates tetracaine adsorption on the surface of the protein molecule. Using the QCM-D method and zeta potential, it was determined that under optimal conditions of complex formation from 10 to 13 TET molecules was immobilized per LGB molecule. This means that TET molecules are located not only in the binding pocket as postulated in the literature, but also on the surface of the β-lactoglobulin molecule. The molecular modeling method confirmed that TET binds to the β-barrel, with the polar amino group present in the ligand molecule facing the exit from the β-barrel. Protonated form as well as deprotonated form have a similar effect, however, the protonated form is bound deeper in the β-barrel. The conducted research enabled the development of a procedure for creating LGB monolayers on the Au surface with a controlled degree of surface coverage and orientation of adsorbed protein molecules, which is important for the creation of functional layers with dedicated physicochemical properties.

Physics and Chemistry in Cultural Heritage Protection

Quantitative assessment of risk to heritage objects due to microclimatic conditions in the environment

(assoc prof. Łukasz Bratasz, Marcin Strojecki PhD, Leszek Krzemień PhD, Magdalena Soboń MSc)

As part of the research task, a theoretical model of deposition of suspended dust particles in the air was developed, taking into account the most important mechanisms of deposition, i.e. Brownian diffusion, gravity, turbulence and the thermophoretic effect associated with the difference in air and surface temperatures. Consideration of all the above-mentioned mechanisms was very important from the point of view of using the developed model for the interpretation of experimental results collected during the previously implemented NCN project. As part of the project, based on long-term measurements of suspended dust in 12 temples in Poland, processes related to transport and deposition of dust were analysed, and the deposition rate was experimentally determined. As a result of research, it was also shown that particulate matter suspended in the air penetrate from the outside through the building envelope and deposit on internal architectural surfaces and items of interior equipment. This effect is particularly significant in the case of historical religious buildings characterized by a relatively leaky building structure, which, in consequence, causes high (close to 90%) penetration of particles in the micro- and submicron range.

The developed model is an extension of the existing model of homogeneous turbulences, by taking into account the important parameter of surface roughness. In the developed model, all important parameters affecting the intensity of air turbulence were included as the synthetic parameter which is the friction velocity between the surface and the air containing dust particles. The second parameter influencing the deposition velocity in a similarly significant way is the temperature difference between the surface on which deposition occurs and the air in which the dust particles are suspended.

Using the parameterized model and determined experimental deposition velocity values, the contribution of individual processes to the total deposition rate was estimated. Using that result, various types of heating systems were evaluated in terms of effective deposition rate, ie. excluding the effects of geometry.

The calculated values of the deposition velocity and the theoretical model developed will be used for estimating the time needed for dust accumulating on internal surfaces to become visible.

In the second part of the task, comparative measurements of water vapor sorption of wood samples taken from wooden structural elements of the port food warehouse located in Trondheim, Norway were performed. The measurements carried out using a vacuum microbalance were aimed at identifying the type of salt used for preserving food, estimating their weight content and determining the impact on the condition of the original building elements. The results obtained from the sorption method were correlated with the results obtained using other techniques, i.e. measurement of amplitude suppression of acoustic signals (acoustic emission - EA) and measurement of moisture content using a classic capacitance meter.

The results obtained from the sorption method were correlated with the results obtained using other techniques, i.e. measurement of amplitude attenuation of acoustic signals (acoustic emission - EA) and measurement of moisture content using a classic capacitance meter. The most important conclusion from the analysis of the results obtained from all three methods used in the research is confirmation of their effectiveness in assessing the condition of wooden structural elements contaminated with salts and stored in adverse microclimatic conditions.

"Sonata Bis" Research Projects of the National Science Centre

2-Oxoglutarate dependent oxygenases in the biosynthesis of pharmacologically active alkaloids - structure, catalytic mechanisms and rational redesign

(Project leader: prof. Tomasz Borowski)

"Sonata Bis" research project NCN 2014/14/E/NZ1/00053 [2015-2019]

Within this project we pursue a tightly integrated structural, biochemical and computational studies on two groups of 2-oxoglutarate dependent dioxygenases (ODD) involved in biosynthesis of alkaloids. Structures of these proteins are not known, as are the detailed mechanisms of their catalytic reactions.

For one of the examined proteins, previously obtained structures were refined and deposited in the PDB database, computational studies (QM/MM) were performed with the aim to identify factors determining regioselectivity and product specificity for this enzyme, a publication was prepared and submitted. Attempts were also made to co-crystallize this protein with another substrate (two different isomers), the obtained diffraction data allowed for determination of structures that are currently being refined. With the genetic engineering methods several mutants of the protein were prepared and they are currently under study. The first diffraction data for two mutants were obtained and are currently analyzed. The above studies were conducted in cooperation with the group of prof. Maksymilian Chruszcz from the University of South Carolina. Pilot studies using the stopped-flow method were also carried out for this enzyme.

For another protein, attempts were made to crystallize the protein with the substrate, the co-substrate binding curves were determined by the MST method. Measurements of sample dispersion were also carried out using the DLS method.

For the next protein, several attempts were made to measure the substrate binding constant by capillary electrophoresis. Protocols for protein production and purification were optimized for three mutants of this protein, measurements of protein thermostability were carried out using the nanoDSF method, the binding constants of the co-substrate and substrates were determined using the MST method, sample dispersion tests were carried out using the DLS method and the first crystallization tests were undertaken.

For another protein, crystallization tests were carried out and binding constants of the co-substrate, its analog and several different substrates were determined by the MST method. Measurements of sample dispersion by DLS method and thermal stability by nanoDSF method were also carried out. Some of the above experiments were done in cooperation with BIOCEV (Prague) and MCB (Kraków).

Within computational studies the reaction mechanism of the CAS enzyme was scrutinized with the purpose to identify factors determining reaction specificity of ODD enzymes. Obtained insights will be used to devise mutants with altered specificity.

Conformational properties of fructans and interaction of selected saccharides with proteins studied by molecular dynamics simulations

(Project leader: assoc prof. Wojciech Płaziński)

"Sonata Bis" research project 2015/18/E/ST4/00234: Molecular mechanics force field for structure, dynamics and conformation of carbohydrates involving furanoses [2016-2020]

In previous tasks related to this project, a united-atoms force field belonging to the GROMOS family was developed, tested and validated; this force field is dedicated to simulations of biomolecular systems containing furanoses (i.e. sugars containing a five-membered ring). The next stage of the research was the application of the above parameters to examine a number of properties exhibited by natural furanoses. Research included several natural fructans, i.e. polymers composed of furanose units. The three most common fructans are: levan (D-fructofuranose units connected by a $\beta(2 \rightarrow 6)$ bond), inulin (D-fructofuranose units connected by a $\beta(2 \rightarrow 1)$ bond) and arabinan (L-arabinofuranose units connected with an $\alpha(1 \rightarrow 5)$ bond).

The results included a series of hydrodynamic parameters exhibited by natural fructans (e.g. gyration radius, persistence length). The relationship between the glycosidic bond conformation at the dimer and oligomer level and the influence of hydrogen bonds on this conformation was also checked. The effect of orientation of ring substituents not involved in glycosidic bond on the conformational parameters of the fructan chain was analyzed. It was found that such orientation has an influence on the fluctuation of the abovementioned parameters, while the mean values remain almost unchanged. Compared to structural analogs formed by pyranoses with $(1 \rightarrow 6)$ bonds, fructans show a similar or greater level of flexibility, which indicates their high conformational heterogeneity.

As part of testing the applicability of the developed united-atoms GROMOS force field, a series of simulations were carried out to investigate various aspects of carbohydrate-protein interactions. For comparative purposes, the research also included competitive force fields (all-atom type) of the CHARMM and GLYCAM/AMBER families. The main studied feature was the free energy of carbohydrate-protein binding accompanied by some basic structural and dynamic parameters of the respective complex. Despite the wide applicability of the abovementioned force fields, they have not yet been systematically validated and compared to each other in terms of protein-carbohydrate interactions.

Selected systems included the following non-functionalized mono- and disaccharides: cellobiose, D-glucopyranose, lactose, sucrose, D-arabinopyranose, D-ribopyranose, L-fucopyranose; the corresponding complexes are collected in the PDB database under the following records: 1i92, 1i82, 1af6, 2aac, 2arc, 1ogd, 1z3v. Completed tasks included the preparation of simulation systems and sets of parameters describing interactions within carbohydrate molecules, carrying out a standard procedure for optimizing geometry, equilibration, etc. The next step of the research was to perform simulations by using the so-called thermodynamic integration scheme, performed in 21 steps (100 ns simulation for each integration step) for systems containing a complex of protein with carbohydrate and sole carbohydrate molecule. Finally, a number of programs have been created to analyze the results and check their convergence.

"Sonata" Research Projects of the National Science Centre

Lipase-Mediated Biosynthesis of Novel Lactose Esters. Physicochemical and Anticancer Studies

(Project leader: Maciej Guzik PhD)

"Sonata" research project NCN 2015/17/D/ST4/00514 [2016-2019]

Stage 1 cont.: Precursors preparation for biocatalytic synthesis of sugar esters

The whole process of obtaining bacterial PHA was improved by applying additional polymer purification protocol after fermentation in bioreactor. The method is based on filtration and separation of the polymer on activated charcoal column. That procedure allowed to obtain extra pure substrates and materials for future synthesis. During preparations of precursors for further biocatalytic reactions with a sugar several issues occurred with purification and hydrolysis of modified monomers of 3-hydroxynonanoic acid methyl ester. Especially it was difficult to achieve higher efficiencies of reactions which block 3-OH groups of PHN monomers. In that situation another type of reaction was proposed: the substrate was derevatised with fluorsulphonyl-2,2,2-trifluoropropane. Analysis confirmed the desired pure product was successfully obtained.

Stage 2 cont.: Biocatalysis- Screening of various reaction media and effect of lipase immobilization

In order to increase the efficiency of lactose tranestrification reaction by our model substrate which is methyl ester of nonanoic acid reactors with other sugars (which build the disaccharide): glucose and galactose were set. The reactions were catalysed by previously selected immobilized lipases (*Themomyces lanuginosa* - TL-IM, *Candida antarctica* lipase B - CALB). In this time a method of extraction the reagents from batch reactors was optimized (by using solution of MeOH 50%/ 50% H2O or pyridine). Then separation and identification of quantitative and qualitative methods using HPLC-MS (with triple quadrupole), GC and GC-MS were upgraded. The mentioned analysis confirmed a presence of expected sugar fatty acid esters after 24h of the reaction and revealed drop in the substrate concentrations giving 42% and 37,6% glucose conversion for reactors with TL-IM and CALB, respectively, after 24h (reaction medium 2-methyl-2-butanol, 2M2B).

To verify whether it is possible to widen reaction media spectrum we set up reactors with: DMF, DMSO, acetone, toluene, pyridine, system 2M2B 80%/20% pyridine, tert- buthanol beside already examined 2M2B and system of 2M2B 80%/20% DMSO. Combinations of all sugars: lactose, glucose, galactose were tested. The experiments confirmed that 2M2B is the best reaction medium but additionally revealed that pyridine and system 2M2B 80%/20% pyridine are also reliable media for conducting biocatalysis. That happened because these solvents improve solubility of the sugar and the sugar fatty acid ester also making them easier to extract from whole reactors.

Continuing a cooperation with Eucodis Bioscience GmbH(Austria) the best working towards fluorinated PHN monomers lipase EL70 was applied. In that case the homogenous enzyme was immobilized on modified mesoporous silica supports (Materium, Canada). The lipase was covalently attached to the supports by crosslinking their –OH groups with divinyl sulphone or -NH2 groups with glutaraldehyde. All these operations gained efficiency of glucose transesterification to its nonanoic acid ester to 88,5% and 98,4% of conversion for Spheres-OH and Spheres-NH2 respectively.

Formation mechanisms and functionality of nanoparticle multilayers with incorporated biomolecules

(Project leader: Maria Morga PhD)

“Sonata” research project NCN 2015/17/D/ST4/00569 [2016-2019]

The research goals of the SONATA project realized in 2019 comprised: (i) determining adsorption and desorption kinetics of polypeptide monolayers at solid/liquid interfaces; (ii) determining conformation of a polypeptide adsorbed at solid substrates (mica, Si/SiO_2) under examined environmental conditions using the streaming potential method, QCM-D method and optical reflectometry method; (iii) quantitative description of obtained experimental data using 3D electrokinetic model and eRSA model; (iv) developing a reliable and repeatable procedure of constructing stable polypeptide layers of defined structure, coverage, and electrokinetic properties; (v) developing a reliable and repeatable procedure of constructing multilayered nanostructures composed of macroion/nanoparticles and nanoparticles/nanoparticles; (vi) determining the influence of the first layer and the type of a macroion or a nanoparticle (size, zeta potential) on the structure of obtained multilayered materials and their electrokinetic properties.

In the course of the research, the unique measurements of the kinetics of polypeptide adsorption were conducted under *in situ* conditions using the streaming potential method, QCM-D and reflectometry method on solid substrates of mica and silicon wafers. The results obtained using the reflectometry method were carried out in cooperation with the University of Geneva with the group of Prof. Michał Borkovec. Electrokinetic studies allowed to determine the conformation of macroions on the surface. It has been proved that PLL molecules adsorb in the *side-on* conformation on the surface. In addition, the use of the electrokinetic 3D model and the extended eRSA model enabled quantitative analysis of the topological parameters of the obtained monolayers, i.e. coverage and electrokinetic properties, as well as stability under varying measurement conditions. Reflectometry measurements allowed for determination of the adsorbed polypeptide masses, equal to 0.18 mg m^{-2}, 0.32 mg $m^{-2}$and 0.46 mg m^{-2} for $I = 10^{-3}$, $I = 10^{-2}$ and $I = 0.15$ M NaCl, respectively, thus confirming the experimental data obtained by the streaming potential method.

The research concerning the formation of multilayer structures in the macroion/nanoparticle and nanoparticle/nanoparticle systems allowed to determine the dependence of the adsorption kinetics of the independent layers and their stability influenced by the type of nanoparticles used in the study. For α-Fe_2O_3/SiO_2 nanoparticles, of the size of 22nm/32nm, the maximum coverage of silica nanoparticles (θ_{smx}), for low hematite coverage (θ_h), is expressed by the relationship: $\theta_{smx} = 3.17\theta_h$. For α-Fe_2O_3/Ag nanoparticles of the size 40nm/11nm, one can observe that there is no increase in AFM height profiles for the second and third layer. This may suggest that the adsorption of silver nanoparticles does not occur directly on the hematite nanoparticles (adsorption centers), but rather between the hematite nanoparticles - silver nanoparticle penetrate somewhat between the created homogeneous monolayer of hematite nanoparticles. Deposition of the third layer of α-Fe_2O_3 nanoparticles is inhibited as a result of repulsive interactions between α-Fe_2O_3 nanoparticles of the first and third layers.

The obtained results contribute to the development of quantitative models describing formation of multilayers and determination of the interaction mechanisms of biomolecules and nanoparticles forming multilayered nanostructures. In addition to cognitive significance, the acquired knowledge will allow to design new materials of desired properties, e.g. biocidal coatings, sensors and substrates for selective protein adsorption.

Bio oliogosaccharides under external forces

(Project leader: Agnieszka Brzyska PhD)

"Sonata" research project NCN 2015/19/D/ST4/01979 [2016-2019]

We focused mainly on the analysis of conformational changes induced by external forces in alginates. The alginate acids are a component of the cell walls of many algae and seagrass. The alginic acid is a linear polysaccharide composed of D- mannuronic (M) and L-glucuronic (G) blocks. These sugar molecules are linked by a β-1-4 glycosidic linkage. The calculations were carried out for monosaccharides (M and G) and for the different oligosaccharide sequences (MMM ..., GGG ... and GMGM ...) present in alginic acid acid polysaccharides.

We also analyzed saccharides found in the (glycosylphosphatidylinositol) GPI anchor glycolipid. The glycosylphosphatidylinosyl anchors are complex glycophospholipids and attach to the C-terminus of proteins. Their primary function is to ensure stable association of proteins with the lipid layer of the cell membrane. We examined the enforced structural changes in the monosaccharides found in the GPI anchor glycolipid (GPI-anchored), i.e. mannose (Man) and glucosamine ($GlcNH_2$). We also investigate the structural rearrangements induced by the external tensile forces in the tetramer with the sequence Man α1-2 Man α1-6 Man α1- 4 $GlcNH_2$ α1-6 characteristic for this glycolipid.

In our calculations we mainly used the EGO (*Enforced Geometry Optimization*) method. The EGO approach allows the simulation of the AFM experiment on a single saccharide molecule. The tests in the above-mentioned scope were conducted in two calculation schemes, i.e. (1) in the standard EGO approach, where extending external forces are applied to terminal glycosidic oxygen atoms in the range of external forces from usually 0.020 au to 0.070 au and (2) in the EGO scanning protocol in which the molecule constantly experiences increasing strength from 0.00 to 0.07 au in steps of 0.005 / 0.01 au.

The theoretical descriptions of structural changes for the above-mentioned systems were developed. In addition, some mechanisms of conformational transitions induced by extending external forces have been proposed for monomeric structures. The obtained results were compared with the available AFM experimental data.

Influence of ZrO_2 crystallographic structure on activity of Cu/ZrO_2 and Cu/ZrO_2-ZnO catalysts doped with Ga, Mn, Ni in low temperature steam reforming of bio-ethanol.

(Project leader: Michał Śliwa PhD)

"Sonata" research project NCN 2016/23/D/ST4/02492 [2017-2020]

The catalytic activity of copper-based catalysts donated with Ga and Mn were investigated in steam reforming of ethanol. The ratio of EtOH/H_2O was 1:3. The catalysts (150 mg mixed with quartz) were reduced at 250 ^oC for 2h in 5%H_2/Ar flow. Next, the temperature of the reactor was increased to 350 ^oC and the vapor containing EtOH/H_2O mixture diluted in Ar were introduced using CEM. For both catalysts, the initial EtOH conversion is high (100%). The level of the conversion decreases faster for Cu/ZrO_2/MnO catalysts in comparison with Cu/ZrO_2/MnO. This same relation is observed for hydrogen yield.

The steam reforming of ethanol was performed for EtOH/H_2O = 1:10 over Cu/ZrO_2/NiO and Cu/ZrO_2/MnO catalysts which had been calcined at 550 ^oC. The reaction was carried out at 350 ^oC The XRD analysis showed that t-ZrO_2 is present in these two catalysts. For both catalysts, the ethanol conversion is above 90% and it stays constant during entire catalytic experiment. The addition of Ni results in decrease of the yield to CH_3CHO while the yield to hydrogen stays at similar level to Cu/ZrO_2/MnO catalyst.

