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

HR EXCELLENCE IN RESEARCH

DETAILED RESEARCH REPORT

year 2022

Contents

Statutory Research

Catalytic Materials and Processes for Sustainable Development

Experimental and computational studies on selected enzymes of application potential

(Professor Tomasz Borowski, Professor Maciej Szaleniec, Maciej Guzik DSc, Anna Miłaczewska PhD, Mateusz Tataruch PhD Eng., Tomasz Witko PhD, Agnieszka Wojtkiewicz PhD, Anna Kluza PhD, Zuzanna Wojdyła PhD, Justyna Andrys MSc, Justyna Prajsnar MSc,)

For the EctC enzyme, a series of molecular dynamics simulations was performed for various variants of the substrate occupation of the functional dimer of the enzyme and two variants of the conformation of the tryptophan residue, which is directly adjacent to the substrate molecule bound in the active site. The analysis of the obtained trajectories in terms of reactive substrate conformations and the obtained profiles of potential of mean force allowed to propose key enzyme-substrate interactions for the catalyzed reaction. The results have been published. For EctC, a QM/MM study was also conducted on the mechanism of the enzymatic reaction.

For the DODA enzyme, molecular dynamics simulations were performed for two different orthologs of this protein and for several variants of enzyme-substrate complexes. Based on the obtained results, cluster models for quantum chemical (QM) calculations were prepared. Currently, QM studies are being conducted on the mechanism of the reaction catalyzed by DODA.

For CAS, QM/MM modeling of possible reaction paths for O-cyclization of the substrate (proclavamate) was carried out. The most probable regioselectivity of the hydrogen abstraction (HAA), which initiates the reaction, was indicated, and the possible scenarios of closing the O-cyclic ring - direct formation of the C-O bond and fragmentation of the radical / cycloaddition - were compared (within mechanical embedding). To better understand how the enzyme provides the selectivity of the reaction, initial modeling was performed for an experimentally unobserved reaction – hydroxylation, which is typical for this family of enzymes. This modeling is the basis for analyzing the role of the inherent properties of the substrate/active site and the binding pocket in determining the selectivity of the reaction.

Experimental work focused on homologues of L-DOPA extradiol dioxygenases (DODA proteins) and ectoine synthase (EctC). Previous crystallographic studies on DODY homologues have shown that in the case of Escherichia coli and Beta vulgaris homologues, the N-terminal fragment of the polypeptide chain blocks the substrate binding site. In addition, in order to optimize the production of enzymes from B. vulgaris and Portulaca grandiflora organisms, it was decided to change the genetic construct (and thus the location of the histidine tag used for purification). Therefore, the DODA protein genes from B. vulgaris and P. grandiflora were truncated and cloned into the plasmid pMCSG9, while the E. coli DODA gene was only truncated. All obtained constructs were tested for protein production in E. coli and purification. The proteins that were produced (DODA from B. vulgaris and E. coli) were screened to find the optimal crystallization conditions. So far, the crystal structure of the shortened DODA enzyme from E. coli in a complex with 4-nitrocatechol has been obtained.

The production of EctC from Sphingopyxis alaskensis and Paenibacillus lautus in E. coli bacteria was tested. EctC from S. alaskensis was produced in E. coli bacteria, purified on a StrepTag column, and crystals were obtained from several different crystallization conditions. The EctC structure was obtained from S. alaskensis in the form of an apo-enzyme. For DODA and Ectc proteins, research is continued to obtain enzyme-substrate or enzyme-substrate analog complexes. The obtained results were used as preliminary research for the submitted grant application.

Research of biological systems at cellular level

(Tomasz Witko PhD, Daria Solarz MSc)

As part of the task, interactions between mammalian cells namelly mouse embryonic fibroblasts (MEF 3T3) with materials made of polymers of bacterial origin (polyhydroxyalkanoates) were investigated. Cells grown on polymeric materials were stained and then imaged using a confocal microscope. The obtained data were analyzed and prepared for publication. As part of the performed studies, the interaction of mammalian cells with the surface of the flat PHB polymer was determined at the level of individual cellular structures (actin, microtubules, intermediate filaments). Research was also carried out on materials with a 3D structure and composites. MEF 3T3 cell viability was tested against test materials containing different concentrations of diclofenac. Thanks to these studies, it was possible to determine that PHO materials can be enriched with therapeutic concentrations of diclofenac without harming the viability of the body's cells. In addition, the behavior of cells on the surface of the material was determined, taking into account the penetration and settlement of the pores of the material. The result of the conducted research is a published scientific paper (Dressings on Wound Healing in Mice, DOI: 10.3390/ijms232416159).

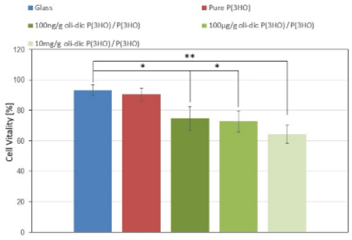


Fig 1. Cytotoxicity of materials containing different concentrations of diclofenac against mammalian cells (MEF 3T3).

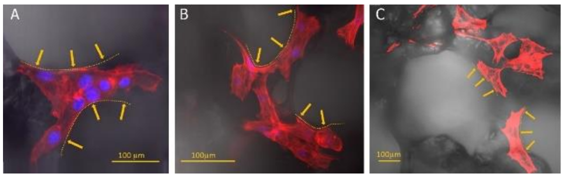


Fig 2. Images from a fluorescence microscope showing the arrangement of cells at the edges of the pores of the material.

Imaging of mammalian cells was also performed on materials with a 3D structure made by electrospinning. The materials were obtained in cooperation with the University of Belgrade. The tested materials were PLA-PHO composites with a ratio of 3:1, 1:1, 2:3, 1:3. Confocal imaging of cells on the materials was performed and it was found that mammalian embryonic fibroblasts penetrate all the tested materials very well, which makes them potentially useful in medicine. The result of the conducted research is a publication (Electrospun polylactid/polyhydroxyoctanoate (PLA/PHO) biomaterials support fibroblasts adhesion and migration) being prepared for publication.

As part of the cellular studies performed as part of the task, a comparison of the effects of dipterocarpol derivatives (1DH-DPC) on normal (MEF3T3) and cancer (A549) cells was also carried out. These studies became part of a patent application filed in 2022 (A.M. Wojtkiewicz, M. Szaleniec, M. Guzik, J. Prajsnar, G. Pacek, T. Witko, O. Adamczyk, D. Solarz-Keller, "Dehydrogenated derivative of dipterocarpol, method of its production by enzymatic means and application” (P.441592), 29.06.2022).

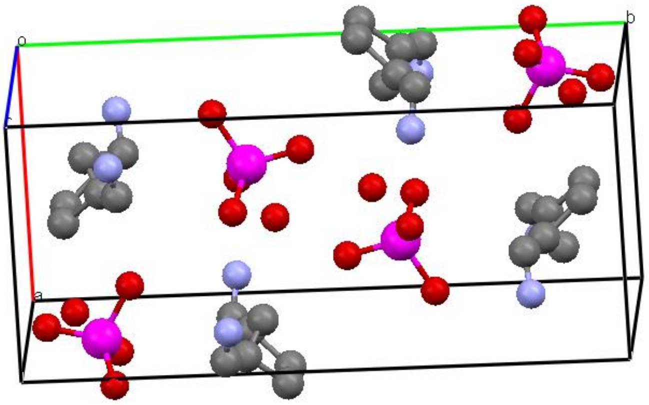
New peroxo and polyoxo compounds of Mo(VI), W(VI) and V(V). Synthesis, structural studies and applications in oxidation processes.

(Professor Wiesław Łasocha, Adrianna Sławińska MSc, Katarzyna Pamin DSc, Daria Napruszewska MSc)

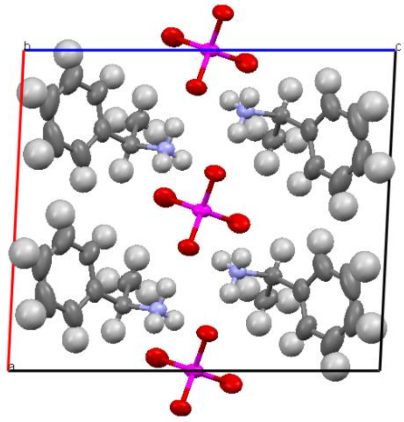
The conducted works concerned the synthesis, structural and physicochemical studies of new organic-inorganic peroxomolybdates and polyoxomolybdates. The most important research directions and the results obtained are presented below.

1. A number of syntheses were carried out using various isomers of cyclohexaneamine, pyridine alkyl derivatives, aniline and amantadine. Syntheses under reflux and with the use of autoclaves were used. Tests were carried out for solutions acidified with hydrochloric or acetic acid. The obtained solid phases were examined using powder diffraction and elemental analysis methods. The most important results include obtaining new amantadine and tert-butyl-pyridine octamolybdates, as well as 1,3-diaminomethyl-cyclohexane (1) and methylbenzylamine (2) mono-molybdates.
2. Possibilities of synthesis of Mo(VI) peroxo compounds using dicarboxypyridine acids were investigated. Compounds with potassium and sodium cations were synthesized for the purpose of summarizing and verifying physicochemical studies, mainly catalytic and pharmacological.
3. A number of preparative studies were carried out on obtaining Cu, Co, Ni molybdates for the purpose of comparative catalytic studies. Methods for the synthesis of copper trimolybdate and Anderson-type ammonium-hydronium nickel-molybdate were refined.
4. Verifying tests of catalytic oxidation of cyclic hydrocarbons and cyclohexanone in the Baeyer-Villiger (BV) reaction were carried out for selected compounds from the group of peroxomolibdates.
5. Selected peroxo compounds were tested at the Medical College of the Jagiellonian University as part of pharmacological tests on cancer cell lines. The best compounds show activity comparable to that of cis-platinum used in practical therapies in medicine.

The figure below shows the unit cells for compounds 1 and 2. Magenta, black, blue and red balls represent Mo, C, N and O, light gray balls represent H atoms.



1)



2)

Crystallochemical data.

(**1**) $[C_6H_{10}\ (CH_2NH_3)_2]\ .\ MoO_4.\ H_2O$, SG: Pbnm, a,b,c,αβγ; a 8.764(3), 19.744(5), 7.475(2), 90., 90., 90., V = 1293.58(14)$Å^3$, Z=4.

(**2**) $[C_6H_5CH(CH_3)NH_3]_2\ .\ MoO_4$,SG: C 2, a,b,c,αβγ; 11.7866(3), 6.0073(2), 13.6893(6), 90., 92.817(3), 90., V = 968.108 $Å^3$, Z=4.

