JERZY HABER INSTITUTE OF CATALYSIS
AND SURFACE CHEMISTRY
POLISH ACADEMY OF SCIENCES

KNOW | Leading National Research Centers

DETAILED RESEARCH REPORT

year 2017

Contents

Leading National Research Centre

Marian Smoluchowski Krakow Research Consortium "Matter-Energy-Future"

Leading National Research Centre [2012-2017]
(coordinator: Professor Małgorzata Witko)

In July 2012 the Institute as a member of Marian Smoluchowski Kraków Research Consortium „Matter-Energy-Future" received a status of The Leading National Research Center in physics for the period 2012-2017. Apart from the Institute, the Marian Smoluchowski Kraków Research Consortium consists of: Faculty of Chemistry of the Jagiellonian University, Faculty of Physics, Astronomy and Applied Computer Science of the Jagiellonian University, Faculty of Physics and Applied Computer Science of AGH University of Science and Technology, and The Henryk Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences.

During the year 2017 the Institute undertook a series of activities aiming at intensifying joined research activities of employees of the institutions partaking in the Consortium. Three research grants supporting joined research projects of the employees of the Institute with researchers of other partner institutions of the Consortium were pursued:

1. „Single step synthesis of ethyl acetate from bioethanol on catalysts based on palladium salts of heteropolyacids" (Dr. eng. Urszula Filek; cooperation with Faculty of Chemistry, Jagiellonian University)
2. „Functionalization mechanism of plazmonic nanoparticle surface with selected aminoacids and mercaptoamines" (Dr Magdalena Oćwieja; cooperation with The Henryk Niewodniczański Institute of Nuclear Physics Polish Academy of Sciences and Faculty of Chemistry, Jagiellonian University)
3. „Analysis of adsorption kinetics based on final structure of adsorption monolayers" (Dr. hab. eng. Jakub Barbasz; cooperation with Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University)

Six most talented students of the PhD studies lead by the Institute were receiving one year-long PhD stipends, "Smoluchowski Doctoral Scholarships".

The Institute hosted as visiting professors researchers from abroad, who gave lectures and seminars for employees and PhD students of the Institute. The researchers who visited the Institute were the following:

1. Dr. Francesca Ravera, Institute of Condansed Matter Chemistry and Technologies for Energy, Padova, Italy
2. Prof. Georg Papastavrou, University of Bayreuth, Bayreuth, Germany
3. Dr. Wuge Briscoe, University of Bristol, Bristol, Great Britain
4. Dr. Jean Daillant, Soleil Synchrotron, Gif-sur-Yvette, France
5. Prof. Hubert Motschemann, Regensburg University, Regensburg, Germany
6. Prof. Vicente Cortes-Corberan, Spanish National Research Council, Madrid, Spain

The Institute participates in a FOKUS action entitled "ArtMet", which is coordinated by the Faculty of Chemistry of the Jagiellonian University and is devoted to interdisciplinary research of metal objects.

Statutory Research

Catalytic Materials and Processes for Sustainable Development

Catalytic Processes Using Bio-Resources.
Palladium Catalysts Reactivity Modified by Tungsten Hydrogen Bronzes

(Professor Alicja Drelinkiewicz, Erwin Lalik PhD, Robert Kosydar PhD, Aleksandra Pacuła PhD, Monuika Góral-Kurbiel PhD, Michał Kołodziej MSc, Tomasz Szumełda MSc)

The project seeks to establish an effect of hydrogen bronzes H_xWO_3 formed in the palladium nanoparticles - WO_3 oxide or H_2WO_4 (c-WO_3) systems on the processes with hydrogen participation: catalytic hydrogenation of regents with C=C/C=O (furfural, cinnamaldehyde) and hydrogen evolution from aqueous solutions of electrolyte

The work included synthesis of two series Pd/WO_3 and Pd/C-WO_3 catalysts with Pd loading 0.5-4 wt% (impregnation by Pd(ac)$_2$, reduction with H_2 at 250°C), characterized with methods BET, XRD, XPS, SEM, HRTEM. The formation of hydrogen bronzes (determination of H/WO_3, Q kJ/mol WO_3) was studied with calorimetric method (22^0C, p atm). The evolution of hydrogen from 0.5 M H_2SO_4 solution was studied using CV method (Pd/WoO_3 – carbon Vulcan XC72(1/1 w/w).

The catalysts of Pd/WO_3 series of low surface area (6-8 m^2/g) consists of mono-clinic WO_3, the ones of Pd/c-WO_3 displayed higher surface area (13-30 m^2/g) and o-rombic WO_3, formed during pre-reduction with hydrogen. In catalysts of both series, the metal Pd particles of similar 5 -6 nm size appeared and apart from the Pd metal (ca. 70 %) also palladium of lower electron density ($Pd^{\delta+}$) was observed related to the presence of oxide matrix. The hydrogen uptake by the Pd/WO_3 catalyst was slow process. The measured values of H/WO_3 (0.33) and heat of the process (11.1 kJ/mol WO_3) are consistent with the literature ($H_{0.35}WO_3$, 9.6±0.8 kJ/mol WO_3) and they did not depend on the Pd loading (0.5-4 wt %Pd). The uptake of hydrogen was much quicker for Pd/c-WO_3 catalysts and the higher H/WO_3 (0.39-0.44) and heats (15.5-18.8 kJ/mol WO_3) were measured which increased with the Pd loading. Such difference in hydrogen bronzes formation process has been related to different structures of WO_3 oxide matrix.

In catalytic hydrogenation of cinnamaldehyde (toluene, 25^0C, p atm) the Pd/WO_3 catalysts displayed slightly higher activity than Pd/C-WO_3 ones. The selectivity of reaction (C=C vs C=O hydrogenation) changed with the pd loading. The highest selectivity to C=C hydrogenation (95 %) observed at the lowest Pd loading, 0.5%Pd/WO_3 exceeded even selectivity (85 %) displayed by the 2%Pd/SiO_2 reference catalyst. The rate and selectivity to C=C increased when the mixture Pd/WO_3 + WO_3 (or Pd/WO_3 + H_2WO_4) was used. The higher hydrogen uptake measured for these mixtures proved a hydrogen "spill-over" process through the catalyst-oxide interphase. These effects shows high mobility of hydrogen bonded in form of hydrogen bronzes which allows its participation in hydrogenation reaction, especially enhancing the C=C hydrogenation.

In hydrogenation of furfural, bio-reagent, (izopropanol, 35^0C, 6 bar) the 4%Pd/WO_3 catalyst displayed much lower activity than 4%Pd/c-WO_3, exhibiting high activity comparable to that of 2%Pd/C. The selectivity to unsaturated alcohol (furfuryl) on 4%Pd/c-WO_3 catalyst (75 %) was much higher than that (50 %) on Pd/C. In the presence of Pd/c-WO_3 + H_2WO_4 mixture the other product, furfuryl izopropyl eter was formed related to acidic properties of the oxides system.

In a process of hydrogen evolution (electrolysis of water in fuel cells) the Pd/WO_3 samples displayed better performance (potential, current density) than Pd/C which increased with the Pd loading.

The obtained results may be used in designing the systems consisting of hydrogen which is able to participate in various catalytic reactions. Textural features of tungsten oxide are crucial for hydrogen reactivity.

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

(Professor Wiesław Łasocha, Dariusz Mucha PhD, Barbara Bożek MSc, Adrianna Sławińska MSc)

The aim of the research was to obtain new peroxocomplexes and polyoxo compounds of metals: Mo(VI), W(VI) and V(V) with amines, and selected inorganic cations. These compounds were subject to structural, physicochemical and catalytic investigations.
The research planned for 2017 included in particular:
a) Examination of the phase system formed by: molybdenum oxide MoO_3, or molybdic acid - water - cations such as cobalt(II), nickel(II), copper(II).
b) To study the possibilities of synthesis of tin (II) and copper (I) molybdates(VI).
c) Synthesis of new molybdates of alkyl aniline derivatives.

Among the results obtained, it is worth mentioning:

Ad a) Obtaining a series of mixed compounds of the type $(Co_xZn_{1-x}) Mo_3O_{10}.nH_2O$. A new method of synthesis of mixed cobalt zinc trimolibdates with the use of microwaves was elaborated. Their chemical homogeneity and morphology were examined using SEM techniques and catalytic properties were tested.

Ad b) Attempts have been made to synthesize Sn(II) and Sn(IV) molybdates by the double exchange method using sodium and silver molybdates. Sn(II) ions from the water solutions of sodium molybdate(VI) immediately precipitated molybdenum blues, silver molybdate was exchanged to form AgCl and hexagonal MoO_3. Sn(IV) chloride was reacted with sodium molybdate to form NaCl, other solid products were not obtained in crystalline form. No molybdenum blue formation was observed during the CuCl exchange reaction with Na_2MoO_4. The obtained crystalline products are at the stage of in-depth analyzes, these phases have not been described in available databases (PDF, ICSD) yet.

Ad c) Special work was carried out on the elaboration of general regularities binding synthesis and structural studies results of methyl-aniline derivates. We have examined so far the mono-, di- and 2,4,6-trimethyl-derivatives of aniline. For these compounds, phase diagrams containing: the type of structure as a function of the position of methyl groups and selected 5 standard synthesis conditions were developed. Research was also carried out on various reaction conditions to study the possibility of synthesis of 2-n-propylaniline molybdates. Three monophasic polycrystalline precipitates were obtained, analytical data indicate that new penta-, octa- and decapolymolybdates of 2-n-propylaniline were obtained.

For some of the obtained compounds, catalytic tests were carried out, they were tests of cyclooctane oxidation using oxygen from the air (tests at IKiFP PAN) and oxidation of cyclooctene using tbhp-tertbuthydroperoxide (tests performed in University of Aveiro, Portugal).

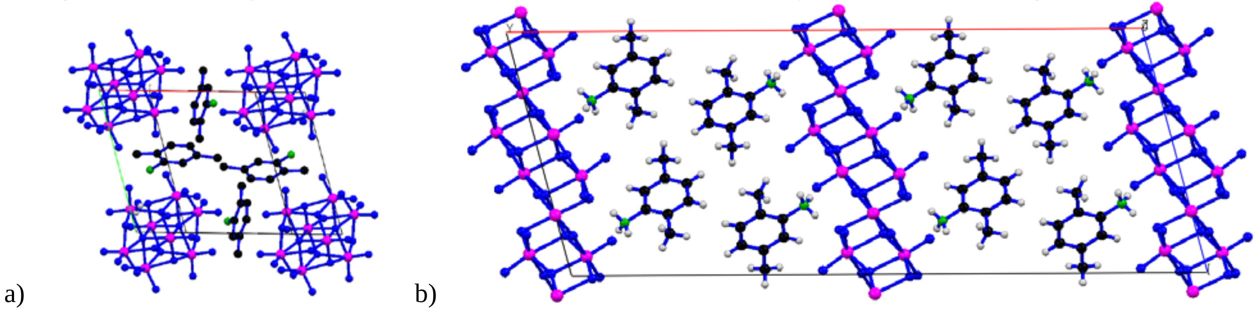

a) b)

Figure 1. Examples of new 2,5-dimethylanilinium polymolybdates obtained as part of presented research: a) elementary cell of β-octamolybdate, b) projection of pentamolybdate structure. Colors: red, green, black, gray and blue mean molybdenum, nitrogen, carbon, hydrogen and oxygen.

Catalytic Oxide Systems for Production High Value Added Products – Components of Fuels and Plastics

Optimisation of Redox Properties of Bi-Functional Copper Systems Supported on ZrO_2 Carrier – Catalysts for CO Hydrogenation and Methanol Oxidation, Leading to Obtain Fuel and Plastics Components

(Dorota Rutkowska-Żbik DSc, Michał Śliwa PhD, Katarzyna Samson PhD, Małgorzata Ruggiero-Mikołajczyk PhD, Łukasz Kuterasiński PhD, Wojciech Rojek MSc, Jerzy Podobiński MSc)

The main goal of the research task consisted of the optimization of bi-functional copper catalysts deposited on metal oxides of varying acidity and reducibility for the hydrogenation of carbon dioxide to methanol and dimethyl ether.

As the first step, a reference CuO/ZrO_2 system was selected, which was obtained via co-precipitation method using copper and zirconia nitrates and NaOH as precipitation agent. Its catalytic activity was confirmed in the preliminary catalytic tests in CO2 hydrogenation reaction towards methanol and dimethyl ether (DME); the reaction was carried in high-pressure fixed-bed flow reactor, coupled on-line with gas chromatograph equipped with FID and TCD detectors to detect reagents, in temperature range 200-350°C. As the second step, the influence of selected promoters (3% of Ag, Mn, Ru, and Ce) on the physic-chemical parameters and catalytic activity of the system was examined. The obtained samples were characterized with the following methods: XRD, low temperature N_2 sorption, H_2-TPR, NH_3-TPD, and SEM microscopy. Table 1 shows the selected physic-chemical parameters of the studied catalysts. Catalytic activity of the samples was measured in the hydrogenation of CO2 according to the procedure described above and oxidation of methanol (in temperature of 300°C, under atmospheric pressure, carried in fixed-bed flow reactor, coupled on-line with gas chromatograph equipped with FID and TCD detectors to detect reagents), which is considered the probe reaction to quantify redox and acidic properties of oxide catalysts.

The above mentioned studies are in line of the research effort aiming at utilization of carbon dioxide, which is classified as one of the greenhouse gases. Due to the continuous industrial development and heavy dependence of energetics on coal, the amount of CO2 emitted to the atmosphere is still increasing. On the other hand, the growing demand for fuels and energy fosters a search for their alternative sources and/or development of new, more effective technologies of exploitation of traditional resources. Therefore, the interest in the use of carbon dioxide, which is an attractive chemical substrate, considered a safe and economical source of carbon. Its transformations may yield numerous organic compounds, which may be used as fuels or its additives, such as methane, methanol, higher alcohols or dimethyl ether.

Table 1. Selected physico-chemical parameters of CuO/ZrO_2-X catalysts, where X = Ag, Mn, Ru, Ce

Sample	S_{SA} [m^2/g]	V_{pores} [cm^3/g]	ϕ_{pores} [Å]
CuO/ZrO_2	139	0,16	79,6
CuO/ZrO_2-Ag	193	0,15	34,5
CuO/ZrO_2-Mn	150	0,51	16,5
CuO/ZrO_2-Ru	151	0,12	34,4
CuO/ZrO_2-Ce	185	0,14	34,6

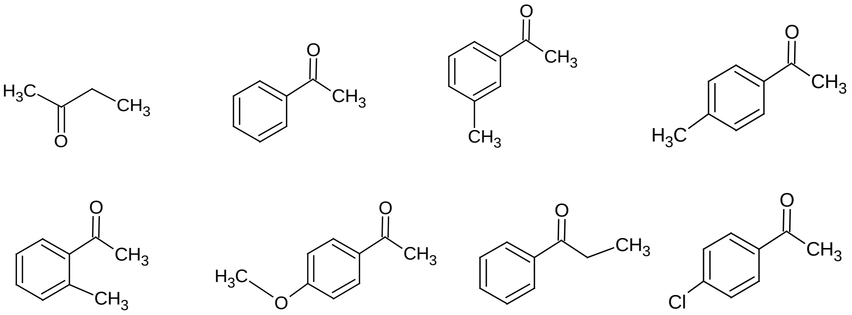
Hydrogenation of Ketones in the Presence of Metallic Catalysts Supported on Micro- and Mesoporous Carriers

(Professor Ewa Serwicka-Bahranowska, Dorota Duraczyńska PhD, Alicja Michalik-Zym PhD, Bogna D. Napruszewska MSc, Robert P. Socha PhD, Małgorzata Zimowska PhD

The research aimed at synthesis, characterization and application of supported ruthenium catalysts in the heterogeneous hydrogenation of selected ketones. Two types of carriers were used, i.e., mesoporous silica of the SBA-15 type and gel-type methacrylate-styrene resin containing 1,2-ethylenediamine (so-called FCN resin).

In the case of a silica support, ruthenium was deposited on the carrier by wet impregnation method. The obtained catalysts (1 wt.%Ru/SBA-15 and 2 wt.%Ru/SBA-15) were characterized with the aid of XRD, XPS, SEM, HRTEM techniques and nitrogen adsorption/desorption isotherms. The catalytic properties were explored in the hydrogenation of 2-butanone to 2-butanol. The product of this reaction is used in industrial practice as a solvent in chemical and textile processes. The test reaction was carried out under mild conditions of pressure and temperature (1 bar H_2 and room temperature); water was used as solvent. Both catalysts exhibited high catalytic activity and after just 30 minutes of the process the catalyst 1 wt.% Ru/SBA-15 reached 30% and 2 wt.% Ru/SBA-15 51% 2-butanone conversion. Under the same conditions, the reference catalyst containing 2 wt.% Ru deposited on high surface area silica (2 wt.% Ru/SiO_2) reached 30% conversion after 30 minutes of the catalytic test. Those materials constitute very few examples of catalysts showing catalytic activity in the hydrogenation of 2-butanone to 2-butanol under very mild pressure and temperature conditions.

The polymer-supported ruthenium catalyst, 2 wt.% Ru/FCN, was obtained by adding an appropriate amount of $RuCl_3$*H_2O aqueous solution to the THF suspension containing swollen polymer followed by reducing the Ru^{3+} ions to the metallic Ru using $NaBH_4$. The catalyst was characterized using the following methods: XRD, XPS, FTIR, SEM, EDS and HRTEM. Catalytic properties were investigated in the hydrogenation reactions of selected ketones: ethyl methyl ketone, acetophenone, methylacetophenone (CH_3 group in the ortho, meta and para position), p-chloroacetophenone, p-methoxyacetophenone and ethyl-phenyl ketone (Scheme 1).



Scheme 1. The structures of studied ketones

Hydrogenation reactions of selected ketones were carried out under mild pressure and temperature conditions (40°C, pressure of H_2 = 1 bar) in biphasic IO/H_2O (1:1) (IO = isooctane) solvent system for 4 hours. The studied catalyst showed activity in the catalytic hydrogenation of selected ketones. In all reactions the hydrogenation of C=O group yielding the appropriate secondary alcohol was observed. In the case of acetophenone the hydrogenation of aromatic ring occurred as well.

Selective Hydrogenation of Benzene over Metallic Catalysts

(Professor Ewa Serwicka-Bahranowska, Katarzyna Pamin PhD, Jan Połtowicz PhD, Alicja Michalik-Zym PhD, Małgorzata Zimowska PhD)

Reaction of benzene hydrogenation is a consecutive proxess in which cyclohexene, the desired product, appears as an intermediate. The challenge consists in choosing a catalyst and the reaction conditions in such a way as to hinder subsequent cyclohexene hydrogenation. Ruthenium is one of the most promising metallic catalysts employed in this process.

Previous results obtained in our laboratory for supported ruthenium catalysts pointed to the dependence of the reaction course on the degree of ruthenium dispersion. Patent literature (Asahi Chemical Ind.) indicates that unsupported ruthenium black is the catalyst enabling benzene hydrogenation to cyclohexene with high efficiency. However, no systematic study on the effect of Ru black particle size on the catalytic performance in benzene hydrogenation is available. Therefore, the present study aimed at shedding light on this issue by preparing a series of ruthenium black catalysts of different metal particle size and determining their activity and selectivity in the selective hydrogenation of benzene to cyclohexene.

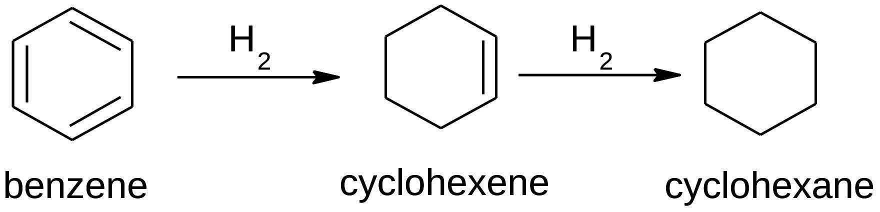


Figure 1. Scheme of benzene hydrogenation

Modification of Ru black particle size was achieved by variation of synthesis parameters such as: type of base used for the precipitation of ruthenium hydroxide, the manner of addition of the precipitating base to the reaction mixture, speed of mixing, or reaction temperature. In the second stage of synthesis, consisting in reduction of the hydroxide precursor to metallic Ru, the agitation speed of the reaction mixture was varied.