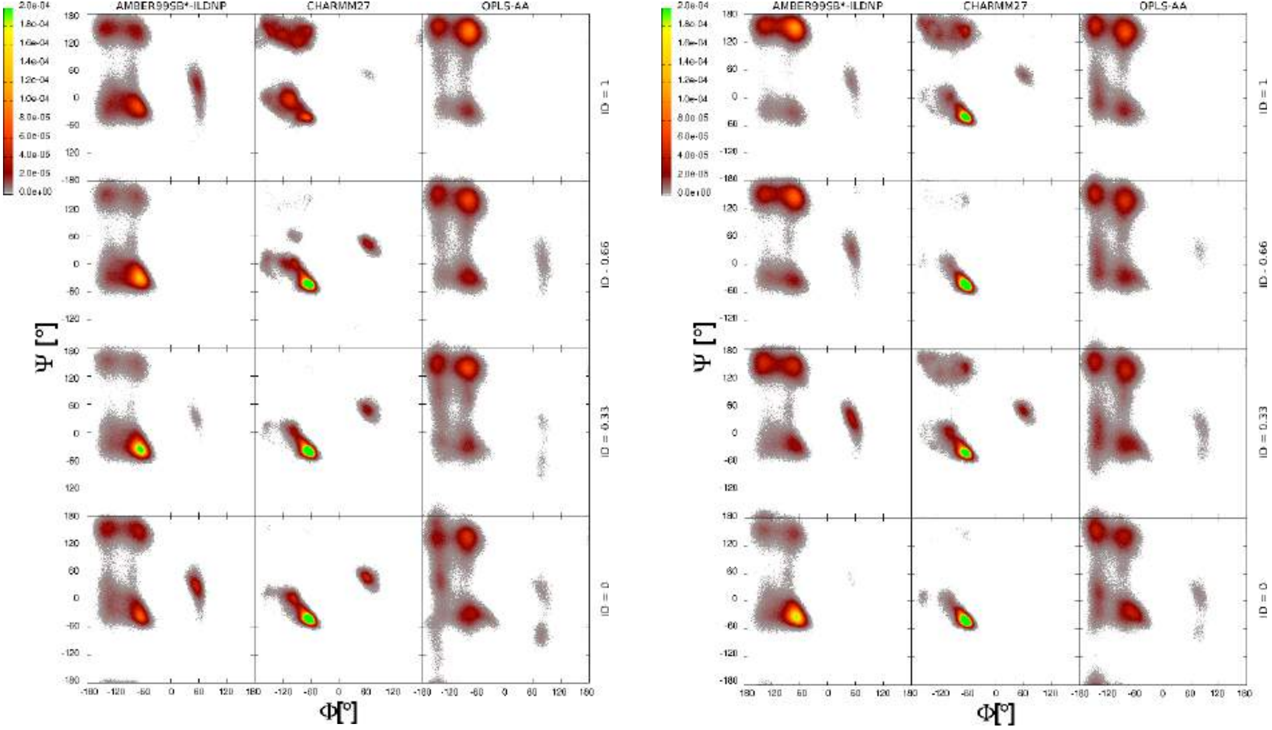
From a single molecule to smart material - understanding the polypeptide complexes formation and properties

(Project leader: Piotr Batys PhD)

"Sonata" NCN 2018/31/D/ST5/01866 [2019-2022]

The effect of the force field on the secondary structure of poly-L-lysine (PLL) and poly-L-glutamic acid (PGA) obtained from molecular dynamics (MD) simulation has been determined. In particular, the OPLS-aa, CHARMM27, and AMBER99SB*-ILDNP force fields have been investigated. The MD method allowed to determine the effect of pH on chain length, radius of gyration and the number of internal hydrogen bonds within the polypeptides, as well as the secondary structure. The results of MD simulation were verified experimentally using circular dichroism, dynamic light scattering and combinations of electrophoresis with laser Doppler anemometry methods. The experimental measurement allowed determining the dependence of the secondary structure, particle sizes and zeta potentials in the pH range 3-11, at constant ionic strength.

The obtained results turned out to be extremely important from the applicability point of view. The differences between the investigated force fields are enormous, despite the fact that all of these force fields are considered sufficiently accurate for protein modelling. More importantly, all three force fields are used in the literature to simulate PLL and PGA, although there is a lack of work considering their validation and a critical look at the results obtained. Experimental validation indicates that the CHARMM27 force field systematically overestimates the α-helix contribution, while OPLS-aa the β-structure contribution. Both of these force fields seem to be insensitive to pH changes. From the tested force fields, only AMBER99SB*-ILDNP was capable to correctly reproduce the changes in the secondary structure associated with the changes in the polypeptide' ionization degree (changes in pH).



a) b)

Figure 1. Ramachandran plots as a function of the force field and the ionization degree (ID), for (a) PGA and (b) PLL.

"Opus" Research Projects of the National Science Centre

2-oxoglutarate dependent oxygenases catalyzing atypical oxidative transformations - structural and mechanistic studies.

(Project leader: Tomasz Borowski)

"Opus" research project NCN 2014/15/B/NZ1/03331 [2015-2019]

This research project focuses on three novel types of 2-oxoglutarate dependent dioxygenases whose structures and catalytic mechanisms are still unknown. They catalyze: oxidation of 2-oxoglutarate to ethylene (EFE), oxidative deamination of kanamycin (KanJ) and formation of an endoperoxide bridge (in the synthesis of a mycotoxin – verruculogen; FtmF).

Within the last year, the expression protocol of the FtmF protein has been optimized for *E. coli* and *P. pastoris*, this study was conducted in collaboration with Professor Karin Hoffmann-Sommergruber from the Medical University of Vienna. Crystallization tests with addition of substrate were carried out for purified preparations of the FtmF protein.

For the EFE homologue, which does not produce ethylene, i.e. EFE from *Penicillium chrysogenum*, the protein purification protocol was optimized and protein crystallization tests were carried out in collaboration with the group of prof. Maksymilian Chruszcz from South Carolina University. Moreover, for the EFE homologue from the *Penicillium digitatum* organism, the expression and protein purification protocols were optimized and protein crystallization tests were performed.

In the case of the KanJ enzyme, the refinement of structures was completed, and they were deposited in the PDB database. The first publication on the structural and biochemical characterization of the KanJ protein was prepared and submitted.

The publication collecting the results of modeling of the mechanism of the FtmF protein was also completed, including docking results, molecular dynamics simulations and quantum-chemical calculations. The publication was accepted and published in *Dalton Transactions*.

Theranostic nanocarriers for MRI imaging

(Project leader: prof. Piotr Warszyński)

"Opus" research project NCN 2015/17/B/ST5/02808

Neurodegenerative diseases (e.g. Alzheimer's, Parkinson), psychiatric disease (e.g. schizophrenia), cancer, bacterial infection and many others are still a challenge for medicine. One of the major drawbacks of current conventional therapies is lack selectivity of preparations, since administered drugs are more or less equally distributed within the body. This results in many of the negative side effects due to the impact of the drug in the whole body, also on the healthy part of it. It seems reasonable to find the route of controlled delivery of therapeutic, which would result in an action of the active substance only in strictly defined, pathologically changed place in the body. The solution of this problem is the drug targeting, i.e., delivery of the therapeutic component selectively and simultaneously monitoring its distribution. The term “theranostic” is defined as a material that combines the modalities of therapy and diagnostic imaging. Thus, theranostics deliver therapeutic drugs and diagnostic imaging agents at the same time within one dose. The ultimate goal of the theranostics is to gain the ability to image and monitor the diseased tissue, delivery kinetics, and drug efficacy with the long-term hope of gaining the ability to tune the therapy and dose with heretofore unattainable control.

The main project objective is to develop the scientific background for a completely new strategy for theranostics by applying biodegradable and biocompatible nanocarriers that are able to deliver the therapeutic components to the site of its action, image a diseased tissue and monitor the delivery without simultaneously imposing side effects. The use of theranostic nanocapsules may be the ideal solution for future personalized therapy e.g. for cancer treatment.

In 2019 we focused on the synthesis of theranostic polyelectrolyte nanocarriers containing ^{19}F as MRI contrast agent. The nanocarriers were synthesized by the sequential adsorption of charged nanoobjects (layer by layer technique) on nanoemulsion droplets. For the preparation of theranostic nanocarriers we used biocompatible polycation poly L-lysine and polyanion NAFION as fluoropolymer MRI contrast. The size of obtained theranostic nanocarriers was in the required range of 100-300 nm. We obtained the full resonance spectrum of NAFION with five resonances in MRI measurements. These signals were assigned to chemical groups containing equivalent fluorine nuclei. Relaxation times T1 and T2 of individual groups were measured and the number of ^{19}F nuclei estimated for each resonance. For imaging purposes, a peak at +42 ppm was selected because of the relatively high MR signal, well separated from other resonances. MRI imaging measurements showed the possibility of using this type of capsules as theranostic carriers.

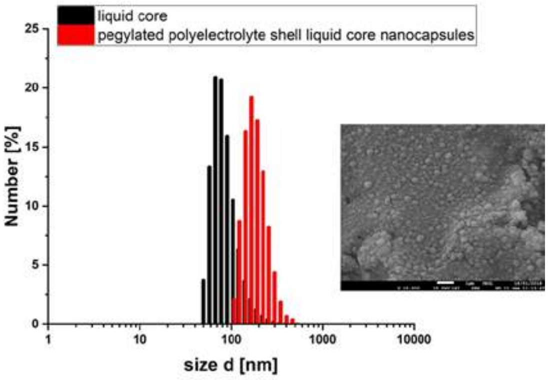


Fig. The size of emulsion droplets (liquid core) and NAFION polyelectrolyte shll nanocapsules measured by DLS method and their visualization by Cryo-SEM.

A New Generation of Hierarchical Y and Omega Zeolite Catalysts: Advanced IR and NMR Studies and Molecular Modeling

(Project leader: prof. Ewa Brocławik)

"Opus" research project NCN 2015/17/B/ST5/00023 [2016-2020]

Si-OH-Al groups in dealuminated zeolites FAU and mazzite were found more acidic than hydroxyls in HMOR and HZSM-5 which are known as strongly acidic zeolites. Very strongly acidity of obtained Si-OH-Al groups was evidenced in low temperature IR studies of CO sorption. Huge red-shift of stretching OH frequency caused by the interaction with CO probe was registered which can be taken as the measure of the acid strength, higher than in other zeolites. Catalytic activity in the α-pinene isomerization also increased distinctly upon desilication due to the production of mesopores, the increase of accessibility and preserving very high strength of acid sites. Recorded 27Al MAS NMR spectra revealed 6 kinds of Al in parent dealuminated zeolite Y (of Si/Al = 31) and in the samples desilicated using NaOH and NaOH/tetrabutylamine hydroxide (TBAOH). They comprised—two tetrahedral forms, AlIVa (typical of a bulk zeolitic framework) and AlIVb (similar to amorphous aluminosilicates), penta-coordinated AlV, and three kinds of hexa-coordinated forms, AlVIa, AlVIb, and AlVIc. The distribution of Al among these sites depended on the stage of zeolite treatment. Desilication removed both Si and Al from zeolite, but while the extracted Si species were prone to self-aggregation and remained in solution, the Al species preferred to be adsorbed onto the zeolite surface. In consequence, the contribution of bulk zeolitic AlIVa decreased upon desilication and the contribution of modified surface AlIVb forms increased. This is important since the effective enrichment of the concentration of surface Al forms is frequently claimed to be the reason behind a hindering of excessive mesopore formation. Quantitative IR study evidenced that Al extracted from zeolite is then reinserted back as tetrahedral Al and showed an ion-exchange capacity. After the exchange of Na+ to NH4+, decomposition of ammonium ions and the subsequent dehydroxylation of the produced hydroxyls, result in the formation of Lewis acid sites. The quantitative IR studies evidenced that the stoichiometry of dehydroxylation was typical of zeolites—loss of two protonic sites produced one Lewis site. The latter findings seemed to carry the main new message from this study. Finally, calcination of the desilicated zeolites decreased the contribution of both AlIVa and AlIVb and produced mostly hexa-coordinated Al forms, mainly AlVIb and AlVIc. It is possible that these hexa-coordinated Al species corresponded to Lewis acid sites formed by dehydroxylation of protonic sites.

These findings completed the studies on a new generation of hierarchical Y and omega zeolite catalysts planned in the project (closing in January 2020).

Nanoparticle Monolayers of Controlled Heterogeneity and Structure as Efficient Antifoulong Substrates

(*Project leader: prof. Zbigniew Adamczyk*)

"Opus" research project NCN 2015/07/B/ST5/00847 [2016-2019]

Deposition mechanism of positively charged gold nanoparticles at silica/silicon substrates was investigated scanning electron microscopy (SEM), and atomic force microscopy (AFM). The gold nanoparticles were synthesized in a chemical reduction method using sodium borohydride as reducing agent. Cysteamine hydrochloride was applied to generate a positive surface charge of nanoparticles. The stability of nanoparticle suspensions under controlled pH and ionic strength was determined by dynamic light scattering (DLS). The electrophoretic mobility measurements showed that the zeta potential of nanoparticles decreased with ionic strength and pH from 56 mV at pH 4.2 and I=10^{-4} M to 22 mV at pH 8.3 and I=$3x10^{-3}$ M. The surface enhanced Raman spectroscopy (SERS) confirmed chemisorption of cysteamine on nanoparticles and the contribution of amine moieties in the generation of nanoparticle charge. The gold nanoparticle monolayers of a coverage determined by SEM and AFM were deposited on silicon/silica wafers under diffusion-controlled transport. The topology of the substrate and the nanoparticle monolayers (see Fig. 1) comprising the root mean square (*rms*) parameter was also quantitatively determined as a function of the particle coverage. For the bare wafer the surface *rms* was equal to 0.15 nm and systematically increased with the nanoparticle coverage. These results were adequately interpreted in terms of a general theoretical model developed for surface covered by features of arbitrary shape. Additionally, the stability and electrokinetic characteristics (zeta potential vs. pH) of the monolayers were acquired using streaming potential measurements. It was shown that the inversion of the negative zeta potential of the bare substrate (overcharging) occurs at the nanoparticle coverage equal to 0.15, whereas for larger coverages positive zeta potential values were asymptotically attained. Additionally, the desorption kinetics investigated by the streaming potential method confirmed the stability of the gold particle monolayers for a broad range of ionic strength and pHs. It was argued that these results enable to develop efficient methods of gold sensor preparation exhibiting well-controlled surface roughness and electrostatic charge comprising both negative and positive values.

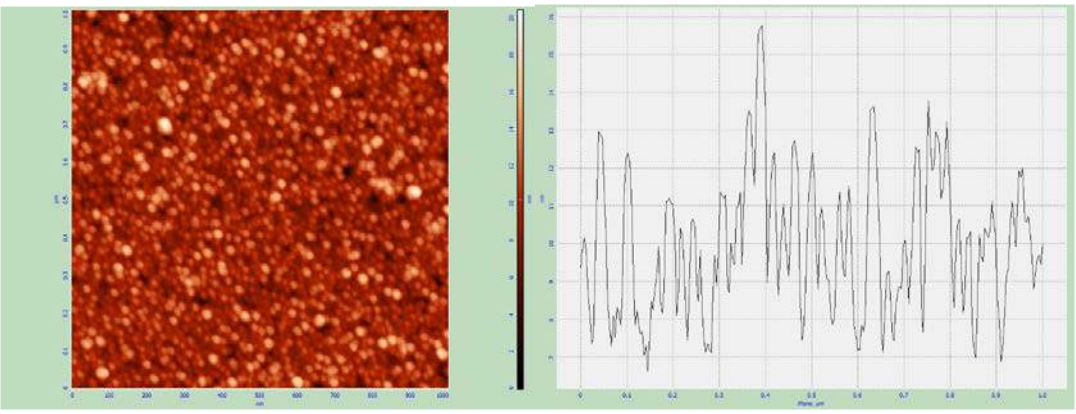


Figure 1. The Au nanoparticle monolayer (particle size 9.5 nm) on the silicon/silica wafer derived from AFM imaging, surface concentration of particles N = 1800 μm^{-2}, the coverage θ = 0.13. The right hand side shows a local profile of the interface for this particle coverage.

Magnetic nanoparticles on periodic iron oxide templates: control of magnetism using particle - substrate interaction and external electric field

(Project leader: assoc prof. Nika Spiridis)

Project: NCN OPUS 2016/21/B/ST3/00861 [2017-2020]

In the cobalt-magnetite and cobalt-hematite layer systems, the correlations between chemical and magnetic properties were investigated using methods with chemical and magnetic sensitivity: conversion electron Mössbauer spectroscopy (CEMS) and X-ray photoemission electron microscopy (X-PEEM).

Epitaxial magnetite $Fe_3O_4(111)$ films (typically 10 nm) were grown on MgO(111) substrates with a Pt(111) buffer by reactive deposition of Fe. Hematite, α-$Fe_2O_3(0001)$ films were prepared by oxidation of the magnetite $Fe_3O_4(111)$ films. The film growth and oxidation (annealing for 5 hours at 500^oC and oxygen partial pressure of $1x10^{-5}$mbar) processes were controlled *in situ* under UHV by CEMS to ensure a perfect magnetite or hematite stoichiometry. Low temperature CEMS measurements showed that the magnetite films have bulk properties (including the Verwey transition), whereas the hematite films do not exhibit the Morin transition down to 100 K.

Cobalt films, flat and wedged, were grown by MBE at room temperature. The wedged films covered a thickness range 0.2-3 nm and included a reference area without Co. All films were covered with a thin MgO or metal layer for *ex situ* measurements.

The X-PEEM experiments were performed at the PEEM/XAS beamline at the Solaris synchrotron. Series of images were taken along the wedge as a function of the X-ray energy covering the L2,3 edges for iron and cobalt for left and right circular polarization of the beam, so that XMCD-images and spatially resolved XAS spectra could be extracted for interesting sample areas.

A different degree of Co oxidation was found for magnetite and hematite. The oxidation of cobalt is accompanied by an interfacial reduction of oxides, which is more pronounced in the hematite XAS spectra. The XMCD images showed a ferromagnetic coupling between magnetite and cobalt and a ferromagnetic domain structure at the iron edge for the Co-hematite interface, identical with the cobalt domain structure.

In situ studies of the domain structure for the Co-magnetite system using magnetic force microscopy (MFM) were also performed.