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

(Associate Professor Dorota Rutkowska-Żbik, Professor Małgorzata Witko, Professor Mirosław Derewiński, Associate Professor Renata Tokarz-Sobieraj, Anna Micek-Ilnicka DSc, Michał Śliwa PhD, Katarzyna Samson PhD Eng., Małgorzata Ruggiero-Mikołajczyk PhD, Łukasz Kuterasiński PhD, Mariusz Gackowski PhD, Robert Kosydar PhD, Erwin Lalik PhD, Małgorzata Zimowska PhD, Jerzy Podobiński MSc, Natalia Ogrodowicz MSc, Joanna Kaim MSc)

The aim of this year studies was to check how the type of the oxide-type support influences the properties and catalytic activity of copper containing materials in low temperature hydrogenation of furfural in the gas phase.

As the support for the active phase, the following oxides were used: SiO_2 (amorphous silica, SSA = 213 m^2/g), CeO_2 (SSA = 2,60 m^2/g) and ZrO_2 (SSA = 0,87 m^2/g). The copper phase was introduced via wet impregnation using copper(II)nitrate. Next, the samples were dried at 120 °C for 10 h and calcined at 500 °C for 3 h in the air flow to obtain catalysts containing oxide-based species. The resulting materials, in which copper content reached 1%, 2%, 5% and 10% wt. %, were characterised by a set of physico-chemical methods. XRD analysis allowed for identification of the CuO phase in samples where copper was introduced onto CeO_2 and SiO_2 (for these materials it was also possible to determine the mean diameters of crystals which were in the range 26,1 – 29,8 nm, irrespectively of the amount of the introduced copper). SEM imaging with EDS analysis confirmed the existence of the copper-enriched regions on the samples' surfaces. Introduction of the active copper phase resulted in a small decrease of the specific surface area (up to 10%). Redox properties of the materials were probed by the H_2-TPR technique showing one reduction peak (in the range 248–301 °C). The analysis of the obtained reduction profiles indicates that replacement of silica for ceria or zirconia impacts positively the reducibility of CuO as demonstrated by lowering of the reduction temperature. The FTIR spectra of CO adsorbed on $Cu_{2.0}SiO_2$, $Cu_{2.0}CeO_2$ and $Cu_{2.0}ZrO_2$ show a maximum at ca. 2122 cm^{-1}, which is attributed to Cu-CO vibrations. Its frequency is almost independent on the used support. Its intensity is ca three times higher for the sample with CeO_2 as the support suggesting the highest dispersion of copper in this catalyst. The higher dispersion, the larger surface area and higher number of the adsorption centers. The acidity of the catalysts was additionally studied by NH_3-TPD. Copper-silica system was characterized by the highest concentration of acid sites, although they are mostly of weak or medium strength. The lowest concentration of acid sites was found for the system with ZrO_2.

Catalytic activity of the samples was tested in hydrogenation and decarbonylation of furfural in the gas phase at temperature range 120-200 ºC. The activity tests demonstrated that at low temperatures (120 °C and 160 °C) copper systems are not active, irrespectively of the support used. The lack of activity can result from too low content of the active phase needed for the reaction or low specific surface area of samples containing CeO_2 and ZrO_2, leading to insufficient dispersion of copper on their surfaces. At 200 °C the activity of the catalysts is low – the furfural conversion is in the range 9–36%. The investigated copper-based systems catalyze mostly hydrogenation of furfural and not its decarbonylation. The highest selectivity towards furfuryl alcohol (96%) was found for $Cu_{1.0}SiO_2$, the lowest (70%) - for $Cu_{5.0}CeO_2$.

To sum up, our experiments shown that the type of the support used for the active metal phase influences activity of the studied catalysts and their selectivity to different reaction products.

Physics and Chemistry of Surfaces and Nanostructures

Mechanisms and kinetics of protein immobilization on solid/electrolyte surfaces: Determination of the mechanisms of human serum albumin corona formation at polymer microparticle carriers

*(Małgorzata Nattich-Rak DSc, Marta Sadowska PhD, Monika Wasilewska PhD,
Paulina Żeliszewska PhD, Katarzyna Kusak MSc, Professor Zbigniew Adamczyk, Associate professor Magdalena Oćwieja)*

Coronavirus attachment to abiotic surfaces is a crucial issue controlling their inactivation and removal by filtration, which can decrease the epidemics proliferation. However, because of limited availability of intact virus samples, no systematic experimental works were devoted to a thorough physicochemical analysis of this process. It is postulated in the work that reliable information about mechanisms of virus attachment to abiotic surfaces can be acquired using a polymer nanoparticle with protein corona system, which can well mimic physicochemical parameters of virions.

Therefore, the main objective of this work was to elucidate the mechanism of protein corona formation and deposition kinetics of the virus-like particles formed in this way at solid/electrolyte interfaces. Attention is focused on the polystyrene nanoparticle/human serum albumin (HSA) conjugate, which exhibits pronounced advantages. The formation of albumin corona at the particles was studied using the dynamic light scattering, the electrophoretic and the solution depletion methods involving the atomic force microscopy. It was confirmed that the particles had a diameter of 120 nm, which corresponds to the external diameter of the SARS-CoV-2 virion with the spike protein.

The deposition kinetics of the particles was determined by atomic force microscopy under diffusion and by quartz microbalance under laminar flow conditions. It was shown that the particle deposition rate at the gold sensor abruptly vanished at pH above four (see Fig. 1) which correlated with their zeta potential. This effect proved that electrostatic interactions played a decisive role in the deposition of the polymer particles with protein corona.

The experimental results acquired in this work showed that it is feasible to prepare stable polymer particles with protein corona (pseudo-virus) suspensions characterized by well-defined physicochemical characteristics. Such particles can be used as reference systems for predicting kinetics of intact virus deposition on abiotic surfaces and for their efficient removal by various filtration procedures.

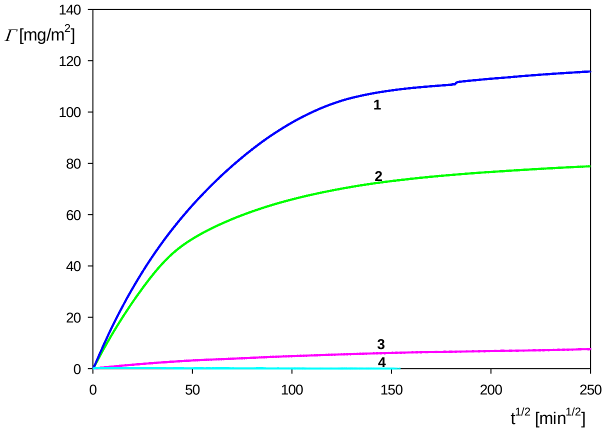


Fig. 1. Kinetics of the polymer particle with HSA corona deposition at the gold sensor under flow conditions derived from QCM measurements for various pHs, the corona coverage 0.80 mg m^{-2}, 10 mM NaCl, c_b = 50 mg L^{-1}, flow rate 2.5×10^{-3} ml s^{-1}. The solid lines show the experimental data derived from the Sauerbrey model for the third overtone: 1. pH 4.0, 2. pH = 4.5, 3. pH = 5.5, 4. pH = 7.4.

Monolayers of metal nanoparticles of controlled structure and electrokinetic properties deposited on the surfaces of oxide carriers

(Associate professor Magdalena Oćwieja, Małgorzata Nattich-Rak DSc, Aleksandra Pacuła DSc, Dorota Duraczyńska PhD, Marta Sadowska PhD, Monika Wasilewska PhD, Paulina Żeliszewska PhD, Julia Maciejewska-Prończuk PhD, Patrycja Gnacek MSc, Katarzyna Kusak Eng., Robert Kosydar PhD)

The development of efficient methods for the synthesis of nanomaterials composed of two or more nanostructures characterized by different physicochemical properties is of interest to many scientists. Particularly interesting and important seems to be the preparation of microparticles with "raspberry-like" morphology. The unique properties of such systems make them materials with important practical applications. In particular, microparticles composed of an oxide core and plasmonic nanoparticles are used, as catalysts, substrates in surface-enhanced Raman spectroscopy (SERS) and carriers of active substances in biology and medicine. In addition, raspberry-like structures can be successfully used, as substrates for obtaining hybrid particles with more complex morphology.

Therefore, the main objective of the undertaken research work was to develop efficient methods for the preparation of monolayers of gold (AuNPs), platinum (PtNPs), and ruthenium (RuNPs) nanoparticles with controlled composition and electrokinetic properties on the surfaces of oxide particles (including silica (SiO_2), aluminum oxide (Al_2O_3), and titanium oxide (TiO_2)). The plasmonic nanoparticles were obtained by chemical reduction method using sodium borohydride. The use of trisodium citrate and cysteamine as stabilizing agents made it possible to obtain nanoparticles characterized by negative and positive surface charges. The titanium and aluminum oxide particles were obtained by the sol-gel method, while the silica particles were obtained by the Ströber method.

The aminopropyltriethoxysilane (APTES) modification was applied to obtain silica particles with a positive surface charge. In the next stage of the work, physicochemical characterization of all obtained colloidal particles was carried out to determine their morphology, size distribution and stability under controlled conditions of pH, ionic strength and temperature. The electrostatically-driven deposition of plasmonic nanoparticles on the surfaces of oxide supports was carried out under diffusive transport conditions. Using the electrophoretic light scattering (ELS) technique, the changes in the zeta potential of the oxide particles as a function of the concentration of plasmonic nanoparticles were determined. The morphology and structure of obtained structures were examined by electron microscopy Imaging.

The application potential of PtNPs/SiO_2 systems was investigated in the hydrogenation reactions of cinnamaldehyde and acetophenone. The results obtained proved that the PtNPs/SiO_2 catalysts formed from the positively charged PtNPs stabilized with cysteamine are inactive in these hydrogenation reactions. However, satisfactory results were obtained for the RuNPs/SiO_2 systems. In addition, the effectiveness of PtNPs/SiO_2 and AuNPs/SiO_2 systems was checked as substrates for the detection of phenylalanine (a model substance), erlotinib and chlorpromazine by surface-enhanced Raman spectroscopy (SERS). On the base of the results, it was proved that the systems, in which the surface concentration of monolayers of negatively charged plasmonic nanoparticles deposited on the SiO_2 surface is in the range of 20-23%, allow recording the spectra of the analytes in the concentration range of 10-6 - 10-5 mol/dm3. It was also observed that the structures of PtNPs/SiO_2 and AuNPs/SiO_2 obtained using the plasmonic nanoparticles stabilized with cysteamine less effectively amplified the signal from the cationic analytes than nanoparticles stabilized with the citrate anions. On this basis, it was concluded that electrostatic interactions between analytes and plasmonic nanoparticles play an important role in the mechanisms of substance detection by SERS.