XRD analysis of the synthesized series of Ru black showed that the crystal sizes varied in the range 5 - 15 nm. The catalysts were tested in the reaction of benzene hydrogenation in a 1000 ml titanium reactor, under 50 bar pressure, at temperature 150^{o}C and mixing rate of 1500 rpm. The results showed that the yield of cyclohexene increased with the decreasing ruthenium particle size.

These results are the basis for further work aimed at optimization of the ruthenium black particles dispersion.

Catalytic Oxidation of Cyclohexanone to ε-Caprolactone with Hydrogen Peroxide over Natural Basic Minerals

(Professor Ewa Serwicka-Bahranowska, Robert Karcz PhD, Bogna D. Napruszewska MSc, Joanna Kryściak-Czerwenka PhD, Joanna Olszówka PhD, Elżbieta Bielańska PhD)

ε-Caprolactone is a important substrate for production of biocompatible and biodegradable polymers – polycaprolactone and its derivatives. Currently it is manufactured on an industrial scale by means of a Baeyer-Villiger oxidation of cyclohexanone with organic peroxyacids, which causes environmental concern due to the hazardous nature of the oxidants and copious amounts of acid by-products.

The use of hydrogen peroxide as cyclohexanone oxidant, coupled with an appropriate catalyst, represents a more green alternative. It has been demonstrated that materials with basic properties, such as synthetic hydrotalcites, are efficient catalysts for this process. Our experience proves that also natural basic minerals may show activity in cyclohexanone oxidation. For this reason we undertook investigation of a series of basic minerals, obtained from different deposits in Poland, for their catalytic properties in the studied reaction. In addition, the effect of grinding in a planetary mill on the catalytic activity has been assessed.

8 mineral samples were the subject of investigation: 2 dolomites, 3 magnesites, 2 chlorites, and 1 serpentinite. The best performance among the as received samples was observed for the Jelenia Góra dolomite ($CaMg(CO_3)_2$), which yielded 14% ε-caprolactone. Selected minerals were subjected to grinding in the planetary mill, for 10 and 30 min periods. It has been found that short milling time was beneficial for the catalytic performance (e.g., in the case of Jelenia Góra dolomite 23% ε-caprolactone yield was obtained), but the longer milling time was not always advantageous. To explain correlation between catalytic activity and physicochemical properties of the minerals the materials were thoroughly characterized with XRF, XRD, SEM, particle size distribution (PSD), BET surface area, measurement of contact angle, FTIR and UV-Vis spectroscopies. It has been found that in all cases milling causes a decrease of minerals crystallinity and thermal stability. However, as revealed by PSD measurement, short milling provided material with decreased particle size, whereas prolonged milling resulted in agglomeration of particles. The latter effect suppressed the beneficial influence of milling on the catalytic activity. Moreover, it has been observed, that the presence of Fe impurity in the mineral sample led to the rapid deterioration of the catalytic properties.

It may be concluded that basic minerals are active, cheap and eco-friendly heterogeneous catalysts for liquid phase oxidation of cyclohexanone. Catalytic activity of minerals can be enhanced by milling. However, optimization of mechanochemical treatment is required, to avoid aggregation of catalyst particles resulting in hindrance of catalytic activity.

Electrochemical Properties of Gold as a Material for Connectors in Solid Oxide Fuel Cells

(Michał Mosiałek DSc, Robert P. Socha PhD, Professor Paweł Nowak, Grzegorz Mordarski PhD)

One of the unresolved problems connected with the construction of high-temperature solid oxide fuel cells is the choice of the proper material for the connector, joining cathode with the external electrical circuit. Most frequently used material, heat-resisting steel, liberates during the work of the fuel cell volatile chromium compounds, which degrade of the cell. The proper material seems to be gold, which shows high electrical conductivity, high plasticity and good corrosion resistance. The aim of this work was to characterize the electrochemical properties of gold at the conditions of the cathode of high-temperature solid oxide fuel cells, with the special attention to gold susceptibility to migration under the influence of the electrical field.

The investigation concerned the interaction of gold with most frequently used solid electrolytes as well as with oxygen, the main component of the atmosphere in the cathode compartment of the cell. Following electrolytes were used in the investigations: $Ba_{0.95}Ca_{0.05}Ce_{0.9}Y_{0.1}O_3$, $Zr_{1-x}Y_xO_{2-0.5x}$, $Ce_{0.8}Sm_{0.2}O_{1.9}$, $Ce_{0.8}Gd_{0.2}O_{1.9}$. The properties of the gold electrode was investigated in two- and three-electrode measuring systems, with the use of both pseudo-point as well as porous gold electrodes, and with the application of chronoamperometry, cyclic voltammetry and electrochemical impedance spectroscopy as the measuring methods.

The chronoamperometric investigations showed that gold under the influence the negative polarization undergoes to some extent migration, when contacted with the investigated electrolytes. It manifests itself as the increase of current in the initial period of the experiment (up to 4 h), and the appearance of the gold deposit on the surface of the electrolyte around the electrode. After the initial period of time current stabilizes and stays constant for 20 hours. It means that the appearance of deposit, which increases the area of contact between the gold electrode and the electrolyte, causing at the same time the decrease of the current density, prevents further deposit growth. Such behavior was observed only at the polarization more cathodic than –0.3 V. At the polarizations less cathodic the current was constant from the beginning and no formation of the gold deposit was observed.

Information on the interaction between gold and oxygen was gained from the analysis of the voltammetric and impedance measurements, performed at different temperatures and oxygen partial pressures. No oxidation of gold at the conditions of the cathodic compartment of a cell was observed. However, the reaction of the oxygen reduction occurs with significant rate, which is advantageous for the application of gold as a material for connectors. Basing on the analysis of the dependence of the polarization resistance on the oxygen partial pressure it was established that the reaction proceeds in the mixt kinetics regime. From the analysis of the dependence of the polarization resistance on temperature the activation energy 1.52 eV was calculated.

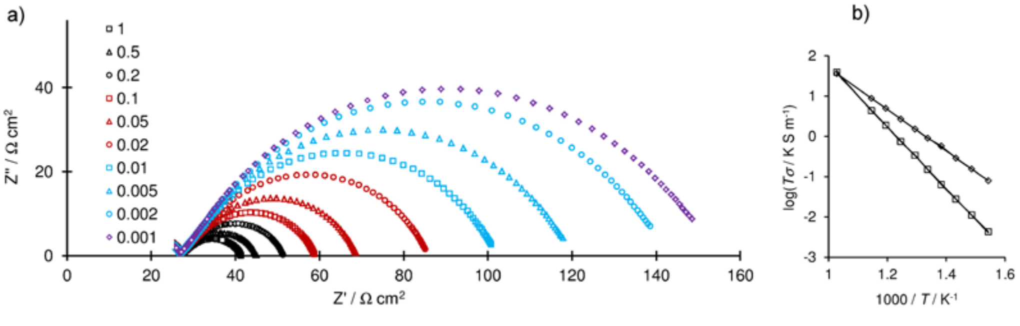


Figure 1. Exemplary impedance spectra of Au electrode contacted with $Ce_{0.8}Sm_{0.2}O_{1.9}$ electrolyte at the temperature of 700 °C. Numbers in the legend means P_{O2} (a), dependence of the resistivity on T: polarization resistance – squares, electrolyte resistance – diamonds (b).

Enzymatic Processes – Basic and Applied Research. Selected Enzymes and Photocatalytic Set Free CO – Computational Research

(Professor Tomasz Borowski, Maciej Szaleniec DSc, Maciej Guzik PhD, Agnieszka Wojtkiewicz PhD, Mateusz Tataruch PhD, Sangita Kachhap PhD)

The purpose of our basic research pursued with computational methods is to obtain insights into mechanisms of enzymatic reactions and structures of selected proteins and/or their complexes with ligands.

One of the studied enzymes is **Lcar** (N-carbamoyl-L-amino acid amidohydrolase) – an enzyme employed in industry for kinetic resolution of racemic mixtures of N-carbamoyl-L-amino acid. The studied enzyme is a functional homodimer that binds Co^{2+} ions in the active site. Despite the fact that the crystal structure of this protein is known there are still several open questions: if the enzyme binds one or two metal ions per active site, if it is a cooperative enzyme, how is the substrate bound to the enzyme and what is the detailed reaction mechanism. Classical molecular dynamics simulations (each 80 ns long) were performed for **Lcar** homodimer for two variants: with one or two cobalt ions per active site. The 12-6-4 LJ nonbonded model was used to describe metal ion-protein/water interactions. Analysis of the trajectories revealed that in both cases the geometry around the metal ion and the composition of its first coordination shell changed with respect to the starting crystal structure, which suggests that the 12-6-4 LJ model does not give a reliable description of the metal site of **Lcar**. Hence, the bonded model parameters were derived (explicit metal-ligand bonds) and are currently used in MD simulations.

Another enzyme studied is **DapB** (Dihydrodipicolinate reductase) – a bacterial enzyme that catalyses NAD(P)H-dependent reduction of dihydrodipicolinate to tetrahydrodipicolinate. Crystal structure solved for the complex with an inhibitor was the starting point for a series of MD simulations (each > 55 ns) done for various plausible starting geometries of the enzyme-substrate complex and different ionisation state of a key residue in the active site. Binding energies computed with the MMPBSA method allowed us to choose the most likely configuration of the reactive complex, which was used to prepare a cluster model used in quantum-chemical studies on the reaction mechanism.

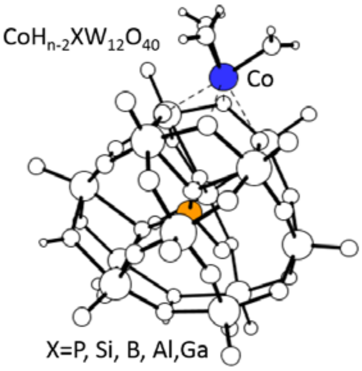
Another protein studied is **Pru p 3** (Nonspecific lipid transfer protein) – a major allergen from peach. This protein binds fatty acids and binding of oleic acid causes a significant increase of affinity to IgE antibodies isolated from patients allergic to peach. Classical MD simulations (150 ns each) were done for the apo form of **Pru p 3** and its complexes with oleic acid or anion of the latter. Analysis of protein-ligand interactions revealed amino acid residues responsible for ligand binding and identified regions of the protein that undergo the most significant structural changes in response to ligand binding.

Two metalloenzymes studied previously in our group (**QDO** and **Ni-ARD**) catalyse oxidative cleavage of their substrates with release of CO molecule. Hence, it was interesting to study reaction mechanism of a photochemical reaction thereby a synthetic compound (from the same class as substrates of the two enzymes) reacts with dioxygen with a release of CO. Within the computational studies, done at (TD-)DFT level, we have located key stationary points and minimal energy crossing points for low-lying excited states (S1, T1, T2). The results allowed us to put forth the likely mechanism of photoactivation of the compound and its reaction with dioxygen.

Experimental and Theoretical Description of Heteropoly Compunds, Modified at the Central and Compensating Ions Position

(Professor Małgorzata Witko, Renata Tokarz-Sobieraj DSc, Anna Micek-Ilnicka DSc, Urszula Filek PhD, Robert Gryboś PhD)

The main goal of the experimental and theoretical research was to describe the electronic structure as well as physico-chemical and catalytic properties of cobalt salts of heteropoly compounds. Studied systems possessed a Keggin anion structure with selected central ions (P, Si, B, Al, Ga) and with tungsten in the addenda atom position.



As a continuation of research on heteropoly compounds with various central cations, cobalt salts with a general formula $CoH_{(n-2)}XW_{12}O_{40}$ (n=3, 4, 5; X= P, Si, B, Al), were synthesized. One Co2+ counter cation was introduced for each Keggin anion: $CoHPW_{12}O_{40}$ (CoHPW), $CoH_2SiW_{12}O_{40}$ (CoHSiW), $CoH_3AlW_{12}O_{40}$ (CoHAlW), $CoH_3BW_{12}O_{40}$ (CoHBW). For commercially unavailable compounds (HAlW, HBW), a new synthesis method utilizing cobalt nitrate was developed.

Physico-chemical properties of the new compounds were measured. Derivatographic analysis revealed high thermal stability, comparable with respective acids. Temperatures of decomposition were, for example: CoHPW -578^{o}C (HPW-618^{o}C), CoHSiW -600^{o}C (HSiW-539^{o}C) i CoHAlW -489^{o}C (HAlW-474^{o}C). Infrared spectra show bands characteristic for a Keggin anion. Detailed analysis of the bands shows strong interaction between the introduced Co^{2+} cation and the Keggin anion, as evidenced by large shifts in W-O_b-W bands (901 cm^{-1} for CoHPW salt vs. 883 cm^{-1} for the HPW acid) and W-O_c-W bands (772 cm^{-1} in salt vs. 788 cm^{-1} in acid).

Catalytic properties were tested in a reaction of dehydration (in He stream) and oxidation in air) of ethanol. Samples weights were 0.1g, reagent flow through the reactor was 35 ml/min, WHSV- 2,0 h^{-1}. The identified alcohol conversion products were: ethylene, diethyl ether and acetaldehyde. Additionally, during oxidation reaction, trace amounts of ethyl acetate were detected. Experimental data shows that introduction of Co2+ cation reduces the acidic properties of the compounds, while increasing the redox properties, which change in the order:

CoHPW>CoHSiW>CoHAlW~CoHBW

In parallel to experiments, theoretical DFT calculations were performed, using both cluster (single Keggin anion with protons and co cations) and periodic models. Obtained results allowed for quantifying redox properties (energies of frontier orbitals, band gap width). Obtained series correspond well with experimentally observed catalytic activity. Acid properties were modeled by calculating H+ binding energy. The calculations allowed to determine how the energy of proton binding by the Keggina anion (which is a measure of the strength of acidic centers) affects: a) the chemical nature of the central ion (charge of the inner tetrahedron), b) the presence of cobalt as a compensating cation, c) the degree of hydration of cobalt ions. Two types of acid sites were considered: (i) directly coordinated to Keggin anion and (ii) created as a result of water dissociation on cobalt cation.

Physics and Chemistry of Surfaces and Nanostructures – Experiment and Theory

Novel Materials with Different Pore Structure: Synthesis, Physicochemical and Catalytic Properties, and Spectroscopic Studies

(Professor Bogdan Sulikowski, Mariusz Gackowski PhD)

Synthetic zeolites are very important catalysts in the chemical industry and oil refining, so their properties are extensively studied since few decades. The most important advantages of zeolitic catalysts are caused by the fact that active sites (Brønsted & Lewis centres) are situated inside micropores. However, diffusional limitations are frequently observed when large molecules are processed. Moreover, such mass transfer limitations may cause a fast catalyst deactivation. To improve the catalytic efficiency of zeolites their hierarchical forms containing mesopores could be produced, using predominantly alkaline solutions.

While desilication has been studied for various zeolites, such as mordenite, ferrierite, Beta, up to now most of work has been focused on ZSM-5 zeolite. However, out of over 200 zeolite structures known, zeolite Y is of paramount importance. This zeolite, manufactured on a large industrial scale, is used for production of cracking and other specialty zeolitic catalysts. Desilication of standard zeolite Y turns out to be much more difficult task that desilication of other structures due to the high concentration of AlO_4^- groups protecting the zeolite framework against the OH^- attack. Treatment with alkaline solutions applied to the standard zeolite Y with Si/Al=2.5 led largely to its amorphization, similarly to direct dealumination by acid solutions.

We have performed a detailed study of desilication of zeolite Y subjected earlier to dealumination. Thus, parent zeolite Y with increased Si to Al ratio was desilicated with various alkaline solutions (NH_3 aqua, NaOH, tetrabutylammonium hydroxide - TBAOH and NaOH/TBAOH mixtures). The acid properties of the samples were characterised extensively using first of all FT IR and solid-state NMR spectroscopies. In particular, the concentrations of Brønsted and Lewis sites, their strength as well as heterogeneity of the Si-OH-Al groupings were followed by IR spectroscopy. On the other side, the status of Al and Si in the samples was studied parallelly using NMR. The addition of tetrabutylammonium ions to NaOH proved to be a crucial modification of the zeolite Y desilication procedure.

Finally, the catalytic properties of the zeolites obtained were evaluated in the isomerization of α-pinene to yield limonene and camphene. The latter constitute valuable feedstock for the chemical and food industries. The reaction was carried out in the liquid phase. Our earlier studies led to the selection of a few zeolites, having different structure, chemical composition and the architecture of pores. Development of a highly active and selective catalyst based on zeolite Y requires however further rationalization, by understanding on a molecular level the processes taking places whilst a zeolite under question is being desilicated. Quantitative deconvolution of the NMR spectra has led to estimation of the framework composition (that is its Si/Al ratio) and distribution of aluminium between the *framework* and *nonframework* positions.

Analysis of the catalytic data obtained on these zeolites, coupled with the data acquired on other aluminosilicates with different framework architecture, will allow one to arrive at general relationships existing between the zeolite composition, framework Si/Al ratio, aluminium distribution, the presence of acid sites, the presence of micro- and mesopores, and the course of α-pinene isomerization.

Structural, Electron Properties and Dynamics of Surface and Nanostructures Studied with Microscopic and Spectroscopic Techniques Radiation in the Ultra High Vacuum Conditions.

Magnetic Properties of the CoO/Fe(001) System: Exchange-Bias in Function of Layer Thicknes and Temperature

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

High-temperature reorganization of the adsorbed oxygen on the W(110) surface was investigated using low-energy electron diffraction (LEED) and low-energy electron microscopy (LEEM). Using these two techniques, we have resolved two out of three high-temperature tungsten oxide phases reported in the literature. We verified the structural properties of oxygen adsorbed on tungsten by comparing bright- and dark-field LEEM images. In particular, we determined the relationship between atomic steps and the occurrence of a specific structural domain in different phases. Finally, we described the temperature-induced structural transformation that was directly observed, both in real and reciprocal space, for two oxygen surface phases formed on W(110). By careful examination, it was proven that under specific circumstances, this transition can be conducted in both directions, and it is possible to halt it at any stage.

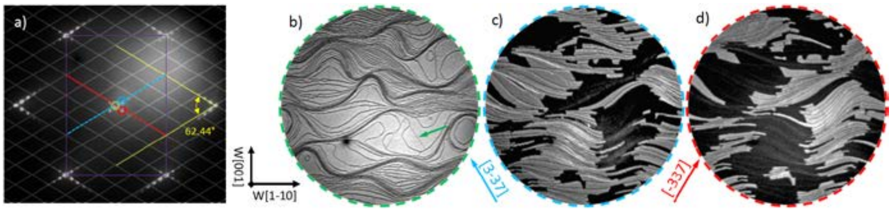


Figure 1 One of the identified oxide phases: (a) LEED pattern with a W(110) surface unit cell (violet rectangle) and specific spots used for bright-field (b) and dark-field imaging (c, d).

We studied the magnetic properties of epitaxial double-wedge CoO/Fe bilayers grown on MgO(001). We collected a comprehensive set of data derived from the hysteresis loops measured as a function of temperature in a wide range of CoO and Fe thicknesses using Kerr microscopy imaging. We focused on relatively high temperatures and CoO layers with small thicknesses to address the onset of the exchange bias. We identified a characteristic CoO thickness of 30-40 Å, above which the films can be considered bulk-like. However, a considerable exchange bias is still observed even for CoO films as thin as 12 Å if the temperature is sufficiently low. From the results of the XPS measurements, we deduced the presence of an interfacial iron oxide layer of mixed stoichiometry and a uniform composition of the CoO layer along the CoO wedge.

We performed first-principles calculations of the adsorption of atomic oxygen and magnesium on the Fe(001) surface. Two adsorption scenarios were considered. In the first process, the Mg atoms were adsorbed on the Fe(001) surface with preadsorbed O atoms, whereas in the second scenario metallic Mg preadsorbed on the Fe(001) surface was exposed to oxygen. For both O and Mg atoms, fourfold hollow sites were found as the energetically most favorable adsorption sites on the clean Fe(001) surface. The result of both adsorption scenarios was the formation of a MgO adlayer on the Fe(001) surface with a sharp MgO/Fe interface. In particular, the deposition of Mg atoms on O/Fe(001) showed that magnesium can pull out O adatoms from the Fe surface. Structural, electronic, and magnetic properties were analyzed as a function of O and Mg coverages. The calculated electronic structure and magnetic moments showed that the full MgO monolayer affects the properties of the Fe surface much weaker than an incomplete MgO adsorbate layer.