Development of Modern Generation Technology of Stable Biological Surface Film for Various Bio-Medical Applications (Antibacterial or Regeneration Properties)

(Project leader: Marcel Krzan PhD)

"Opus" research project NCN 2016/21/B/ST8/02107 [2017-2020]

During the project, we want to develop technology for generation and application of stable thin films of surface formulated from mixtures of hypoallergenic, non-toxic biological polymers, mainly biosurfactants (proteins, polysaccharides, glycolipids and phospholipids). The scientific goal of the project is to investigate the competitive adsorption processes occurring in multi-component solutions, where the main substrates are various surface-active bio-polymers. During the project, we want to investigate which of the available biopolymer formulations have sufficient surface activity to be used in cosmetic products. We want to determine the minimum described above necessary to carry out wetting or to create the surface effect of surfactant concentrations. To achieve this effect, we plan detailed research on the tested solutions of biopolymers. We want to set the above-described minimum concentrations necessary to carry out wetting or to create a surface surfactant concentration effect. To achieve this effect, we plan detailed research on the tested solutions and mixtures of biopolymers.

In 2019y we continued the analysis of interrelations between surface activity / dilational elasticity and thin foam film / foam stability in developed mixtures of bio-polymers with high surface activity. We also started to studied into emulsion systems based on developed bio-surfactants mixtures.

We published in 2019y two papers in Colloid Surface A: (IF 3.3) and Colloid Surface B: (IF 4.0). The obtained results were presented during national and international scientific conferences, as “Fizykochemia bez granic” at Lublin; ECIS European Colloids and Surfactant conference, Leuven, Belgium; Bubble and Drops conference, Sofia, Bulgaria; European Student Colloid conference 2019, Varna, Bulgaria; and Annual European Rheology Conference AERC 2019, Prohors, Slovenia (9 presentations, 3 oral and 6 posters). Obtained experimental data were also used to prepare 4 new publications in ISI / JCR journals, which were submitted before end of December 2019.

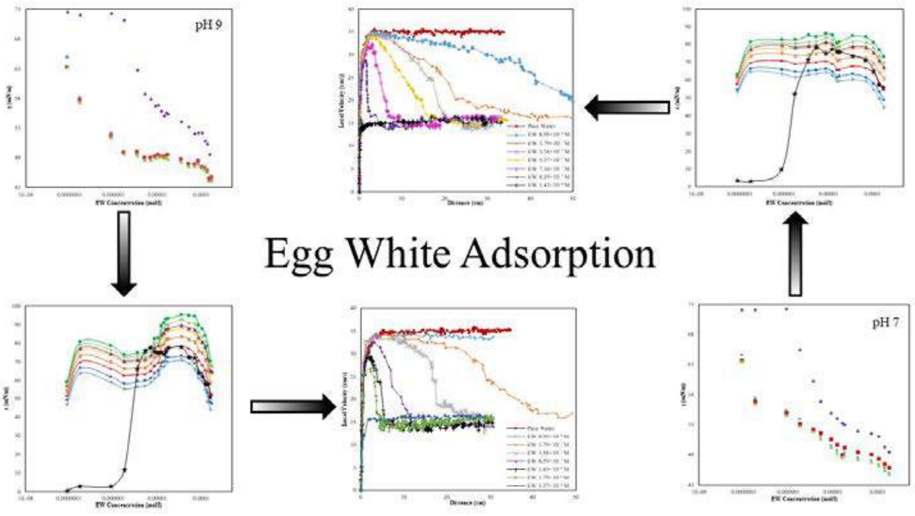


Fig. 1. The dynamic surface tension, surface dilational elasticity and profiles of bubble local velocities of Egg white solutions at pH 7 and pH 9 (Ref. M. Dabestani, M. Krzan, S. Yeganehzad, R. Miller, Characterisation of Egg White adsorption layers under equilibrium and dynamic conditions, Coll. Surf. A, 568 (2019) 29-35)

The mechanism of regioselective oxidative dehydrogenation of 3-ketosteroids catalyzed by Δ^1-cholest-4-en-3-one dehydrogenase from Sterolibacterium denitrificans

(Project leader prof. M. Szaleniec)

"Opus" research project NCN 2016/21/B/ST4/03798 [2017-2020]

The aim of the project is to evaluate the reaction mechanism of steroids dehydrogenation catalyzed by Δ^1-cholest-4-en-3-one dehydrogenase (AcmB). The enzyme is produced by denitrifying bacterium, *Sterolibecterium denitrificans,* and belongs to the FAD-dependent 3-ketosteroid dehydrogenases. It catalyzed the oxidative dehydrogenation od 3-ketosteroids that leads to formation of a double bond between C1 and C2 atoms of a steroid ring A. The project focus on elucidation of the enzyme's structure together with characterization of its catalytic properties. Furthermore the theoretical calculations are applied for study of the reaction mechanism.

The third year of project was a breakthrough in study of AcmB. Thanks to cooperation with a group of professor W. Minor it was possible to crystalize the enzyme, measure its diffraction pattern and solve its structure. Currently the structure refinement is underway, especially in the regions of mobile loops. However, the electron density of the active site was good enough to allow construction of the cluster model that was used to conduct quantum chemical modeling of the classical reaction mechanism. This type of mechanism was already confirmed as the most probable based on the theoretical results obtained for KSTD from *R. erythropolis.*

In parallel to structure determination of AcmB we continued the mechanistic study of ketosteroid dehydrogenation using models derived from KSTD from *R. erythropolis.* In cooperation with a group of professor V. Moliner the QM:MD methodology was used to study several variants of reaction mechanisms. The potential mean force (PMF) for both proton transfer from C2 and hydride transfer from C1 to FAD were calculated. The obtained models were used in estimation of kinetic isotope effects (within the quasi-transition state theory). Additionally, the methodology was used to investigate the effect of the mutations (which were already reported in the literature) as well as reaction pathway for different substrates.

The theoretical studies were accompanied by a series of kinetic measurements utilizing stopped flow equipment in the steady-state and pre-steady-state regimes. The steady-state measurements allowed comparison of the apparent kinetic constants for a range of 3-ketosteroids. Furthermore the KIE was measured using direct method for substrates selectively labelled at C1 or C2 atoms with deuterium. The results showed a very small (close to 1) KIE. The kinetics yielded also specific activities of the mutated enzyme variants of AcmB, which allowed correlation of the observed activity with the role of mutated residues in the mechanism. The pre-steady-state measurements delivered observed constants of reduction and reoxidation half reaction cycles and determination of the kinetic isotope effects for k_{red} for both AcmB and KSTD. In both cases the obtained values were higher than those obtained in a steady-state approach but lower than those predicted by theoretical modeling. The difference between KIE determined in steady-state and pre-steady-state kinetics was explained by significantly lower k_{ox} by DCPIP in comparison to k_{red} by substrates.

Finally a series of reactor tests was conducted in the optimal conditions for different reoxidation agents (PMS, PMS/DCPIP, DCPIP, DCPIP, $K_3Fe(CN)_6$) using HPLC as a analytic method. Based on this it was possible to select optimal reoxidant in terms of reactor productivity and stability as well as to compare initial activity of the enzyme under different conditions.

Theoretical and Experimental Studies on the Mechanism of Oxidative Dehydrogenation (ODH) of Light Alkanes over Vanadium-containing Hierarchical Zeolite Materials

(Project leader: assoc prof. Dorota Rutkowska-Żbik)

"Opus" research project NCN 2016/23/B/ST4/02854 [2017-2022]

The aim of this joint experimental and theoretical work is to gain understanding in the nature of the V species responsible for their catalytic activity in the oxidative dehydrogenation (ODH) of light alkanes into light alkenes and to determine the mechanism of the ODH process.

The additional series of vanadium catalysts were obtained: based on the FAU zeolite matrix with the amount of vanadium changing in the range 1 – 6 %mas. And analogous series in which desilicated FAU was used. As the reference samples the following systems were selected: the systems in which the same amount of vanadium was deposited ion high-surface silica (cabosil) and in which vanadium was introduced to FAU by ion-exchange method.

The physico-chemical characterization of obtained samples was started in order to verify their chemical composition and qualitative description of the vanadium active centres. The XRD diffractograms are similar and do not contain peaks derived from other crystallographic phases than FAU (or SiO_2 in case of SiO_2-based systems) unless the V content is the highesr, for which peaks derived from V_2O_5 were registered.

Vanadium ions are well dispersed. Introduction of V results in slight expansion of BEA structure and suggests that vanadium is incorporated into BEA structure. The obtained materials exhibit specific surface area of 670-750 m^2/g (FAU-based samples) and 260-290 m^2/g (SiO_2-based samples) depending on the amount of introduced vanadium. In order to study the speciation of vanadium, the obtained samples were examined with UV-VIS spectroscopy. Depending on the synthesis method, the samples exhibit peaks at 270 and 340 nm, characteristic for tetrahedral V atoms. At higher vanadium loadings (ca. 5%), there appear peaks at 430 nm typical for octahedral vanadium atoms.

The catalytic tests of the obtained samples were started in the ODH of propane performed in a fixed bed gas flow reactor in the temperature range between 400 – 520°C. The highest activity was registered for the V_2SiBEA sample, in which only tetrahedral vanadium is observed (see Figure 1): the selectivity to propene reached 80% with 20% conversion (at 450 °C, flow 1s) – see Figure 2.

Additionally, the DFT method (BP/def2-TZVP) was used to describe geometry and electronic structure of BEA zeolite substituted with vanadium atoms placed in all inequivalent T-sites. The quantum-chemical calculations aimed at the determination of electronic and geometry structure of the proposed sites and their properties were initiated. The following form of vanadium ions were considered: V(=O)(-OH).

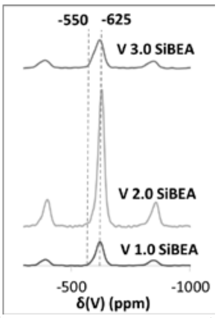


Figure 1. ^{51}V MAS NMR spectra of V_xSiBEA.

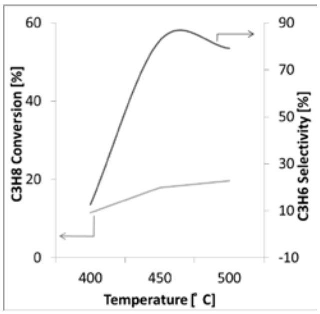


Figure 2. C_3H_8 conversion and C_3H_6 selectivity in propane ODH over $V_{2.0}$SiBEA.

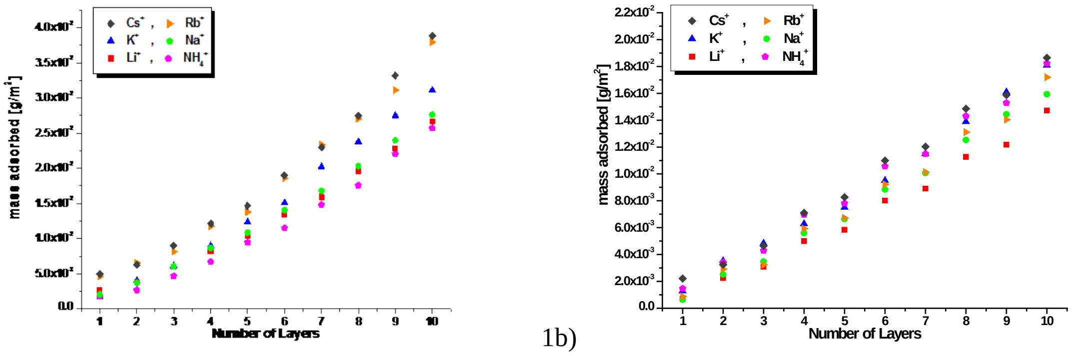
The influence of counterions on the formation and functionality of polyelectrolyte membranes

(Project leader: Marta Kolasińska-Sojka DSc)

"Opus" research project NCN 2016/23/B/ST8/03128 [2017-2020]

The scientific goal of the project is to investigate the dependence of the structure and properties of polyelectrolyte multilayers on selected ions from the Hofmeister series. The impact of the interaction of counter-ions from the liotropic series on the resulting polyelectrolyte films is unknown and thus, understanding the mechanisms responsible for creating such structures may contribute to the design of materials with well-defined parameters, which will improve the properties of selective membranes, biomaterials and nanocontainers in the future.

The ongoing work concerned the comparison of the effect of monovalent cations: Li^+, Na^+, K^+, Rb^+, Cs^+, $NH4^+$ on the formation of PDADMAC/PSS and PAH/PSS multilayer polyelectrolyte films. The quartz crystal microbalance was used to study the kinetics and efficiency of the multilayer deposition process in the presence of selected basic electrolytes: LiCl, NaCl, KCl, RbCl, CsCl, NH_4Cl at ionic strength I = 0.15 M. The results of this part of the work are presented in Fig. 1.



1a) 1b)

Fig. 1 Mass of deposited polyelectrolyte films as a function of adsorbed layers for the systems studied: a) PDADMAC/PSS and b) PAH/PSS

The mass adsorbed in the case of the PDADMAC/PSS (1a) system is bigger than for films with an analogous number of layers but made of PAH/PSS polyelectrolytes (1b), which is consistent with our previous research. In the case of the PDADMAC/PSS multilayers, a more indicated effect of the counterion on the adsorbed film is observed. For 10-layer PDADMAC/PSS systems, the lowest mass is for a film with NH_4^+ counterion while the highest is for films adsorbed in the presence of Cs^+.

Then, permeability to electroactive probes was tested for analogous polyelectrolyte films, built up in the presence of selected monovalent cations on gold disc electrodes. As a redox system an equivalent mixture of 1mM $K_3Fe(CN)_6$ / $K_4Fe(CN)_6$ in 0.15 M NaCl was used.

It has been shown that the permeability of multilayers towards the selected electroactive agent is reduced as a result of covering of the gold electrode with a polyelectrolyte film. The degree of permeability also depends on the counterion used. It has been observed that in the case of 9-layer films, the permeability is directly proportional to the hydration radius of a selected cation. In the case of 10-layer films, the effect of blocking of the electroactive agent by the polyelectrolyte film terminated with negatively charged layer plays an important role and the differences in permeability depending on the counterion used are much more subtle, although still visible.

Dendrimers as a platform for designing biologically active carrier

(Project leader: Assoc. Prof. Barbara Jachimska, Paulina Komorek MSc, Magdalena Szota MSc)

"Opus" research project NCN ST5/02788 [2017-2020]

Due to the specific structure, dendrimers enable the transport of the active substance, both by binding it to surface groups and in free spaces inside the molecule structure. Studies on the immobilization of the active agent in the dendrimer structure were tested by optimizing the conditions for forming stable complexes with the anti-cancer drug fluorouracil. The efficiency of the complex formation was monitored by controlling changes in the carrier zeta potential, measuring UV-vis spectra, CD and the adsorption efficiency of the active complex by QCM-D, SPR. The impact of internal body environment conditions in which a given therapeutic would perform its functions was tested by the interaction of the dendrimer carrier with blood plasma proteins. Studies have shown that the immobilization of drug molecules and proteins significantly reduces the effective surface charge of the dendrimer, which is valuable information for the design of controlled drug delivery systems. In addition, dendrimers are more effective in forming a stable fluorouracil complex than protein, which can provide a protective coating for the active dendrimer complex, thereby eliminating the possible cytotoxic nature of the carrier itself.

Determining the mechanisms of interaction of functional materials with proteins present in plasma together with the analysis of conformation changes and reorganization of protein structures on functional surfaces is of significant cognitive importance. The development of direct methods enabling the measurement of the adsorption kinetics of plasma proteins on the surface of a PAMAM-type dendrimer carrier and determining the optimal conditions for the formation of a dendrimer complex containing an active therapeutic agent seems important due to controlling the pharmacokinetic profile of the drug used.

In search of effective and environmental friendly frothers and emulsifiers – quantitative description of thin liquid film stability in solutions of “green” surfactants

(Project leader: assoc. prof. Jan Zawała)

"Opus" research project NCN 2017/25/B/ST8/01247 [2018-2021]

The presented research project is aimed to investigate potential application of bio-surfactants as new, effective and environmental-friendly stabilizers of foam and emulsion films under dynamic conditions. Scientific tasks of the project are performed according to the new methodology and by use of the new experimental set-up elaborated within the project framework. The experimental set-ups allow description of kinetics of drainage of single symmetrical liquid films (foam and emulsion), formed at the liquid/gas and liquid/liquid interfaces by the colliding bubbles and /or droplets in solutions of biodegradable and environmental-friendly surfactants.

In the reporting period the experimental set-up allowing determination of the time of drainage of a single foam film formed under dynamic conditions, i.e. during a single bubble collision with a free solution surface, was elaborated. The experimental set-up was composed of single bubble generator (elaborated in our research group), glass column and device for determination of a single bubble lifetime at the surface of solutions of various surface-active substances - SAS (CCD camera, coupled with elaborated software). The bubble lifetime was automatically measured as a time span between the moment of the bubble first collision with the solutions surface and the moment of bubble rupture. In addition, to control the initial adsorption coverage at the bubble surface, so-called “bubble trap”, also elaborated in our research team, was applied. On the basis of such experiments, qualitative analysis of influence of the SAS solutions concentration and adsorption coverage at the liquid/gas interface on foam film stability was possible.

Example of the results on bubble lifetimes at the surface of cetyl-trimethyl-ammonium bromide (CTAB) solutions is presented in the Fig. 1. In Fig. 1A the lifetimes for different SAS concentrations are presented for short adsorption times (t_{ads}), while in the Fig. 1B the data for identical SAS concentration but different t_{ads} values are shown. It was found that, as could be expected, the foam film stability increases with increasing bulk solution concentration (see Fig. 1A). This result was obtained only for comparison with corresponding data with t_{ads} control (when “bubble trap” was used). The comparison revealed that independently on the value of initial adsorption coverage at the bubble surface the formed foam films are of similar stability. This was a proof that the adsorption coverage increases during the bubble motion in solution before liquid film formation and, under favorable conditions, can even reach the equilibrium value.