Physicochemical and mechanical properties of "bio" systems
Physicochemical and mechanical properties of biosystems

(Aneta Michna Dsc, Maria Morga PhD, Agata Pomorska PhD, Agnieszka Kurek MSc, Associate professor Jakub Barbasz, Piotr Batys DSc, Leszek Krzemień PhD)

The aim of the task was to study systems called "bio" in terms of their physicochemical properties (such as charge, shape and size distribution of molecules and particles as a function of environmental parameters) and mechanical (such as Young's modules, spatial packing structure).

This task was a continuation of the statutory task from 2021 carried out by members of the "nano and microscale systems" group.

Continuing the statutory research from 2021, we worked on computer modeling of indentation performed using an atomic force microscope, in particular using a "colloidal probe". We used the finite element method for this purpose. In this case, we extended our cell model developed last year based on filling its interior with a viscoelastic fluid, which is a better approximation than commonly used models that do not take into account energy dissipation as a result of friction forces. We also used the so-called cortex as an element limiting the cell from the outside.

The proposed model was as easy to use as the Hertz function to extract cell properties from the measurement, with additional consideration of the cell's internal structure.

In the field of modeling, the paper "The role of the cortex in indentation experiments of animal cells" was published in Biomechanics and Modeling in Mechanobiology1

We also worked on the behavior of macro ions depending on the parameters of the environment in which they are found.

We also worked on methods of analyzing data from atomic force microscopy, in particular on the analysis of the number of DNA strand breaks2,32,3

[1] Krzemien, L., Giergiel, M., Kurek, A., & Barbasz, J. (2022). The role of the cortex in indentation experiments of animal cells. Biomechanics and Modeling in Mechanobiology, 1-11.

[2] Seweryn, S., Skirlińska-Nosek, K., Wilkosz, N., Sofińska, K., Perez-Guaita, D., Oćwieja, M., Barbasz J. Szymoński M. Lipiec, E. (2022). Plasmonic hot spots reveal local conformational transitions induced by DNA double-strand breaks. Scientific Reports, 12(1), 1-11.

[3] Sofińska, K., Cieśla, M., Barbasz, J., Wilkosz, N., Lipiec, E., Szymoński, M., & Białas, P. (2022). Double-strand breaks quantification by statistical length analysis of DNA fragments imaged with AFM. Measurement, 111362.

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

(Professor Nika Spiridis, Professor Józef Korecki, Jacek Gurgul DSc, Kinga Freindl PhD Eng., Ewa Madej PhD Eng., Ewa Młyńczak PhD, Piotr Mazalski PhD, Robert Socha PhD, Dorota Wilgocka-Ślęzak PhD Eng., Adam Dziwoki MSc Eng., Bohdana Blyzniuk MSc, Natalia Kwiatek MSc)

Important research activity of the group were investigations of the spintronic systems based on antiferromagnetic (AFM) iron oxides, in cooperation with two faculties of the AGH University of Science and Technology. Correlations of the chemical structure and spin Hall magnetoresistance (SMR) were studied in epitaxial α-Fe_2O_3(hematite)(0001)/Pt(111) bilayers with hematite thicknesses of 6 and 15 nm. Unlike previous studies that involved Pt overlayers on hematite, the present hematite films were grown on a stable Pt buffer layer and displayed structural changes as a function of thickness. These structural differences (the presence of a ferrimagnetic phase in the thinner film) significantly affected the magnetotransport properties of the bilayers. We observed a sign change of the SMR from positive to negative when the thickness of the hematite increased from 6 to 15 nm. For α-Fe_2O_3(15 nm)/Pt, we demonstrated room-temperature switching of the Néel order with rectangular, nondecaying switching characteristics.

Another studied system with an AFM iron oxide were CoO/FeO bilayer. We investigated the influence of the proximity of an AFM CoO layer on magnetic properties of ultrathin wüstite (FeO) films. Comparative Mössbauer spectroscopy measurements for MgO/FeO/MgO(001) and MgO/FeO/CoO/MgO(001) showed that the neighboring CoO layer can significantly enhance the ordering Neel temperature of wüstite. Importantly, we find that the proximity of AFM CoO strongly influences the exchange interaction at the Fe/FeO interface in the Fe/FeO/CoO heterostructure. We observed 500% enhancement in the exchange bias field and a double increase in the blocking temperature compared to the Fe/FeO bilayer. Our results show that the limitation of the low ordering temperature of a seemingly application-useless antiferromagnet can be overcome by antiferromagnetic proximity.

AFM-based spintronic systems were also the subject of cooperation in the framework of the SPINLAB network. The magnetic properties of NiO/Co/Pt as a function of Co layer thickness were investigated by polar magneto-optical Kerr effect (PMOKE) (magnetometry and microscopy) and Brillouin Light Scattering (BLS) spectroscopy, and the stoichiometry of NiO was analyzed using XPS. These studies showed that the strength of interfacial Dzyaloshinskii–Moriya interaction increases with the amount of stoichiometric NiO.

Fe(001) epitaxial films are used in our group as a model ferromagnetic system in which quantum well states formed by d electrons are responsible for many fundamental phenomena that oscillate with layer thickness, such as magnetic anisotropy or magnetoresistance. In this subject, the group has continued cooperation with Peter Grünberg Institut PGI, Forschungszentrum Jülich. The photoemission data from Fe(001) thin film grown on Au(001) were compare to the photoemission simulations based on different ab initio band structures. In the second step, calculation of the intrinsic anomalous Hall conductivity (AHC) in bcc Fe were performed for different determination methods of the electronic band structure and Fermi level position. In the framework of the same cooperation, the electronic structure of the ferromagnetic semiconductor EuO was studied by means of spin- and angle-resolved photoemission spectroscopy and density functional theory.

As usual, our laboratory performed dozens of XPS analysis aimed at determination of the chemical and oxidation state of catalytic materials. The XPS analysis were recently complemented by the X-ray absorption spectroscopy (XAS) analysis at the National Synchrotron Radiation Center Solaris, where the group is being involved in operation of two beamlines: DEMETER and PIRX. The activities at DEMETER was mainly devoted to commissioning of the PEEM microscope within the new beamline, after it was transferred from PIRX to the undulator beamline DEMETER.

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

(Professor Tomasz Pańczyk, Professor Wojciech Płaziński, Agnieszka Brzyska PhD, Paweł Wolski PhD)

Studies on the interaction of three monosaccharides: β-d-glucuronic acid (GlcA), N-acetyl-β-d-glucosamine (GlcNAc) and N-acetyl-β-D-galactosamine (GalNAc) with carbon nanotubes (fCNT) functionalized with carboxyl groups were carried out. In addition, two naturally occurring glycosaminoglycans, i.e. hyaluronic acid (HA) and chondroitin (Ch), were investigated as potential factors modifying the surface of carbon nanotubes.

Adsorption of hydrophilic molecules on the surface of carbon nanotubes is one of the most widely used methods to improve the hydrophilicity and biodistribution of inherently highly hydrophobic carbon nanomaterials. Despite structural defects and the introduction of various functional groups into the structure of nanotubes, intentionally or unintentionally, these surfaces still require modification. Adsorption studies of GlcA, GlcNAc, GalNAc, HA and Ch molecules have shown that these molecules have the ability to bind to the surface of fCNTs, and show very significant differences in the geometry and energetics of this process depending on the type of molecule as well as the degree of protonation of surface carboxyl groups. Studies based on classical molecular dynamics simulations using the GLYCAM/AMBER force field showed that in the group of monomers, GlcNAc and GalNAc molecules bind most strongly to the fCNT surface and they always adsorb on the same side in relation to the fCNT surface. The preferred side of the GlcNAc and GalNAc molecules depends on the protonation state of the surface carboxyl functional groups. It has been shown that changes in the pH of the environment leading to the protonation or deprotonation of carboxyl groups will simultaneously cause a change in the orientation of monosaccharide molecules on the fCNT surface. This phenomenon may have important implications in the design of molecular nanodevices.

GlcA molecules bind to the surface of fCNTs much weaker, although they also show a preferential orientation to the surface (opposite to GlcNAc and GalNAc molecules). Their orientation does not depend on the state of protonation of surface carboxyl groups. On the other hand, HA and chondroitin molecules adsorb to form helical structures and in these cases significant deformations of glycosidic bonds were found. A series of conformationally locked rearrangements were observed, such as deformation of several glycosidic bonds in the glycan backbone or distortion of the GlcA rings. All of these changes work towards tighter glycan-fCNT contacts and are a consequence of the strong, attractive interactions between the two molecules. The architecture of the glycan-fCNT contact is consistent with that previously observed for interactions of HA and its building blocks with non-functionalized CNTs; they confirm the involvement of CH-π interactions as the main driving force of glycan-CNT adsorption.

The binding energy of HA and Ch decamers with the fCNT surface depends on the number of oligosaccharide molecules. An increase in their concentration on the surface significantly weakens the bond with the surface. This is particularly evident in the adsorption of four chondroitin molecules on the surface with deprotonated carboxyl groups. In these cases, the Ch oligomers are partially detached from the surface and directed to the bulk. This proves the limited possibilities of using glycan oligomers to create compact functional layers on the surface of carboxylated carbon nanotubes. However, the use of GlcNAc and GalNAc monosaccharides for this purpose seems to be possible.

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

(Associate Professor Paweł Weroński)

The surface roughness of a particle monolayer is one of its fundamental characteristics, conventionally described in terms of power spectral density (PSD). We derived a general equation for this function, valid also for systems of a finite size. We demonstrated similarity of the equation to those applied in the theory of scattering. Specifically, the PSD is a linear function of the squared form factor and static structure factor. The form factor is a Fourier transform of the particle height profile. The structure factor is equal to the product of particle number density and Fourier transformed pair-correlation function calculated in the finite area, increased by one. The relationship suggests that roughness measurements of particle monolayers can be considered as a type of spectroscopy, in a wider sense of the term.