The first stage of commissioning of the PEEM/XAS beamline at the National Synchrotron Radiation Center Solaris has been successfully accomplished and the beamline is ready to accept proposals and user in the framework of Central European Research Infrastructure CERIC-ERIC.

Studies of the Influence of Microscopic and Physicochemical Parameters on the Ability to Control the Properties of Systems with Biomedical Importance.

Forced Structural Changes in Selected Oligosaccharides

(Professor Tomasz Pańczyk, Wojciech Płaziński DSc, Barbara Jachimska DSc, Agnieszka Brzyska PhD)

The aim of the studies was an analysis of influence of various microscopic and physiocochemical parameters on the ability of controlling the phenomena occurring in systems with biomedical importance. Particularly, the studies were focused on the compilation of already gathered information in order to produce multifunctional models, utilizing phenomena observed due to the coupled influence of the microscopic parameters. The studies involved carbon nanotubes and dendrimers as drug delivery systems but also the phenomena of the tautomeric balance in aldo- and ketohexoses. There were also attempts of explanation of systematic preferences to form either five- or six-membered rings, exhibited by different types of saccharides. Moreover, we studied the fundamental conformational degrees of freedom that are related to furanose and pyranose monomers (e.g. rotation of hydroxymethyl and lactol moieties, ring inversion and pseudorotation) by applying the *ab initio* potentials combined with molecular dynamics technique.

The extensive studies on the application of carbon nanotubes or dendrimers as nanocarriers of drugs carried out so far are fully justified because of highly important practical meaning of these systems. The key problem is the design of a system which is soluble in aqueous media and is able to deliver and release of drugs in response to natural signals. Our studies, carried out so far, are very promising, however, we noticed also some drawbacks of the discussed systems. It was found that tuning of the systems for the individual chemical (pH changes) or physical (external magnetic field) signals not always leads to the desired properties of the carriers. It was found in the case of carbon nanotube functionalized by polyethylene glycol coupled with folic acid residues that these molecules partially incorporate to the nanotube interior and block the release of doxorubicin. But application of magnetic nanoparticles as extra modifiers leads to fully functional systems. The studies focused on conformational features of carbohydrates led to developing a novel computational method that combines advantages of both classical molecular dynamics simulations and the quantum-mechanics calculations. The two-step method relies on selecting a set of conformations that are representative for saccharide molecule interacting with solution and on using them in the subsequent quantum-mechanical calculations. Such approach allows to reduce the errors resulting from overestimation the hydrogen bond-rich structures which exhibit low energy but are essentially absent in the presence of polar solvent. On the other hand, applications of dendrimers in drug delivery systems have motivated an upsurge in this promising field. Dendrimer structure potentially allows two types of encapsulation: one in the dendrimers localized in the multilayer shells, and the other in the microcapsule interiors, resulting in a dual delivery and/or release system. For the host-guest system of dendrimer-drug complexes, the interaction between dendrimers and drugs are important for an understanding of the molecular mechanism of the delivery system and providing insights to optimize the system.

The systems composed of carbon nanotubes/dyes/drug or PAMAM dendrimers reveal useful properties as drug delivery systems. They offer ability of triggering drug release in response to the pH change. Practical realization of such functional composite systems needs, however, further studies. Conformational rearrangements involving the pyranose and furanose rings are essential for carbohydrate-protein interactions and require consideration when applying the computational methodologies aimed at predicting the 3D carbohydrate structure.

Nanostructures of Soft Matter

Topology of Protein Monolayers at the Solid/Electrolyte Interfaces and Mechanisms of Their Interactions with Ionic and Macromolecular Ligands.

Determining Binding Efficiency of Divalent Cations to Serum Albumin Monolayers

(Professor Zbigniew Adamczyk, Jakub Barbasz DSc, Marta Kujda PhD, Aneta Michna PhD, Maria Morga PhD, Małgorzata Nattich-Rak PhD, Marta Sadowska PhD)

Serum albumin (HSA) binds many endogenic and egzogenic ligands, controls their concentration and facilitates their efficient transport to various tissues. In addition to the important physiological role, HSA is often used for drug delivery and for medical device coating (hemodializer membranes and peacemakers, orthopedic titanium implants, catheters) which prevents adhesion of other proteins, platelets and bacteria. Albumin monolayers are exploited as biosensors for a quantitative detections of various ions such as Cd^{2+}, Ni^{2+}, Zn^{2+}. However, experimental studies of these ligand binding were only performer in the bulk by applying spectroscopic and fluorescence methods. Despite essential significance, no results were reported dealing with ion ligand binding to albumin monolayers of controlled coverage and structure adsorbed at solid surfaces. Therefore, the main goal of this work was obtaining quantitative characteristics of Cu^{2+}, Zn^{2+} cation binding to albumin monolayers adsorbed at mica surfaces that represents an model substrate of well-defined surface properties. Unique electrokinetic techniques were applied in these investigations, which enabled to perform *in situ* measurements of ion binding. The experimentally determined zeta potential values were converted to the ligand coverage using the nonlinear Gouy-Chapman model. Typical isotherms obtained in this way are presented in Figure 1. The isotherms were theoretically interpreted in terms of the Langmuir model characterized by the equilibrium adsorption constant K_a equal to 3.77×10^{-5} and 2.73×10^{-4} cm for Zn^{2+} and Cu^{2+} ions, respectively. This corresponds to the binding energy of the monolayer $\Delta\phi_m$ equal to -8,0 and -10 kT, respectively.

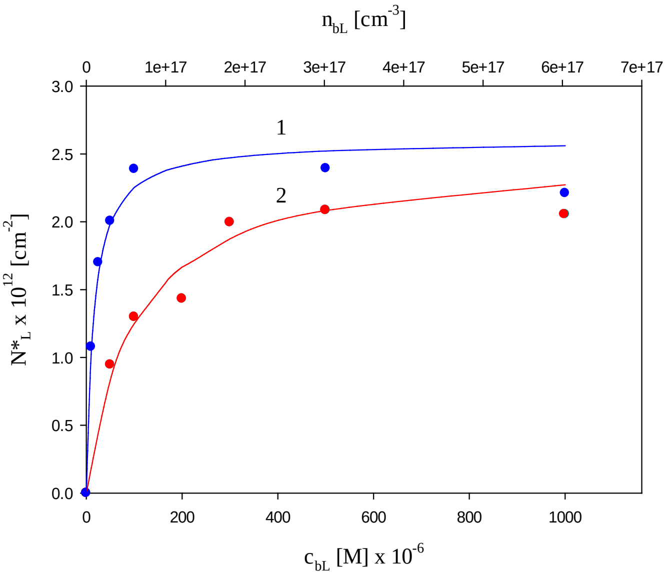


Figure 1. Adsorption isotherm of: 1) Cu^{2+} (●) i 2) Zn^{2+} (●) ions at the albumin monolayer adsorbed at mica shown as the dependence of the surface concentration N_L^* [cm^{-2}] on the bulk concentration c_{bL} [M] (lower horizontal axis and n_{bL} [cm^{-3}] (upper horizontal axis). The solid lines represent theoretical results derived from the Langmuir isotherm (ionic strength 10^{-2} M, pH 5.7, T = 298 K).

Determination of Mechanisms of Synthesis, Structure, and Transport Parameters of Supported Colloidal Particle Multilayers.

Kinetic Aspoects of Spherical Particles Multilayers Formation Using "Layer-byLayer" Method

(Paweł Weroński DSc, Jakub Barbasz DSc, Piotr Batys PhD)

Multilayer adsorption of colloidal particle at solid-liquid interface leads to a spontaneous formation of porous thin film that has a significant effect on ion transport from the bulk solution to the adsorption surface. That allows for using colloidal particle multilayers as functional nanomaterials such as membranes or heterogeneous catalysts. A simple and effective method of synthesis of colloidal particle multilayers is the “layer by layer” technique. Usually, we have assumed that maintaining a constant adsorption time during the synthesis of subsequent layers lets us obtain a constant surface coverage. This assumption, however, is not obvious.

The main aim of our research was to verify the above assumption by theoretical description of the adsorption kinetics of subsequent layers. The “layer by layer” adsorption process can be described theoretically with the random sequential adsorption model. The model allows also a quantitative description of subsequent particle adsorption probability at given surface coverage. To determine the relationship between the particle adsorption probability on one hand and surface coverage as well as multilayer structure on the other, we developed an adequate numerical algorithm and computer software. Next, we simulated five model multilayers of spherical particle at the surface coverage of subsequent layers θ_m = 0.1, 0.2, 0.3, 0.4 and 0.5. Numbers of single layers in the multilayers were equal 44, 25, 20, 18, and 17, respectively. The mean multilayer thickness of all the structures was about 30 particle radii. For subsequent layers of each multilayer, we then carried out calculations of colloidal particle adsorption probability as a function of surface coverage, $P(\theta)$.

Our results let us formulate interesting conclusions. The important parameter determining the character of the relationship $P(\theta)$ is the maximum surface coverage of single layer θ_m. In the case of large maximum surface coverage of single layer, θ_m = 0.5, the function $P(\theta)$ calculated for subsequent layers of the multilayer exhibits a similar behavior. With a decrease of the parameter θ_m, however, the function exhibits increasing variability with the layer number. This variability is especially significant for first layers of the multilayer. The results obtained for the parameter θ_m = 0.1 suggest that particle adsorption probability at the synthesis of the first and second layers are different by several hundred percent. Under such conditions, the assumption of constant surface coverage at a constant adsorption time of subsequent layers seems completely groundless.

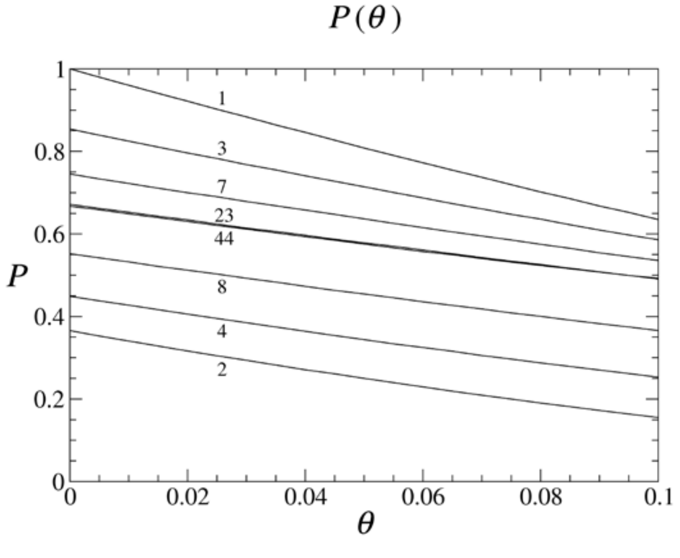


Figure 2. Particle adsorption probability at the parameter θ_m = 0.1, calculated for the layers 1, 2, 3, 4, 7, 8, 23, and 44.

Kinetics of Three-Phase Contact Formation at Quartz Surface in Solutions of Surface-Active Substances

(Jan Zawała DSc, Dominik Kosior PhD, Anna Niecikowska PhD, Agata Wiertel-Pochopień MSc, Marcel Krzan PhD)

The aim of this research task was to determine mechanism of difference in quartz particles flotation recovery observed during laboratory flotation tests, carried out in (i) hexyloamine solutions of different concentrations (single-bubble Hallimond Tube) and (ii) pure and mixed solutions of non-ionic and cationic surface-active substances (microflotation). Experiments related to influence of mixed flotation reagents on the quartz particles recovery were carried out in cooperation with Department of Mining Engineering, Dumlupinar University, Turkey, while influence of short-chain amine on quartz flotation was performed in cooperation with Faculty of Geoengineering, Mining and Geology, Wrocław University of Technology. The partner institutions provided results of laboratory flotation tests. Our duty was to perform model experiments, which results can be used to explain mechanisms responsible for different quartz recovery observed under different physicochemical conditions. The experiments carried out in our laboratory were aimed to determine quartz surface contact angles and the kinetics of collision and attachment of single air bubbles to the quartz smooth surface and under conditions corresponding to the flotation tests.

In the case of quartz flotation in pure liquid of cationic surfactant (n-hexadecyl-trimethylammonium bromide – CTAB) the recovery of quartz particles was increasing significantly, from ca. 10% to almost 100%, when the CTAB concentration increased from 1×10^{-7} M to 1×10^{-3} M. Application of the non-ionic surface-active substance (n-octanol) as an additional flotation reagent (in flotation mixture) allow for significant decrease of the CTAB concentration (ca. 4 orders of magnitude) needed to achieve comparable flotation recovery. On the basis of fundamental research performed in our laboratory it was found that, depending on the solution composition, two different factors are responsible for rupture of the wetting film and bubble attachment to the quartz surface. In the case of pure CTAB solutions these factors are (i) destabilizing (attractive) electrostatic interactions between surfaces of bubble and quartz (for CTAB concentrations 1×10^{-7} M - 2×10^{-6} M) and (ii) increase in the quartz surface hydrophobicity, related to the CTAB molecules adsorption at negatively charged quartz/liquid interface (for CTAB concentrations > 3×10^{-6} M). In the case of mixed non-ionic/cationic surface-active substances solutions, non-ionic n-octanol was responsible only for modification of the liquid/gas (bubble) interface properties and change in the bubble motion parameters, i.e. shortening its bouncing time and prolongation of bubble-solid surface time of contact. The rupture of the liquid film was driven by the electrostatic interactions, which was possible due to CTAB molecules presence. The CTAB concentration was small, nevertheless sufficient for bubble interface charge reversal from positive to negative and liquid film destabilization (rupture).

In the case of flotation of quartz particles in hexylamine solutions it was found that quartz recovery was increasing strongly with amine concentration and the best recovery was obtained for solution pH close to the pK_a value. The results of experiments performed in our laboratory confirmed these observations – the variations in time of the three-phase contact formation by the single bubble colliding with quartz surface correlated very well with flotation tests results. After analysis of the results, which included analysis of influence of amine concentration and solution pH on bubble velocity, shape deformations and kinetics of collision and attachment as well as percentage of ionized and non-ionized hexylamine forms in the solutions it was concluded that co-adsorption of these forms both at the bubble and quartz surfaces is responsible for the wetting film destabilization.

Functional Polyelectrolyte Multilayers Films

(Professor Piotr Warszyński, Marta Kolasińska-Sojka PhD, Tomasz Kruk PhD)

The main objective of the task was to investigate the influence of ion valency from the electrolyte used on the formation of functional thin multilayer films containing selected polyelectrolytes and to determine the permeability of the obtained multilayers for selected electroactive compounds. Depending on the type of electrolyte and polyelectrolyte, films with different parameters can be obtained, which we use to control the structure and physicochemical properties (thickness, roughness and permeability for selected macromolecules) since they determine the functionality of the studied systems. Due to such multistage control, we can optimize the occurrence of the desired functionality, such as appropriate anti-adhesion or biocidal parameters, conductivity or photoactivity, and in the case of nanocapsules - controlled release of the active compounds.

As part of the planned work, the influence of monovalent and divalent cations used as electrolytes (NaCl, $MgCl_2$) on two polyelectrolyte systems: PAH/PSS (polyallylamine /poly(styrene sulfonate) and PDADMAC/PSS (polydiallyldimethylammonium chloride/poly(styrene sulphonate)). Additionally, the effect of ionic strength on such designed systems was analyzed by applying multilayers deposition from ionic strength I = 0.015M and I = 0.15M of electrolyte. The permeability of the investigated multilayer films was studied for two types of electroactive probes: positively charged $[Ru\ (NH_3)_6]^{2+}$ and negatively charged equimolar solution of $[Fe(CN)_6]^{4-}$ with $[Fe(CN)_6]^{3-}$.

We have observed that the amount of polyelectrolyte adsorbed in the presence of Mg^{2+} ions is bigger compared to the one deposited in Na^+ solution with the same ionic strength. This effect was more pronounced in the case of films containing PDADMAC as polycation. The results of cyclic voltammetry indicate that the permeability of polyelectrolyte multilayers is influenced not only by the type and size of salt ions involved in the film formation, but also by the type (charge) of the electroactive probe. In the case of positively charged $[Ru(NH_3)_6]^{2+}$, most of the multilayers studied did not show barrier properties. Our results confirmed that the barrier properties of multilayer films depend on the steric effect, i.e. on the accumulation of molecules in the film and the repulsion of charged molecules through analogously charged layers within the film. The most important achievements include the experimental confirmation that the properties of polyelectrolyte membranes can be controlled also by the valence of electrolyte ions applied in polyion solutions used for the formation of multilayer films. Moreover, our results show that the permeability of polyelectrolyte membranes is inextricably linked to charge compensation and the nature of the molecule that is transported through the polyelectrolyte film.

The results of the work are planned to be used in the development of technologies for the production of biocompatible and bioactive coatings with controlled permeability. Polyelectrolyte films can be used to build biocompatible microcapsules with controlled physicochemical properties for use as carriers of active substances. In addition, the possibility to incorporate other components into the microcapsule shell, such as nanoparticles, allows obtaining a set functionality, e.g. the use of fluorescent or magnetic particles lets the use of such nanocomposites as theranostic carriers.

Properties of Degradable Surfactants as Emulsifiers/Nanoemulsion Stabilizers.

Description of Adsorption of Esterquats and/or Amidequats Type Degradable Surfactants on Free Surface

(Professor Piotr Warszyński, Ewelina Jarek PhD, Marzena Noworyta Eng)

The main objective of the research is the determination of adsorption properties of degradable surfactants: esterquats and/or amidequats. In the molecular structure contains ester or amide bond that can undergo hydrolysis. We used the extended “surface quasi two-dimensional electrolyte” (STDE) model of ionic surfactant adsorption for the theoretical description of the surface tension isotherms of four esterquat surfactants: regular esterquats, N,N,N-trimethyl-2-(dodecanoyloxy)ethaneammonium bromide (DMM-11) and N,N,N-trimethyl-2-(dodecanoyloxy)-1-methylethaneammonium bromide (DMP2M-11) with –O-CO- bond and betaine esters with –CO-O- bond: dodecyloxycarbonyl-methyl-*N*,*N*,*N*-trimethylammonium bromide (DMGM-12), and dodecyloxycarbonyl-1-ethyl-*N*,*N*,*N*-trimethylammonium bromide (DMALM-12) (cf. Figure 1.). We demonstrated that depending on the surfactant structure, partial hydrolysis of ester bond can lead to increase of the relative surface activity, more pronounced in the basic conditions (pH 10), that can be explained by formation of surface active hydrolysis products dodecanoate anion or dodecanol. In 2017 year we used quantum chemistry calculations methods (ab initio Hartree-Fock and DFT) with various basis sets and solvation models to determine the relative rates of surfactant hydrolysis and substantiate some of the model parameters used for the description of surface tensions isotherms.

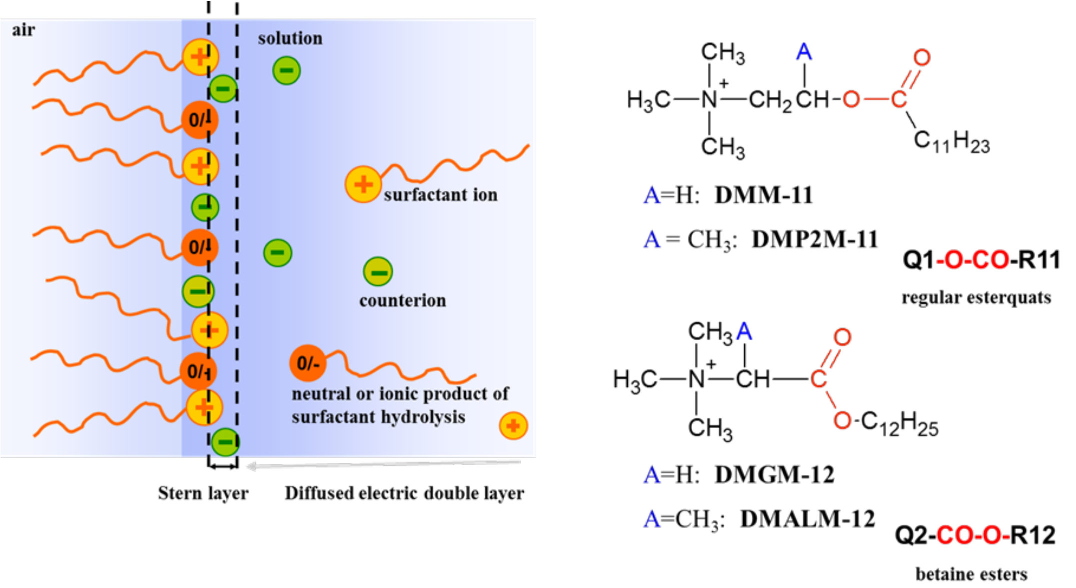


Figure 1. Schematic illustration of the main concept of the model of the hydrolysis driven adsorption of the studied esterquats

Calculations reveal that the reaction of esterquat hydrolysis is irreversible. An entropic barrier connected with the transition state (OH addition at carbonyl atom) reflect the increase of the hydrolysis rate at high pH. The presence of side CH_3 group (Fig.1 A=CH_3) causes decreasing of positive charge on carbonyl carbon (i.e. electrophilicity) and causes steric hindrance for OH addition. The DFT calculations correctly reflect trends in the hydrolysis rate between the respective pairs of surfactants (i.e. DMM-11 and DMP2M-11, DMGM-12 and DMALM-12).They also give correctly the gradation of dissociation constants for the hydrolysis products being week electrolytes. Additionally in 2017, using the pendant drop shape analysis method, we also determined adsorption kinetics (dynamic surface tensions) of amidequat type surfactants with different molecular structure and surface active products of their hydrolysis at various pH and ionic strength. We also attempted to use studied esterquats for formation of nanocarriers.