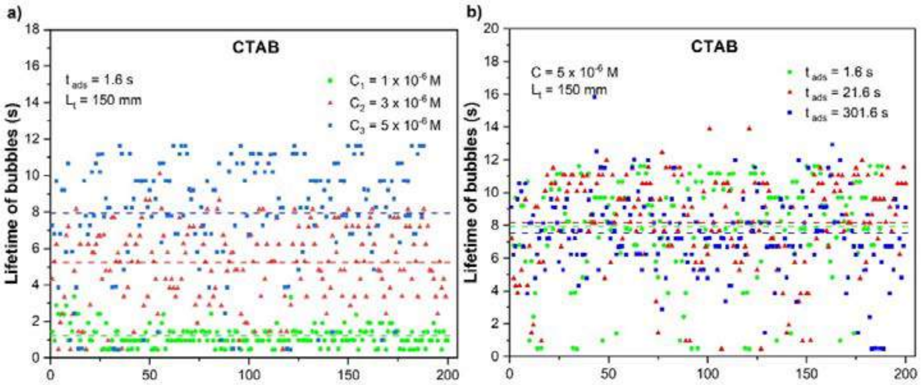


Fig. 1. Lifetimes of single foam films formed under dynamic conditions in solutions of CTAB

PH stimuli responsive surfactants and copolymers for nanovehicles formation

(Project leader: prof. Piotr Warszyński)

"Opus" research project NCN 2017/25/B/ST4/02450 [2018-2021]

The main project objective, realized by the consortium of two research groups from Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences and from Wrocław University of Science and Technology, Faculty of Chemistry, is to develop scientific background of a novel strategy of formation of pH sensitive nanostructures: thin films or nanocapsules. Using newly-synthesized amphoteric surfactants with hydrophobic chains of different lengths, mixed with polyelectrolytes and/or amphiphilic polyelectrolytes with hydrophobic groups grafted on the polyelectrolytes backbone by pH-labile linker, we will construct nanostructures which decomposition can be trigger by change of pH. Application of this type of components will allow to obtain nanostructured thin films with controlled hydrophobicity, or antimicrobial properties sensitive for changes of environment.

We synthesized amphiphilic polyelectrolytes on the basis of poly(4-styrenesulfonic-co-maleic acid) (PSS/MA) with hydrocarbon chains (C12 or C16) linked by pH-sensitive, amide and ester groups. Obtained degree of hydrophobization was 15% or 40%. Using the pendant drop shape analysis method, we determined adsorption kinetics (dynamic surface tensions) and equilibrium interfacial tension of solutions of amphiphilic copolymers at water/decane interface. It appeared that a given concentration copolymers with amide linker exhibited lower interfacial tension than ones with ester linker, presumably to higher self-aggregation ability of the latter. Additionally adsorption of amphiphilic copolymers at liquid/solid interface was studied by QCM-D techniques. The polymers were adsorbed on top of polyelectrolyte multilayer formed with the combination of PSS and PDADMAC. After adsorption the polymeric films were exposed to the condition of high pH. The observed loss of film mass could be attributed to the cleavage of hydrophobic chains due to base catalyzed hydrolysis of amide of ester bonds.

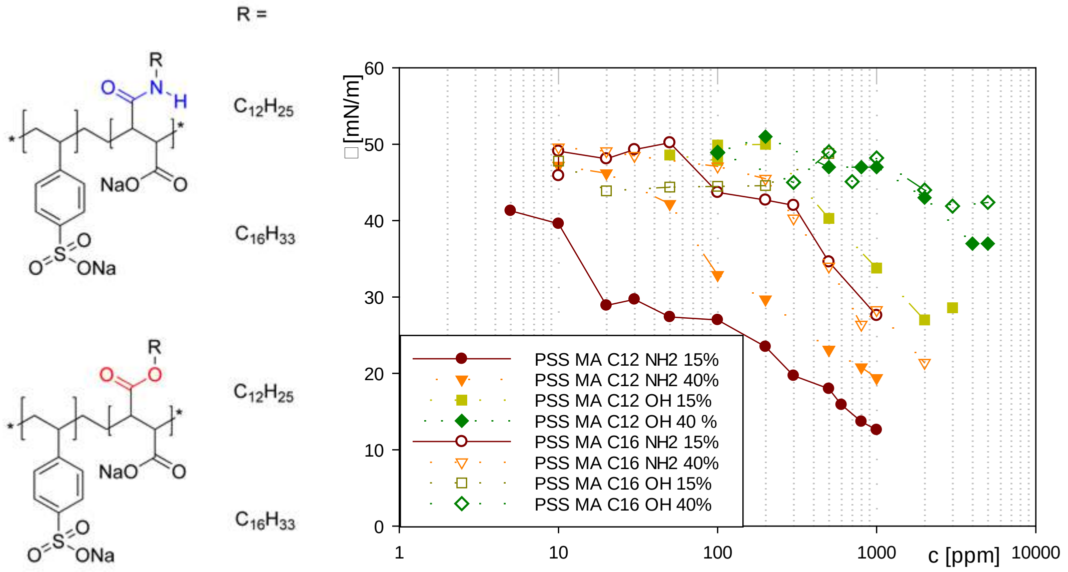


Fig. Molecular structure and interfacial tension isotherms of amphiphilic polyelectrolytes synthesized on the basis of poly(4-styrenesulfonic-co-maleic acid) with amide and ester linker.

Studies of the interactions of carbon nanotubes with telomeric DNA by means of the molecular dynamics simulations

(Project leader: prof. Tomasz Pańczyk)

"Opus" research project NCN 2017/27/B/ST4/00108 [2018-2021]

Analysis of mechanism of interaction of telomeric DNA fragments, particularly non canonical forms, that is i-motif, iM, or G-quadruplex, Gq, requires good understanding of phenomena occurring also without the presence of carbon nanotubes. Therefore, the starting point of the consideration was the structure in which iM and Gq form in the same place of the DNA. Additionally each of the structures was capped by canonical double helix (TTAGGG):(CCCTA) repeated sixfold. As reference structures two simplified cases were studied: iM with the remaining part of the cytosine-rich helix and bare iM. The iM was studied as protonated one iMp (corresponding to acidic pH) and nonprotonated iMn (representative to neutral pH). Analysis of the root of mean squared deviation rmsd as a function of time, choice of structure and pH led to conclusion that iMp is highly stable but its deprotonated version iMn undergoes spontaneous deterioration. In order to study the thermodynamic stability of the iM the two dimensional metadynamics was applied. Two stable states have been detected that is the native iM structure and partially unfolded hairpin structure.

The next stage of the studies was analysis of the work of unfolding of iM and Gq with the rmsd restraints using steered molecular dynamics. It was possible, using this method, to determine the potentials of mean force using Jarzyński equation. The calculations led to conclusion that enforced unfolding of iM does not affect the state of Gq but the unfolding of Gq leads to some deformations of iM. Interestingly, the disordered guanine-rich strand reveals stronger stabilizing effect than the perfect Gq structure itself.

The collected data concerning stabilities of iM and Gq, also in a form of iG structure, could then be used in analysis of stability of these structures when iM is in contact with a functionalized carbon nanotube fCNT. Main results collected in this stage of the studies are: conclusion that iM adsorbs strongly on the fCNT surface and the presence of functional groups has rather minimal effect on its behavior. iM migrates on the fCNT surface rather freely and adjust the bottom orientation towards the CNT surface. iMp turned out to be very stiff structure when compared to iMn which undergoes deformations. It also turned out that narrow (10,0) nanotubes lead to a faster deterioration of iMn that the wider (20,0) CNT.

Adsorption of nonprotonated iMn turned out to be quite strong and the enforced desorption of iM led, in some cases, to the unfolding of iM prior to its desorption. The final conclusion, which can be drawn from that stage of the studies, is that there are no clear premises to prove the role of single walled CNT as factor stabilizing the iMn. Such effect was described in literature. Our results suggest that that effect can be related to proton transfer between carboxyl functional groups and cytosines leading to formation of semi-protonated pairs C-C+ which lead to strong increase of the stability of iM even at the neutral pH.

Biopolymers as templates for preparation of nanostructured hydrotalcite-like materials and their calcined mixed oxides derivatives for catalytic applications

(Project leader: prof. Ewa Serwicka-Bahranowska)

"Opus" research project NCN 2017/27/B/ST5/01834 [2018-2021]

The project sets the frame for fundamental research into designing nanostructural hydrotalcite-like compounds (Htlc) with aid of commonly available, cheap and environmentally friendly natural biopolymers (e.g., starch, gelatin), used in the capacity of soft biotemplates. The approach represents an alternative to the use of the more expensive, more complex and less green procedures based on synthetic templating agents (e.g., surfactants, block copolymers).

Research concentrated on establishing chief factors determining the physico-chemical properties of Mg-Al hydrotalcite materials (Ht) prepared with the use of starch as a structure controlling biotemplate. The syntheses were carried out at room temperature, by co-precipitation at pH=10. The investigated synthesis parameters included the nature of the precipitating agent ($NaOH/Na_2CO_3$ or $NH_3aq/(NH_4)_2CO_3$), the nature of starch (potato, corn, cassava), the method of starch addition to reagents, the method of drying and the effect of washing. The materials were examined with XRD, SEM/EDS and FTIR. Results showed that the use of the ammonia-based precipitant enabled preparation of nanocrystalline solids with very fine (< 50 nm) particle size, comparable with those obtained by means of the inverse micellar route, but at much lower cost and with significantly less effort. Under the adopted experimental conditions all investigated types of starch had a similar effect on the crystallinity and the grain size of the Ht precipitates (Fig. 1). Freeze-drying was the preferred method for achieving the possibly low Ht crystal size. Ht with smallest nanocrystals was obtained when starch was present in all solutions used during synthesis. Synthesis in the presence of starch gel hindered incorporation of carbonate anions, and the layer charge was preferentially compensated by nitrates from metal salts solution. Washing with water induced a rapid recrystallization of the as received Ht materials and caused exchange of nitrates with carbonates. FTIR spectra showed that an interaction exists between the biopolymer template and the Ht particles, resulting in a higher degree of order within the starch component adhering to Ht surface.

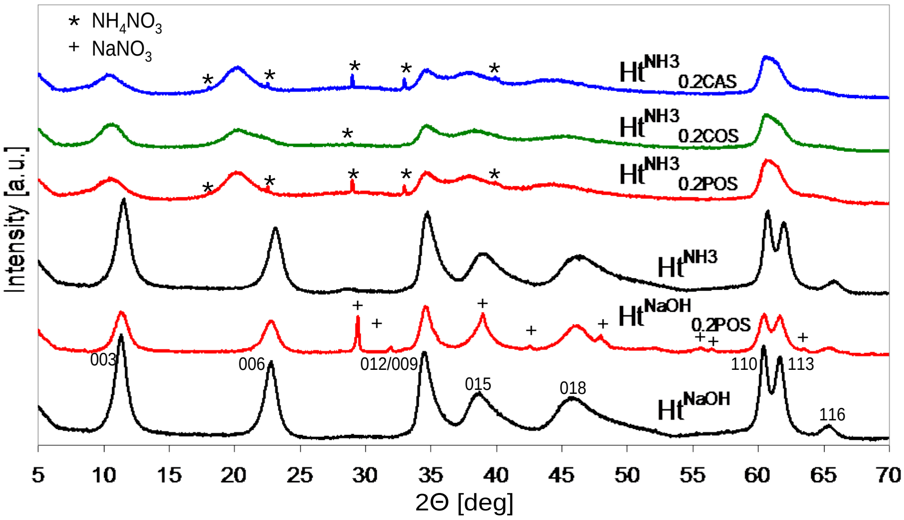


Fig. 1. XRD patterns of Ht materials obtained with the use of different precipitating agents and in the presence or absence of various starches (POS = potato starch, COS = corn starch, CAS = cassava starch).

New polysaccharide-based biomaterials as an effective platform for adsorption and release fibroblast growth factors: applications in diagnostics and treatment of diseases of affluence

(Project leader: Aneta Michna DSc)

"Opus" research project NCN 2018/31/B/ST8/03277 [2019-2022]

The main objectives of the project are: to develop a quantitative description of adsorption of biocompatible polyelectrolytes (polycations and polyanions), possessing various shapes, charges and polydispersity, and forming anchored layers at solid /liquid interfaces; to determine the adsorption rate and the stability of the biomaterials formed by sequential adsorption of oppositely charged polyelectrolytes; to explain the mechanisms of binding of two types of fibroblast growth factors (FGF) to the biomaterials (used as a platform for FGF adsorption); to determine the FGF desorption rate, and to calculate the protein-carrier binding energy.

The polycations of different shape and polydispersity (generation 8 PAMAM dendrimers (PAMAMG8), polyallyldimethylammonium chloride (PDADMAC), branched polyethyleneimine (bPEI)) were thoroughly characterized in bulk during the time of the reporting period. The diffusion coefficients and the electrophoretic mobilities of the polycations were measured in various electrolytes (NaCl, KCl, $MgCl_2$, $CaCl_2$), ionic strengths (10^{-2} M, 0.15 M) at pH range 3-12. The performed measurements allowed us to determine the hydrodynamic diameters and the zeta potentials of the polycations as well as their isoelectric points.

It was found that the hydrodynamic diameter of the polyelectrolytes was practically constant and independent of the electrolyte type, the ionic strength and solution pH. The determined hydrodynamic diameters were equal to: 10.8 nm for PAMAMG8, 36 nm for PDADMAC, and 14 nm for bPEI. The zeta potential of the polycations decreased with the ionic strength increasing for all types of electrolytes. Furthermore, the zeta potentials of PAMAMG8 and bPEI decreased with pH increasing. The obtained zeta potential-pH dependence allowed us to evaluate the isoelectric points (IEP) of the polycations. IEPs were equal to 10.4 and 10.8 for PAMAMG8 and bPEI, respectively.

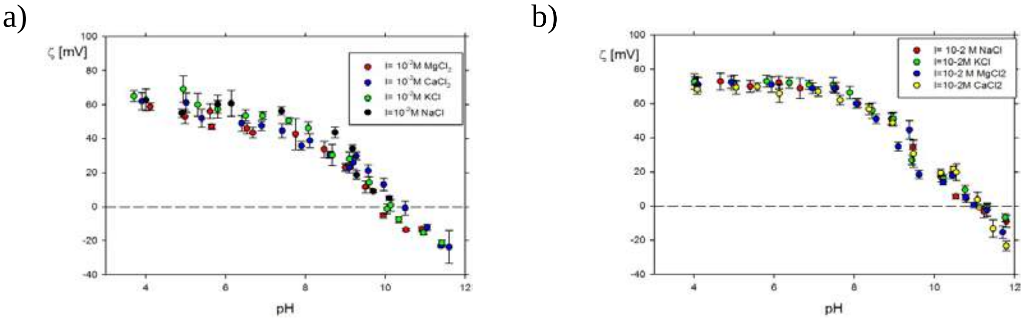


Fig. 1 The dependences of zeta potentials (ζ) of: a) dendrimers (PAMAMG8) and b) branched polyethyleneimine (bPEI) on pH in various electrolytes. The results were obtained for the ionic strength of 10^{-2} M.

Moreover, a unique streaming potential cell was constructed that will be used to determine: the zeta potential of the Si/SiO_2 coated with biomaterials (polyelectrolyte mono and multilayers), the stability of the biomaterials, and to evaluate the kinetics of adsorption and desorption of FGF.

Using quartz crystal microbalance with dissipation (QCM-D), it was found that the ionic strength of 10^{-2} M, pH 4.0, 5.8 and 7.4 as well as simple electrolyte (NaCl, KCl) did not have any influence on the PAMAMG8 adsorbed mass. For these conditions the mass of PAMAMG8 monolayer was practically constant and was equal to 2.4 mg /m^2.

Novel composites of smectite minerals and TiO_2 nanoparticles prepared by inverse microemulsion method for photocatalytic applications

(Project leader: prof. Ewa Serwicka-Bahranowska)

"Opus" research project NCN 2018/31/B/ST5/03292 [2019-2022]

The scientific aims of the project are: a) to obtain TiO_2/clay composites according to a novel design based on the synthetic procedure employing exfoliated organoclays and inverse microemulsion of TiO_2-based nanoparticles, and (b) to test the newly engineered materials as photocatalysts for purification of water from organic pollutants. The proposed novel synthetic procedure offers a number of possibilities for control of composite properties via a) changing the nature of clay, b) changing the organocation, c) modification of oxide naoparticles size by appropriate choice of the microemulsion synthesis parameters. Moreover, inverse microemulsion method enables synthesis of multicomponent oxides, which opens way for chemical modification of TiO_2 nanoparticles.

In the first months of project implementation investigations concentrated on the comparative study of TiO_2/clay composites synthesized by conventional pillaring of Na-montmorillonite and by the novel strategy based on combining organomontmorillonite and TiO_2 nanoparticles prepared by inverse microemulsion. It has been found that in the latter case TiO_2 forms anatase particles, of structural and textural properties significantly different than those found in the pillared counterparts. As a result, the pore size distribution profile of TiO_2/clay composite obtained by inverse microemulsion method is more homogeneous than the one characteristic of TiO_2-pillared sample, with maximum shifted from ca. 2.5 nm to about 6.7 nm, thus indicating a more open, mesoporous network, which should facilitate diffusion of reagents in the photocatalytic tests in aqueous medium (Fig. 1). Anatase crystal size in the novel composites was estimated to be around 7 nm, which corresponds very well with the dominant pore size and suggests that the uniform size of TiO_2 grains formed within inverse micelles is a chief factor determining the mesoporosity of composites prepared with via inverse micellar route.

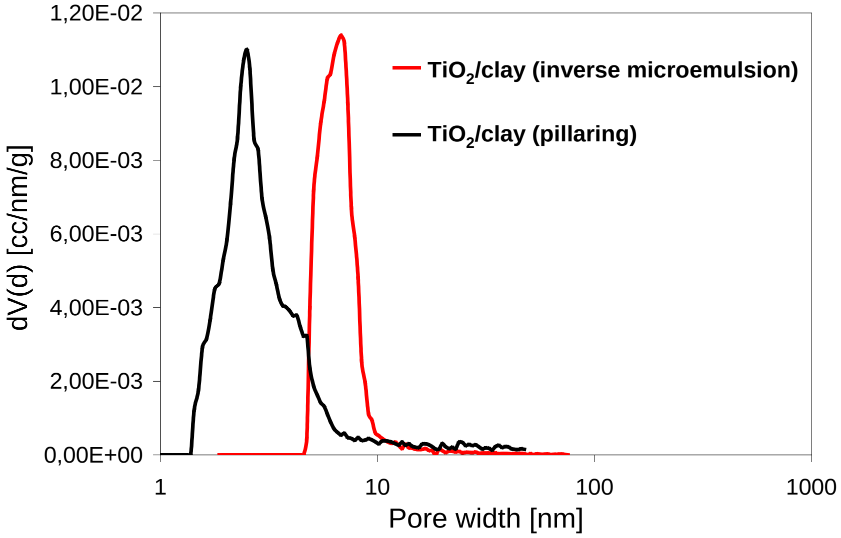


Fig. 1. Comparison of DFT pore size distribution profiles of TiO_2/clay composites prepared by pillaring and by inverse microemulsion methods.