We also demonstrated that our results can be generalized to virtually any monolayer image. Therefore, our research provides a framework for a quantitative, cheap, and easy method, based on statistical analysis of the digital monolayer image, for determining the surface roughness and particle pair-correlation function. The approach can be an attractive alternative to standard, expensive, experimental methods such as GISANS or GISAXS. The PSD provides also quantitative information on key parameters of the system: the particle radius, number density, and dimensions of analyzed area. Therefore, the theoretical model provides also a general framework for an automatic, potentially real-time parametrization of particle monolayers. Unlike in the methods currently used in image analysis, relying usually on individual particle identification, we do not have to localize each of the particles here. Instead, we just need to calculate the discrete Fourier transform of the monolayer image and to least-squares fit the monolayer parameters.

We showed that our analytical results are consistent with numerical ones derived for a system with parameters corresponding to typical experimental conditions. We also demonstrated a practical application of our approach for spectral analysis of a monolayer image found in the literature.

Functional multilayer polyelectrolyte films

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

Continuing last year's task, we have focused on various aspects of the characterization of nanocomposite materials, including the optimization of the methodology for the production of multilayer films and nanocapsule coatings with controlled physicochemical properties and specific functionality, as well as studies of interactions between selected polyelectrolyte films, obtained under different pH conditions and in the presence of various counterions, and the supported lipid bilayer deposited on the surface of such films. The studied systems were obtained by sequential adsorption from aqueous solutions, and the process of deposition of polyelectrolyte multilayers and lipid bilayers was monitored in situ using a quartz crystal microbalance (QCM-D).

The diversity of physicochemical parameters during the formation of such polymer-lipid systems determines their structure and functionality, which allows to optimize the occurrence of the desired features, such as appropriate (anti)adhesive or biocidal parameters and selective passage of active substances when films are used as membrane elements. In addition, an important aspect in the design of biomembranes is the ability to control the deposition of lipid bilayers on nanostructured carriers.

The formation of supported lipid bilayers by adsorption and rupture of liposomes has been studied with respect to their deposition conditions and films made of different polyelectrolytes used as a support to isolate the bilayer from the solid surface. Liposomes were prepared from zwitterionic 1-palmitin-2-oleyl-sn-glycero-3-phosphocholine (POPC) and negatively charged 1-palmitin-2-oleyl-sn-glycero-3-phosphoethanolamine (POPE) in phosphate buffer at different pH values and adjustable ionic strength. Multilayer polyelectrolyte films (PEM) were obtained by sequential adsorption of oppositely charged polyelectrolytes from their solutions - layer-by-layer deposition (LBL). The mechanism of lipid bilayer formation on the PEM support was studied using a quartz crystal microbalance with dissipation monitoring (QCM-D) and atomic force microscopy (AFM). QCM-D made it possible to track adsorption kinetics, and due to AFM measurements, the morphology of lipid vesicles and embedded lipid bilayers on PEM films was verified. In addition, the polyelectrolyte multilayers were characterized by ellipsometry to determine their thickness and swelling. It was confirmed that the kinetics of the formation and the quality of the formed deposited lipid bilayers were influenced by both the pH and the ionic strength of the liposome suspension and the type of polyelectrolyte used as a support for the deposited lipid bilayer.

The optimum conditions were determined for the rapture of liposomes deposited on the surface of the polyelectrolyte film, their fusion and the formation of a supported lipid bilayer. These were: pH = 9.5 and ionic strength of 0.15 M NaCl in phosphate buffer. It was confirmed that the type of polyelectrolyte support played a key role in the formation and the quality of the final lipid bilayer. Homogeneous lipid bilayers were formed on the surface of the $PEI(PGA/PLL)_3$ films deposited under alkaline conditions, i.e. pH = 10.

Theranostic nanocarriers of anticancer drugs

(Associate Professor Krzysztof Szczepanowicz, Tomasz Kruk PhD, Joanna Odrobińska-Baliś PhD, Anna Pajor-Świerzy PhD)

Despite significant progress in cancer research, many people still die due to the lack of effective methods for diagnosing and treating cancer. Late diagnosis and multi-drug resistance of neoplastic cells are severe clinical problems. The main problem is the low selectivity of chemotherapeutic agents, which mainly results from systemic administration as well as poor solubility of chemotherapeutics in an aqueous medium (there are primarily hydrophobic substances). Therefore, it seems reasonable to use carriers of anti-cancer drugs to improve the biodistribution of therapeutic substances in the system and thus increase their therapeutic index. Nanotechnology creates new perspectives, as it enables the transport of drugs to the target, and nano-drug preparations may have unique pharmacokinetic but also pharmacodynamic properties.

In 2022, we were focused on the development of the method of preparation of theranostic nanocarriers for targeted anticancer therapy. The theranostic nanocarriers of anticancer drugs were formed by the sequential adsorption of charged nanoobjects method (called layer-by-layer). The following anticancer drugs: salinomycin and docetaxel, were selected for encapsulation into multilayered core@shell nanocarriers formed with poly-L-lysine (PLL), gadolinium labeled poly-L-lysine (PLL-Gd), poly-L-glutamic acid (PGA) and pegylated-PGA (PGA-g-PEG). The size of prepared nanocarriers ranged between 80-200 nm. The properties of developed nanocarriers were optimized for passive targeting based on the EPR effect.

At the next stages of the research, the effects of encapsulated anticancer drugs, as well as empty nanocarriers, on the viability of the selected cell lines will be examined. Moreover, the ability to monitor them by MRI will be investigated.

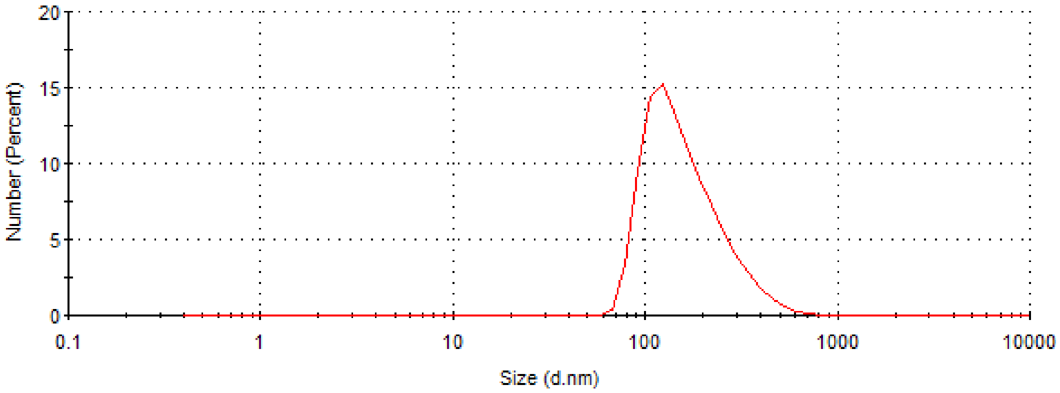


Figure 1. The size distribution of Gd-labeled polyelectrolyte nanocarriers.

Kinetics of drainage of liquid films under dynamic conditions – experiment and numerical simulations

(Associate Professor Jan Zawała, Dominik Kosior PhD, Georgi Gochev DSc, Andrzej Baliś PhD Eng., Agata Wiertel-Pochopień MSc Eng., Dorota Gaweł MSc, Mariusz Borkowski MSc)

The aim of this research task was to investigate the stability of single liquid films formed at various interface under dynamic conditions, i.e. during single bubble collision with a liquid/fluid and liquid/solid interfaces. Outcome of this phenomenon is determined by dynamics of motion of a fluid object in the continuous (liquid) phase prior to its collision and liquid film formation, which depends mostly on state of an adsorption layer at the moving interface. Such adsorption coverage, due to hydrodynamic reasons, is called dynamic adsorption layer (DAL).

In the reporting period the influence of motion induced DAL structure on stability of a single foam film formed by a bubble colliding at solution surface (liquid/gas interface), was investigated. Thank to freshly developed experimental set-up involving Dynamic Fluid-Film Interferometry (DFI) technique, analysis of stability of a single foam film could be performed (for the first time) not only on the basis of qualitative data (lifetimes of a bubble at solution surface) but also quantitative results (evolution of a film thickness in time). Moreover, the DFI technique allow for imagining the single foam film topography at every subsequent stage of its drainage to so-called critical thickness of rupture (see Fig. 1AB). During experiments the stability of a single foam films at the surface of n-octanol solutions of various concentrations was quantified. The solution surface was located at two different distanced from the capillary orifice (point of a single bubble formation), i.e. L = 3 and 40 cm. Every subsequent stages prior the bubble rupture (coalescence) at the solution surface was analyzed, namely: (i) bubble free rise due to buoyancy, (ii) collisions and bouncing during foam film formation and (iii) foam film drainage. It was found that, in certain concentration range, lifetimes of a single bubble were significantly longer for L = 3 cm, comparing to L = 40 cm, what suggested faster drainage rate for longer capillary-solution surface distance. The results indicated that, as a consequence of full architecture of the DAL developed for L = 40 cm, adsorption coverage (θ) at the bubble top pole was significantly depleted, what caused much faster liquid film drainage. For L = 3 cm, the distance covered by a bubble was too short for the DAL formation (θ was practically uniform at the liquid/gas interface). The quantitative data on variations of a single foam film thickness in time (obtained in DFI experiments) were used for verification of the above hypothesis. It was found that during the initial stage of a foam film drainage its rate was significantly higher for L = 40 cm in comparison to L = 3 cm (Fig. 1C). It was a direct proof of differences in mobility of a liquid/gas interface and confirmation of correctness of the research hypothesis. This effect, however, was observed only in a narrow concentration range of n-octanol. Presumably, it could be a consequence of too low CCD camera frequency used in the DFI experiments (only 20 fps). Such low frequency did not allowed for obtaining the data up to 300 ms, where, most probably, differences in a foam film drainage were the highest. In further studies faster (color) camera (with frequency at least 200 fps) should be used.

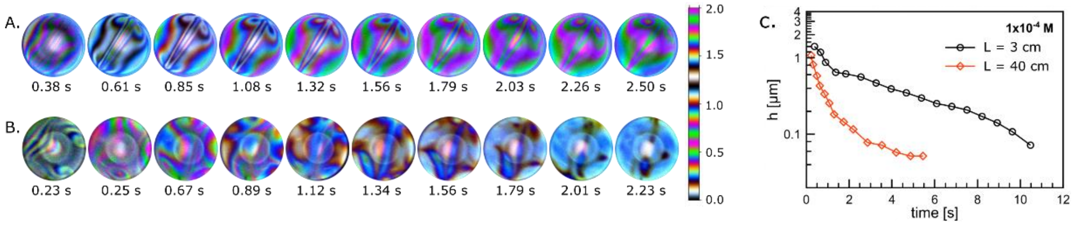


Fig. 1. Sequences of photos showing initial stage of drainage of liquid film formed by a single bubble colliding with air/n-octanol solution interface (concentration 1×10^{-4} mol/dm^3) for (A) L = 3 cm, (B) L = 40 cm. The figure on the right (C) shows quantitative data on liquid film thickness measured from analysis of evolution of colors of the interference patterns.