Physics and Chemistry in Cultural Heritage Protection

Quantitative Assessment of Risk to Heritage Objects Due to Microclimatic Conditions in the Environment

(Professor Roman Kozłowski, Leszek Krzemień PhD, Arkadiusz Kupczak PhD, Marcin Strojecki PhD)

Research into moisture response and mechanics of artistic materials has so far predominantly focused on objects of fine and decorative art. This has been an important drawback when it comes to historical materials relevant to library and archival collections – paper, board, parchment and leather. The task, therefore, consisted in expanding the quantitative assessment of climate-induced risk of physical damage to encompass parchment – the library material particularly vulnerable to moisture which can be regarded as the worst case concern guiding requirements for safe environmental conditions for the library and archival collections.

Data for moisture-related dimensional change (strain) were obtained for a set of modern and historical parchments (Figure 1). The measurements were done in two perpendicular directions in each sample to take into account the possible effect of collagen fibre preferred orientation, and the larger response was considered.

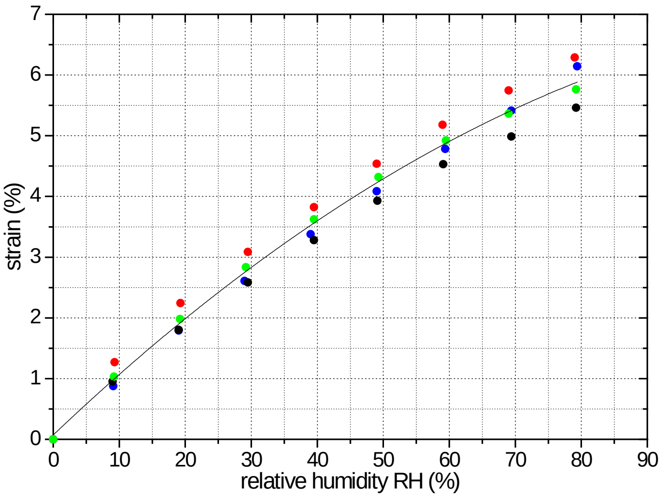


Figure 1 The exemplary experimental data for moisture-induced dimensional change (strain) of four historical parchment specimens are compared with the average curve calculated from the least-square regression of the data to the second order polynomial of the form: strain in % = $1.0335 \cdot 10^{-1} \cdot RH - 3.796 \cdot 10^{-4} \cdot RH^2$.

The yield strain of parchment at tension - that is the strain beyond which parchment undergoes permanent deformation - was determined as the upper limit of the elastic range in the tensile stress-strain curves recorded in the laboratory. The average value of 1.9% was obtained from the measurements in the relative humidity (RH) range between 20 and 90% which is of interest for the preservation of library collections indoors. The rate of water vapour uptake or release by parchment was high when compared to the duration of RH fluctuations – the new equilibrium moisture content in the material on an RH change was established in less than one hour. Therefore, RH variations can be translated into the strain vs time history by assuming that parchment reaches its full dimensional response at each instant of the variation. When the tensile strain of parchment calculated for the RH variations exceeds the critical value of 1.9 % during the shrinkage induced by the drying episodes in the indoor climate, parchment sheets restrained in their movement by mounting into a rigid protective system or attachment to boards of a book binding may undergo excessive tension and deformation.

The developed algorithm describing risk of damage mechanism for parchment will be added to the HERIe software - a web-based tool for quantitative assessment of risk of climate-induced physical damage to heritage objects (available at herie.mnk.pl).

"Sonata Bis" Research Projects of the National Science Centre

Computer Aided Design and Prediction of Properties of Drug Delivery Systems Based on the Structure of Carbon Nanotubes

"Sonata Bis" research project NCN 2012/07/E/ST4/00763 [2013-2017]
(project leader: Professor Tomasz Pańczyk)

In the next stage of the project the studies were focused on a carbon nanotube which is linked, using hydrolytically and pH labile linkers, with the nanoparticles having magnetic cores. The studies took into account the presence of factors which enhance biocompatibility of the carbon nanotube (adsorption of PEG on the surface) and making the system selective to cancer cells (the presence of folic acid residues). At the neutral pH, i.e. during the storage or circulation in the bloodstream, the drug molecules (carmustine in this case) are tightly kept inside the nanotube due to covalent bonds (hydrazone) with the functionalized magnetic nanoparticles. In the case of contact with the cancer cells the process of a selective interaction of the carrier with the folate receptor occurs and the endocytosis initiates. Due to very low pH of endosomes the hydrazone linkers become cleaved but the release of drug cannot occur yet because of strong noncovalent dispersion interactions of the nanotube and magnetic nanoparticles. This stage can be visualized in situ using MRI. Application of an external strong magnetic field leads to intense turns of the nanoparticles and uncapping of the internal space of the nanotube. Moreover, application of the alternating magnetic field can lead to mechanical destruction of the carrier and subsequent rapid release of the drug. The considered drug – carmustine – is commonly used in the treatment of brain tumors and it is administered in a form of implants. Application of the discussed carrier can enable intraveous administration of that drug since carbon nanotube can cross the blood brain barrier spontaneously. The discussed system is definitely the most important achievement of the project but due to limited space its detailed analysis is impossible here.

The previously studied carrier of doxorubicin, utilizing the effect of protonation of dyes molecules at the acidic pH during the endocytosis process, revealed too strong blocking of drug molecules by the folic acid – PEG conjugates. In the next stage the new concept and its theoretical verification concerning the improvement of this kind of drug carriers has been shown. The idea of that improvement was the incorporation to the carbon nanotube some nanostructual objects: they were either light fulerene particles C320 or heavy colloid nanoparticles (also magnetic ones). Their role was to push out the folic acid – PEG chains from the CNT interior and weakening their structure at the CNT ends after reduction of pH. Indeed, the dyes molecules exerted a pressure on the nanoparticles and the magnitude of that pressure was enough for pushing the nanoparticles out of the CNT, however, the transfer of the nanopartices to the bulk was not observed. Analysis of the effects related to the application of the external magnetic field led to the conclusion that its generates rapid turns of the nanoparticles around their body frames, what, in turn, leads to the rotation of the whole system due to principle of inertia conservation.

The obtained results are of significant importance in the area of development of drug delivery systems. The considered systems are able to release the drug molecules spontaneously when they enter acidic environment of tumor tissue or endosomes. The molecular architecture of these systems is quite simple. So, it is possible to directly apply the obtained results in fabrication of pH sensitive drug carriers.

2-Oxoglutarate Dependent Oxygenases in the Biosynthesis of Pharmacologically Active Alkaloids - Structure, Catalytic Mechanisms and Rational Redesign

"Sonata Bis" research project NCN 2014/14/E/NZ1/00053 [2015-2020]
(project leader: Professor Tomasz Borowski)

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

Experimental works covered optimalization of conditions for bacterial growth and protein overexpression for two proteins (varied parameters included: bacterial strain, expression plasmid, broth, aeration, temperature and time) as well as optimalization of a protocol for protein purification. The protocol for one of the proteins was successfully optimized, whereas the one for the other requires further work. Crystallization experiments (around 30 trials) were done with the sitting drop method using commercially available screens; under certain conditions, which are being optimized now, crystalline precipitate was obtained. For yet another protein good quality crystals were obtained for complexes with 2-oxoglutarate and succinate. Crystal structures were solved, refined and deposited in Protein Data Bank. The identity of the metal ion bound in the active site was confirmed to be Ni^{2+} with ICP-OES and anomalous signal measurements. Many attempts were made to obtain crystals with the primary substrate bound, however, so far unsuccessful. Mutation sites that potentially could affect reaction selectivity were identified; the work on their expression and purification is in progress. Gene coding a protein sequence was cloned into an appropriate plasmid and this paved the way to high yields of the protein in soluble form. Many crystallization attempts were made, in some conditions micro crystals were obtained.

Within computational studies a series of classical molecular dynamics simulations were done for one of the proteins and its complexes with substrates. Based on the results obtained a plausible mechanism of substrate binding, which explains the observed reaction regiospecificity, was formulated.

Molecular mechanics force field for structure, dynamics and conformation of carbohydrates involving furanoses

"Sonata Bis" research project NCN 2015/18/E/ST4/00234 [2016-2020]
(project leader: Wojciech Płaziński DSc)

Parameterization of the GROMOS force field for furanoses and functionalized pyranoses

The aim of the study was to develop the parameters to simulate the selected types of carbohydrates within the united-atoms force field GROMOS. The research consisted of the two independent stages. Firstly, we have developed the parameters describing the conformation of the exocyclic moieties (hydroxyl, hydroxymethyl and methoxy groups) connected to the furanose ring as well as the influence of these moieties on the flexibility of the furanose ring. Secondly, we have derived the parameters describing the conformation of the carboxyl moiety (in the three possible chemical states, i.e. protonated, deprotonated and alkylated), attached to the pyranose ring.

The parameterization procedure relied partly on the quantum-mechanical calculations and partly on adjusting the parameters to reproduce the available experimental data best. The considered data were taken from the literature and consisted of the NMR-derived vicinal proton-proton coupling constants, the structural data deposited in various databases as well as the thermodynamic parameters measured for carboxylate-containing sugars interacting with metal ions.

The final force field parameters can be used to study a wide spectrum of chemical compounds that are significant for both biology and biochemistry of living organisms. In particular, they can be applied to simulate: uronic acids (e.g. pectins and alginates), ketohexoses (e.g. fructose), aldopentoses (e.g. ribose and deoxyribose), aldotetroses (e.g. threose) and many others. Moreover, it is possible to use the derived force field in order to investigate more complex molecular systems, containing not only carbohydrates but also other types of biomolecules.

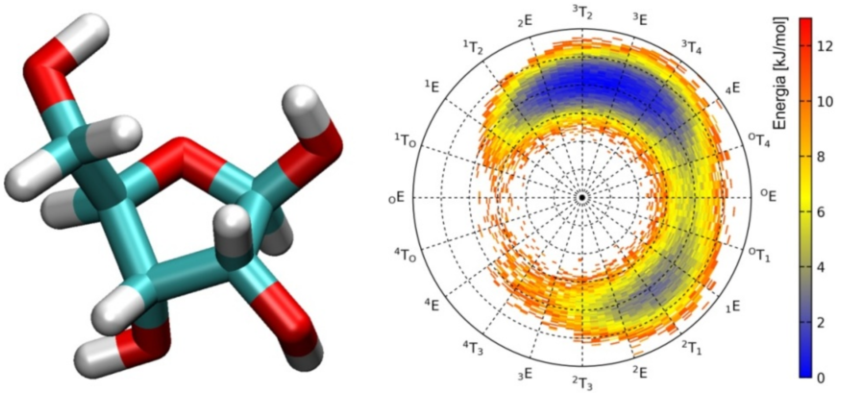


Figure 1. The molecule of β-D-ribofuranose and the corresponding, calculated free energy profile that illustrate the preferred geometry of the five-membered ring.

"Sonata" Research Projects of the National Science Centre

Targeted Drug Delivery Systems - Synthesis and Functionalization of Nanocarriers

"Sonata" research project NCN 2011/03/D/ST5/05635 [2012-2017]
(project leader: Krzysztof Szczepanowicz DSc)

The nanocapsules can be used in the specific drug delivery systems as they can penetrate the cell membrane. Moreover, they can be functionalized to achieve “intelligent targeting”, i.e. delivery to the specific cells or organs. It would allow minimizing one of the main drawbacks of currently used pharmacological formulation that is lack of selectivity, therefore, it should eliminate side effects of applied therapeutics due to the elimination of their toxic effects in the whole body.

The main aim of this project was the development of scientific background and elaboration of the technique of therapeutic agents incorporation inside biocompatible nanocapsules with functionalized shells for targeted drug delivery. That aim will be achieved in a few fundamental steps. First of them will be the development of the method for encapsulation of active components followed by capsule functionalization for targeted drug delivery in order to achieve “intelligent targeting”, i.e. delivery to the specific cells or organs. The final step will be biological tests: cytotoxicity and verification of selective action of prepared nanocarriers with model cells. The work is carried out simultaneously in two centers which possess necessary facilities in this type of study: Jerzy Haber Institute of Catalysis and Surface Chemistry Polish Academy of Sciences and the Department of Biochemistry, Biophysics and Biotechnology, Jagiellonian University.

In 2017, this project was completed. The set goal was implemented in the following stages:

- The method of nanocapsules preparation using the LbL technique on nanoemulsion cores was developed,
- A method of the preparation of nanoparticles of polyelectrolyte complexes containing active agents (eg RNA, DNA) was developed,
- Modification and functionalization of the nanocapsule shells for passive and active targeting was developed,
- The influence of the obtained nanocapsules on model cells was tested.

The Mechanism of Regioselective Oxidation of Cholesterol Derivatives by a Novel Molybdenum Enzyme, Steroid 25-OH Dehydrogenase from *Stereolibacterium denitrificans*

"Sonata" research project NCN 2012/05/D/ST4/00277 [2013-2017]
(project leader: Maciej Szaleniec DSc)

The aim of the project is the elucidation of the reaction mechanism for hydroxylation of cholesterol derivatives by steroid C25 dehydrogenase (S25DH). The mechanism was investigated using cluster models and DFT or DFT+D2 method.

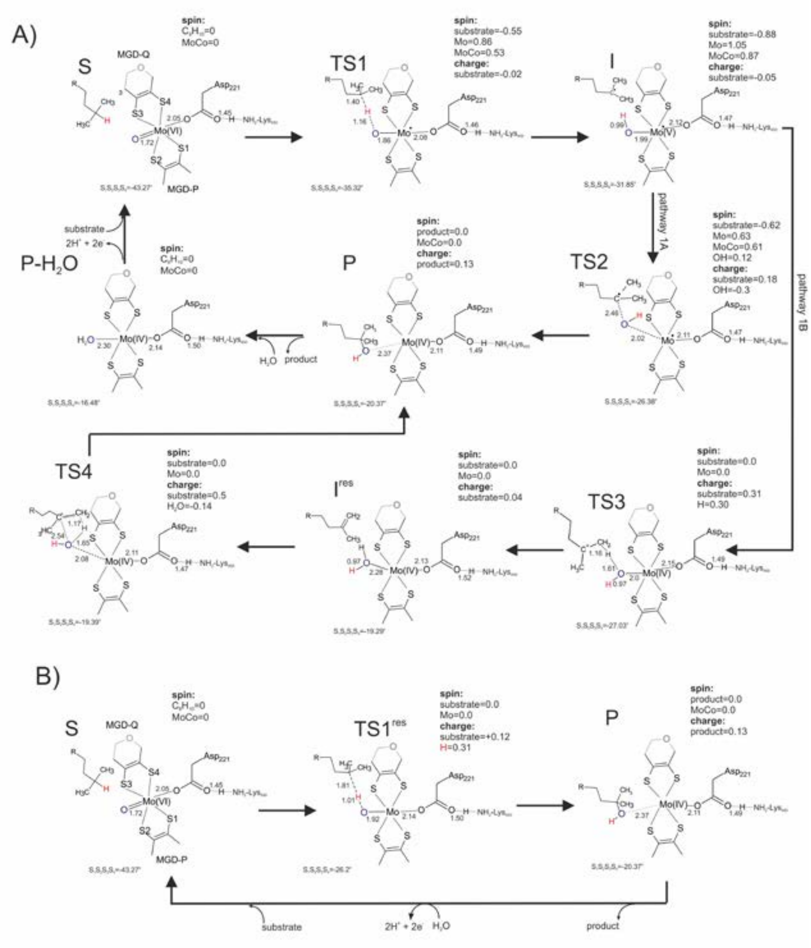


Figure 1. 3 mechanistic varians of cholest-4-en-3-onu oxidation. A) pathway initiated by homolytic C-H cleavege, B) pathway initiated by heterolytic C-H cleavege

The conducted modeling demostrated, that the radical C-H cleavege is the most probable scenario of cholest-4-en-3-on activation. This leads to radical intermidiate product **I** and quasi-radical transition state associated with OH rebound process (**TS2**). After product dissotiation a spontanious coordination of a water lingand by reduced Mo(IV) site occures. Moreover within this pathway an alternative proton-assisted electron transfer (PA-ET) can occure (**TS3**) Swhich leads to a dehydrogenated intermidate product **I**$^{\mathbf{res}}$. As such product was not observed experimentally it was assumed that it would have to be subjected to hydratation process (**TS4**). However, such step is associated with a very high energy barrier due to high stability of **I**$^{\mathbf{res}}$ and therefore kinetically improbable. The experimetal kinetic isotope tests also corroborated proposed hypotesis. The calculated intrinsic KIE for **TS1** was 7.4 (7.07 for a big model), while for **TS2** 1.66 (1.59 for a big model). Meanwhile in the alternate pathway that assumed heterolytic C-H cleavage KIE associated with such activation was **2.8**, while for dehydrogenation step (**TS3**) 3.02 or 1.57 for transferring D or H, respectively, from primary C atom. The calculated iKIE associated with water addition (**TS4**) was 1.34 (assuming transfer of D_2O). As a result the iKIE on all alternate pathways was lower than the apparent exp. KIE but that calculated for pathway 1A.

Finally, it was demonstrated by QM:MM modelling that product spontaneously leaves Mo coordination sphere of the reduced Mo(IV) site.

Mechanism and Kinetics of Bubble Coalescence at Undisturbed and Vibrating, with Controlled Frequency and Amplitude, Liquid/gas Interfaces

"Sonata" research project NCN 2013/09/D/ST4/03785 [2013-2017]
(project leader: Jan Zawała DSc)

The project was focused on experimental and numerical studies of influence of controlled vibrations of liquid/gas interface on air bubbles coalescence at free surfaces of liquids of different physicochemical properties. The research tasks of the project were aimed to determination of additional threshold acceleration of the liquid/gas interface, causing significant prolongation of lifetime of the bubbles colliding with these interfaces (coalescence times), comparing to undisturbed liquid surfaces. Gas bubbles were formed in a control way at capillary orifices of different inner diameters. The experiments were carried out in distilled water and pure silicone oils of precisely determined physicochemical parameters (viscosity and surface tension). Due to different surface tensions the radius of bubbles (R_b) formed at identical capillaries (inner diameter 0.025 - 0.15 mm) in the liquids studied were different and varied within the range of 0.51-0.87 mm for water and 0.31 - 0.60 mm for oils, respectively.

It was found that for each bubble size there is specific amplitude and frequency of the liquid/gas interface vibrations (additional acceleration) below which the bubble coalescence time is comparable with resting liquid surface and above which can be prolonged even by several orders of magnitude. Moreover, it was determined that larger the bubble, smaller the acceleration necessary to achieve the effect of coalescence time prolongation. This observation was additional confirmation of theoretically postulated mechanism of probability of bubble rebound from the interface, in which size of the intervening liquid film formed between the bubble and the interface is a parameter of crucial importance.