"Preludium" Research Projects of the National Science Centre

Synthesis of biopolymer-based nanoparticles

(Project leader: Marta Szczęch MSc)

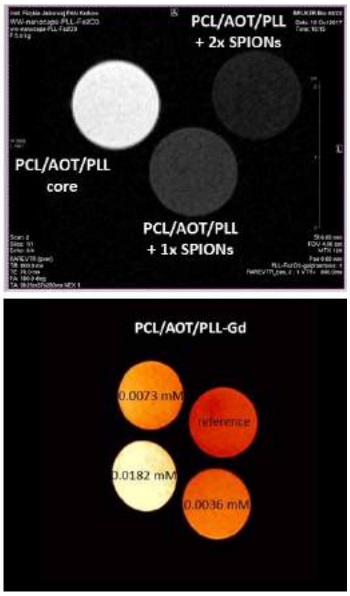
“Preludium” research project NCN 2016/23/N/ST5/02783 [2017-2019]

Hybrid (multifunctional) nanoparticles (HNPs) are a very promising group of nanosystems with a great biomedical application, especially in nanomedicine. One of the types of these nanosystems are theranostic nanoparticles that allow to delivering of both therapeutic and imaging agents in the same dose. This multifunctional aspect is a huge advantage of the HNPs but there are still some obstacles that limited their application in clinical trials e.g. ineffective delivery of the hybrid nanosystems to the specific part of the body, low content of imaging agents or limited biodistribution.

The aim of the “PRELUDIUM” project was to develop the scientific background of the synthesis methodology of biocompatible and biodegradable hybrid nanoparticles based on bio-polymers as nanocarriers for therapeutic substances (hydrophobic) with incorporated magnetic nanoparticles for MRI.

The project “PRELUDIUM” finished in October 2019. It allowed achieving the following goals:

- ✓ method of the preparation of biocompatible and biodegradable polymeric nanoparticles (polycaprolactone (PCL), polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA) was developed using the Phase Inversion Composition technique (PIC), Spontaneous Emulsification Solvent Evaporation technique (SESE), and Spontaneous Emulsification Solvent Diffusion, technique (SESD).
- ✓ method of the preparation of ‘core-shell’ nanoparticles by modification/functionalization of obtained polymeric nanoparticles was developed,
- ✓ method of encapsulation of hydrophobic active agents (drugs) such as Coumarin-6 (a fluorescent dye, and an example of the model hydrophobic drug), and Paclitaxel (Taxol, an example of the anticancer drug) was developed,
- ✓ method of the preparation of theranostic nanocarriers by incorporation of MRI contrast agents i.e. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) and Gadolinium complexes with Poly-L-lysine was developed,
- ✓ method of the preparation of nanocarriers for passive targeting drug delivery systems was developed,
- ✓ method of the preparation of magnetically responsive drug delivery nanosystems was developed,
- ✓ cytotoxicity tests of obtained nanocarriers and the evaluation of encapsulated drug activity were performed.



The MRI analysis of obtained hybrid nanoparticles based on PCL NPs.

Functional hybrid nanomaterials based on poly(amidoamine) PAMAM dendrimers

(*Principal Investigator: Karolina Tokarczyk MSc; Supervisor: assoc prof. Barbara Jachimska)*

"Preludium" research project NCN 2016/23/N/ST4/02532 [2017-2019]

The development of controlled systems for delivering biologically active substances to the body is particularly crucial in nanomedicine. The main element of drug delivery systems is the use of nanocarriers that should be well characterized at the molecular level before the use. Methods optimizing the delivery of active substances are developing very dynamically, which results in searching for new models of supplied active substances that are taken into account. Dendrimers are a promising group of drug carriers because of their unique structure. They can be used in diagnostics and medical therapies.

Using the research methodology proposed in the project, the mechanism of action of hybrid systems at the molecular level was investigated. This is an important aspect of developing therapeutic strategies. Interactions of G4 PAMAM dendrimers with the active substance, 5-fluorouracil were analyzed, taking into account the interactions of complexes with blood plasma protein, bovine serum albumin. Thanks to this, it was possible to study the effectiveness of hybrid systems in the internal environment of the body in which the therapy will perform its function. The interaction of the therapeutic agent with the dendrimer carrier and the efficiency of immobilization is an important parameter for the design of theranostic systems. Characterization and optimization of the physicochemical properties of nanosystems were performed, both in solution and on the solid-liquid interface. The hydration degree of created systems and the influence of electrostatic and hydrophobic interactions on the complex formation process were determined. The obtained results form the basis for research on drug delivery systems based on the structure of dendrimers with an improved pharmacokinetic profile.

The results obtained as part of this research project have enriched the basic knowledge about the mechanisms of complex formation based on PAMAM dendrimers. The effect of blood plasma proteins on the properties of dendrimers as well as the effect of dendrimers on protein stability and structure were determined. The efficiency of the nanocarrier operation was studied while maintaining the internal environment conditions of the body, thanks to which the possibilities of designing nanohybrid systems in terms of the required physicochemical properties in the aspect of their further applications in nanomedicine as intelligent pharmacological carriers were expanded.

Effect of controlled adsorption time on kinetics of three-phase contact formation at solid surfaces with different hydrophobicity

(Agata Wiertel-Pochopień MSc, Supervisor: assoc. prof. Jan Zawała)

"Preludium" research project NCN 2017/27/N/ST4/01187 [2018-2020]

The goal of this project is to determine the effect of controlled time available for adsorption of surface-active substances (SAS) at an air bubble (gas/liquid) interface on kinetics of its attachment and three-phase contact (TPC – gas/liquid/solid) formation at a solid surface of different hydrophobicity.

Experiments on adsorption kinetics of the surface-active substances with identical hydrophilic group – alkyltrimethylammonium bromides C_nTAB (octyltrimethylammonium bromide - C_8TAB, dodecyltrimethyloammonium bromide - $C_{12}TAB$, hexadecyltrimethyloammonium bromide - $C_{16}TAB$), was performed to determine the values of solutions surface tension as a function of their concentrations. Then, using these data, adsorption time needed to achieve precise value of adsorption coverage at bubble surface was calculated on the basis of Ward-Tordai equation. Based on the adsorption kinetics it is possible to control a degree of adsorption coverage at the gas/liquid interface without changing the bulk solutions concentration. Additionally, flotation of quartz particles in solutions of C_8TAB, $C_{12}TAB$, $C_{16}TAB$, $C_{18}TAB$ (octadecyltrimethyloammonium bromide) and their mixtures with n-octanol of concentration $5{\cdot}10^{-4}$ M were carried out. Fig. 1 shows dependence of quartz flotation recovery on C_nTAB concentrations in pure and mixed solutions. Addition of n-octanol as a nonionic surfactant increases recovery of quartz (synergistic effect), e.g.: at $C_{16}TAB$ concentration of 5×10^{-7} M the recovery was equal to ca. 25% and in mixtures was equal to ca. 90% (see Fig. 1C).

It was found that there is a correlation between time of the three phase-contact formation by air bubble colliding with quartz surface, contact angle of quartz and flotation recovery of quartz particles in $C_{16}TAB$ solutions.

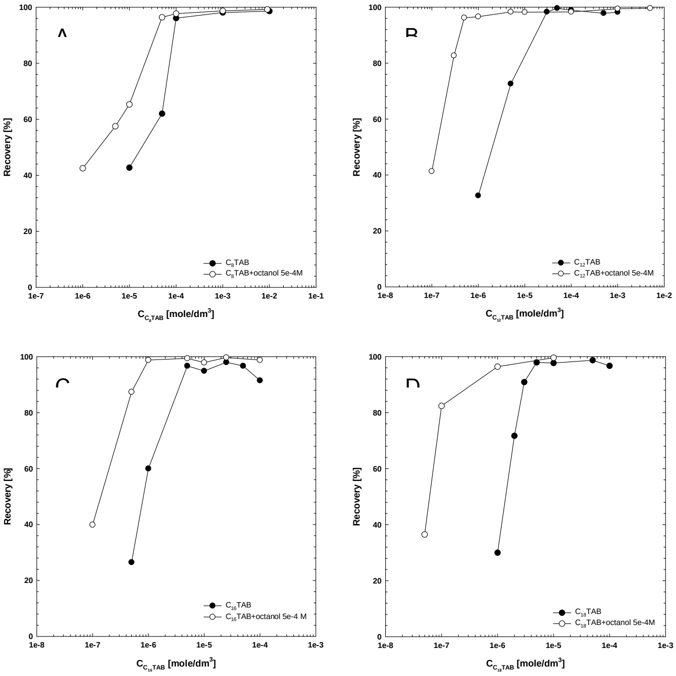


Fig. 1. Quartz recovery in pure (A) C_8TAB, (B) $C_{12}TAB$, (C) $C_{16}TAB$ and (D) $C_{18}TAB$ solutions and their mixture with n-octanol solution of concentration $5{\cdot}10^{-4}$ M

Tungsten-containing aldehyde oxidoreductase from Aromatoleum aromaticum - study of catalytic reaction mechanism

(*Agnieszka Winiarska MSc, supervisor: prof. Maciej Szaleniec*)

"Preludium" research project " NCN 2017/27/N/ST4/02676 [2018-2020]

The bacterium *Aromatoleum aromaticum* under anaerobic conditions is capable of denitrifying degradation of number of organic compounds including environmental pollutants, e.g. phenols, aromatic hydrocarbons (toluene, ethylbenzene), aromatic alcohols (e.g. benzyl alcohol) as well as alkyl and aromatic amino acids. In the phenylalanine degradation pathway, the bacterium uses aldehyde oxidoreductase enzyme (AOR) that contains tungsten, the heaviest element with biological function. Tungsten aldehyde oxidoreductase from *Aromatoleum aromaticum* (AOR_{Aa}) catalyses the oxidation of aldehydes to the corresponding carboxylic acids. The broad substrate spectrum of the enzyme suggests that the role of AOR_{Aa} is to remove toxic aldehyde by-products accumulating during the fermentative degradation of various compounds.

The research planned in the project is aimed to provide knowledge about the structure of the active enzyme centre and its reactivity, which will enable determination of the AOR_{Aa} reaction mechanism.

In 2019, during an internship conducted as part of a project at the Microbiology Laboratory at the Phillips University of Marburg, in a 30-liter fermenter the bacterium *Aromatoleum aromaticum* was grown to obtain AOR protein for further studies. In addition, the new AOR expression system was transferred to IKiFP, containing protein with a tag enabling more efficient purification by means of affinity chromatography.

In the active centre of AOR_{Aa}, the tungsten cofactor performs the main catalytic function. The exact coordination of tungsten is difficult to determine by X-ray diffraction or even by spectroscopic methods. This however is a necessary prerequisite to determine the mechanism of the catalysed reaction. Therefore, as part of the project, modelling of various possible versions of the tungsten cofactor by DFT and molecular dynamics methods was carried out. The geometries of the obtained models were compared with the known structure of the cofactor from *Pyrococcus furiosus* (which is characterized by unresolved coordination lacking oxygen ligands). On this basis, the most likely models containing tungsten in oxidation state +VI and +IV were selected, which next will be used in modelling the reaction mechanism.

Further information on the mechanism of the reaction can be obtained from experimental determination of kinetic isotope effect. As part of the project, the isotope effect was determined by: i) the direct method, by separate determination of the reaction rates with deuterated and non-deuterated benzaldehyde, and ii) by competitive method, i.e. comparison of the conversion rates of both substrates in the mixture. In both methods, the determined kinetic isotope effect was small and amounted to about 1.5, which indicates that C-H/D activation is not the only process controlling the observed kinetics.

"Beethoven" Research Projects of the National Science Centre

Domain wall dynamics and magnetic texture behavior in magnetic films with Dzyaloshinskii-Moriya interaction

(Project leader: Piotr Mazalski PhD)

“Beethoven” research project NCN 2016/23/G/ST3/04196 [2018-2021]

In the frame of project ultrathin magnetic layers, in which the Dzyaloshinskii-Moriya interaction (DMI) is expected – layers grown using sputtering techniques and molecular beam epitaxy. One of the interesting layers with unsymmetrical type of interfaces was trilayers Au/Co/NiO where ferromagnetic layers is in contact with nonmagnetic layer (Au) and antiferromagnetic layer (NiO). NiO layer in contact with Co layer allow to induce and observe very interesting magnetic dependences and related with them magnetic domain structure. Using thermal annealing it was possible to induce reorientation of magnetization from perpendicular to the surface layer to in plane orientation. Additionally by annealing process it is possible to demagnetize the sample (without using any magnetic field) and obtain submicrometer size magnetic object like “bubble/skyrmion”. It was also started studies of multilayers sample like Pt/Co/Ir and Ir/Co/Pt where strong dependences of DMI coefficient were detected. Such kind of samples will be used for magnetization dynamics studies. Presently, new layer sequences also with alternative magnetic/nonmagnetic sublayers are under preparation. It was also started preparation for ion irradiation of the trilayers sample to modify DMI strength and preparation for dynamics studies of magnetic domain structure. A special set up for dynamics studies of magnetization in the layers was developed and will be run in the early 2020.

Additionally micromagnetic simulations of magnetization distribution were performed taking into account variation of magnetic anisotropy and DMI coefficient in the simulated layers.

Part of the results were presented in international conferences (“oral” and “invited”) and used for prepared publication.

"Miniatura" Research Projects of the National Science Centre

Hydrotalcite-like heterogeneous catalysts for Baeyer-Villiger type oxidation of steroids to corresponding lactones with hydrogen peroxide

(Project leader: Robert Karcz PhD)

"Miniatura" research project NCN: 2018/02/X/ST4/01605 [2018-2019]

Project was completed stepwise. Synthesis and physicochemical characterization of the Mg-Al hydrotalcite-like compounds was performed as a first step. Prepared materials were characterized using PXRD, DSC-TG, SEM, EDX and FTIR. Materials were prepared using well-established method of co-precipitation at constant pH. Briefly, solutions a) containing Mg and Al salts and b) sodium hydroxide were added dropwise to a stirred solution of sodium carbonate, keeping pH at c.a. 10. After completing the addition mixture was left for 15 min and solid product was collected and washed with water. Results of performed physicochemical studies confirmed successful preparation of hydrotalcite-like materials possessing varying Mg/Al ratio (2,3,4,6). Prepared materials were applied as catalyst for Baeyer-Villiger oxidation of epiandrosterone in unoptimized conditions, using hydrogen peroxide as oxygen donor. On the basis of this results Mg_4Al catalyst was chosen for further studies as reference catalyst. In the same time method of reaction mixture analysis was optimized. The reaction conditions optimization was done by performing series of experiments witch changing conditions like: reaction temperature, catalyst amount, type and concentration of reagents. As a substrate for this part of research epiandrosterone was chosen (Fig. 1). Initial reaction conditions were chosen on the basis of literature review. During this step reaction mixtures were analyzed using along with GC-FID also GC-MS and HPLC-ESI-MS. After finding optimal conditions for epiandrosterone lactonization a series of catalyst possessing varying basicity was applied as catalyst for this reaction, followed by experiments regarding other steroid substrates. It was revealed that catalytic activity of Mg-Al catalysts increases along with growth of catalyst basicity (Mg/Al ratio).

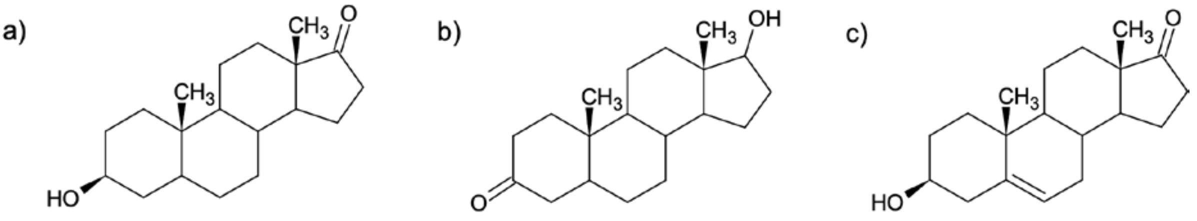


Figure 1. Structures of a) epiandrosterone, b) stanolone, c) dehydroepiandrosterone.

Stanolone was chosen as a substrate due to important structural alteration – oxo group is present in A ring, and HO- group in the D ring – placement reversed to that of epiandrosterone. Dehydroepiandrosterone possess double bond in ring B. Results of catalytic experiments revealed high conversion of stanolone in studied nitrile/H_2O_2 system (K=95%), but MS analysis revealed that main products possessed high m/z ratios (c.a. 340). This result indicates occurrence of consecutive oxidation reactions. Optimization of reaction conditions with this substrate will be addressed in further research. In case of Baeyer-Villiger oxidation of unsaturated substrate dehydroepiandrosterone products of epoxidation of double bond were also found in reaction mixture - both epoxidized lactones and ketones.

Investigation of docking of aliphatic hydrocarbons on zeolite active centers by molecular dynamics - scientific internship.

(Project leader: Agnieszka Drzewiecka-Matuszek PhD)

"Miniatura" research project NCN 2018/02/X/ST4/01794 [2018-2019]

Nowadays, quantum-chemical calculations have become a convenient complement to experimental research. They are helpful in explaining the results obtained, as well as they can be a starting point in choosing new paths for modification of chemical compounds in order to improve their properties, e.g. catalytic ones. Because of technological progress and increase in the computing power of computers, larger systems can be studied in conditions more and more close to experiment and using advanced quantum mechanical methods, including DFT (Density Functional Theory). In this context, research carried out on periodic systems are important as the impact of the environment is taken into account and, at the same time, artificial isolation of a fragment of the system is avoided.

During my monthly (01/05/2019-31/05/2019) scientific internship in the Theoretical Chemistry Group headed by prof. Carine Michel in Laboratoire de Chimie, ENS de Lyon I was familiarized with the methodology of calculations for periodic systems using the commercial Vienna Ab initio Simulation Package (VASP) program and the CP2K program available under the GPL license. The training included the preparation of initial geometries using the Avogadro program (available under the GPL license) and their optimization using both of the above-mentioned programs. I have also been trained in conducting research on reaction paths, in particular on the methodology of determining transient states (TS) of reactions. I have been acquainted with the methods of searching for reaction transient states: Nudged Elastic Band (NEB) and Dimer, which are implemented in the VASP program. The next stage of the internship were simulations using the molecular dynamics method ab initio (ab initio MD) allowing observation of system changes over time (up to 10 ps) at given pressure and temperature (NPT) or at given volume and temperature (NVT). The changes in geometry over time have been visualized using the MOLDEN and VMD programs. However, the results obtained require further statistical analysis.