Design, synthesis and characterization of hydrogel layers based on bio-colloidal solid particles for medical and food applications

(Marcel Krzan PhD)

Hydrogels, based on natural polymers, such as hyaluronic acid, are gaining increasing popularity because of their biological activity. The antibacterial effect of ozone is widely known and used, but the instability the gas causes severely limit its application. During the work, we proved that it is possible to trap ozone in capsules with walls made of olive oil, which has been preserved by means of an unsaturated bond reaction. This allowed the creation of stable nanocolloid solid particles containing trapped ozone. It allowed the production of stable, therapeutically active ozone derivatives.

In this study, we obtained an innovative hydrogel, based on hyaluronic acid containing micro/nanocapsules of ozonated olive oil. By a combination of the biocompatible polymer with a high regenerative capacity and biologically active ingredients, we obtained a hydrogel with regenerative properties and a very weak inhibitory effect against both bacterial commensal skin microbiota and pathogenic Candida-like yeasts. We assessed the stability and rheological properties of the gel, and determined the morphology of the composite, using scanning electron microscopy (SEM) and particle size by the dynamic light scattering (DLS) method. We also performed Attenuated total reflectance Fourier transform infrared (FTIR-ATR) spectroscopy. The functional properties, including the antimicrobial potential, were assessed by the microbiological analysis and in vitro testing on the HaCat human keratinocyte cell line. The studies proved that the obtained emulsions were rheologically stable, exhibited an antimicrobial effect and did not show cytotoxicity in the HaCat keratinocyte model.

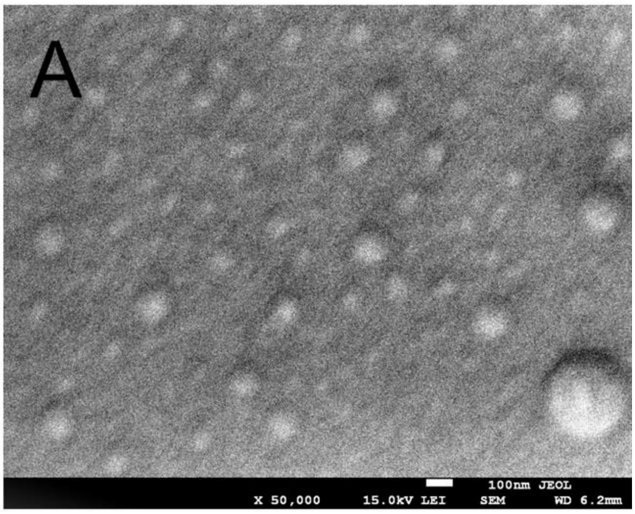
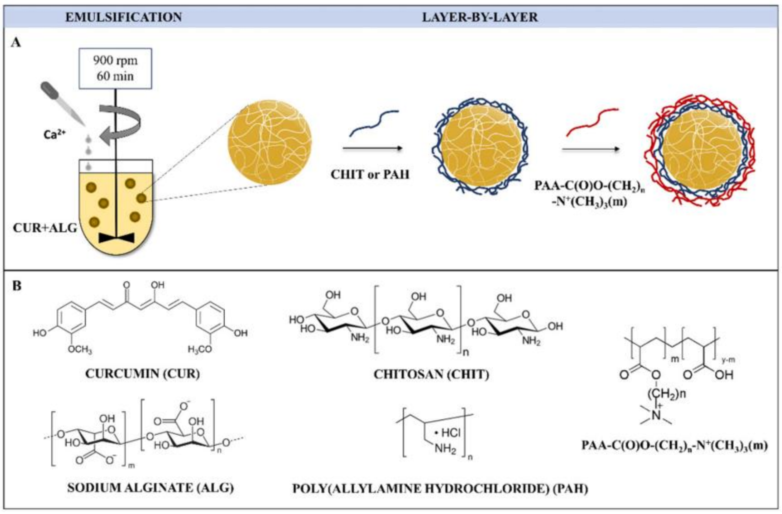


Figure. 1 Scanning electron microscope image of a hydrogel containing ozone nanocapsules (Hyal/O$_3$ at × magnification 50,000)

Hydrogel microcapsules with nano-structured coatings

(Professor Piotr Warszyński, Lilianna Szyk-Warszyńska PhD, Ewelina Jarek PhD, Tomasz Kruk PhD, Marzena Noworyta MSc)

The design of multifunctional microcarriers has attracted significant attention because they combine various functions within a single system. In 2022. we developed hydrogel microcarriers encapsulated with substances of natural origin, including resveratrol (RES), curcumin (CUR), and epigallocatechin gallate (EGCG), which have chemotherapeutic and chemopreventive properties. Alginate (ALG) based microparticles were loaded with RES, CUR, and EGCG using the emulsification method and then coated with polyelectrolyte (PE) films, such as chitosan (CHIT) or poly(allylamine hydrochloride) (PAH). The morphology and mean diameter of obtained microcarriers were characterized by scanning electron microscopy (SEM), and encapsulation efficiency was determined by UV-Vis spectroscopy. The composition of particles was confirmed using Fourier transform infrared (FTIR) spectroscopy, and the physicochemical properties of functional PE layers were studied using quartz crystal microbalance with dissipation monitoring (QCM-D). The cytotoxic effect of designed delivery systems was studied by evaluating their impact on the proliferation, mitochondrial metabolic function, and lipid peroxidation level of *5637* human bladder cancer cells. Our work demonstrates that the physicochemical and biological features of fabricated microcarriers can be controlled by the type of encapsulated chemotherapeutic agent and PE coating. The proposed delivery systems of naturally occurring substances could open a new path in the efficient treatment of bladder cancer.



For the curcumin (CUR) containing hydrogel microcapsules coated with a polyelectrolyte shell consisting of chitosan (CHIT) or poly(allylamine hydrochloride) (PAH) we applied an additional outer layer with antimicrobial function. For this purpose, poly(acrylic acid) (PAA) was decorated with different hydrophobic side chains (n-hexane and n-dodecane side entities) and different degrees of substitution (m) of quaternary ammonium groups (abbreviated as PAA-C(O)O-$(CH_2)_n$-$N^+(CH_3)_3$ (m); n = 6, 12; m = 8–14%). The grafting approach of PAA with the alkylene quaternary ammonium salt moiety was performed under mild reaction conditions using Steglich esterification followed by quaternisation. The structure of antimicrobial decorated PAA was confirmed by 1H NMR and FTIR, and the mean diameter of all multifunctional microparticles was characterised by SEM. The release of CUR from the microcarriers was described using a hybrid model, i.e., a combination of first-order kinetics and the Korsmeyer-Peppas model. The antimicrobial activity of functionalised PAA and multilayered CUR-loaded hydrogel microcarriers with quaternary ammonium function was assessed against *Staphylococcus aureus* and *Serratia marcescens* by the agar diffusion assay method. Only a limited inhibition zone of PAA was observed, but in the case of both antimicrobial decorated PAA and the corresponding multilayered nanocarriers, the inhibitory activity increase was achieved against both strains of bacteria.

Influence of the presence of crystal lattice defects on the corrosion resistance of metals with hexagonal close-packed lattice

(Konrad Skowron PhD Eng., Michał Mosiałek DSc, Grzegorz Mordarski PhD Eng.)

Grit blasting is the standard method used to modify the roughness of titanium surfaces for dental implants and other applications where increased coating adhesion and bond strength are important. Positron annihilation lifetime spectroscopy (PALS) showed that grit blasting induces a high density of non-uniformly distributed dislocations and clusters of 2-3 vacancies in the surface layer with a depth of about 240-270 μm. The average dislocation density decreases with the distance from the surface and the total extent of the observed changes depends on the grit blasting parameters. The concentration of vacancy clusters exhibits a maximum at the depth of about 90 μm.

Grit blasting with steel slags combined with H_2SO_4 etching resulted in the deterioration of corrosion resistance in 0.15 M NaCl solution in comparison to the reference specimen. This effect can be related to micro pits formed during etching, as it was revealed by the SEM investigation. The application of X-ray photoelectron spectroscopy indicates that etching leads to a decrease in TiO_2 fraction and an increase in the amount of Ti (II and III) oxides on the surface. This also may be responsible for the worsening of the corrosion resistance of Ti studied in 0.15 M NaCl. Regardless of the observed decrease in corrosion resistance in this medium, it is still at an acceptable level for many applications.

The electrochemical impedance spectroscopy results show that polished Ti has similar polarization resistance values to this of the reference sample. Removal of the top layer (about 50 μm thick) most likely exposed a structure characterized by lower lattice strain values than those directly beneath the grit-blasted surfaces. Previous research on Ti subjected to surface mechanical attrition treatment (SMAT) showed an improvement in corrosion resistance of grade 2 Ti in 0.15 M NaCl despite the fact that PALS measurements indicated even higher dislocation density than in the present case. However, unlike in the present case, the higher value of the shorter lifetime component suggested that for the SMATed samples, there was much less annihilation of positrons in undisturbed crystal lattice regions which points out not only a higher density of dislocations but also their more homogeneous distribution.

Annealing of point defects at 150 °C slightly improved the corrosion resistance of the titanium studied. It resulted in a slight improvement of the corrosion resistance, reflected in higher values of polarization resistance values, as well as most noble corrosion potentials in comparison to the remaining samples. The obtained results show vacancy cluster annealing may not be sufficient for grain boundary equilibration to such an extent that it has a clear effect on corrosion resistance.