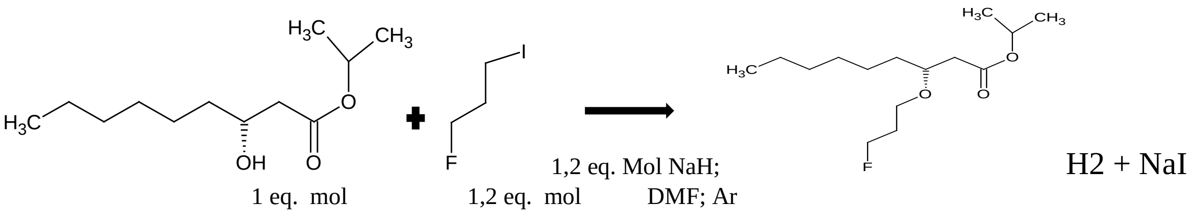
In addition, the influence and interrelations between (i) colliding bubble radius (R_b), (ii) liquid surface tension and (iii) values of threshold acceleration of the vibrating liquid/gas interface on probability of colliding bubble lifetime prolongation (increase of foam film stability) were determined. It was shown that threshold acceleration related to the control vibrations of the liquid/gas interface is strictly related to the bubble radius and physicochemical properties (surface tension) of the liquids studied. Independently on the kind of the liquid used, the value of threshold acceleration in function of vibration frequency was increasing with the R_b decrease. Moreover, for the bubbles of comparable sizes, colliding with interfaces of different liquids, it was found that threshold acceleration is much smaller for the liquid of smaller surface tension. Results obtained confirms the hypothesis about crucial role of the liquid films size in probability of prolongation of the bubble lifetime at surface of pure liquid. This prolongation is related to deformation of the bubble surface during collision (liquid film formation), which in turns determines radius of the separating liquid film. The bubble deformability is strictly related to the value of Laplace pressure (Δp). Higher the Δp (smaller the R_b), higher threshold acceleration is needed (bubble has to strike the surface with higher velocity, i.e. force) in order to achieve sufficient deformation degree, i.e. to form liquid film of sufficiently large radius to prevent its rupture (coalescence).

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

"Sonata" research project NCN 2015/17/D/ST4/00514 [2016-2019]
(project leader: Maciej Guzik PhD)

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

This stage concerns chemical modification of polyhydroxynonanoate (PHN) monomers – protection of 3-hydroxyl group. The hydroxyl protection aims at introduction of anticancer features into a molecule and also protects from repolymerisation. This chemical reaction consists of the formation of the C-O-C ether linkage with a halogen alkyl or a phenyl group by means of the SN1 reaction involving a catalyst, e.g. NaH.



The product was successfully obtained and purified with Versa-Flash chromatography. The efficiency of this process was 11.75%. However, the reaction conditions and further processing of the product still need optimisation. The second example C3-OH protection was a chemical reaction with a phenyl derived from the diaryl-iodo-trifluoro-methanesulfonic salt with the participation of a copper catalyst (CuOTf, toluene, at 100 ° C. - diarylation reaction). An unpurified product was obtained in a yield of 67.7%. Analyses were carried out using a RP-HPLC-MS system with C18 column. All reactions were conducted at the Institute of Pharmacology of the Polish Academy of Sciences in cooperation with Dr. Jakub Staroń.

Stage 2 c.d.: Lipase screen

The stage considered continuation and development of tasks from the previous project year, i.e. optimization of reaction conditions and detection of lactose transesterification products using immobilized lipases, PHN monomers and their derivatives on the example of methyl ester of nonanoic acid (C9), and esters with allylic protection (monofluoride alkyl). On the basis of literature reports and screening tests, it was found that the most effective reaction occurs in the 2-methyl 2-butanol environment at a molar ratio of ester to lactose 1: 3 at 55 °C. Maximum concentrations can be obtained up to 48 hours of reaction, resulting in up to 25.5% conversion relative to lactose concentration in the case of the C9-lactose ester. The best-acting enzymes are the amyl lipase *Thermomyces lanuginosa*, *Pseudomonac cepacia* and *Candida antarctica* lipase B. Regarding the esterification of sugar by the alkyl halide, the best-acting lipases are: EL 03; EL 04; EL 09; EL 17; EL 20; EL 30; EL 70 from 32 tested lipases from Eucodis Bioscience; giving up to 16% conversion and *Candida antarctica* lipase B giving 5.8% conversion.

Stage 3: Chemical synthesis of nonanoic acid lactose ester

Preliminary screen of chemical reaction with lactose was based on patent by F. Scholnicki, M.K. Sucharski and W.M. Iinfield, Eastern Regional Research Center Philadelphia, Pennsylvania 19118, in which lactose was esterified with nonanoic acid using a K_2CO_3 catalyst in DMSO at 50 °C. However, this reaction is nonspecific, i.e. provides mono-, di-, tri-esters and causes difficulty in monoester purification. It was more effective to bring C9 to the chloride form with oxalyl chloride in DCM with a drop of DMF as a catalyst. In this form, the acid reacts with the previously dried lactose in DMF. The lactose monoester was separated from the diester fraction and lactose alone on a preparative RP-C18 Versa Flash in a 70% MeOH / 30% H2O system to give 19.9% conversion. Efficiency is planned to be increased by changing the method of drying lactose and reaction media.

Formation Mechanisms and Functionality of Nanoparticle Multilayers with Incorporated Biomolecules

"Sonata" research project NCN 2015/17/D/ST4/00569 [2016-2019]
(project leader: Maria Morga PhD)

The research goals of the SONATA project realized in 2017 included: (i) revealing mechanisms of nanoparticle bilayer formation on mica and silicon solid substrates; (ii) determining the stability of obtained nanoparticle bilayers of well defined structure and electrokinetic properties; (iii) developing a reliable and repeteable procedure of constructing well-defined nanoparticle bilayer films (Fe_2O_3/Ag, Fe_2O_3/SiO_2) and macromolecule monolayers (poly-L-lysine - PLL, poly-Larginine - PARG, polyallylamine hydrochloride - PAH) on mica and silicon surfaces; and (iv) development of an efficient measurement procedure for quantitative characterization of nanoparticle bilayers as well as macroion-nanoparticle nanofilms under in situ conditions.

Since it is assumed that electrokinetic properties affect the formation and stability of multilayers, it is especially important to find correlation between physical properties of nanoparticles as well as biomolecules and the stability of colloidal multilayers obtained in the self-assembly process. Therefore, in the course of the research, using the RSA modeling and the 3D electrokinetic model, the correlation between the coverage of obtained nanostructures and their zeta potentials was found**.** Knowing the deposition kinetics of oxide particles (α-Fe_2O_3 and SiO_2) as well as macroions and metallic particles with specific surface properties, a series of in situ electrokinetic studies were carried out to determine the mechanisms of bilayer formation in the nanoparticle-nanoparticle and macro-nanoparticle systems. An additional element of novelty, unprecedented in the literature, was determination of the impact of the first layer (precursor layer) on the zeta potential and stability of obtained bilayers. For this purpose, monolayers with different degree of coverage: $\theta = 0.03$, $\theta = 0.07$, $\theta = 0.15$, $\theta = 0.17$, $\theta = 0.20$, up to the saturated monolayer $\theta = 0.34$ in ionic strength 10^{-2}M NaCl, were formed in the electrokinetic cell and investiaget in situ using the streaming potential method. For such monolayers, the process of bilayer formation was carried out and examined for Fe_2O_3/Ag, Fe_2O_3/SiO_2, and PLL-Ag bilayers.

It was found that in all cases the zeta potential of the bilayers reaches a value close (for coatings $\theta \leq 0.03$, 0.05) or equal (coverage $\theta \geq 0.07$) of the zeta potential of the dense monolayer of particles forming the outer layer. This indicates that the role of the substrate and the supporting layer becomes rather minor even for such a low coverage of the first layer. This is an unusual behavior, contardicting assumptions of the classic DLVO theory, for which negatively charged particles of the second layer are deposited on the negatively charged surface. These effects have been described in a quantitative manner based on heterogeneous charge distribution in the first substrate layer (hematite, PLL). This leads to the presence of adsorption patches (sites) that can attract negatively charged silica or silver particles forming the second layer.

Additionally, knowing the desorption kinetics from the desorption experiments, the presence of irreversibly bound silica fraction was confirmed, considering the Fe_2O_3/SiO_2 bilayer, and the energy minimum depth was calculated to be: $\phi_m = -18.1\ kT$. Using DLVO theory, it was showed that electrostatic interactions are the dominant forces responsible for stability of formed bilayer. This indicates that the particles were attached to only one hematite particle. On the other hand, the silica particles attached to more than one hematite NP were irreversibly adsorbed.

Besides for significance in basic sciences, the studies provide a basis for developing a robust method of preparing multilayer materials with well-controlled surface properties.

Bio Oligo/Polysaccharides under the External Forces

"Sonata" research project NCN 2015/19/D/ST4/01979 [2016-2019]
(project leader: Agnieszka Brzyska PhD)

The main goal of this theoretical project is to study and describe the possible enforced conformational/structural changes in biomolecular structures i.e., oligo- and polysaccharides. In the reported period the research was focused on the description of the mechanism of the enforced structural changes in the bio-oligosaccharides with respect to

(i) type and location of the substituents in the pyranose ring,
(ii) type and location of the glycosidic bonds.

(i) We concentrated on molecules containing pyranose rings with two hydroxyl groups at ring positions 1 and 4. We used the Enforced Geometry Optimization (EGO) method to study possible enforced intramolecular transitions and/or inversions in the pyranose ring in the starting chair conformations ($^{1}C_{4}$ and $^{4}C_{1}$) with both hydroxyl groups in the axial configuration. The results obtained provide new insight into the mechanism of the conformational transitions which strongly depends on substituents present in a pyranose ring.

(ii) We considered the unsulfated λ-carrageenan-based oligomers (up to pentamers – see Figure 1) with the two types of glycosidic linkage between the saccharide units, i.e. α (1→4) and β (1→3). Four different types of the *permanent* enforced conformational transition in the carrageenan-based oligomers were identified ($^{4}C_{1}\rightarrow{}^{1}C_{4}$, $^{4}C_{1}\rightarrow{}^{1}S_{3}$, $^{4}C_{1}\rightarrow B_{3,O}$, $^{4}C_{1}\rightarrow{}^{2}S_{O}$). Generally, the type of the conformational conversion depends directly on the galactopyranose ring position in the oligosaccharide chain. The complex responses to the external stretching forces are related to the different glycosidic bond types in the examined structures. Additionally, the type of the terminal oxygen atoms (axially or equatorially oriented) of the oligomeric structure also determines the mechanical resistance to the external forces.

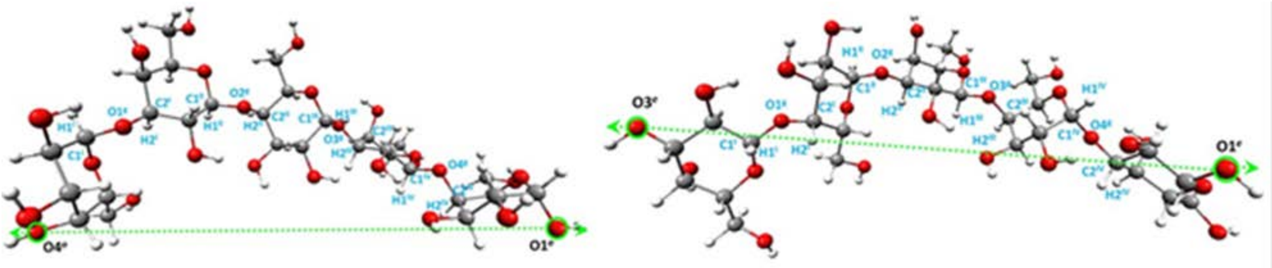


Figure. 1 Two possible pentameric structures and the stretching force modes. The superscripts *a* and *e* denote the axial and equatorial position of the terminal –OH groups.

We conclude that interpretation of force-extension curves can be very tricky; not every plateau corresponds to a conformational transition and not every transition can be clearly seen. Such situations are highly probable for more complex molecular systems, e.g. for heteropolymers with repeating units connected through the same and different types of glycosidic bonds.

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

"Sonata" research project NCN 2016/23/D/ST4/02492 [2017-2020]
(project leader: Michał Śliwa PhD)

Metal nitrates and precipitation agents, necessary for catalyst synthesizes, have been purchased together with small laboratory equipment.

DSC-TG measurements (Figure 1) were performed in order to evaluate the number of water molecules present in gallium nitrate hydrate and zirconium (IV) oxynitrate hydrate. DSC-TG experiments show that direct heating of commercial reagents $Ga(NO_3)_3{\cdot}xH_2O$ and $ZrO_2(NO_3)_2{\cdot}xH_2O$ produces a mass loss of 75.4 % and 63.9 %, respectively. The observed mass loss corresponds to $x = 7$ and $x = 6$, respectively for $Ga(NO_3)_3{\cdot}xH_2O$ and $ZrO_2(NO_3)_2{\cdot}xH_2O$. For the DSC-TG runs, the hypothesis has been made that gallium oxide and zirconium oxide are the residues obtained at the end of the analysis and N_2O_5 and H_2O are released upon nitrate heating.

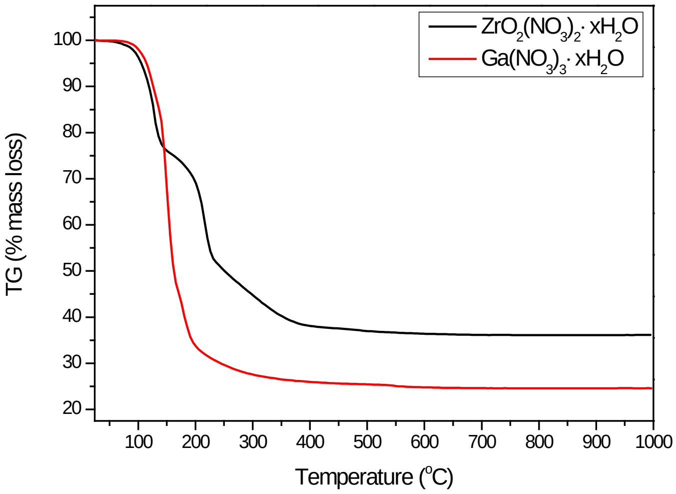


Figure 1. TG curves for metal precursors.

Temperature program reduction (H_2-TPR) was performed for standard CuO. TPR runs were carried out for four different CuO concentrations in the quartz flow reactor connected on-line to mass spectrometer. The desirable concentration of CuO was achieved upon its dilution with Al_2O_3. Based on the recorded TPR profiles, the calibration curve for hydrogen on four concentration levels has been prepared. During TPR measurements the following m/z lines were monitored: 2 (H_2), 18 (H_2O).

The catalytic set-up for catalyst testing in steam reforming of ethanol has been designed. The set-up will consist of: Bronkhorst vapor delivery system (CEM) equipped with mass flow controllers (MFC) for gases and liquids, heated lines to fixed-bed quartz flow reactor, VICI-valco switching valves, oven and on-line GC (HP 5890 Series II) equipped with TCD and FID detectors.

"Opus" Research Projects
of the National Science Centre

Synthesis and Physicochemical Characterization of the Electrocatalysts Containing Non-noble (Fe, Mn) and Noble (Pd) Metals Deposited on Porous N-Doped Carbon Materials with Different Morphologies and Examination them in Oxygen Reduction Reaction

"Opus" research project NCN 2013/11/B/ST5/01417 [2014-2017]
(project leader: Aleksandra Pacuła PhD)

Catalytic performance of the composites for the ORR was evaluated in alkaline solution (0.1 M KOH) by means of cyclic voltammetry (CV) and linear sweep voltammetry (LSV).

Electrochemical measurements were performed using an electrochemical cell in conventional three-electrode configuration. Glassy carbon (GC) disk covered with the studied sample was used as a working electrode, whereas Pt wire and Ag/AgCl/KCl$_{sat}$ electrode were used as counter and reference electrodes, respectively.

The composites derived from Mg-Fe-Al LDHs (Fig. 1a) were more active in the ORR than those derived from Mg-Mn-Al or Mg-Al LDHs (Fig. 1b). The number of electrons involved in the ORR over the carbon materials was between 2 and 3. The ORR proceeded mostly according to 2-electron (undesired) than according to 4-electron (desired) reaction pathways. Newly prepared composites cannot be considered as alternative catalysts for ORR. However, they possess interesting properties, which can be attractive for other purposes. They can serve as the supports for other active species in alternative catalytic applications.

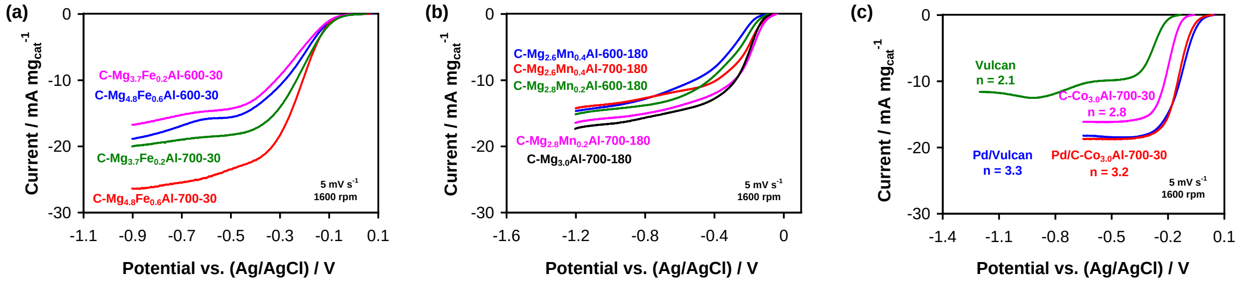


Figure 1. The comparison of polarization curves recorded in 0.1 M KOH saturated with oxygen using rotating disk electrode (RDE) (with rotation rate of 1600 rpm, at potential sweep rate of 5 mV/s) for the electrode materials (deposited on GC) derived from Mg-Fe-Al (a) Mg-Mn-Al and Mg-Al (b) and Pd-containing composites and the corresponding supports (c).

Pd nanoparticles deposited on the carbon materials derived from Mg-Fe-Al, Mg-Mn-Al and Mg-Al appeared to be inactive in ORR. For comparison, Pd nanoparticles deposited on the commercial carbon material (undoped with nitrogen) appeared to be active in the ORR. Similar result was obtained after supporting of Pd nanoparticles on the carbon material (doped with nitrogen) derived from Co-Al LDHs (Fig. 1c). It revealed that physicochemical properties of the carbonaceous supports have an important influence on dispersion and exposure of deposited Pd and therefore its electrochemical activity. New carbon materials derived from Mg-Al, Mg-Fe-Al and Mg-Mn-Al LDHs are not better supports for Pd than the commercial carbon material.

Innovative Total Oxidation Catalysts Derived from Organosmectites Intercalated with Reverse Micelles Containing Oxide/hydroxide Nanostructures

"Opus" research project NCN 2013/09/B/ST5/00983 [2014-2017]
(project leader: Professor Ewa Serwicka-Bahranowska)

The project aimed at design of a new class of active and thermally stable transition metal oxide combustion catalysts, as an alternative to widely used platinum group metal catalytic systems. The innovative approach to catalyst design consisted in a combination of two synthetic procedures: one, involving the preparation of organoclays, and the other, employing inverse micellar route for manufacturing of oxide nanoparticles.

Natural montmorillonite and synthetic Laponite were used in the capacity of layered silicate components, both as sodium forms and as organic derivatives obtained by exchange of Na^+ with $(C_{16}H_{33})N(CH_3)_3^+$ cations. Oxide nanostructures (e.g., Mn-Al-O, Mn-Cu-Al-Zr-Ce-O, TiO_2) were prepared from precursors synthesized by inverse micellar method. The resulting composites were calcined at 450 and 600°C. The materials were characterized with XRF, XRD, SEM, HRTEM, FTIR, XPS, FTIR and Raman spectroscopies, H_2 TPR, and N_2 sorption in -196°C, and tested in the catalytic combustion of toluene. Results of the catalytic tests demonstrated that all catalysts prepared according to the novel design are more active in combustion of volatile organics than the reference samples obtained in a conventional way, without employing the inverse micellar procedure. Moreover, the catalysts prepared in the organic medium were more active than their counterparts obtained in aqueous system. Oxide nanoparticles obtained from precursors prepared via inverse micellar route were characterized by small sizes determined by the volume of micelles, and displayed high crystallinity, resulting from the impact of additional heat input accompanying combustion of the organic component of the composites during calcination. It has been demonstrated that the micellar hydroxide-like cores may have multielemental composition, which is of importance for engineering of catalytic materials as it enables optimization of compositional, structural, textural, redox, and, in consequence, catalytic properties of the composites.