The obtained preliminary results and acquired skills will be used in the continuation of the research project on testing ODH reactions in the presence of zeolite catalysts substituted with vanadium and can also be used in future projects and in the implementation of research tasks of the group "Heterogeneous catalysis: theory and experiment" at the Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences. New research skills will also increase my research potential, which is going to have a positive impact on my further scientific development.

Searching of novel bacterial ketosteroid dehydrogenases for oxidative dehydrogenation of steroids

(Project leader: Agnieszka M. Wojtkiewicz PhD)

“Miniatura" research project 2018/02/X/ST4/01963 [2018-2020]

The aim of the project is the identification of a physiological role for potential FAD-dependent 3-ketosteroid dehydrogenases (KstD) from two strains: *Sterolibacterium denitrificans* Chol-1S (two genes) and *Pseudomonas putida* KT2440 (three genes). In particular the project will help to answer following questions: i) is the expression of mentioned genes leading to catalysts with KstD activity; ii) are active enzymes regioselective in the direction of Δ^1 or Δ^4 products; iii) are substrates of the reaction only non-branched four-ring steroids as androstendione or branched at C17 as cholestenone or 3-ketopetromyzonole or two- or five-ring systems as well; iv) are functional catalysts differ in pH optimum or in the preference of an electron acceptor.

In the first stage of studies the necessary materials for the project were bought: components for genome DNA isolation from bacterial strains and for latter genes visualization. Oligonucleotides used as primes for genes isolation from genomic DNA were designed and ordered (for two sequences from *S. denitrificans* (SMB21450, SMB25049) and three from *P. putida* (Pp_2607, Pp_2600, Pp_2605)). Amplification of mentioned genes was confirmed by horizontal electrophoresis. For each gene a product of 1.7 kbp was obtained. Amplified genes were isolated for further construct preparation and bacteria transformation.

In the next step genes will be transferred to *E. coli* DH5α, while bacteria will be cultivated for enzyme isolation. Obtaining of functional enzymes will lead to answer for above questions.

In parallel, using theoretical approaches, the analysis of five studied sequences was prepared. The sequences were aligned to the sequence of KstD1 from *Rhodoccocus erythropolis*. KstD1 is the closest homologue with crystallographic structure. After alignment the conserved amino acid residues were identified as present or absent in five studied sequences. Such alignment confirmed, that at least three sequences poses conserved residues and may be functional enzymes with KstD activity.

Physicochemical characterization of stimuli-responsive smart-materials. Short scientific visit in Lutkenhaus Lab at Texas A&M University.

(Project leader: Piotr Batys PhD)

"Miniatura" research project NCN 2018/02/X/ST5/02470 [2018-2019]

The scientific internship under the MINIATURE project took place from April 24 to May 22, 2019 in the Lutkenhaus Lab research group at the Faculty of Chemical Engineering at Texas A&M University, USA. The purpose of the internship was to gain experience in experimental research on smart, bio-compatible materials based on polyelectrolytes.

During the internship a series of experiments were carried out using differential scanning calorimetry (DSC) and computer simulations using the molecular dynamics (MD) method. The properties of different hydrated polyelectrolyte complexes were compared, with a particular emphasis on the PAH-PSS and PDADMA-PAA systems and polypeptides-based complexes. Experimental characterization using the DSC method allowed to identify different states of water molecules in hydrated polyelectrolyte complexes and shown the drastic differences between water properties in complexes made of different polyelectrolytes, despite the same degree of hydration. MD simulations of corresponding systems enabled to better understand the experimentally observed differences and associate them with the differences in hydrogen bonding between polyelectrolytes and water molecules. The simulations also provided detailed information on the dynamic properties of water molecules as a function of hydration and temperature. In addition, it was shown how the specificity of functional groups of various polyelectrolytes influences on the strength and amount of hydrogen bonds. This will allow to predict the properties of water molecules in hydrated polyelectrolyte complexes which are directly related to the properties of the entire systems. The presented results have a huge impact on a better understanding of the properties of polyelectrolyte complexes and will facilitate their control by appropriate selection of polyelectrolyte, as well as temperature and degree of hydration.

Optimization of magnetic measurements of ultrathin epitaxial ferromagnetic metals layers by magnetic circular dichroism (XMCD)

(Project leader: Kinga Freindl PhD)

“Miniatura" research project 2018/02/X/ST3/02465 [2018-2019]

In the framework of this project and under a cooperation agreement between the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS (ICSC PAS) and the SOLARIS National Synchrotron Radiation Center (NSRC “Solaris”) calibration measurements of synchrotron radiation for the PEEM/XAS beamline were carried out in the past year. To this end, X-ray absorption spectroscopy (XAS) were performed in an external magnetic field generated by a liquid nitrogen cooled electromagnet. This electromagnet, previously designed at ICSC PAS, was modified, characterized and installed in the XAS measurement chamber. Thanks to this device it was possible to measure XAS spectra in a wide range of X-ray incidence angle for thick epitaxial iron layers of single crystal properties.

The appropriate shape and orientation of the substrates were tested and selected for carrying out the intended research. The next step of the substrate preparation before the experiment was the evaporation of high melting metallic stripes on the MgO substrates, so that the layers after evaporation would be grounded and the sample current could be measured by the total electron yield mode of XAS measurements (TEY XAS). After the evaporation of the grounding stripes on the MgO substrates the remaining sample preparation took place in an UHV preparation chamber connected to the XAS measurement chamber. In this way, thick iron layers with single crystalline properties were evaporated on the MgO substrates with grounding strips. The iron samples allowed to measure the X-ray magnetic circular dichroism signal for Fe as a function of the X-ray incidence angle on the sample. Based on these measurements, calibration of the polarization degree of X-rays on the PEEM/XAS beamline was obtained for various geometries of the optical system.

Then, in order to implement the project's tasks, the beamtime proposal was prepared and submitted to NSRC “Solaris” for XAS measurements of 3d metals thin films of deposited on the ferromagnetic Fe single crystal substrate. The proposal was accepted with the highest ranking A and the beam-time for the experiment was allocated, however in a slightly narrowed than requested time frame.

"Iuventus Plus" Programme Research Projects of the Ministry of Science and Higher Education

Determination of the influence of surface properties of silver nanoparticles on their cytotoxic activity towards the human cells of immune system and the cells of connective tissues

(Project leader: Magdalena Oćwieja DSc)

Project „Iuventus Plus" MNiSW IP2015055974 [2016-2019]

Numerous literature data showed that biological activity of silver nanoparticles strongly depends not only on their size, morphology and surface charge but first of all on chemical structure and properties of stabilizers deposited on their surface. The aim of the undertaken studies was to develop efficient methods of functionalization of surfaces of silver nanoparticles by such molecules which allow to control the processes which are induced in normal and tumor cells. The biofunctionalization with the use of amino acids seemed to be the most promising approach. Therefore, the main aim of the experimental works conducted in the considered period of time was to evaluate the impact of silver nanoparticle functionalization by cysteine and arginine on their toxicity towards histiocytic lymphoma (U-937) and human promyelocytic (HL-60) cell lines, as well as lymphocytes T (HUT-78) and B (COLO-720L) and fibroblasts.

Silver nanoparticles obtained via chemical reduction method using sodium borohydride were functionalized by cysteine or arginine under acidic conditions. Then, physicochemical characteristics of silver nanoparticles was conducted with the use of various experimental methods. It was established that an average size of both types of silver nanoparticles was equal to 12±5 nm. The zeta potential of the silver nanoparticles was strongly dependent on pH and ionic strength. It was found that the silver nanoparticles stabilized by cysteine exhibited an isoelectric point at pH 5.1 whereas the isoelectric point of the silver nanoparticles functionalized by arginine appeared at pH 9.4.

The influence of silver nanoparticles on the viability of selected cells of the human immune system was assessed determining the mitochondrial activity (MTT assay) and disruption of cell membrane (LDH assay). The lipid peroxidation of cell membrane was also evaluated applying MDA assay. Special attention was focused on the determination of the immunogentity of silver nanoparticles. It was studied measuring the release of interleucine 6 (IL-6) and secretion of nitic oxide. The proapoptotic properties of silver nanoparticles were determined via secretion of caspase-9 and necrosis factor TNF-α. Additionally, it was determined the impact of silver ions delivered in the form of silver nitrate and all reagents used during the silver nanoparticle synthesis on the viability of the cells.

It was found that the silver nanoparticles independently on the type of functionalization caused disintegration in the cell membranes and the disruption in the activity of mitochondria. It was demonstrated that destruction of cell membranes is related to the lipid peroxidation which in turn arises form generation of reactive oxygen species and oxidative stress. For selected concentration range, the biological activity of both types of silver nanoparticles was higher than silver ions. It was showed that positively charged silver nanoparticles stabilized by cysteine were much more toxic than the same nanoparticles characterized by negative surface charge. This effect was especially noticeable in the case of disruption of cell membrane and DNA damage. Additionally, it was observed that the HUT-78 and COLO-720L cells were more sensitive on the silver nanoparticle treatment that HL-60 and U-937 cells. It was not confirmed that the functionalization of silver nanoparticles by amino acids reduces the toxicity of silver nanoparticles in comparison to those ones which were not modified.

Research Projects
of the National Centre for Research and Development

New Functionalised Polymers for Biomedical Applications

(Project leader: Maciej Guzik PhD)

"Leader" programme research project NCBiR 0090/L-7/2015 [2017-2019]

In 2019, work continued to modify polyhydroxyoctanoate (PHO). Referring to 2018 - it was possible to confirm using other spectroscopic techniques (NMR, IR) the positive course of a chemo-enzymatic reaction using modifiers, i.e. betamethasone and diclofenac. In addition, the research on polymer modification and oligomers derived from PHO decomposition was extended by three other methods - two based on modification of the entire polymer (the first - by enzymatic catalysis - a lipase B from *Candida antarctica* and the second - consisting of nucleophilic substitution catalyzed by 4-dimethylaminopyridine and N,N′-dicyclohexylcarbodiimide) as well as one involving a simultaneous controlled degradation and modification of resulting oligomers. In the case of polymer enzymatic modification, a technique for extracting the modified polymer was developed and the positive course of the enzymatic reaction for modifiers, i.e. diclofenac, betamethasone, dexamethasone, S-ibuprofen, gentamycin and vancomycin was confirmed using spectroscopic techniques (NMR, IR). The mass average and number average molecular weight of modified polymers were also determined and their wettability was determined. The modification reaction catalyzed by DMAP and DCC was also carried out (with oxytetracycline). The successful conduct of the chemical reaction was confirmed using IR, and the wettability and average molecular weight of the modified polymer were determined. Modification and simultaneous decomposition of the polymer was carried out using a hydrolyzing agent which was p-toluenesulfonic acid. The most optimal modification conditions for diclofenac were selected using NMR. These blends were used to infiltrate ceramic sponges made of calcium triphosphate (TCP) for bone tissue regeneration. The dose of anti-inflammatory, nonsteroidal drug was selected based on the dose in half, one and two tablets commercially available in stores. Cell studies were carried out on the pre-ostebolast line (MC3T3-E1) for prepared composites. An indirect cytotoxicity test showed that blend composites containing diclofenac-modified PHO oligomers are not toxic. However, the direct cytotoxicity test, which consisted of weekly incubation of cells directly on prepared scaffolds, showed that only materials containing half the dose contained in one analgesic tablet did not show a cytotoxic effect. The morphology of the cells on the scaffolds was imaged using scanning electron microscopy (SEM).

In the reporting period, the biofilm culture method was selected - two variants were considered: a) in a CDC flow reactor and b) in a sandwich model. Selected strains were tested in a wound healing model and with unmodified polymer as biofilm forming support. Work is continuing on determining the parameters of PHO films modified with diclofenac. The same preparation was subjected to cytotoxicity tests on fibroblast cells. The results showed that a concentration of 100 ug per g of polymer did not show a negative effect on the selected cell line. Fibroblast migration experiments on diclofenac modified medium have also been performed. No differences are shown relative to the unmodified polymer. The results of these observations allowed the material (PHO-diclofenac) to be selected for planned *in vitro* tests.

Outperforming functionality: composite/mixed matrix porous materials in membrane-based processes

(Project leader: Aleksandra Pacuła DSc.)

Project funded by NCBiR: Agreement nr EIG CONCERT-JAPAN/1/2019 [2019-2022]

The X-MEM project is funded within EIG CONCERT-Japan – Connecting and Coordinating European Research and Technology Development with Japan, within joint call on Functional Porous Materials. The X-MEM project is a consortium of 6 international partners: University of Cantabria (Spain) – project coordinator, Yamaguchi University (Japan), Ege University (Turkey), Atilim University (Turkey), Hacettepe University (Turkey) and Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences (ICSC PAS) (Poland).

The X-MEM project includes the preparation of the composites and the characterization of their physicochemical properties in order to use them for the membrane formation. The functionality of obtained porous composite membranes will be examined in the following processes: cell growth in bioreactors *in vitro* (treatment for neurodegenerative (motor neuron) disorders), reverse electrodialysis (the method of utilizing energy produced via salinity gradient process applying natural water with high salt concentration (seawater) and natural water with low salt concentration (river water)), photocatalytic removal of non-biodegradable water pollutants (degradation of PFAS = polyfluoroalkyl substances).

In general, the X-MEM project focuses on the preparation of the components dedicated for the membrane formation used in the devices, which can serve the society and satisfy people's demands for health, energy and environment.

ICSC PAS is involved in 6 tasks: task 1 – Preparation and characterization of novel metal-TiO_2 composite catalysts embedded in zeolite nano-space lattice; task 2 – Preparation, functionalization and characterization of electrically conductive carbon-based materials and characterization of additional commercial graphene-based nanomaterials; task 3 – Thin-film coating with carbon-based materials of porous biocompatible polymer membrane supports; task 4 – conductivity tests of membranes; task 5 – Technical analysis of the scaling up potential for the membrane devices developed; task 6 – Diffusion and dissemination. All the partners are involved in task 5 and task 6.

In 2019, ICSC PAS was active in task 6 (Diffusion and dissemination). The activity of ICSC PAS focused on the communication between partners including the participation in kick-off meeting via Skype (11/07/2019) and face-to-face kick-off meeting in Santander (18/10/2019); the preparation of a PowerPoint presentation and delivering the speech at the meeting in Santander (20-minute oral presentation).

"Homing" Programme Research Projects of the Foundation for Polish Science

Development of methodology of synthesis and stabilization of metal nanoparticles for conductive materials.

(Project leader: Anna Pajor-Świerzy PhD)

Homing POIR.04.04.00-00-3EE4/17 [2017-2019

In recent years, metal nanoparticles have generated considerable interest because of their unique properties, which are interesting not only from the scientific point of view but also because of the possibility of their using in many branches of industry. One of the applications of metal nanoparticles are conductive inks that can be used for the fabrication of "printed" electronic devices. Silver NPs, due to their high conductivity and oxidation stability, have been the most commonly used so far for the fabrication of electronic circuits and devices. Although Ag NPs have obvious advantages, their high price limits their application on a large industrial scale. Among the metals, which can be used as a replacement of silver are tin and nickel, due to their much lower cost and good electrical conductivity. However, Sn and Ni NPs are easily oxidized in air, resulting in poor conductivity. As a suitable option to overcome the high cost of Ag and to prevent the spontaneous oxidation of Sn and Ni, Sn-Ag and Ni-Ag core-shell particles can be synthesized and investigated.

The general objective of the “Homing” project was a development of the method of synthesis of metallic nanoparticles stable against aggregation and oxidation, which can be used as a functional component of inks and pastes for the printing process of conductive tracks.

During the research carried out in 2019, the process of obtaining conductive inks based on core-shell (Ni-Ag) metallic nanoparticles was optimized and after that they were used in the printing process of conductive patterns. The process of fabrication of metallic printed tracks involved four stapes. In the first stage, bimetallic nanoparticles (Ni-Ag) were obtained. Then, the nanoparticles suspension was concentrated by centrifugation and dispersion in an appropriate amount of distilled water. In the next stage, a layer of ink containing nanoparticles was deposited on a glass substrate by using a hand coater or screen printing method, and finally, thermal sintering was performed after the drying process.

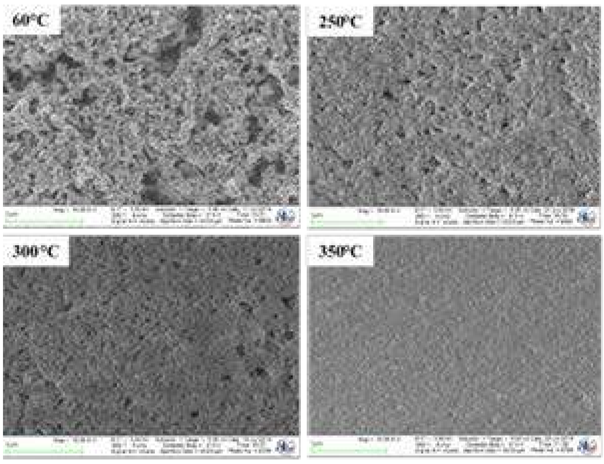


Fig. 1. SEM images of metallic coatings after sintering at various temperatures.

To obtain patterns with high conductivity, the ink properties (by using appropriate wetting agents), as well as the sintering process conditions (temperature, time), have been optimized. The lowest resistance value (highest conductivity) was obtained for inks based on Ni-Ag nanoparticles with a size of about 280 nm, using TEGO KL 245 as a wetting agent, after 30 minutes of heating at 350°C. After considering the thickness (~2.5 µm) of the deposited layer, a conductivity of 11% of bulk nickel was obtained. The morphological properties of metallic coatings were investigated using Scanning Electron Microscopy (SEM, Fig. 1). The formation of a uniform layer of particles after the heating process at 350°C was observed, which results in high conductivity.