Physicochemical properties of functional nanocarriers based on dendrimers and proteins

(Professor Barbara Jachimska, Magdalena Szota PhD, Kamil Rakowski PhD)

Due to the specificity of their structure, protein systems are adapted to carry various types of ligands. The structure of many proteins potentially allows for two types of immobilization of the therapeutic agent, either on the outside of the protein or within the structure. In the structure of the BSA molecule, two main active centers are defined, the so-called Sudlow I and II. It is known from the literature that it is potentially possible to successfully incorporate a ligand into both or one site, depending on the type of ligand. The protein can simultaneously play a dual role in the delivery or release of the active substance. The research concerned the effectiveness of creating a BSA-based carrier for the 5-fluorouracil (5-FU) molecule. 5-Fluorouracil (5FU) is a broad-spectrum anticancer drug against solid tumors. The research was carried out in many ways, using complementary measurement techniques to determine the physicochemical properties of the system. The critical conditions for complex formation were determined, which enabled both optimization of the system and significant correlations between the form of the drug (5FU, AN1, and AN3) and the effective localization of the active substance in the structure of the protein molecule. The presence of two amino groups in the 5FU structure contributes to the deprotonation of the molecule at high pH values ($pH > 8$). This was confirmed by UV-Vis and 1H NMR spectroscopic studies in a wide range of pH. The drug locations in the BSA structure for both deprotonated forms were simulated using MD. The location of the active centers of the protein was determined depending on the forms in which the drug molecule occurs.

Physics and Chemistry in Cultural Heritage Protection

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

(Łukasz Berger Eng., Magdalena Soboń MSc Eng., Marcin Strojecki PhD, Arkadiusz Janas PhD, Professor Łukasz Bratasz)

As part of the research task, work was carried out on further development of software for the quantitative assessment of risks of heritage objects - HERIe. The purpose of the development of a digital preventive conservation platform supporting the decision-making process in the field of preservation of museum collections is to include other types of degradation processes than those already considered (fires, mechanical damage caused by fluctuations in relative humidity) leading to the loss of collection value. In 2022, research was undertaken to implement modules to predict colour changes in photosensitive materials exposed to light. The research was conducted in cooperation with the Canadian Conservation Institute and consisted of the synthesis of available reflectance spectra obtained for 108 samples of historically produced synthetic dyes, which were exposed to several dozen different light doses produced by a LED type source. A data visualization method has been developed so that the user can estimate the colour change of the test sample depending on the amount of colour remaining (depending on the light intensity, number of hours, number of days and years of past exposure, and the number of photos taken with the flash) and the predicted colour change for future exposure. In order to facilitate the user's assessment of threats to collections with various dyes, a module of free selection of several dyes was developed to predict and visualize the colour change of a representative for a collection set of dyes. In addition, archival information on the tested dyes was collected and a selected sample was linked to a page with information about the sample in the catalogue of dyes from 1901.

The result of the activities carried out by the Research on Cultural Heritage group was the extension of the HERIe digital platform for preventive conservation. The HERIe platform actively supports the international community of museologists and conservators in assessing the safety of the conditions of exhibition and storage of collections, enabling remote access to the quantitative assessment of risks to heritage resources. The platform contains modules corresponding to environmental risks to heritage objects like air pollution, light, inappropriate temperature or relative humidity. The developed software, the first of its kind in the world, is available free of charge on the internet under the name HERIe and the address herie.pl for all those involved in the protection of collections. The platform is widely used by this environment of people associated with the protection of collections. From 2020, the development of the HERIe platform is also carried out as part of the European Union project IPERION HS "Integration of European Research Infrastructure platforms in heritage science" (2020-2023), supported by the Horizon 2020 program "Integration and opening of existing national and regional research infrastructures with a European scope”.

Development grant

Cu-containing FAU-type zeolite as a modifier of ANFO-based explosives

ICSC PAS development project no. 2/GR/2020 [2021-2023]
(Łukasz Kuterasiński PhD Eng.)

The topic concerning the manufacture, modification and utilization of explosives still is particularly important due to their peaceful applications. One of the most commonly used explosives in the civil industry (particularly in mining) is ANFO (Ammonium Nitrate Fuel Oil) due to its easy and cheap procedure of production as well as its good blasting properties.

The blasting properties of prepared explosives can undergo modification by the change of their chemical composition. It can be also realized by the addition of certain modifiers, which are able to catalyze the decomposition of explosives, reduce the emission of post-blast fumes, and influence explosive sensitivity. This action allows preparing explosives with desired blasting properties, e.g. the velocity of detonation (VOD), explosion heat, and strength or toxicity of pot-blast fumes.

In the undertaken research, we studied Cu-FAU as ANFO modifiers in terms of controlling the chemical composition of post-blast fumes due to the commonly known application of Cu-zeolites as $DeNO_x$ catalysts. The choice of zeolite of FAU-type structure implies its wide application in the chemical industry due to its unique chemical and porous structures.

The FAU-type zeolite was added to the ANFO via a simple mixing procedure. Weight loading of the zeolite in the resulting ANFO-based material reached 5% wt. Prior to blending ANFO with zeolite, the parent zeolite in a protonic form (HFAU) was modified with copper (also up to 5% wt.) using a wet impregnation technique.

The prepared ANFO samples underwent physicochemical characterization concerning crystallinity, structure (X-ray diffraction, Fourier Transform Infrared Spectroscopy), the state of the surface, morphology (Atomic Force Microscopy, Scanning Electron Microscopy), thermal properties (Thermal Gravimetry and Differential Scanning Calorimetry), blasting properties (involving velocity of detonation, density, the pressure and temperature of detonation, the heat and strength of the explosion, as well as the analysis of post-blast fumes).

Analysis of either X-ray diffraction patterns and FTIR spectra indicated the presence of crystalline ammonium nitrate. The introduction of Cu-FAU to ammonium nitrate resulted in the appearance of new signals assigned to the zeolite phase. The intensity of either XRD reflexes or IR bands attributed to zeolite increased with the rising zeolite content in ANFO.

The modification of ANFO with Cu-zeolite caused significant changes in the surface of the obtained ANFO. Atomic Force Microscopy analysis indicated the appearance of numerous grains of irregular shapes assigned to the zeolite phase with simultaneous reduction of the surface folding of the prepared ANFO sample. The use of Cu-FAU decreased the thermal effect accompanying the AN decomposition from 5.82 mW/mg (for the pure ANFO) to 2.82 mW/mg.

Based on thermodynamic calculations of blasting properties, it was concluded that the application of Cu-FAU as a modifier of ANFO revealed the enhancement of the detonation properties of ANFO-based explosives, which corresponded with their changes in the status of the surface and thermal properties. It was found that in all cases, the addition of Cu-FAU to ANFO caused the growth of detonation pressure, temperature, and the heat of the explosion up to 15%, 33%, and 50%, respectively. A similar tendency was found for the VOD, which grew up to 5%. Simultaneously, the total volume of post-blast fumes (including CO_x, NO_x, and water vapor) underwent a reduction of 6%.

"Sonata Bis" Research Projects of the National Science Centre

Synergistic effects of mixed biosurfactants solutions in stability of liquid films under dynamic conditions - fundamental research with practical importance in flotation separation process

"Sonata Bis" research project NCN 2020/38/E/ST8/00173 [2021-2025]
(Project leader: Associate professor Jan Zawała)

The project is related to fundamental research on determination of mechanism of synergistic effects in solutions of chosen mixed flotation reagents. The experiments planned to be carried out are aimed to describe the reasons and conditions of synergism in relations to formation and stability of liquid films formed at various interfaces under dynamic conditions. New amino acid based surfactants and their mixtures with simple non-ionic surface-active substances were chosen for this purpose.

In the reporting period the investigations were aimed to explain spectacular effect of enhancement of foamability of solutions of amino acid surfactants (AASs with twelve carbon atoms in hydrocarbon chain) mixed with simple non-ionic fatty acid (n-octanol – C8). Three AASs based on alanine (C12-ALA), leucine (C12-LEU) and phenylalanine (C12-PHE) were chosen for experiments. It was found that in particular concentration range of a main mixture ingredient (AASs), the height of a formed foam can be even two times higher comparing to the height of the foam formed in pure mixture components alone. Such synergistic effect was analyzed on the basis of equilibrium surface tension values (σ) of the mixed solutions and pure mixture components, both measured experimentally and calculated theoretically as a sum of magnitudes of a surface tension decrease caused by pure mixture constituents. Only slight differences between experimental and theoretical values of σ in those systems were revealed. Moreover, comparison of experimental and theoretical σ values suggested that, in the case of C12-ALA and C12-LEU, the effect observed during foam formation should be synergistic, while for C12-PHE antagonistic. In the latter case this conclusion was in direct contradiction with experimental observations (the synergistic effect were observed for all studied AASs). To get deeper insight into the mechanism of this effect at the molecular level, the Molecular Dynamic Simulations (MDS) were performed. It was revealed that atypical variations of mixed solutions surface tension are a consequence of bulk aggregates formation, which could be formed only in mixed AASs/n-octanol solutions, but not in pure solutions either of AASs or n-octanol alone. Aggregates formation caused depletion of the surface concentration at the liquid/gas interface under equilibrium conditions. As a result of the fact that the foam formation is very dynamic phenomenon associated with quite fast formation of fresh interface, during foamability experiments the bulk aggregates could be gradually dissolved, and the released molecules were adsorbing at the formed bubbles. Therefore, the aggregates could be considered as internal reservoir of stabilizing agents, which stabilizing features are turned on under dynamic conditions. The aggregates formation postulated on the basis of MDS was confirmed during Dynamic Light Scattering (DLS) experiments. It was found that the size of aggregates, formed only in mixed solutions, was ranging between 200 and 300 nm. On the basis of MDS it was also found that atypical (and different from other AASs) variations of σ in C12-PHE solutions is a consequence of much stronger hydrogen bonds formation between AASs surfactants molecules comparing to AASs/n-octanol molecules.

Our investigations allow for verification of meaning of value of so-called Critical Synergistic Concentration (CSC), determined on the basis of foamability experiments. It was revealed that the CSC value can not be determined using general algorithms based on the σ, because in the case of AASs, the σ variations do not correlate with the solution foamability.

"Sonata" Research Projects of the National Science Centre

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

"Sonata" research project NCN 2018/31/D/ST5/01866 [2019-2023]
(Project leader: Piotr Batys DSc Eng.)

Streaming potential and quartz crystal microbalance measurements, combined with all-atom molecular dynamics (MD) simulations, were used to study the pH dependency of the adsorption of poly-L-lysine (PLL) and poly-L-arginine (PARG), on α-quartz surface. It was shown that the observed adsorption behavior rises from an interplay of the change in the number of possible peptide-surface ion pairs between the charged moieties and repulsive electrostatic interactions between the polypeptide molecules. For low pH values, polypeptide adsorption was the strongest and stable monolayers were formed. However, electrostatic repulsion between the polypeptides led to a relatively low maximum surface coverage. On the other hand, higher pH led to more weakly bound, but significantly denser, polypeptide films with limited stability. Simulations indicate that electrostatic interactions are the main driving force for adsorption, while hydrogen bonding and non-specific interactions also contribute. Additionally, the important role of the counterions of the negatively charged quartz surface that form a positively charged ion adlayer was highlighted. Ion release of the condensed sodium ions at the charged surface occurs via displacement by polypeptide adsorption. The revealed mechanisms provide systematic guidelines to engineering active surfaces of charged polypeptides with controlled surface coverage and reversible binding.