1 MSc student and 1 PhD student participated in project implementation, thus receiving education and training in the engineering of advanced catalytic materials.

2-Oxoglutarate Dependent Oxygenases Catalyzing Atypical Oxidative Transformations - Structural and Mechanistic Studies

"Opus" research project NCN 2014/15/B/NZ1/03331 [2015-2017]
(project leader: Professor Tomasz Borowski)

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, oxidative deamination (of kanamycin) and formation of an endoperoxide bridge (in the synthesis of a mycotoxin – verruculogen).

Experimental studies covered optimalization of crystallization conditions for EFE and KanJ proteins as well as optimalization of protein production protocol for homologous EFE enzyme showing different enzymatic activity. For *Pseudomonas Syringae* EFE good quality crystals were obtained and three crystal structures (at 1.5 Å resolution) were solved and refined. X-ray fluorescence scans were done to identify the metal ion bound in the active site. For the homologous EFE protein the protocol was varied in a search for conditions under which the protein could be obtained in soluble form in high yields. For this purpose various *E. coli* strains were tested.

For the *Streptomyces kanamyceticus* KanJ protein the crystallization conditions were optimized. Substrates (2-oxoglutarate, kanamycine, ribostamycine) were introduced into crystals either via co-crystallization or soaking, the phase problem was solved with the use of iodide compounds also introduced into crystals via soaking. X-ray fluorescence scans confirmed the metal bound in the active site is nickel.

Computational studies covered quantum-chemical investigations on reaction mechanisms of two enzymes: FtmF and KanJ. Results obtained for FtmF confirmed the catalytic role of a tyrosyl group present in the active site and explained the observed regiospecificity of the enzymatic reaction. Moreover, MD simulation results revealed the likely site for ascorbate binding, whereas QM studies confirmed it is an efficient reducing agent for closing the catalytic cycle of FtmF. For KanJ MD simulations were done for several variants of enzyme-substrate complexes. QM studies, that followed, revealed significant differences in the reaction mechanism depending on the ionization state of a key amino group.

Theranostic Nanocarriers for MRI Imaging

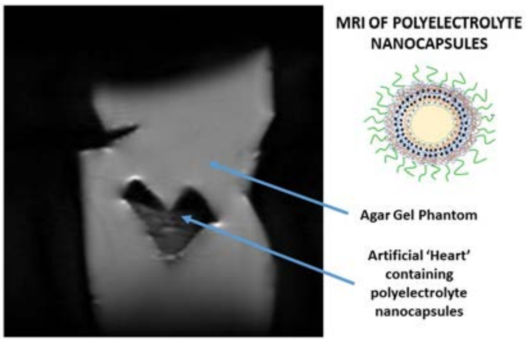
"Opus" research project NCN 2015/17/B/ST5/02808[2016-2019]
(project leader: Professor Piotr Warszyński)

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.

Nanomedicine is the field of research with huge expectations for the development of personalized medicine based on the application of nanoparticles (NPs) as nanoscale materials interact effectively with biological systems. In particular, the simultaneous use of nanodiagnostics in vivo imaging and nanotherapeutics for drug delivery may allow overcoming many intractable health challenges. Although in the past decades a large effort has been devoted to the development of functionalized targeted drug delivery systems, some challenges still remains unsolved: delivery drugs to specific cells, the real time monitoring of drug delivery and monitoring and control the drug release. 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.

In 2017 we were focused on the synthesis of theranostic polyelectrolyte nanocapsules. The nanocapsules were synthesized by the method developed in our Institute i.e. direct encapsulation of emulsion droplets in the polyelectrolyte multilayer. Theranostic drug delivery system was prepared by the sequential adsorption of polyelectrolytes (layer by layer technique) using biocompatible polyelectrolytes (Poly L-lysine as the polycation and Poly Glutamic acid as the polyanion) and magnetic one or two layers of Fe_2O_3 nanoparticles for MRI contrast. The size of obtained nanocapsules was ~100nm. Synthesized nanocapsules were visualized by MRI as the solutions and in agarose gel phantoms.



MRI of theranostic polyelectrolyte nanocapsules in gel phantom

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

"Opus" research project NCN 2015/17/B/ST5/00023 [2016-2020]
(project leader: Professor Ewa Brocławik)

The preparation of hierarchical zeolites containing additional system of mesoporesvis desilication in alkaline solutions, found the most effective and non-expensive way of producing of mesopores in zeolites. Most of existing attempts concerning zeolite desilication was performed for ZSM-5 using NaOH as the desilicating agent while here, desilication of commercial zeolite FAU with the Si/Al ratio of 30, obtained by dealumination of typical zeolite Y, was performed.

Variable parameters of desilication were: the kind of desilication agent (NaOH, tetrabutylammonium hydroxide (TBAOH), or a mixture of NaOH/TBAOH), temperature of process, and post desilication treatment - calcination in air or at vacuum. Desilicated zeolites were the subject of characterization studies. The status of Si and Al were followed with MAS NMR and XPS. The pore structure was studied by N_2 adsorption, and the acidity was determined by IR spectroscopy. The concentration of both Bronsted and Lewis sites was examined by quantitative experiments of pyridine adsorption, whereas the nature of Lewis sites and the acid strength of Si-OH-Al groups was studied by low temperature CO sorption. The heterogeneity of Si-OH-Al groups was also analyzed. The accessibility of sites to bulky reactants was studied by quantitative IR studies of bulky probe molecules: 2,6 ditertbutyl pyridine. The effect of the desilication of zeolites as well as modification of their structural and acidic properties on catalytic activity was studied in the isomerization of α-pinene.

$Si-OH_1-Al$ groups in parent zeolite FAU-30 were found to be homogeneous and very strongly acidic. They were more acidic than hydroxyls in HMOR and HZSM-5 which are known as strongly acidic zeolites. The treatment of FAU-30 with diluted NaOH at mild conditions (room temperature) caused a decrease of the Si/Al ratio to ca. 10, a complete destruction of zeolite structure and loss of acidity. On contrary, the treatment with TBAOH resulted in only small Si extraction and slight increase of Si/Al. Furthermore, desilication with NaOH/TBAOH preserved zeolite structure and microporosity, but distinctly increased the volume of mesopores of diameter ca. 3 nm. Si/Al decreased to ca. 18. XPS showed the same ability of Si in surface zone and inside the bulk to extraction. Very strongly acidity of obtained homogeneous 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 ($\Delta\nu_{OH...CO} = 354\ cm^{-1}$) 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. We have also obtained very good, nearly quantitative agreement between the experimental OH frequency red-shift upon CO adsorption in dealuminated zeolite Y and the values computed by extracting anharmonic OH frequencies from ab initio molecular dynamics simulation performed for the realistic zeolite models and at experimental temperature.

Hierarchical zeolites generated by mild desilication (after former dealumination) should show much better catalytic activity than traditional zeolites due to both high acidity and better accessibility of active sites for reagents – we hope to establish a general procedure for synthesis of such materials.

Nanoparticle Monolayers of Controlled Heterogeneity and Structure as Efficient Antifoulong Substrates

"Opus" research project NCN 2015/07/B/ST5/00847 [2016-2019]
(*project leader: Professor Zbigniew Adamczyk*)

Deposition mechanism of positively charged gold nanoparticles at silica/silicon sensor was investigated by quartz crystal microbalance (QCM), 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 transmission electron microscopy (TEM) revealed that the average size of nanoparticles was equal to 12±3 nm. 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=$3\text{x}10^{-3}$ 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 influence of suspension concentration, ionic strength and flow rate on the kinetics of nanoparticle deposition on the sensor was quantitatively determined. The significant increase in the maximum coverage of gold monolayers with ionic strength (see Figure 1) was interpreted as due to the decreasing range of the electrostatic interactions among deposited particles. Moreover, the negligible hydration of formed monolayers, their structure and high stability were determined by comparison of the QCM results with those obtained by AFM and SEM. The experimental data was adequately interpreted in terms of the extended random sequential adsorption (eRSA) model that considers the bulk and surface transfer steps in a rigorous way. The obtained results are useful for a facile fabrication of gold nanoparticle-based biosensors capable to bind target molecules via available amine moieties.

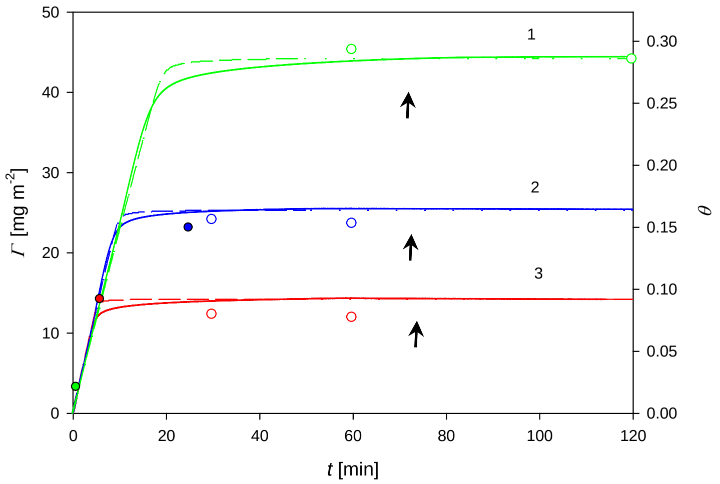
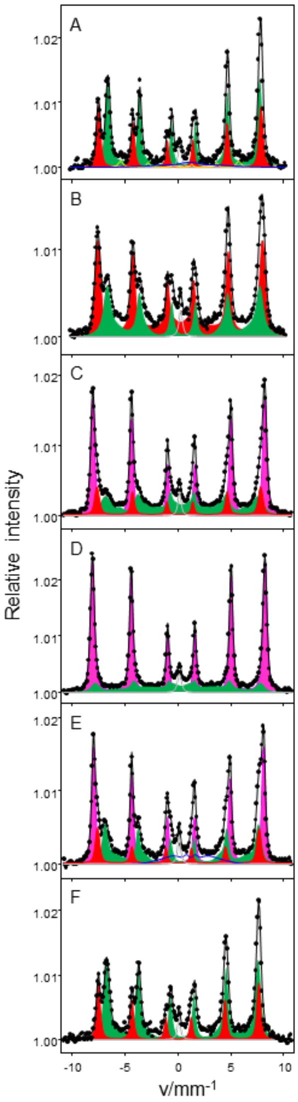


Figure 1. Kinetics of gold nanoparticle deposition at the Si/SiO$_2$ sensor determined by QCM (bulk suspension concentration 50 mg L^{-1}, pH 5.7 and T=298) for ionic strengths: 1) $3\text{x}10^{-3}$ M, 2) 10^{-3} M and 3) 10^{-4} M. The solid lines show the experimental results and the dashed lines present the theoretical results calculated from the eRSA model including the coupling between bulk and surface transport. The point show the results obtained from AFM (●,●,●) and SEM (○,○,○). The arrows show the beginning of the desorption runs.

Magnetic Nanoparticles on Periodic Iron Oxide Templates: Control of Magnetism Using Particle Substrate Interaction and External Electric Field

"Opus" research project NCN 2016/21/B/ST3/00861 [2017-2020]
(project leader: Nika Spiridis DSc)



Conducted research is a part of the project's task which aims at controlled preparation of monocrystalline iron oxide surfaces with a well-defined periodicity and specific electronic and magnetic structures on Pt (111). Contrary to studies of phase transformations in bulk and nanoparticle iron oxides systems, a few reports concerning ultrathin epitaxial films relay on surface science methods, such as LEED, LEEM, STM. The most important conclusions on the magnetite-hematite transformation concern the kinetics, oxidation pathways and a role of a substrate. In particular, it was reported that whereas for Ag(111) the magnetite to hematite transformations can be reversed by UHV annealing for Pt(111) reduction of α-Fe_2O_3 to magnetite requires additional iron. Magnetite – hematite transformation on Pt(111) was studied using a combination of surface methods and *in situ* conversion electron Mössbauer spectroscopy (CEMS) that probes the entire film volume.

A 5-nm magnetite film was prepared by reactive deposition of iron (^{57}Fe) in oxygen atmosphere 8x10^{-6} mbar with the substrate kept at 520 K. Typically, within the used preparation recipe, deposition was followed by UHV annealing at 830 K which resulted in a regular Fe_3O_4(111) termination. The magnetite film was oxidized in several steps at an oxygen pressure 1-2x10^{-5} mbar, with a final exposure of 21500 L, and then it was reduced by annealing under UHV. Figure shows CEMS spectra after selected characteristic preparation steps. The spectrum for the as-prepared magnetite film (A) reveals dominating bulk-like components from Fe^{3+} at tetrahedral (red) and Fe$^{2.5+}$ at octahedral sites (green). Additionally, small surface (blue) and interfacial (yellow) components and a component from iron diluted in the Pt substrate (gray) can be identified. With progressing oxidation, first, for exposure < 4500 L, magnetite transforms towards maghemite (B), then, at 7500 L, the onset of hematite (magenta) is observed (C), which coexists with magnetite/maghemite, and, finally, at 21500 L, the entire film volume converts to hematite (D). Prolonged (hours) annealing under UHV conditions gradually (E) transforms hematite back to magnetite (F). This process strongly depends on temperature and its onset is observed at 630 K.

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

"Opus" research project NCN 2016/21/B/ST8/02107 [2017-2020]
(project leader: Marcel Krzan PhD)

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 biological film compositions will be enhanced by the addition of biopolymers with huge biological (antibacterial, fungicidal or regenerative for skin) activity, such as chitosan, bacterial cellulose, hyaluronic acid or levan. Scientific aim of the project is connected with analysis

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. In order 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. In order to achieve this effect, we plan detailed research on the tested solutions and mixtures of biopolymers.

During the first year of the project we performed measurements of dynamic and equilibrium surface activity, surface elasticity as well as testing the rising velocity of gaseous bubbles in solutions of selected biopolymers. We studied i.e. saponin (herbal saponin extract), egg white powder, whey protein and mixtures thereof. During the experiments we examined the effect of pH changes on adsorption processes. On the basis of the obtained results we developed of a series of compositions of mixtures of bio-polymers with high surface activity and promising biological activity. We also determine which concentrations ranges of such compositions are necessity for the generation of stable foam fraction. Our assumption were verified in preliminary tests in the foam column apparatus and using the double-syringe method.

The obtained results were presented at the 7th Bubble and Drops international workshop, Lyon 26-30 June 2017 (two presentations, oral and poster) and submitted to the 17th Food Colloids Conference, 2018, Univ. of Leeds, UK.

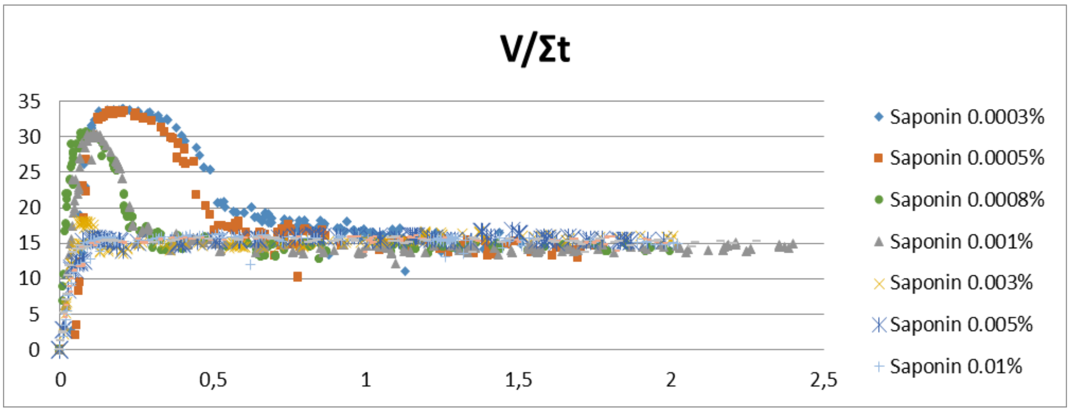


Figure 1. Bubble rising velocities in saponin solutions (herbal extract saponin)

The Mechanism of Regioselective Oxidative Dehydrogenation of 3-Ketosteroids Catalyzed by Δ^1-Cholest-4-en-3-one Dehydrogenase from *Sterolibacterium denitrificans*

"Opus" research project NCN 2016/21/B/ST4/03798 [2017-2020]
(project leader: Maciej Szaleniec DSc)

The aim of the project is the evaluation of reaction mechanism of steroids dehydrogenation by Δ^1-cholest-4-en-3-one dehydrogenase (AcmB). The enzyme is produced by denitrifying bacterium, *Sterolibecterium denitrificans* and belongs to FAD-dependent 3-ketosteroid dehydrogenases. The enzyme catalyzes oxidative dehydrogenation od 3-ketosteroids that leads to a double bond formation between C1 and C2 of steroid ring A. The projects includes the production of enzyme in a large amount and then the introduction of its structure (crystallographic and microscopic techniques) and its catalytic properties (kinetic tests), which will finally enable modeling of a possible reaction mechanism with DFT methods.

In order to obtain the enzyme, the overexpression system in *E. coli* DH5α using StarGate system was established. The protein was purified by affinity chromatography on Ni-NTA resin.

In order to crystalize the enzyme the conditions and buffer system for crystallization were optimized. However, the diffraction on the obtained crystals resulted with unsatisfactory low resolution which and the structure of AcmB could not be solved. As an alternative approach a homology model of AcmB was prepared based on 3-ketosteroid dehydrogenase KSTD from *R. erytropolis*. The model described well the FAD-binding region and the active site. However, due to longer amino acid sequence of AcmB (52 residues) the substrate binding region is still ambiguous due to lack of proper template. It seems that the extra loop in the substrate binding region and may be responsible for wider substrate specificity of AcmB.

Another aspect of structural studies of AcmB was studies at a spontaneous enzyme aggregation and its unknown tertiary structure. The protein aggregation was studied using size-based methods such as n-PAGE, SEC-HPLC, DLS and AFM. It was shown that the degree of aggregation depends from enzyme concertation. AFM imagining detected presence of the monomeric enzyme in solution, while spectrophotometric studies revealed that aggregation can be inhibited by the presence of reduction factors such as 2-mercaptoethanol or ditiotretiol.

The kinetic parameters of steady state reaction were determined for progesterone and three new testosterone derivatives. The measurements were prepared using a stopped flow method at saturating concentration of 2,6-dichloroindophenol (DCPIP). The apparent reaction rates k_{cat} of new substrates were at the same level as the reaction rate determined for progesterone. The app. Michaelis constant K_m of new substrates were 10-fold higher than K_m for progesterone, that indicates lower affinity of new substrates for the enzyme active site.

The kinetics of half-reductive (FAD reduction by substrate) and half-oxidative ($FADH_2$ oxidation by DCPIP or O_2) reactions were also studied with stopped-flow. Experiments were conducted under anaerobic conditions due to the re-oxidation of flavin by the oxygen. Reduction rate of the flavin was determined for progesterone and testosterone derivatives. All reduction rates k_{red} were high (150 – 1100 s^{-1}). Dissociation constant K_d for evaluated substrates could not be determined, which indicates very good affinity of both substrates to the active site of the enzyme. Determined k_{ox} were 8 and 0.2 s^{-1} for the DCPIP and oxygen, respectively. The determined reaction rates of k_{cat} and k_{ox} were of similar value, which may indicate that the possible reaction limiting step is either flavin re-oxidation or the product dissociation.

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

"Opus" research project NCN 2016/23/B/ST4/02854 [2017-2020]
(project leader: Dorota Rutkowska-Żbik DSc)

The aim of this work is to gain understanding in the nature of the V species introduced in V-containing zeolite materials with hierarchical micro- and mesoporous structure (mostly derived from BEA zeolite) 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.

Two series of vanadium catalysts were obtained, with the amount of vanadium changing in the range 0.5 – 4.0 %. Series I was obtained using two-step post-synthesis method, consisting of the de-alumination of the parent BEA zeolite, followed by the introduction of vanadium into the formed vacant sites (samples denoted as VxSiBEA). Series II (denoted further as VxHAlBEA) was obtained by impregnation of BEA zeolite of initial Si:Al ratio of 17. The physico-chemical characterization of obtained samples was started in order to verify their chemical composition and qualitative description of the vanadium active centers.

The system to measure the prospective catalytic activity of the obtained samples in the ODH of light alkanes was designed. It should be composed of 1) a fixed-bed flow stainless steel heated reactor; 2) steel tubes equipped with mass flow regulators; 3) coupled to a gas chromatograph (GC) with TCD and FID detection.