Exchange of PhD Students
and Academic Staff Programme Projects
of the Polish National Agency for Academic Exchange

International scholarship exchange of PhD candidates and academic staff

(Project coordinator: prof. Tomasz Borowski)

NAWA PPI/PRO/2018/1/00006/U/001 PROM [2018-2020]

In accordance with the assumptions of the Project, the following scholarship competitions under the PROM Program were conducted and resolved in 2019:

1. Competition for scholarships to finance the costs of participation in short forms of education abroad (academic conference, academic school, scientific workshops) to doctoral students of IKiFP PAN;
2. Competition for scholarships to finance the costs of short-term internships at foreign research units for doctoral students of IKiFP PAN;
3. Competition for scholarships to finance the costs of participation in short forms of education abroad (scientific conference, academic school, scientific workshops, scientific courses, scientific training) for doctoral students of IKiFP PAN / 2nd edition /;
4. Competition for scholarships to finance the cost of short-term internships in foreign research units for PhD students of IKiFP PAN / 2nd edition /;
5. Competition for scholarships to finance the costs of short-term internships at IKiFP PAN for PhD students from foreign research units;
6. Competition for doctoral students from foreign research units for scholarships to finance the costs of participation in the 51st National Catalytic Colloquium;
7. Competition for scholarships for doctoral students from foreign scientific units to finance the costs of participation in the "School on molecular computational biochemistry" at IKiFP PAN;
8. Competition for scholarships to finance the costs of short-term internships at IKiFP PAN for PhD students from foreign research units / 2nd edition /;
9. Competition for scholarships for doctoral students from foreign scientific units to finance the costs of participation in the "Workshop on data collection and structure solving in macromolecular X-ray diffraction" at IKiFP PAN;
10. Competition for scholarships to finance the costs of short-term internships in foreign research units for representatives of the scientific staff of IKiFP PAN.

As a result of all of the above competitions, 67 participants were recruited to the Project and they received travel scholarships. The project assumed that exactly 67 people would take part in short forms of education under the PROM Program, so the project participants' rate was achieved at 100%.

Summarizing:

- 15 people took part in the School in the field of molecular computational biochemistry (12 doctoral students and 3 lecturers from abroad) organized by IKiFP PAN in Krakow on 11-14.03.2019;
- 16 doctoral students of IKiFP PAN took part in short forms of education (scientific conferences, workshops, trainings, schools);
- 8 doctoral students of IKiFP PAN took part in short-term internships at foreign research units;
- 12 people took part in the Workshop on protein crystallography (11 doctoral students and 1 lecturer from abroad) organized by IKiFP PAN in Krakow on 12-16.07.2019;
- 7 foreign doctoral students took part in research internships at IKiFP PAN;
- 1 doctoral student from abroad took part in a scientific conference in Krakow;
- 8 representatives of the scientific staff of IKiFP PAN took part in internships at foreign research units.

Polish Returns Programme – CRAQUELURE

(Project leader: assoc prof. Łukasz Bratasz)

“Polish Returns” Programme PPN/PPO/2018/1/00004/U/00001 Polish National Agency of Academic Exchange [2018-2022]

The goal of the "Polish Returns" program and thus the CRAQUELURE project is to create a project group conducting research on the development of a physical model of paint layers in paintings based on funds obtained from external sources as well as conducting other research in the field of heritage science. Therefore, the first year of project implementation was devoted to submitting applications for research funding and employment of new employees funded from the project the project.

During this period, several applications for external financing were submitted to:

- The Getty Foundation for the development of the Herie tool that allows the calculation of mechanical hazards in heritage objects, in particular the development of a model for cracking images on canvas and the development of a module identifying hazards for parchment objects - the project has been approved by the Foundation.
- European Commission, program H2020, competition H2020-MSCA-ITN-2019, application number 860866, for funding research and doctoral thesis - the project did not receive funding.
- the European Commission, to include the Institute in the consortium implementing the CollectionCare Project "Innovative and affordable service for the Preventive Conservation monitoring of individual Cultural Artefacts during display, storage, handling and transport" under the H2020 program, competition number H2020-NMBP-ST-IND- 2018 - project 814624 obtained funding.
- European Commission, program H2020, competition INFRAIA-01-2018-2019, RIA action, application number 871034. The IPERION HS project obtained funding.
- NCN, Sonata competition - project under evaluation.

At the beginning of 2019, two competitions for assistant positions specializing in mechanics and specialist in image processing were announced. The evaluation procedure selected two candidates for the position of assistants: Dr. Lindsay Oakley and Dr. Kamil Kutorasiński. Dr. Kutorasiński developed a computer model of the formation of cracks in canvas paintings and conducted a series of parametric simulations to determine the most important parameters responsible for the formation of cracks. It was found that the moisture shrinkage of all image components described in the literature - canvas, glue, and ground layer - allows to explain the cracks in the corners of the images but cannot explain the development of cracks in the central parts of the painting. The new hypothesis was proposed that cracks in the central part of the images are the result of "drying" shrinkage (annealing), which is the result of slow approaching of the ground to thermodynamic equilibrium and deeper packing of the polymer structure. To confirm this hypothesis, a number of samples imitating real images were prepared, which were subjected to humidity cycles in a climatic chamber. This research continues.

In June 2019, Dr. Kutorasiński resigned because of a more favorable job offer. Therefore, an additional competition for the position of assistant was announced and Dr. Mohammad Yaghouba Abdollahzadeh Jamalabadi, who started work in mid-December.

Switchable surfactants at air-water interface and their role in photo-responsive foam

(Project leader: Marcel Krzan PhD)

Project NAWA PPN/BIL/2018/1/00093 [2019-2020]

In this project we introduce a new class of photo-switchable surfactants and their 2D assemblies at the liquid/gas interface, which make the interface as well as macroscopic foam responsive to light irradiation. This opens exciting new possibilities for foams such as self-healing capabilities or the possibility to adapt foam properties by photo-switching of the surfactants. We have synthesized amphiphilic arylazopyrazole (AAP) derivatives which are superior analogues of azo-benzene surfactants due to the less spectral overlap between *trans* and *cis* isomers, a more favorable photo-stationary state (>95 % switching in both directions), and increased thermal stability of the *cis* isomer. Such molecules have anionic or cationic head groups as well as different chain lengths, which allows for tuning lateral interactions and surface rheology as well as structure.

In our works we have investigated merocyanine MC and spiropyrane SP sulfonate surfactants at the air-water interface using vibrational sum-frequency generation SFG spectroscopy and surface tensiometry and discussed their use as new surfactant to render aqueous foam active to light stimuli via photochromic reactions of the surfactants at the interface and in the bulk solution. Using SFG spectroscopy we have addressed the changes in surface excess and surface charging state as a function of concentration, pH and light irradiation on a qualitative level and we have compared our conclusions to the stability of macroscopic foams. At the air-water interface, we have demonstrated that the surfactant can be switched between a merocyanine (MC) form, which stabilizes under dark conditions while the spiro form (SP) is formed after irradiation with 465 nm blue light. To tune the net charge of the surfactant we changed the pH value: At a pH of 5.3 the O-H group of the MC form can be partially deprotonated and SFG spectroscopy showed that the intensity O-H stretching bands from interfacial water molecules under dark conditions was increased substantially, which we associated to an increase in the surface net charge. At a pH of 2.7 we observed a different behavior where the O-H intensities were found to be higher under blue light irradiation, while they decreased when the samples and interfaces were kept in the dark. The changes in foam stability of aqueous foams from solutions with SP/MC surfactants showed an excellent correlation to the surface net charge and surface excess that we have gained from SFG spectroscopy and surface tensiometry. We propose to associate these changes with the variation of in the disjoining pressure, which can be activated by light irradiation and can be tuned by pH adjustments. Clearly, structure-property relations from interfacial level to the macroscopic foam exist and can be used to tailor foam properties on a molecular level and to render foams responsive to light stimuli.

Data obtained during the Project were presented on international conference: Bubble and Drops conference, Sofia, Bulgaria. In Oct. 2018 wec also submitted the first paper in Langmuir ACS journal (IF 3.68).

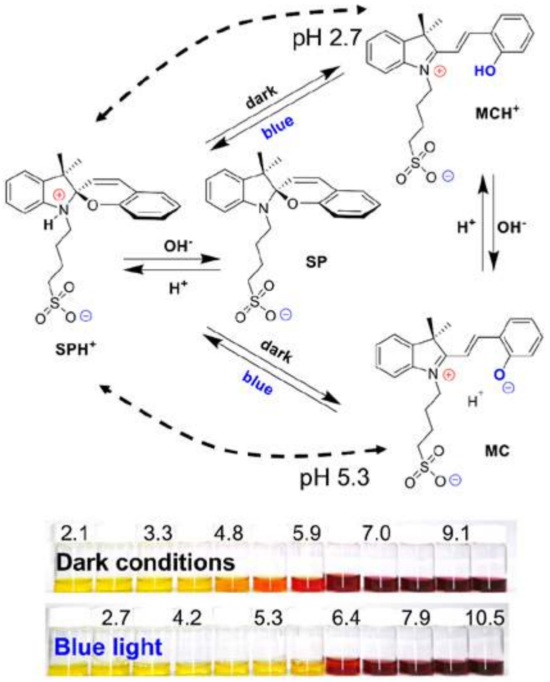


Figure: *Structures of merocyanine (MC), protonated merocynine (MCH^+) and spiropyrane (SP) as well as protonated spiropyrane (SPH^+) sulfonates as a function of pH and light irradiation. Counterions have been omitted for clarity. Note that at pH 5.3 the hydroxyl of the MC form is expected to be almost fully deprotonated (mixture of MC and MCH^+), while for pH 2.7 only the MCH^+ form is present. In addition, photographs of the surfactant solutions for different pH values of the aqueous solution. The latter were either irradiated with 465 nm blue light or kept under dark conditions as indicated in the figure. pH values were as indicated in the photographs.*

Methane selective oxidation to methanol of over metallozeolites catalysts

(Project leader: assoc. prof. Dorota Rutkowska-Żbik)

Project NAWA PPN/BIL/2018/1/00069 [2019-2020]

The aim of the current bilateral collaboration project between the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS and the J. Heyrovský Institute of Physical Chemistry Czech Academy of Sciences is a targeted preparation of highly structured metal (Fe, Mn) ion/oxo centres stabilized in zeolites of ferrierite (FER) and beta (*BEA) topology, which may serve as active catalysts for CH_4 oxidation to CH_3OH by N_2O.

Ion exchange method was employed to obtain the Fe-FER samples of various distribution of Al in the zeolite network in which Fe content was changed in the range of 0-3 wt%. The systems were characterized by UV-Vis and FTIR spectroscopy leading to the determination of iron location in the zeolite framework. Then the possibility of the "α oxygen" (Fe=O group) formation was checked via dissociation of N_2O over the iron centers and their content was measured in the temperature programmed reduction experiment. The oxidative properties of the "α oxygen" were probed in the oxidative dehydrogenation of propane. The Fe-FER sample of Fe/Al = 0.09 was degassed at 450°C in He flux of 40 ml/min during 3 h and activated at 250°C in 40 ml/min flux of N_2O (leading to the formation of the α oxygens being the active centers of the reaction) during 0.5 h. Next, propane was adsorbed at room temperature on the catalyst prepared in abovementioned way, followed by the temperature programmed desorption experiment in which mass signal from propene was enregistered (temperature was ramped up from room temperature up to 450°C). C_3H_6 desorption was detected at temperature ca. 190°C – see Figure 1.

The Mn-FER samples with various Al distribution in the zeolite framework were also synthetized and their physico-chemical characterization with UV-VIS and FTIR techniques started. The task will be completed in 2020.

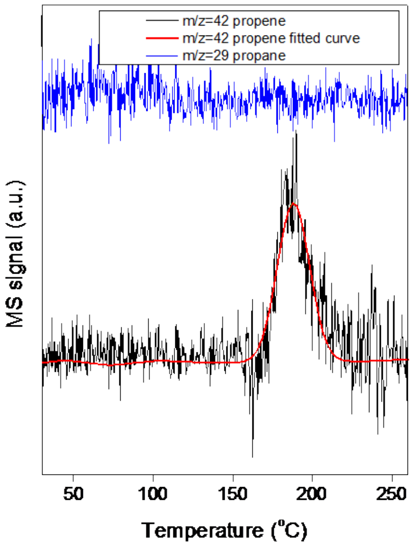


Figure 1. TPD of the Fe-FER samples with generated "α oxygens" after propane adsorption.

Understanding molecular aspects of the protein misfolding process: in situ spectroscopic and microscopic studies

(Project leader: assoc. prof. Barbara Jachimska, Paulina Komorek MSc)

Project NAWA PPN/BIL/2018/1/00103 [2019-2020]

The protein folding process is one of the fundamental biological processes, which significantly changes with age. The age-related pathological process leads to an uncontrolled production, reorientation, and storage of proteins in cells and tissues. These proteins aggregate to form fibrils. The protein fibrils often accumulate in the nerve system, being responsible for the development of neurodegenerative disorders such as Alzheimer or Jacobs-Kraft disease. It was recently found that protein fibrils also accumulate in liver, joints, kidneys, leading to the development of lethal diseases. In the last years, significant scientific attention is directed to this challenging sociological problem. Due to large structural analogy to fibrils formed by amyloid protein, they are called amyloid fibrils. The scientific investigations aim at the distinction between the synthesis, correct and pathological folding of proteins. Numerous structure analyzing techniques are employed to study amyloid fibril structures. The structure of protein fibrils is known and displays large structural arrangement of a native correctly folded protein. However, the mechanism of their formation, dynamic structural changes leading to protein misfolding and aggregation remain unknown.

Horizon 2020 Programme

CollectionCare - Innovative and affordable service for PC monitoring of individual Cultural Artefacts during display, storage, handling and transport

(Project leader: assoc. prof. Łukasz Bratasz)

Horizon 2020 No. 814624 [2019-2022]

The research team formulated a basic physical model of glue-sized canvas paintings as a basis for including this category of objects into the HERIe software. The Finite Element model of the painting was developed in the COMSOL Multiphysics software. It was used to identify possible sources of non-uniform distribution of stress/strain over the painting due to non-uniform restraint on moisture-induced painting's movement to elucidate mechanism of cracking, especially presence of cracks in the corners whose direction is perpendicular to the painting's diagonal. It was assumed that the painting is built of a wooden frame, canvas sized with animal glue – a composite - and animal glue based ground layer (the gesso), a stiff material similar in mechanical properties to aged brittle oil paints, having function of a substitute for the design layer. A broad parametric study was performed to see how the model was behaving as various input parameters were varied. The primary parameter varied was stiffness of the wooden frame as it was identified to be one of the most important parameters controlling concentration of stresses in the corners of a painting. The stiffness of the frame was varied between 10^7 and 10^13 Pa. It was assumed that relative humidity (RH) changed from 70% to 30%.

It was found that there could be a significant concentration of stress in the painting's corners. It is visible that stress concentrates very closely to the painting corner but quickly drops with the distance from the corner to values typical of painting's centre. It is also evident that the maximum stress is reached for the frame of moderate stiffness of the order of 10^10 Pa, the value close to stiffness of typical woods in longitudinal direction, and is greater in case of gesso of the non-zero moisture coefficient of expansion. The stresses are predominately tensile along diagonal with some compressive component in perpendicular direction in the case of gesso layer responsive to moisture whereas in the case of gesso non responsive to moisture, the first principal stress is perpendicular to diagonal of the paining and with small compressive component in the perpendicular direction.

Although stress concentration is visible in painting corners, the level of stress reaches a critical value only in first 2- 3 mm from the corner. Stresses in the gesso layer not responsive to RH changes are even smaller. The general conclusion from the work performed so far is that the stresses building up in the painting's model with assumptions described above cannot explain common observations that surface of paintings is often covered with dense network of cracks.

To inform further modelling by direct observations of crack development, several 'mock' paintings will be constructed and subjected to RH cycles.

Additionally, in the first reporting period, the research team cooperated in identifying best degradation models and damage functions existing in the literature, specific to the field of preventive conservation.

Energy-X: Transformative chemistry for a sustainable energy future

(Project leader: assoc. prof. Dorota Rutkowska-Żbik, prof. Małgorzata Witko)

Horizon 2020 no 820444 [2019-2020]

Climate change makes it imperative that we discover ways of producing energy and chemicals sustainably and replacing the fossil fuels that form the basis for our energy and chemical industry. The vision of ENERGY-X is to enable the sustainable production of synthetic fuels for energy storage and of feedstock for the chemical and materials industry. In the longer-term, the science and technology developed will allow to change the current production system moving to a distributed production. The Energy-X project aims at the formation of an international consortium and to propose a framework of scientific research enabling people on Earth access to energy and materials in a way human influence on natural environment is minimized and climate changes are mitigated.

The institute is mainly involved in Work Package 6 "Coordination with regional and national funding") whose aims are to map of European national and regional funding activities and to propose mechanisms and plan for alignment of co-funding. Within the Work Package 6 the map of European (mostly EU) national and regional funding activities was prepared (deliverable 6.1).

Within Work Package 3 "Strategic long-term research roadmap" the institute's staff took part in the scientific workshop resulting in the design of "Research needs report", which was further promoted during EuropaCat 2019 conference in Aachen, Germany.

A part of the work was also devoted to the identification of the most relevant cross-cutting socio-technical challenges related to ENERGY-X and affected stakeholders.

The vision of the Energy-X project was presented during numerous meetings and conferences. This effort was taken also in view of the formation of the future consortium for Large Scale Initiative project which hopefully will replace Flagships initiative in the following EU framework program.

EU COST Actions

A new Network of European BioImage Analysts to advance life science imaging (NEUBIAS)

(investigator: Marcel Krzan PhD)

EU COST Action CA 15124 [2016-2020]

This Action is a program for establishing a network of BioImage Analysts, in order to maximize the impact of advances in imaging technology on the Life-Sciences, and to boost the productivity of bioimaging-based research projects in Europe. The Action aims to provide a stronger identity to image analysts by organising a new type of meeting fostering interactions between all stakeholders including: Life scientists, image analysts, microscopists, developers and the private sector. It will collaborate with European Imaging research infrastructures to set up best practice guidelines for image analysis. The Action created an interactive database for BioImage analysis tools and workflows with annotated image sample datasets, to help matching practical needs in scientific problems with software solutions. It will also implement a benchmarking platform for these tools. To increase the overall level of image analysis expertise in the science, the Action proposed a novel training program with three levels of courses, releasing of open textbooks, and offering of a short-term scientific missions program to foster collaborations, image analysis technology access, and knowledge transfer for scientists and specialists lacking these means.

As a member of the COST CA15124 (I am not a member of the Management Committee) in the last year of the action, I continued collaboration online with other COST action participants. I worked online with other associates in the action of scientists creating new algorithms to facilitate and accelerate research work using automated image analysis. The results of the research - obtained more easily and with much greater precision thanks to the new software have already been presented during scientific conferences ECIS European Colloids and Surfactant conference, Leuven, Belgium and Bubble and Drops conference, Sofia, Bulgaria.