Additionally, physicochemical properties of PARG molecules in NaCl solutions were determined by MD modeling and various experimental techniques. Primarily, the molecule conformations, the monomer length and the chain diameter were theoretically calculated. These results were used to interpret experimental data, which comprised the molecule secondary structure, the diffusion coefficient, the hydrodynamic diameter and the electrophoretic mobility determined at various ionic strengths and pH values. Using these data, the electrokinetic charge and the effective ionization degree of PARG molecules were determined. In addition, the dynamic viscosity measurements for dilute PARG solutions enabled to determine the molecule intrinsic viscosity for ionic strength range between 10^{-5} and 0.15 M. This confirmed that PARG molecules assumed extended conformations and approached the slender body limit at the low range of ionic strength. The experimental data were also used to determine the molecule length and the chain diameter, which agreed with theoretical predictions. Exploiting these results, a robust method for determining the molar mass of polypeptide, the hydrodynamic diameter, the radius of gyration and the sedimentation coefficient was proposed.

Improving the conductivity of printed patterns by optimizing the synthesis process and physicochemical properties of metal nanoparticles.

"Sonata" research project NCN 2020/39/D/ST5/01937 [2021-2024]
(Project leader: Anna Pajor-Świerzy PhD)

In recent years, a significant growth in the use of metallic nanoparticles (NPs), due to their unique physicochemical properties, in the production of conductive inks and pastes, has been observed. Such materials can be successfully applied in the electronics and power industry. Crucial importance is the optimization of the properties and the selection of appropriate methods of the production of metallic NPs and inks or pastes on their basis. This will contribute to improving the conductive and mechanical properties as well as lower the cost of production of conductive coatings. So far, silver nanoparticles have been the most commonly used in the process of producing conductive circuits. However, silver is an expensive material, therefore, alternative conductive components of inks or pastes are required. One of the candidates is nickel, which has good conductive as well as mechanical properties and is much cheaper. However, a significant problem is the instability of Ni NPs regarding the oxidation process. Therefore, it was proposed to cover the nickel core with a silver coating ensuring their good resistance against oxidation, which results in the formation of core-shell structures (Ni-Ag NPs).

The work carried out in 2022 focused on research on lower the sintering temperature of coatings based on Ni-Ag NPs, which is of particular importance in the production of electronic circuits on heat-sensitive substrates. In this context, the effect of the thickness of the silver shell (depending on the concentration of silver ions) in the core-shell structure on the physicochemical properties (size, stability regarding the aggregation process) of NPs and the conductivity of the coatings based on it was investigated. It was observed that with the increase of the thickness of the silver layer in the core-shell structure of Ni-Ag nanoparticles their stability slightly decreases, while the conductivity of their coatings increased in all ranges of applied sintering temperatures (130-200°C, Fig. 1). The use of the optimal thickness of silver shell allowed for obtaining coatings with a high conductivity at a low sintering temperature (130°C).

The obtained results will significantly improve the development of methods for the production of circuits and electronic devices, including flexible printed electronics.

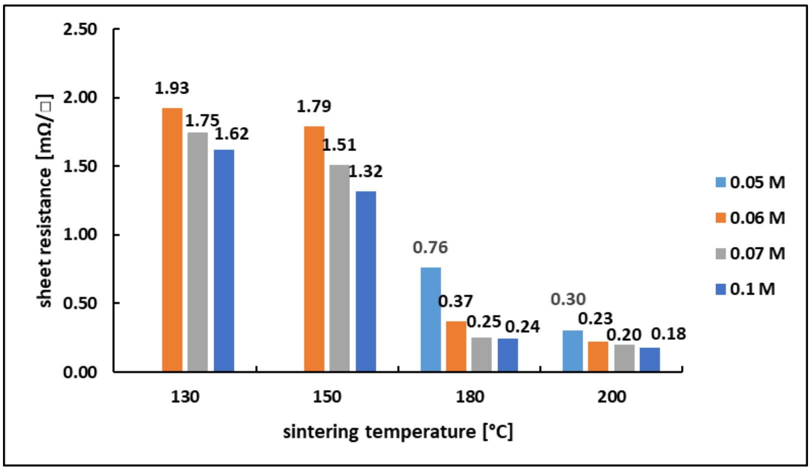


Fig. 1. Dependence of coating conductivity on AgNO$_3$ concentration and sintering temperature (130-200°C) for optimal time (60 min).

"Opus" Research Projects of the National Science Centre

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 [2017-2022]
(project leader: Marcel Krzan PhD)

During the project, we developed technology for generating and applying stable thin films of surface formulated from mixtures of hypoallergenic, non-toxic biological polymers, mainly biosurfactants (proteins, polysaccharides, glycolipids and phospholipids). The scientific goal of the project was to investigate the competitive adsorption processes occurring in multi-component solutions, where the primary substrates are various surface-active bio-polymers. During the project, we investigated which of the available biopolymer formulations have sufficient surface activity to be used in cosmetic products. In addition, we determined the minimum concentrations necessary to carry out wetting or to create the surface effect of surfactant. The developed surface layers were also tested for bacteriostatic and cytotoxicity properties.

In the last year of the project, most of our publications were devoted to using cellulose nanocrystals as foam stabilizers. We demonstrated that carboxylated cellulose nanocrystals enhance foamability and foam stability when mixed with cationic surfactant ethyl lauroyl arginate (LAE), having superior properties over sulfated cellulose nanocrystals (sCNC) concerning surfactant concentration range and foam volume. Mixtures of LAE and cCNC were characterized by their hydrodynamic diameter, zeta potential, surface tension and surface rheological properties. In addition, the influence of electrolytes, namely, sodium chloride, guanidine hydrochloride and sodium salicylate, and the addition of concentrated urea to LAE-cCNC mixtures on foamability and foam stability were investigated. Electrolytes in the concentration of 5 mM showed a moderate effect on foam stability. In contrast, spectacular foam collapse was detected after adding concentrated urea. The preliminary rheological data from the pendant drop oscillations revealed a low elastic modulus upon urea addition and a loss modulus that increased with the frequency, which suggested a viscous interfacial layer.

Influence of counterions on the formation and functionality of polyelectrolyte membranes

"Opus" research project NCN 2016/23/B/ST8/03128 [2017-2022]
(project leader: Marta Kolasińska-Sojka DSc)

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

In the ongoing work, multilayer films were deposited using the technique of sequential adsorption (layer by layer, LbL) of polyions from their solutions. This year's research in the project concerned the completing the comparison of the impact of monovalent cations: Li$^+$, Na$^+$, K$^+$ Rb$^+$, Cs$^+$, NH$_4^+$ on the formation and structure of multilayer polyelectrolyte films, as well as on the permeability of the multilayers to the selected electroactive probes. Using a quartz crystal microbalance, studies of the kinetics and efficiency of the process of deposition of polyelectrolyte films: PAH/PSS and PDADMAC/PSS in the presence of a selected electrolyte with an ionic strength of 0.15M were carried out. The graphs below (Fig. 1) show the dependence of the mass for the PDADMAC/PSS system on the number of deposited layers (A) and the permeability to an equimolar solution of potassium hexacyanoferrates (II) and (III) for 9-layer PDADMAC/PSS films adsorbed in the presence of the selected monovalent countercations.

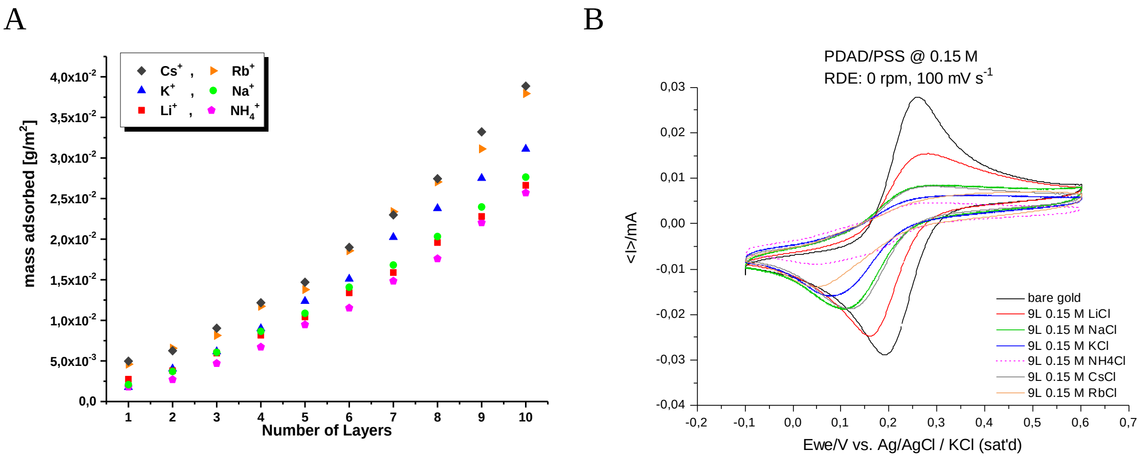


Fig. 1. Dependence of (A) mass on the number of layers in PDADMAC/PSS films deposited in the presence of selected counter-cations; (B) permeability towards an equimolar solution of potassium hexacyanoferrates (II) and (III) for 9-layer PDADMAC/PSS films adsorbed in the presence of the studied countercations.

Our studies showed that both the adsorbed mass and the permeability of the multilayer towards selected electrochemical probes depended on the type of countercation used to form the film.

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

"Opus" research project NCN 2017/27/B/ST5/01834 [2018-2022]
(Project leader: Professor Ewa Serwicka-Bahranowska)

Hydrotalcite-like compounds (Htlc) are analogs of the natural mineral, hydrotalcite. They are a unique class of materials composed of positively charged hydroxide layers and interlayers occupied by anions compensating the layer charge mixed with water molecules. In the center of the layer-forming hydroxide octahedra are divalent and trivalent cations Due to the very large number cations and anions that can fit into the structure of Htlc, these compounds are characterized by a great variety of chemical compositions. Htlc find numerous applications as catalysts, photocatalysts, adsorbents, drugs, drug carriers, plastic fillers, etc. Of particular practical importance are nanostructured forms of Htlc, with particles smaller than 100 nm, characterized by a high surface to interior ratio and a well-developed pore network, properties highly desirable for most applications.