The crystallographic structure of un-modified BEA zeolite was analyzed, what enabled for the proposition of geometry models for BEA zeolite substituted with vanadium atoms placed both in T-sites and in exchange positions. The quantum-chemical calculations aimed at the determination of electronic and geometry structure of the proposed sites and their properties were initiated.

The Influence of Counterions on the Formation and Functionality of Polyelectrolyte Membranes

"Opus" research project NCN 2016/23/B/ST8/03128 [2017-2020]
(project leader: Marta Kolasińska-Sojka PhD)

The scientific goal of the project is to develop the model of the formation of polyelectrolyte and nanocomposite films in the presence of the selected counterions from the lyotropic series, thus to incorporate the Hofmeister ion effects into the properties of polyelectrolyte multilayers. Although the number of studies on various aspects of polyelectrolyte/nanocomposite multilayers is tremendous, there are almost no attempts to combine films properties with the type of electrolyte (lyotropic effect). The mechanism of how the lyotropic series of counterions affect the formation of polyelectrolyte multilayers is not entirely clear and thus, in the case of polyelectrolyte multilayers it is a scientific novelty with many fundamental problems to be solved. For that reason, systematical research on the surface properties of selected systems in the presence of selected counterions from lyotropic series is needed to propose a suitable mechanism.

As a part of the project the effect of counterions from the Hofmeister series on the formation and structure of multilayer polyelectrolyte films is investigated. Multilayer films are deposited using layer-by-layer technique of sequential adsorption of polyions from their solutions. From the planned research plan up to date, the optimization of the deposition of selected multilayers in the presence of KCl has been carried out. It involved the studies of kinetics and efficiency of the deposition process using Quartz Crystal Microbalance with Dissipation for the adsorption of the following polyelectrolyte films: PAH/PSS and PDADMAC/PSS in the presence of KCl basic electrolyte in three different ionic strengths: $I = 0.015M$, $I = 0.15M$ and $I = 1.5M$. Characteristics of wettability and surface energy of the obtained polyelectrolyte films in relation to the electrolyte used during the deposition was done by direct image analysis of the profile of a sessile drop.

The influence of ionic strength on the thickness of the obtained polyelectrolyte films was confirmed - along with the increase of the ionic strength, the thickness of multilayers increases and this effect is much more pronounced for the PDADMAC/PSS system due to the spatial structure of PDADMAC containing rings along its chain. It was also observed that the adsorption kinetics of polyions from KCl solutions is faster by approx. 30% compared to deposition from NaCl solutions. Additionally, wettability studies confirmed the more hydrophobic nature of films terminated with polycationic layer compared to multilayers with polyanion as an outermost layer.

The main and measurable effect of the project is to develop the model of the formation of polyelectrolyte and nanocomposite films in the presence of the selected counterions from the lyotropic series. Basic knowledge and experience collected will help to understand the mechanisms responsible for the structure formation and thus, it will enable the design of materials of strictly defined properties and to relate the counterion conditions with PEM functionality. It will be useful in follow-up studies leading to the implementation of obtained results into the area of selective membranes, biomaterials and nanocontainers, making a significant contribution to them. This is extremely important from a practical point of view, because it will increase the possibility of designing biomaterials, membranes taking into account the desired physicochemical properties and their specific applications. This is why the decent understanding of these, so far – overlooked factors affecting multilayers' structure and permeability is particularly important.

Dendrimers as a Platform for Designing Biologically Active Carrier

"Opus" research project NCN 2016/23/B/ST5/02788 [2017-2020]
(project leader: Barbara Jachimska DSc)

Discoveries in the past decade have revealed that dendrimers are excellent candidates for carriers in host-guest systems, such as drug delivery, gene delivery, molecular imaging, nanomaterial templates, high-performance catalysts and in environmental science. The applications of dendrimers in drug delivery systems have motivated an upsurge in this promising field.

The main scientific objective of this project is to develop a complete, quantitative description of the mechanisms governing the phenomena of protein adsorption on the dendrimer surface. Multidimensional research using advanced in-situ measurement techniques allows conducting a multifaceted study of the protein structure formation. The mechanisms of interaction of functional materials with different types of proteins present in the plasma, together with the analysis of conformational changes and reorganization of protein structures on the functional surfaces have great cognitive value. It will also contribute to a better understanding of the physicochemical mechanisms of creating protein layers on the polymer surface.

The results obtained in this project will contribute to a better understanding of the physicochemical mechanisms of the formation of protein layers with controlled architecture and functionality at interfaces.

"Preludium" Research Projects of the National Science Centre

Polyelectrolyte-Lipids Films with Incorporation Hydrophobic Nanoparticles

"Preludium" research project NCN 2015/19/N/ST4/00917 [2016-2017]

(PhD Student: Magdalena Włodek MSc, supervisor: Professor Piotr Warszyński)

The scientific goal of the project was to develop the mechanism of the formation of polyelecytrolyte – lipids films with embedded nanoparticles. As nanoparticles the commercially available cadmium sulphide quantum dots (QD) with emission spectra ranging from 400nm to 460nm were used.

The aim of this part of the project was to determine the adsorption kinetics of POPC/POPE liposomes containing hydrophobic QDs on the selected, polycation-terminated PEMs and the formation of QD-modified SLBs using the quartz crystal microbalance technique. The polyelectrolyte multilayers were obtained by the sequential adsorption of oppositely charged polyelectrolytes from their solutions - the *layer by layer* (LBL) assembly. Three PEMs with different composition were examined: $PEI(PGA/PLL)_3$, $PEI(PSS/PDADMAC)_3$ and $PEI(PSS/PEI)_3$. Additionally, as the reference, a single layer of PEI atop on supporting surfaces was considered. It was demonstrated that the presence of QDs led to the decrease of critical liposome concentration necessary to initiate the SLB formation due to destabilization of the phospholipid membrane. Moreover, depending on the underlying PEM, presence of QDs could either promote SLB formation (PEI or PDADMAC terminated films) or stabilize deposited liposomes as on PLL terminated multilayers.

Further on, the final structure of QDs-hybrids systems was investigated. The fluorescence emission spectra confirmed the presence of fluorescent nanoparticles within studied systems.. Moreover, the AFM images showed that the incorporation of QDs caused disruption of the bilayer structure and its degree could be correlated with size of nanoparticles.

Basing on our experimental results, we have explained how hydrophobic nanoparticles interact with the lipid bilayer, in particular how they influence the liposomes stability and the tendency to form supported lipid bilayers on polyelectrolyte multilayers.

Synthesis of Theranostic Biopolymer-Based Nanoparticles

"Preludium" research project NCN 2016/23/N/ST5/02783 [2017-2019]
(PhD Student: Marta Szczęch MSc, supervisor: Professor Piotr Warszyński)

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 HPNs but there are also some obstacles that limited its using 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 project is to develop the scientific background of the synthesis methodology of biocompatible and biodegradable hybrid nanoparticles for hydrophobic substances, optimization of magnetic compounds concentration, characterization of the HNPs physicochemical properties, functionalization of their surface properties and finally, evaluation of primary biological tests.

In 2017 we were focused on the preparation of biopolymer nanoparticles with diagnostic agent incorporated into the solid core. To the synthesis, we used the Phase Inversion Composition (PIC) technique at constant temperature. The HNPs were made of biodegradable and biocompatible polymers (poly(ε-caprolactone), PCL) and as a diagnostics, we used iron oxides NPs (Fe_3O_4) that were incorporated into the biopolymeric core. As a result, we obtained HNPs with the mean size 80nm and the maximum amount of the magnetic NPs was 40%. The results provide the basis and can be further considered as a promising theranostic delivery system.

Functional Hybrid Nanomaterials Based on poly(Amidoamine) PAMAM Dendrimers

"Preludium" research project NCN 2016/23/N/ST4/02532 [2017-2019]
(PhD Student: Karolina Tokarczyk MSc, supervisor: Barbara Jachimska DSc)

The purpose of this research project is to understand the role of poly(amidoamine) PAMAM dendrimers as anticancer drug nanocarriers and investigate the interaction mechanism between formed complexes and plasma proteins. Due to the unique, highly branched structure and physicochemical properties, dendrimers have become a popular group of spherical polymers widely studied in terms of biomedical applications. Dendrimers are often used as antibacterial, antifungal and antiviral molecules, as well as pharmacophores nanocarriers. They have the ability to transport substances either by attaching on to the periphery of the dendrimers and in the interior voids of the polymer. The biomedical problems related to the properties of existing therapeutic agents such as: low aqueous solubility, high toxicity or serious side effects require a continuous search for effective and stable therapeutic mechanisms. Thus, properly designed nanohybrid drug delivery devices have served as a promising strategy. Nanoparticles, assisted drug delivery increases their efficiency, reduces side effects and can become the basis of biomedicine.

The methodology involves understanding the binding and release process of anticancer drugs from poly (amidoamine) (PAMAM) dendrimers. We investigate the mechanism of the hybrid systems delivery from a molecular point of view. In the project we use precise, analytical techniques, such as surface plasmon resonance (MP-SPR), quartz crystal microbalance with dissipation monitoring (QCM-D to analyze the properties of created nanosystems. Our research was performed both in bulk and on the surface under precisely defined and controlled conditions: concentration, ionic strength, pH.

The results from this project will help to expand the current knowledge of dendrimers complex formation. They will enhance the capabilities of nanohybrid systems designed with the required physicochemical properties. Expanding knowledge of the interaction between dendrimers with active agents and plasma proteins will contribute to further more advanced research on PAMAM dendrimers, especially as intelligent pharmacophores nanocarriers.

"Fuga" Research Projects of the National Science Centre

High Resolution Analysis of the Spatial Properties of Structured Magnetic Nanostructures

"Fuga" research project NCN 2015/16/S/ST3/00450 [2015-2018]
(Project leader: Piotr Mazalski PhD, supervisor: Nika Spiridis DSc)

In the frame of project, further investigation of ultrathin cobalt layers surrounded by different combination of ultrathin nonmagnetic layers were performed. Structures such as: Al_2O_3/Pt/Co/Pt, Al_2O_3/Pt/Co/Au, Al_2O_3/Pt/Au/Co/Pt, Al_2O_3/Pt/Au/Co/Au were studied. Thickness of the Co layer was d_{Co} = 3 nm. For this thickness, the magnetization is in the plane of the layer. Ion irradiations with different ions fluences were performed in the form of adjacent stripes (with millimeter width) for samples representing each combination of layers. On the basis of these studies, the most interesting parameters of ion fluences were selected and used to irradiate samples over the entire surface. Magnetooptical studies confirmed the possibility to induce multiple perpendicular magnetic anisotropy similar to that obtained previously on samples with a double gradient (of ion fluence and cobalt thickness) and depending on ion fluence and the type of the buffer/cap layer. Samples with irradiated stripes were used for magnetooptical spectroscopy studies and samples irradiated over the entire surface were used for X-ray reflectivity (XRR) and X-ray photoelectron spectroscopy (XPS). Measured spectra allowed us to model the internal structure of the irradiated layers. Results obtained from XRR allowed us to follow changes of the layers thickness and roughness for different samples after ion irradiation. It turned out that samples with gold layers as cap or buffer or both cap and buffer layers have higher roughness and they are more prone to the etching process – material removal – during the irradiation process. XPS studies allowed to characterize the chemical composition in selected irradiated samples with respect to amount of an alloy formed in the layers.

Test studies were also performed on ion irradiated samples where the ultrathin cobalt layer was substituted by an ultrathin iron layer - Pt/Fe/Pt. Magnetooptical measurements revealed that magnetic changes induced by ion irradiation are weaker in relation to changes observed for samples with ultrathin cobalt layers.

Test irradiation were performed using focus ion beam (FIB) to form objects with micrometer sized. Such kind of irradiation allows to modify local magnetic properties and induce interesting magnetic effect depending on the size of irradiated objects.

Works were advanced aiming an upgrade of the UHV system with an additional Kerr chamber allowing in situ measurements of magnetic properties and related with tests of the PEEM and XAS microscope at the Solaris synchrotron.

Effect of electromagnetic Radiation in the Infrared Range on Protein-Surface Interactions

"Fuga" research project NCN 2015/16/S/ST4/00465 [2015-2018]

(project leader: Magdalena Kowacz PhD, Supervisor: Professor Piotr Warszyński)

Adsorption of proteins at a solid surface affects characteristics of the surface (e.g. its biocompatibility) and functionality of the immobilized biomacromolecules. The latter is defined by the type of binding sites, protein conformation and its structural flexibility that enable functional motions to occur. Protein motions are only possible at certain level of hydration. Furthermore, water molecules act as lubricant facilitating sliding along solid surface. In this project we explore the potential of a remote physical trigger – a non-ionizing infrared radiation (IR) to affect protein-surface interactions.

Our results indicate that IR light can protect proteins from surface-induced denaturation depending on the presence of strongly hydrated amino acid residues (Figure 1). Preservation of native fold suggests that proteins can potentially maintain their intrinsic functionality also in the immobilized state. Furthermore, it has been shown by other studies, that enhanced structural stability of proteins counteracts their irreversible adhesion. IR-induced prevention from unfolding affects wettability of the protein coated surfaces and results in their higher hydrophilicity. For protein-decorated nanoparticles it means stronger hydration repulsion promoted by respective protein coronas. IR light can also facilitate displacement of surface active species that became adsorbed to protein apolar compartments and could otherwise promote denaturation. Apart from supporting native conformation, this action of IR increases protein-water interfacial tension and therefore promotes aggregation (hydrophobic attraction) of the protein-coated nanoparticles. Our results indicate that displacement of adsorbed species is mediated by gas nanobubbles nucleated and/or adsorbed on hydrophobic protein patches in response to IR trigger. The net effect of IR on protein-protein and protein-surface interactions, as defined by short-range hydration forces (hydrophilic repulsion or hydrophobic attraction), depends on the relative contribution of the strongly water-holding amino acid residues and apolar compartments on the solvent-exposed protein surface. By its ability to affect protein conformational state and interfacial characteristics (such as effective protein-water affinity) IR radiation can therefore modulate protein interactions. This may have important implications for bioengineering applications.

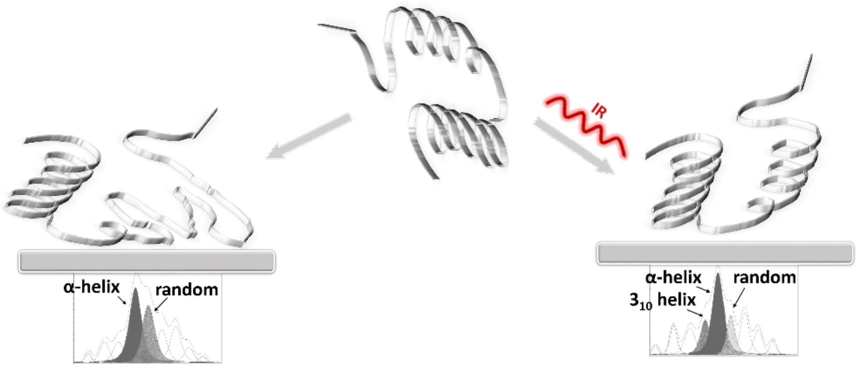


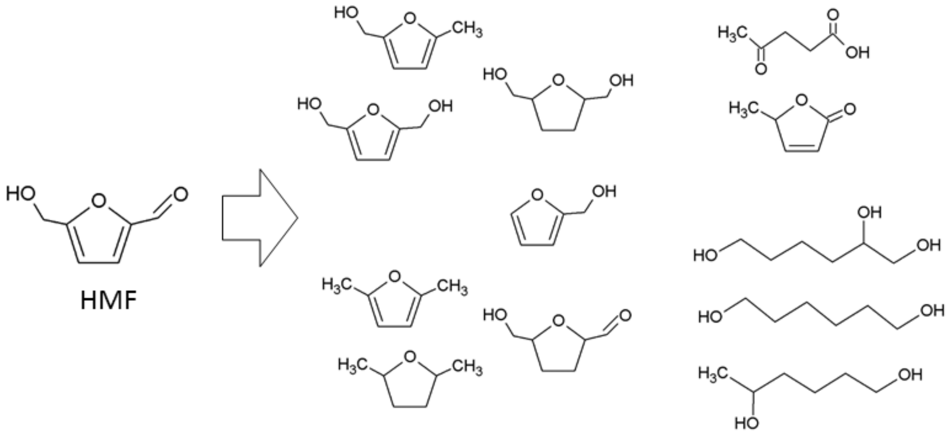
Figure 1. Schematic illustration presenting IR-induced protection of protein structure (preservation of helical conformations) from denaturation on the surface adsorption process

"Miniatura" Research Projects of the National Science Centre

The Role of Hydrogen Molybdenum and Tungsten Bronzes in Hydrogenation of Bioreagent 5-Hydroxymethylfurfural over Pd/MoO_3 and Pd/WO_3 Catalysts

"Miniatura" research project NCN 2017/01/X/ST4/00731 [2017-2018]
(project leader: Robert Kosydar PhD)

Catalytic transformation of 5-hydroxymethylfurfural (HMF) is one of the most actual research tasks as diminishing resources of fossil fuels requires application of other sources of organic compounds needed for production of fuels, chemicals and polymers. It is proposed using of 5-hydroxymethylfurfural derived from conversion of biomass wastes. HMF is a future substrate for producing a number of chemical compounds (Scheme 1) like biofuels, among others by catalytic hydrogenation of HMF. In this project the catalytic behaviour of Pd supported on MoO_3 or WO_3 is being examined in liquid phase hydrogenation of HMF at mild conditions of pressure and temperature. H_2 activation on Pd-centres result in H species formation (in the spillover process). Such hydrogen can diffuse inside the supports forming hydrogen bronzes H_xMoO_3 or H_xWO_3 what may influence the catalytic activity/selectivity. Between October (financial support start: 20th Oct 2017) and December 2017 the analysis of the newest literature concerning this issue as well as first purchases of the reactants (HMF and possible reaction products) were done. The set of gas chromatography measurements of analytic standards was done in order to get calibration data for possible reaction products.



Scheme 1. Selected products of 5-hydroxymethylfurfural (HMF) in the reactions in the presence of hydrogen.

Stabilization of the Catalytic Activity of 1-(*R*)-Phenylethanol Dehydrogenase by Immobilization on Polylysine

"Miniatura" research project NCN 2017/01/X/ST5/00735 [2017-2018]
(project leader: Mateusz Tataruch PhD)

1-(*R*)-Phenylethanol dehydrogenase (*R*-PEDH) is an enzyme which is derived from the bacterium *A. aromaticum* and reduces acetophenone to 1-(*R*)-phenylethanol with 100% stereoselectivity. This biocatalyst catalyzes the reduction of other ketones. The products of these reactions are exclusively *R* forms of aromatic alcohols and they may be used as synthons for drugs synthesis.

The aim of the project is to synthesize an immobilized form of 1-(*R*)-phenyletanol dehydrogenase supported on a water-soluble polypeptide (polylysine) by bis-aryl hydrazone bond conjugation. The main reason for this is a known fact of enzyme increased stability in reactor conditions upon immobilization. In the case of *R*-PEDH, there are no literature reports on the immobilization of this enzyme while similar studies have been carried out in Jerzy Haber Institute of Catalysis and Surface Chemistry PAS (i.e., immobilization on silica carriers with the use of glutaraldehyde and divinylsulfone as linkers).

The grant has started on 20/10/2017. The first stage of the project is the isolation of the enzyme for immobilization. This stage comprises of: i) growth of recombinant *Escherichia coli* strain with gene responsible for the expression of *R*-PEDH, ii) isolation of the *R*-PEDH enzyme using Strep-Tag affinity chromatography. 4 L culture of *E. coli* was prepared in LB medium. 50 ml of bacterial lysate was obtained from the culture. Enzyme activity was verified in reaction with acetophenone using UV-vis assay.

"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

"Iuventus Plus" research project MNiSW IP2015055974 [2016-2019]
(project leader: Magdalena Oćwieja PhD)

Recent literature data indicates that in the field of silver nanoparticle research, particular emphasis is placed on chemical reactions and processes which can occur on the nanoparticle surfaces and, in consequence, can induce their biological activity. The surface charge and oxidation state of silver nanoparticles strongly depend on the chemical structure and properties of stabilizing agents, therefore the impact of these molecules on nanoparticle toxicity seems to be pivotal. Taking this issue into account, the studies realized within the project included a quantitative determination of the cytotoxic effect of three types of silver nanoparticles, characterized by similar shape and size distribution but diverse surface properties, towards histiocytic lymphoma (U-937) and human promyelocytic cells (HL-60).