Computational materials sciences for efficient water splitting with nanocrystals from abundant elements

(investigator: prof. Małgorzata Witko)

EU COST Action no 18234 [2019-2023]

The current action of the European Cooperation in Science and Technology (COST) fits into the trend of the development of new, clean energy sources – derived from electrocatalytic water splitting over nanocrystals from oxides of abundant elements. The multi-scale approach is needed to model the electrocatalytic water splitting, which covers not only the surface reaction, but also reagents' diffusion inside catalyst pores, ion diffusion within the bulk, atom segregation at the interfaces, grain particles movements, etc. This COST Action intends to focus on bridging the knowledge gaps between different theoretical methods (DFT, molecular mechanics and molecular dynamics, Monte-Carlo dynamics, microkinetic modelling) and computer codes in order to facilitate the modelling of all processes which accompany water splitting, in a longer term enabling the discovery of novel electrocatalytic materials.

The project was launched in November. The key personnel of the action were selected (work group leaders, STSM and conference grants manager). The members selected appropriate working groups.

Projects of the Norwegian Research Council

Sustainable Management of heritage Buildings in a Long-term perspective

(*Project leader: Marcin Strojecki PhD*)

Research project SyMBoL No. 274749 of The Research Council of Norway [2018-2021]

In the frame of the SyMBoL project a detailed research plan for a laboratory measurements of the climate-induced physical damage in wooden cylinders mimicking sculptures or massive architectural elements has been prepared. The following elements of the plan were refined:

- technical aspects of specimens preparation including time sequence of the procedure and selection of specific sealants; a microcrystalline wax (R), 40% a solution of Paraloid B72 (P40), a sand sealing spray (CS) and an epoxy resin (E) have been tested evaluating their optical appearance, the induced colour change and the adsorption-desorption behaviour of coated samples.
- specification of patterns of relative humidity variations which reproduce typical cyclic change in churches as well as take into account response times of the objects so that the variations produce maximum possible risk of damage in wood,
- AE sensors positioning and fixing,
- procedure of measuring the fracture energy linking structural weakening of wood to RH variations.

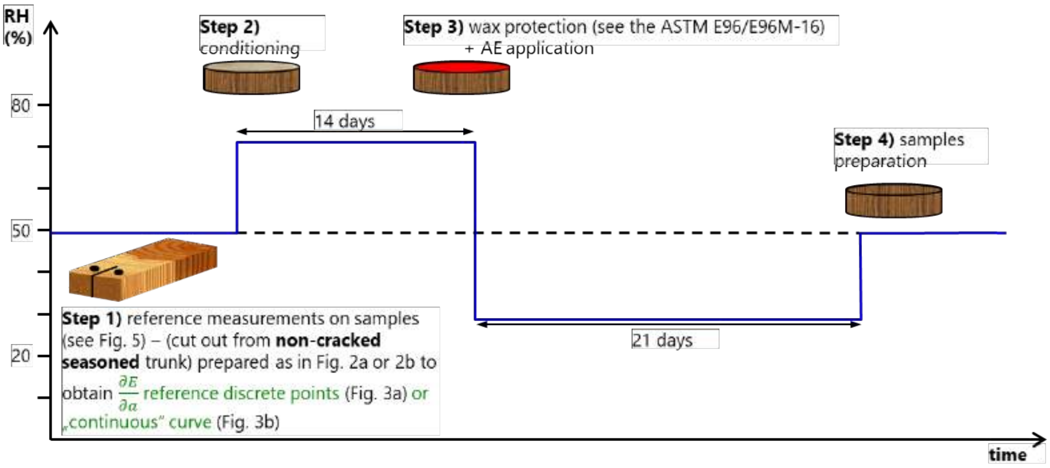


Fig. 1 Measurement protocol of mechanical tests on wooden samples with AE monitoring.

Additionally, improvements in technical description of monitoring of the climate-induced crack propagation in wooden elements in historical churches using the AE method were introduced. The improvement was related to: selection of the sensor-to-object attachment, safety aspects related to long-term continuous operation of the monitoring systems in historical churches, data transfer from the sites monitored to the laboratories using the GSM system. Accompanying monitoring of key indoor climate parameters was planned: temperature, relative humidity, aerosol particles concentrations as well as concentration of indoor CO_2, allowing air exchange rates, times when the churches are open and the approximate number of visitors to be estimated. The corrected plan was prepared and was further analysed by the conservation administration and owners of the churches.

Further development of methodology of modelling dynamic processes of water vapour sorption/desorption and diffusion, and the related strains and internal forces in wooden objects and structures was elaborated. Technical details of calculations of energy release rate and for cracks of various length running radially in massive objects have been analysed. These details refer to proper J-integral calculations i.e. transformation of existing solutions from the Cartesian coordinate system to cylindrical.

Projects of EU Structural Funds Programmes

Cross-institutional PhD Studies „Interdisciplinarity for Innovative Medicine” InterDokMed

(Project leader: assoc. prof. Renata Tokarz-Sobieraj)

Project of Operational Program Knowledge Education Development (POWER)
for interdisciplinary doctoral study programs POWR.03.02.00-00.I013/16 [2017-2022]

The Interdisciplinary PhD Project is implemented as part of the Operational Program Knowledge, Education and Development for 2014-2020, action 3.2 Doctoral studies, Axis III Higher education for the economy and development. The aim of the project is to increase the quality and effectiveness of education at doctoral studies, through the development of an extended program, its implementation and implementation, as part of InterDokMed's Interdisciplinary for Innovative Medicine.

The project is implemented by a consortium of five scientific units with at least A category and KNOW status. The project coordinator is the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS and partners: Maj Institute of Pharmacology PAS, The Henryk Niewodniczański Institute of Nuclear Physics PAS, Faculty of Chemistry, Jagiellonian University and Faculty of Medicine, Jagiellonian University. The choice of implementing units guarantees the implementation of interdisciplinarity through the implementation of scientific research covering more than one area of knowledge (exact sciences / medical sciences), more than one field (chemical / physical / medical sciences) and several scientific disciplines (physics, chemistry, pharmacology, medicine).

In 2019, 47 doctoral students (out of 50 accepted in two recruitments) continued their education. The educational program in 2019 included continuation of the following lectures: "Chemistry in a nutshell", "Physics in a nutshell", "Civilization diseases" and "Pharmacology of civilization diseases", which ended with an exam and classes on "Fundamentals of academic didactics", "Research methods in medicine and health sciences "and" Doctoral seminar ", completed with a credit. In 2019, as part of the planned actions, 5 elective lectures were organized thematically for PhD students of interdisciplinary studies. They were: at the Jerzy Haber Institute of Catalysis and Surface Chemistry PAS: "Analysis of data using artificial intelligence tools" and "Biocatalysis", at The Henryk Niewodniczański Institute of Nuclear Physics PAS: "Biomechanics of the brain", at the Maj Institute of Pharmacology PAS: "Radiopharmaceuticals", at the Faculty of Medicine, Jagiellonian University: "Microbial and bacterial biofilm - their importance in health and disease. " PhD students also conducted elective lectures outside InterDokMed's offer. All PhD students received the second year credit. On 25-26 May and 15-16 June 2019, full-time language workshops were organized for doctoral students. Qualified teachers have conducted classes including: in the first stage - self-presentation / interview; small talks; in the second stage - Academic writing courses. The implementation of such a program creates the possibility of an innovative and modern way of educating a doctoral student, prepared for independent interdisciplinary and multi-domain scientific research, aware of his own values, prepared to continue scientific work and ready to take up tasks in the industrial and biomedical sciences.

Doctoral students carry out their scientific research on an ongoing basis, under the supervision of two supervisors from two different partner units, representing different scientific disciplines. In 2019, doctoral students completed 13 internships abroad, participated in 6 schools / workshops related to their work. They were co-authors of 42 publications from the JCR list and 2 chapters in books. They gave 35 oral presentations and co-authored 111 posters at domestic and foreign conferences. 8 PhD students have won their own projects to fund research.

Interdisciplinary Environmental Doctoral Studies "Physical, Chemical and Biophysical Foundations of Modern Technologies and Materials Engineering" (FCB)

(Project leader: assoc. prof. Anna Micek-Ilnicka)

Project of Operational Program Knowledge Education Development (POWER) for interdisciplinary doctoral study programs POWR.03.02.00-00-I004 / 16 [2017-2022]

The project is financed by the Foundation for the Development of the Education System and is a result of a competition announced by The National Centre for Research and Development (NCBiR) in 2017 for interdisciplinary doctoral studies programs. This project is coordinated by the Faculty of Physics and Applied Computer Science (WFiIS) at the AGH University of Science and Technology (AGH) with the participation of the Faculty of Materials Science and Ceramics (WIMiC) at the AGH, the Faculty of Chemistry at Jagiellonian University (WCh UJ), The Henryk Niewodniczański Institute of Nuclear Physics (IFJ) of the Polish Academy of Sciences and Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences (IKiFP).

FCB doctoral studies are interdisciplinary. The interdisciplinary nature of FCB doctoral studies results from the participation of doctoral students in lectures and seminars in natural sciences in the discipline of chemical sciences and physical sciences. Currently, in accordance with the project assumptions, we learn 75 PhD students at FCB studies, including 5 persons at IKiFP PAN, 34 persons at WFiIS, 15 persons at WIMiC, 9 persons at IFJ, 12 persons at WCh UJ.

The schedule of FCB studies, implemented in 2019, included a series of optional lectures: Physical and chemical foundations of matter construction, Selected issues in physical chemistry, Modern methods of physicochemical research (the lecture was selected by 30 PhD students per 75), Physics for chemists and engineers, and Biophysics, each of the lectures ended with an exam.
Parallel lectures were held by scientists from abroad: Selected problems of modern Biophysics by Prof. Gerardo Goya from the University of Zaragoza and Synthesis of nanosized particles and their applications by Prof. T. Graule from the Laboratory of High Performance Ceramics in Switzerland.

PhD students soft skills were developed by the participation in an online course (Coursera platform) titled Teaching and Learning in University Chemistry Laboratories. This course was designed to preparing laboratory instructions and headings to assess student work and formulating questions to check the knowledge and understanding of the material by the student. At WCh UJ, doctoral students also participated in the training of Fundamentals of academic didactics for the chemistry and related fields. In the Department of Inorganic Chemistry at WIMiC AGH, PhD students conducted didactic classes (30 hours).

PhD students from our IKiFP PAN (5 people) in an academic year. 2018/2019 published 3 publications, they gave 10 oral presentations and presented 12 posters. In addition, doctoral students participated in 2 grants as contractors and 1 doctoral student received the *Preludium* project (2018-2021).

Other International Research Projects

Biocompatible Particle-Stabilized Foams and Emulsions as Carriers for Healing Agents

(Project leaders: Marcel Krzan PhD, Francesca Ravera PhD)

Joint research project of the Polish Academy of Sciences and CNR National Resreach Council [2017-2019]

During the project, we wanted to develop technology for generation and application of stable foam and emulsions formulated from mixtures of hypoallergenic, non-toxic biological polymers, mainly “green surfactants”. Such surface thin film could in the close future become the basis of the cosmetic and dermatological composition for the multiple use products. The application of other biologically active particles, like i.e. chitosan, silk fibroin or liposomes will also be carefully studied. Similar multicomponent film layer (created after foam or emulsion injection on the human body) would be the first line of the skin body defence against various pathogens. Thanks for them the skin will be simultaneously protected, lubricated and regenerated. Similar thin surface film (created from our foams or emulsions) could also be used as a pathogen barrier in the food industry as natural preservatives. The same technology may be utilized in the agricultural production in natural green pesticides. The similar application of the technology let us in close future reduce the number of synthetic surfactants incorporated into the natural environment.

During the last (third) year of the project, we started the study of triglicertyde based emulsions stabilised by saponin (saponin reagent grade, VWR, catalogue no. 0163-100g) and its mixtures with chitosan (HD chitosan, low molecular weight, cosmetic ingredient). The research was carried out simultaneously using the facilities of both institutes in Poland and Italy.

The bilateral project funds allowed us to perform two two-week research internships in Genoa - for dr. Marcel Krzan and dr. Ewelina Jarek. We also had two independent one-week project visits to our Cracow institute of dr. Frances Ravera (Italian PI of the project) and dr. Eva Santini.

The obtained results were published in one paper in Collod Surface B: - Biointerfaces (IF 4.0). Recent data were presented during international scientific conferences, as ECIS European Colloids and Surfactant conference, Leuven, Belgium; Bubble and Drops conference, Sofia, Bulgaria. We also start of prepare new publication in ISI / JCR journal for (for submittion in January 2020). The project was settled and accepted at the Polish Academy of Sciences and on its basis we obtained a new, two-year bilateral project for research continuation.

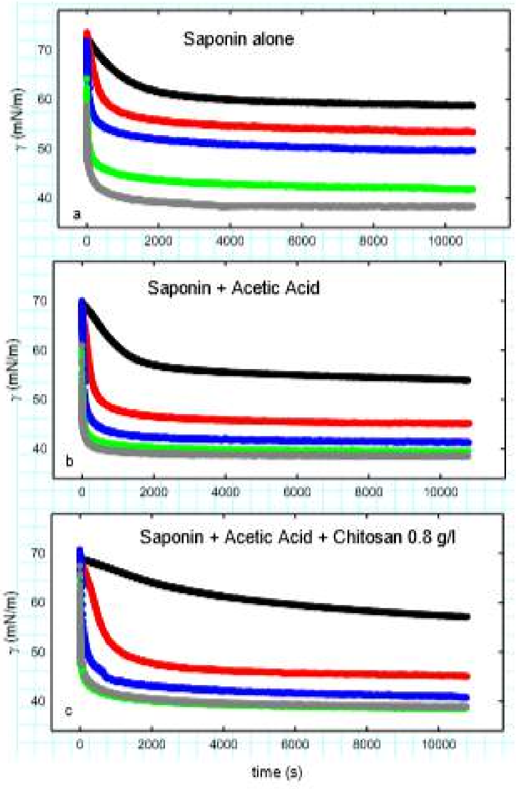


Fig. 1: Dynamic surface tension of saponin solutions in pure water (a), in acetic acid solution at 10 g/l without chitosan (b) and with chitosan at 0.8 g/l (c), for different saponin concentrations: 0.03 g/l (black), 0,05 g/l (red), 0,1 g/l (blue), 0,5 g/l (green) and 0.8 g/l (grey). (Ref. E. Santini, E. Jarek, F. Ravera, L. Ligierri, P. Warszynski, M. Krzan, „Surface properties and foamability of saponin and saponin-chitosan systems”, Coll. Surf. B, 181 (2019) 198-206)

Biocompatible Particle-Stabilized Foams and Emulsions for Biomedical Applications

(Project leader: Marcel Krzan PhD)

Joint research project of the Polish Academy of Sciences and the Bulgarian Academy of Sciences cooperation [2018-2020]

During the project, we want to develop technology for generation and application of stable foam and emulsions formulated from mixtures of hypoallergenic, non-toxic biological polymers, mainly biosurfactants. The medical impact of the biological foams and emulsions, which could be used as the thin cover film, will be further strengthened by the addition of silver nanoparticles. The application of other biologically active particles, like i.e. chitosan, silk fibroin, liposomes or dendrimers will also be carefully studied. Similar multicomponent film layer (created after foam or emulsion injection on the human body) would be the first line of the skin body defence against various pathogens.

During the second year of the project the thin foam film stability measurements were made in solutions of o-called "green surfactant": n-dodecyl-ethyl-arginate, which was enriched with the presence of colloidal silica nanoparticles (with different particle sizes - 6, 9 and 30nm).

The research was carried out simultaneously using the facilities of both institutes in Poland and Bulgaria.

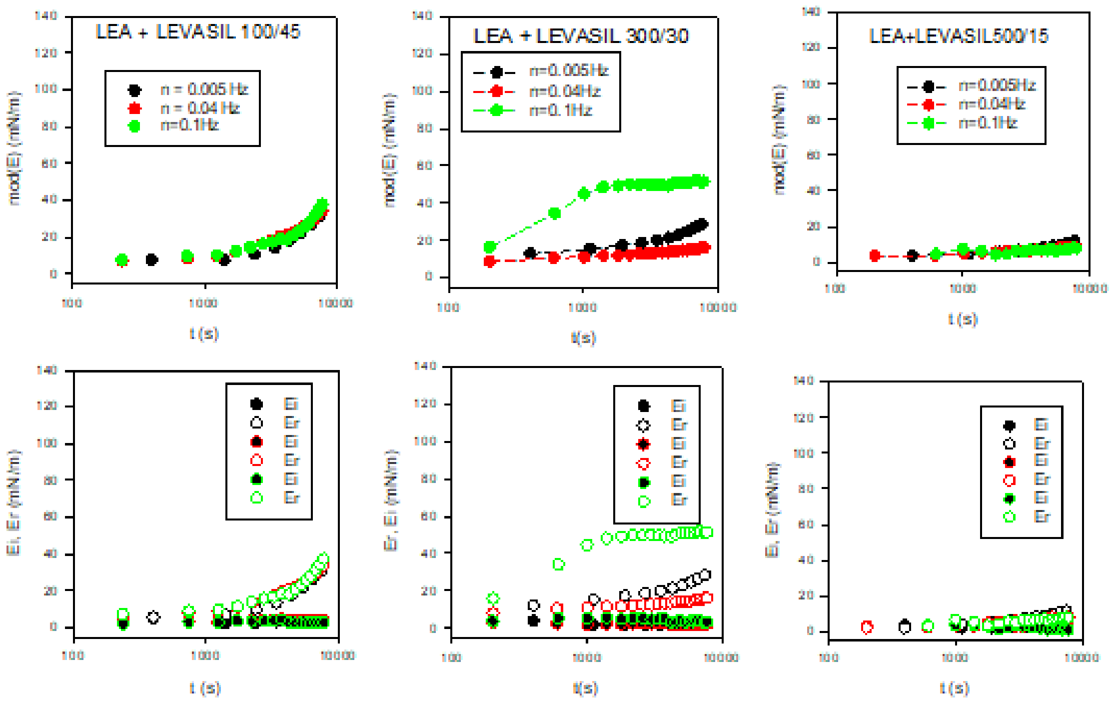


Fig. 1 The variation of complex dilational elasticity modulus (upper parts), with the detailed analysis of real and imaginary components of elasticity modulus (lower parts of figures) in the mixtures stabilized by 100 ppm of lauroyl ethyl arginate, 1% acetic acid and various silica particles (i) 30nm silica nanoparticles (Levasil 100/45%), ii) 9nm (Levasil 300/30%) and iii) 6nm (Levasils 500/15%)).