The silver nanoparticles were obtained in chemical reduction method using silver nitrate as a precursor of silver ions and sodium borohydride as a reducing agent. The surface charge of nanoparticles was tuned by the addition of sodium citrate or cysteamine hydrochloride. Selected physicochemical properties of nanoparticles were determined using dynamic light scattering (DLS), electrophoretic mobility measurements, transmission electron microscopy (TEM), surface enhance Raman spectroscopy (SERS) and atomic absorption spectrometry (AAS). The impact of silver nanoparticles on U-937 and HL-60 cell viability was assessed by MTT and LDH assays whereas the induction of inflammation was determined by the measurements of secretion of nitric oxide (NO) and interleukin-6 (IL-6). The oxidative stress generated in the cells under the influence of silver nanoparticles was evaluated via the process of membrane lipid peroxidation using MDA assay. Additionally, the changes in the total antioxidant activity (TAA) and superoxide dismutase activity (SOD) after the nanoparticle treatment were determined.

The results of studies showed that the cysteamine-stabilized (positively charged) nanoparticles (SBATE) were the least toxic although they exhibited a similar ion release profile as the unmodified (negatively charged) nanoparticles obtained using sodium borohydride (SBNM). Citrate-stabilized nanoparticles (SBTC) induced superoxide dismutase (SOD) activity in the HL-60 cells and total antioxidant activity in the U-937 cells despite their resistance to oxidative dissolution. The highest toxicity of unmodified nanoparticles (SBNM) was manifested in the disruption of membrane integrity, decrease in the mitochondrial functions of cells and the induction of inflammation.

The results revealed that the silver nanoparticles independently on their properties exhibit multidirectional action towards tested tumoral cells. These findings allowed to conclude that mechanisms of silver nanoparticle cytotoxicity is the combination of effects coming from the surface charge of nanoparticles, released silver ions and biological activity of stabilizing agent molecules. On the other hand, the cellular response to the nanoparticle treatment depends on the silver dose and individual features of the tumoral cells.

Applied Research Programme Projects of the National Centre for Research and Development

HERIVERDE Energy Efficiency of Museum and Library Institutions

Applied Research Programme HERIVERDE project NCBiR PBS2/A9/24/2013
[2013-2017]
(project leader: Professor Roman Kozłowski)

In the final phase of the project, a scheme for selection of climate management strategies in institutions preserving heritage assets was developed which included analyses of a building, of prevailing microclimatic conditions and of scenarios for climate control and energy consumption. The work was based on two case studies: Gallery of the 19th-century Polish Art in the Cloth Hall, a division of the National Museum in Krakow as a fully air-conditioned building, and the Krasinski palace, belonging to the National Library in Warsaw as a building with no air conditioning. Microclimatic conditions measured in the two buildings were analysed and the prevailing categories of climate control were determined by referring to the standards accepted in the two institutions and other international guidelines. The HERIe software for quantitative assessment of risk of physical damage to cultural objects due to microclimate variations was used in the data analysis.

Then, alternative scenarios of climate control and energy consumption were developed based on analysis of their effect on the preservation of historic character of a building, on the economic viability, in particular savings due to the increased energy efficiency, and on microclimate adapted to functions of the specific indoor spaces.

For the Gallery in the Cloth Hall, two general scenarios were developed which assumed maintaining full air-conditioning in the Gallery, or resigning from the air-conditioning and using small movable equipment to humidify or dehumidify the space. Within the full air-conditioning scenario, three algorithms of the climate condition control were analysed: strict control (AA category of the American Society of Heating, Refrigerating and Air-Conditioning Engineers ASHRAE), cautious control (environmental guidelines contained in the declaration of the Conservation Committee of the International Council of Museums ICOM and the International Institute of Conservation IIC of 2014) and own recommendations of the National Museums in Krakow allowing decreases of relative humidity to 35% in winter conditions.

For the Krasinski palace, three climate-control scenarios, all rejecting introduction of any equipment for mechanical ventilation or full air-conditioning owing to high historical value of the building were analysed: precise temperature control with separation of the storage zone and rooms for work, passive stabilization of microclimate in the storage rooms - allowing a slow temperature decrease in the cold period, and passive stabilization of microclimate in the storage rooms with an additional air dehumidification so that relative humidity does not exceed 50%.

Results of the measurements and modeling of the total energy consumption per 1 m^3 of the exhibition room volume in the Gallery of the 19th-century Polish Art in the Cloth Hall demonstrated that introducing a well-designed air-conditioning limited to the exhibition rooms and based on the minimal inflow of outdoor air does not necessarily bring an increased energy consumption when compared to rooms with no air-conditioning. In turn, an effective collection protection in the Krasinski palace can be implemented using an energy-efficient strategy of ‘sustainable conservation’, which meets with growing interest in the global museum and conservation community. The strategy recommends control of climate conditions without installing air-conditioning equipment but with the use of passive simple techniques that are easy to maintain.

"Leader" Programme Research Projects
of the National Centre for Research and Development

New Functionalised Polymers for Biomedical Applications

"Leader" programme research project NCBiR 0090/L-7/2015 [2017-2019]
(project leader: Maciej Guzik PhD)

Stage 1: Preparation of polyhydroxyoctanoate (PHO).

This stage concerns preparation of bacterial polymer polyhydroxyoctanoate (PHO). Minimal amount of PHO polymer was produced in a 5L fermentor for preliminary studies with *Pseudomonas putida* KT2440 (160g CDW; 60% PHO). Polymer was purified over activated charcoal in order to cleanse it from impurities (lipopolysaccharide, colour). Polymer was submitted for detailed physicochemical analysis – DMA (t_g=-28,5 ^{o}C), DTG (t_{deg}=269,3 ^{o}C), PALS (t_3=2.818 ns; I_3=29%, R=0,35 nm; V=179 A^3), XRD (Xc ~ 37%), GC (over 99 mol% 3-hydroxyoctanoate). PHO was submitted for microscopic analysis – techniques such as polarised light microscopy, atomic force microscopy and force spectroscopy were performed. Local Young moduli and surface roughness were defined for the surface of polymer casted from ethyl acetate (25.4 MPa; RMS of 2.6−86.8 nm for surfaces of 1−900 μm^2), acetone and chloroform. The results were gather in a manuscript summited to *Biomacromolecules* for assessment.

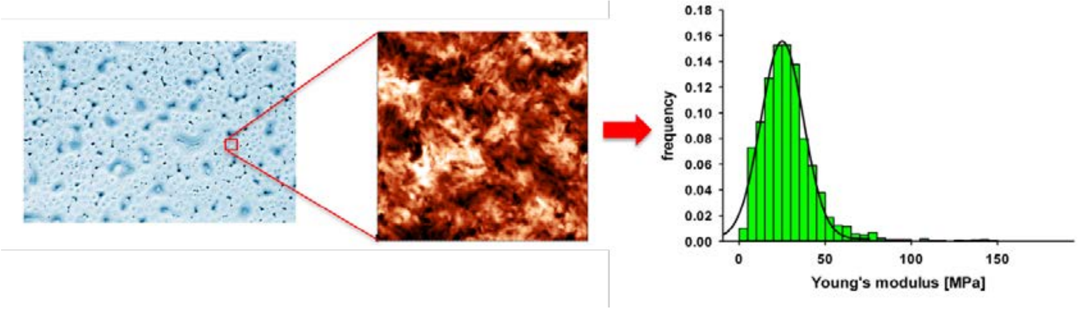


Figure 1. Surface characterisation of PHO.
From left to right: Surface in polarised light; AFM imagining; local young moduli.

Stage 2: Preliminary studies for experiments with cell and microbial cultures

The optical properties of the tested material were assessed. The measuring system (confocal microscope) has been calibrated for imaging with the highest possible quality and resolution. The staining protocols for the tested material were developed for observation in the confocal microscopy. PHO was stained and the optimal concentration of dye (fluorescein) needed to obtain images of optimal quality and resolution was determined. The protocols for pouring substrates made of PHO for the needs of cell cultures were developed. Methods and preparation of materials for performing cytotoxicity tests were selected. The MEF cell cytotoxicity tests were performed on glass substrates. PHO samples were prepared for microindentation studies. A preliminary microbiological examination was performed: the production of extracellular mucus by yeast-like fungi of the genus *Candida* spp. was determined. A colony morphology test was carried out on Congo Red Agar and isolates were selected for further studies on adhesion of mixed biofilm.

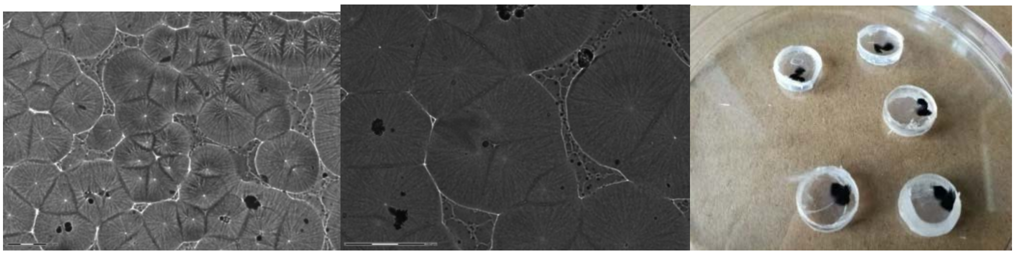


Figure 2. PHA surface poured on a glass substrate using a modified method of drying the material with a shield limiting solvent evaporation and PHA samples at various stages of crystallization (3,7,12,14,17 days) prepared for indentation testing.

EU COST Actions

ECOSTBio Explicit Control Over Spin-states in Technology and Biochemistry

EU COST Action CM1305 ECOSTBio [2012-2016]
(country coordinator: Professor Ewa Brocławik)

The action CM1305 ECOSTBio is a part of the EU project „European Cooperation in Science and Technology" (coordinated by University of Groningen, the Netherlands). European funds were used in aim is to construct a network of both experimental and theoretician research groups to tackle a diversity of chemical problems where spin is an important factor. This will be achieved by the joint creation of a SPINSTATE database of systems with known spin states and spin-related properties. The interactions of theorists and experimentalists will create a synergy, helping theoreticians to validate their models and experimentalists to improve the performances of novel materials with desired properties.

In the year 2017 two scientific meetings took place: sixth scientific workshop in Lisbon, March 30-31, 2017 and seventh scientific workshop in Dublin, December 14-15, 2017. Prof. E. Broclawik participated in both meetings as the Polish representative to the Management Committee for CM-1305.

The cooperation with Slovakia (Bratislava, group of L. Benco) and with Great Britain (Manchester, group of prof. S. de Visser) has been continued and two scientific papers were published in 2017. Cooperation with Germany (Stuttgart, group od prof. Hunger) was also continued and scientific exchange with Republic of Malta has been established.

NEUBIAS A New Network of European Bioimage Analyst to Advance Life Science Imaging

EC COST Action CA15124 NEUBIAS [2016-2020]
(investigator: Marcel Krzan PhD)

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 organizing a new type of meeting fostering interactions between all stakeholders including: Life scientists, image analysts, microscopists, developers and 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 first year of the action I collaborated online with other action participants from various European research groups related to this COST action.

I wanted to set up the best solutions for my Image analysis problems in the case of rising bubbles and ageing of foam cells. I used our new e-mailing list as well as our new interactive net database for image analysis tools. In the results I created new programs and macros, which help me evaluated in new ways the ageing of bubble and foam micro-structures.

Scientific results, obtained thanks to new image analysis measurement tools, were already presented during conference 7th Bubble and Drops international workshop, Lyon 26-30 June 2017 (4 presentations, 1 oral, 3 posters) and submitted for oral presentation on 17th Food Colloids Conference, 2018, Univ. Of Leeds, UK.

Other International Research Projects

Immobilization of Metal Nanoparticles on Organo-Modified Layered Silicates

Polish - Slovak joint research project PAS - Slovak Academy of Sciences

Joint research project of the Polish Academy of Sciences and the Slovak Academy of Sciences cooperation [2016-2018]
(project leaders: Małgorzata Zimowska PhD, Helena Pálková PhD)

The aim of this project was focused on the IR spectroscopy study in both, middle (MIR) and near (NIR) region to investigate the interaction of the organic cationic species of Polydiallyldimethylammonium chloride (PDDA) polymer with natural smectite - Jelszowy Potok (JP). Thermal behaviour of organic cations intercalated between the layers of montmorillonite and the influence of organic cation amount on the changes in the spectra were evaluated.

The organoclays with composition differ in polymer content (JP-PDDA_5% and JP-PDDA_25%) used further for immobilization and variable distribution of ruthenium metal nanoparticles, had been prepared from Na-montmorillonite by delamination of the layered clay mineral in distilled water and subsequent introduction of the organic cationic species of Polydiallyldimethylammonium chloride - PDDA polymer.

The transmission FTIR method in the MIR (4000 - 400 cm^{-1}) and reflectance DRIFT in the NIR (8000 - 4000 cm^{-1}) regions, were used to analyse the changes in the spectra of solid samples. Upon introduction of PDDA cations the C-H vibrational modes of methyl groups appeared in the spectra: stretching νC-H between 2970-2860 cm^{-1} and bending δC-H in the range 1490-1380 cm^{-1}. In accordance with carbon elemental analysis showing higher content of carbon in JP-PDDA_25% sample, the enlarged intensities of the ν and δ C-H bands also confirmed the more pronounced compensating of the layer charge by organic cations. Introduction of PDDA cations into JP significantly modifies the shape of the NIR spectra providing complementary information to MIR region. As a result new bands related to CH first overtones (2ν) in the region 6100–5500 cm^{-1} and combination modes ($\nu+\delta$) below 4500 cm^{-1} have appeared, respectively.

The thermogravimetric (TG) analysis and differential profiles (DTG) of parent layered mineral and organo-clays with 2 different contents of intercalated polymer showed three steps decomposition related to the release of water molecules adsorbed on the clay surface, dehydration of the hydrated, interlayer Na cations and the dehydroxylation of the inorganic clay layers. In polycationic organo-JP the loss of hydration from the cations overlapped with the mass loss step attributed to the thermal degradation of the polycationic surfactant. Due to more hydrophobic character of JP-PDDA_25% sample the mass loss attributed to the release of adsorbed water molecules was lower in comparison with of JP-PDDA_5% composite. However more pronounced mass loss assigned to the release of organic phase was observed for composite with higher loading of polyelectrolyte. These data are in a good agreement with FTIR analysis confirming higher actual amount of polymer chain intercalated between the layers and on the surface of JP platelets of JP-PDDA_25% clay polymer composite.

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

Joint research project of the Polish Academy of Sciences and the CNR National Research Coucil cooperation [2017-2019]
(project leaders: Marcel Krzan PhD, Francesca Ravera PhD)

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 “green surfactants”. We want to use well known proteins (saponin, Bovine Serum Albumine, β-lactoglobuline, etc.) as a model biosurfactants. Such surface thin film could in the close future become basis of the cosmetic and dermatological composition for the multiple use products. The medical impact of the biological foams and emulsions, which could be used as thin cover film will be further strengthened by the addition of silver nanoparticles. The application of other biological active particles, like i.e. chitosan, silk fibroin or liposomes will be also 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 defense against a 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 be also used as a pathogen barrier in 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 amount of synthetic surfactants incorporated into the natural environment.

During the first year of the project we performed measurements of dynamic and equilibrium surface activity and surface elasticity in saponin solutions (saponin reagent grade, VWR, catalog no. 0163-100g) and chitosan (HD chitosan, cosmetic ingredient). On the basis of the obtained results we developed of a series of compositions of mixtures of bio-polymers with high surface activity and promising biological activity (i.e. bacteriostatic properties). We also determine which concentrations ranges of such compositions are necessity for the generation of stable foam fraction. Our assumption were verified in preliminary tests in the foam column apparatus and using the double-syringe method. The research was carried out simultaneously using the facilities of both institutes in Poland and Italy.

The bilateral project measures allowed us to perform two two-week research internships in Genoa - for dr. Marcel Krzan and dr. Ewelina Jarek. We also had project visitors in our Cracow institute in Nov. 2017 - dr. Francesca Ravera (Italian PI of project) and dr. Eva Santini. The obtained results will be presented during scientific conferences in 2018 and reported in a joint publication in the international JCR journal.

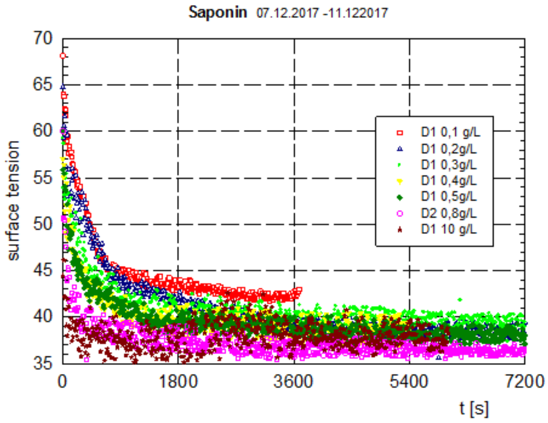


Figure 1. Surface tension variation in saponin solutions

Other Scientific Activity

Roughness of Model Random Surfaces

(investigator: Paweł Weroński DSc)

Surface roughness determines many important physico-chemical processes and phenomena. It is essential in contact mechanics, adhesion, friction, light reflection, surface wearing, structural coloring, microfluidics, sealing, and surface wettability. Therefore, in various branches of science and industry, managing surface roughness is a key problem. It has to be controlled to produce corrosion resistance and anti-reflective coatings, high-quality optics, superhydrophobic surfaces, automotive parts, manipulators, or microdevices, to mention just a few. A simple method for controlling surface roughness, in a wide range, is colloidal particle deposition.

The main aim of our research was to describe theoretically roughness of a model homogeneous surface decorated with randomly distributed disks or pillars. We generated such surfaces with the algorithm of random sequential adsorption. Then, we developed numerical algorithms and computer codes, based on the fast Fourier transform, which let us determine numerically the power spectral density of the generated surfaces. We also developed a quantitative analysis of surface roughness and derived a general equation for the power spectral density of the surfaces. We obtained analytical expressions for the power spectral density in the special case of statistically isotropic, circular areas. We derived limiting equations describing the behavior of the function in the low- middle- and high-wavenumber ranges. We tested our analytical predictions against numerical calculations and found them to be in good agreement.

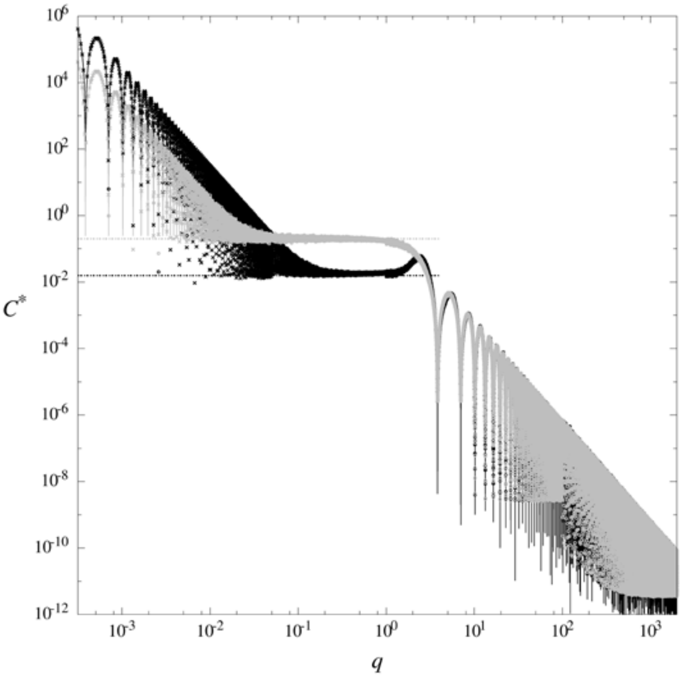


Figure 1. Dimensionless power spectral density of circular areas decorated with randomly distributed pillars or disks. Results in black are for pillars of the height 20 and surface coverage 0.05. Results in gray are for disks of the height 0.2 and surface coverage 0.5. Lines and symbols represent analytical and numerical results, respectively. Solid lines show analytical asymptotic solutions in the low- and high-wavenumber ranges. Dot lines represent analytical solutions in the middle-wavenumber range for arbitrary and small surface coverage.