The project developed a new method for obtaining nanostructured Htlc materials with grain size controlled by the presence of a biopolymer template in the reaction mixture. Commonly available, inexpensive and environmentally friendly starch was used as the biopolymer. The structural template could be easily removed after the synthesis by washing the obtained material with water, or by burning. Another innovation was the use of organic bases, including non-toxic, ecosystem-compatible choline hydroxide, in the process of obtaining Htlc. Two types of solids were prepared: magnesium-aluminum Htlc, which proved to be very active catalysts for the synthesis of ε-caprolactone, a monomer for the production of an important biodegradable polymer, and copper-manganese-aluminum material, which, after thermal decomposition to oxides, showed very high efficiency in purifying air from volatile organic pollutants. In the case of catalysts for removing pollutants from air, the use of organic bases instead of sodium hydroxide makes it possible to obtain materials free of sodium impurity, which greatly improves catalytic activity. An important achievement of the project is also the elucidation, through the use of quantum mechanical modeling, of molecular interactions in solutions containing ammonia and ions of magnesium and aluminum.

The results of the project's research have advanced knowledge in the field of catalytic materials engineering with respect to the application of bio templates, the use of organic bases and the understanding of synthesis processes at the molecular level.

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

"Opus" research project NCN 2018/31/B/ST8/03277 [2019-2023]
(Project leader: Aneta Michna DSc)

The main aim of the ongoing project is the formation of stable biofilms based on polysaccharides and the protein binding and release from the biomaterials under conditions controlled either by diffusion or convection.

The polysaccharides (chitosan, modified chitosan, heparin) were thoroughly characterized in bulk. The hydrodynamic diameters, the zeta potentials and the electrokinetic charges of the polysaccharides were determined in various ionic strengths, pH, and temperature. The chitosan solution density and zero shear dynamic viscosity were also measured, which enabled to evaluate of the intrinsic viscosity increment. Also, molecular dynamics modelling was applied to predict chitosan molecule conformations, the contour length, the gyration radius, the effective cross-section and the density in electrolyte solutions. The experimental results were quantitatively interpreted in terms of the slender body hydrodynamics exploiting molecule characteristics derived from the modelling. It was also confirmed that this approach can be successfully used for a proper interpretation of previous literature data obtained under various physicochemical conditions.

In the next step, chitosan, modified chitosan, carrageenan, poly(diallyldimethylammonium chloride) (PDADMAC), and heparin (HEP) were used to form the multilayers from low macroion concentrations equal to 5 mg L^{-1}. Using the in-depth bulk characteristics of these macroions a proper interpretation of the multilayer formation was carried out. Periodic variations in the apparent zeta potential between positive and negative values of the layers were observed. The stabilities of the films against time (reaching 24 h) were determined using the streaming potential measurements (SPM), optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microbalance (QCM-D). The performed experiments confirm the successful formation of the multilayers from low macroion concentration on silica.

The kinetics of adsorption of a model protein (brain-derived growth factor, BDNF) on PDADMAC/HEP biofilm and the BDNF layer stability for defined ionic strength and pH were studied using SPM, OWLS, and atomic force microscopy (AFM). It was found that BDNF molecules effectively adsorb on HEP-terminated multilayers. The films, obtained in this way, were stable for at least 6 h.

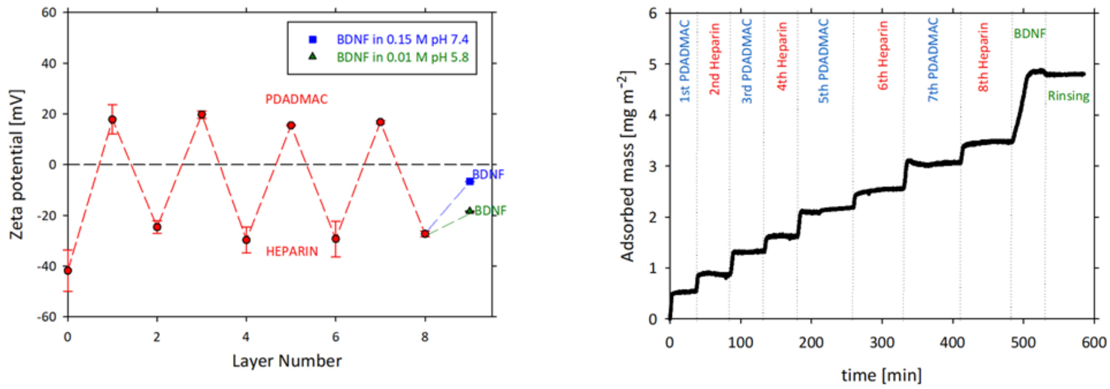

a) b)

Fig. 1 a) The dependence of the apparent zeta potential of silica on the number of macroion layers (SPM), b) Kinetics of adsorption and stability of the macroion layers and kinetics of adsorption/ desorption of the protein on the biofilm terminated by heparin. The bulk mass concentration of the macroions was 5 mg L^{-1}, ionic strength of 0.01 M, pH 5.8. The protein was adsorbed from 1 mg L^{-1}, ionic strength of 0.01 M, pH 5.8 (a, b) and 0.15 M, pH 7.4 (a).

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

"Opus" research project NCN 2018/31/B/ST5/03292 [2019-2024]
(Project leader: Professor Ewa Serwicka-Bahranowska)

The method of TiO_2/clay composite synthesis proposed in the project bases on the use of inverse microemulsion technique for the synthesis of TiO_2 component. Inverse microemulsions, also referred to as reverse microemulsions, are thermodynamically stable and optically transparent dispersions of aqueous microdroplets in a continuous oil phase, stabilized by the presence of surfactant molecules at the water-oil interface. The clay component, montmorillonite (Mt), was prepared in two different ways. In the first case, Mt was used as a cetyltrimethylammonium organoclay (CTA-Mt), to facilitate its exfoliation in the organic reaction medium (1-hexanol). In the other procedure, Na-montmorillonite (Na-Mt) was used. This form of clay mineral is hydrophilic and does not disperse in n-hexanol. To bypass this obstacle we used a completely novel approach, in which Na-Mt component was prepared as inverse microemulsion in 1-hexanol, with clay mineral present as a suspension in aqueous micellar cores. The procedure resulted in the Na-Mt transformation to CTA-exchanged Mt. In consequence, both the composites derived from the parent CTA-Mt and the ones obtained from the Na-Mt component, were compositionally similar. However, preparation of Na-Mt in microemulsion led to better disintegration of clay particles than the exfoliation treatment of CTA-Mt in an organic solvent. This resulted in the TiO_2/Na-Mt series showing better intermixing of clay component with titania nanoparticles (Fig. 1) and ensured formation of composites with better developed pore networks, while retaining similar pore size distribution characteristics. The adopted synthesis method enables formation of essentially mesoporous materials, with significant specific surface area and uniform mesoporosity, determined by the size of titania nanoparticles (4-6 nm). Given that the size of the inverse micelles can be modified by appropriate selection of the synthesis conditions, this method offers the possibility to control the dimensions of TiO_2 nanoparticles used for the preparation of composites.

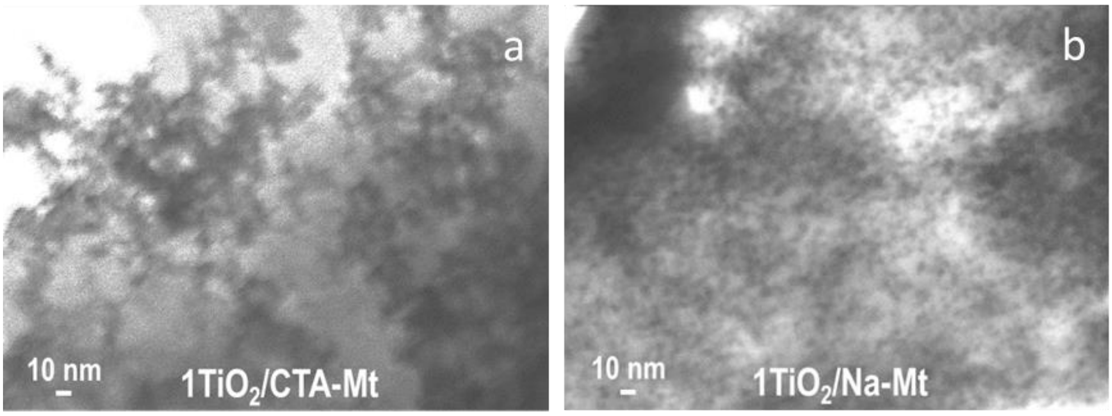


Fig. 1. TEM images of TiO_2/clay composites obtained from: a) CTA-Mt, (b) Na-Mt.

Coarse-grained modeling of carbohydrates

The MARTINI 3 force field parameterization for polymers of glucose and hyaluronan

"Opus" research project NCN 2019/35/B/ST4/01149 [2020-2024]
(Project leader: Associate Professor Wojciech Płaziński)

A set of theoretical models operating at the coarse-grained resolution level was developed and applied for molecular dynamics simulations of mono-, di- and oligo- and polysaccharides composed of glucose monomers and using different types of glycosidic bonds: β(1-4) (cellulose), α(1-4) (amylose), β(1-3) (curdlan), β(1-2) and α(1-6). The developed model is compatible with the MARTINI 3 force field, which makes it possible to use it in systems of mixed composition, containing molecules of other types (carbohydrates + solvents, lipid membranes, proteins, etc.), for which analogous, coarse-rained models are available due to work by other research teams. Parameterization and validation were based on all-atom simulations (within the CHARMM force field in the context of bonded interactions and polymer parameters, such as gyration radius or end-to-end distance) and the experimental data (e.g. log P values and the data referring to the structure of sugar-containing complexes and carbohydrate interactions with lipid membranes). Similar studies have also been initiated in the context of the group of glycosaminoglycans, in particular: hyaluronan.

As part of the second project, a series of calculations based on a hybrid protocol combining classical MD simulations with QM calculations was started to determine the conformational (ring distortion energies) and spectroscopic (spin-spin coupling constants) properties of non-functionalized and 2-*O*-sulfonated iduronate.

External stimuli-assisted Molecular Beam Epitaxy of functional layers and nanostructures

"Opus" research project NCN 2020/39/B/ST5/01838 [2021-2024]
(Project leader: Professor Józef Korecki)

The year 2022 completed 18 months (6 months in 2021, 12 months in 2022) of the project implementation. The all tasks related to the implementation of external stimuli (magnetic field, electric field, strain field) into the ultra high vacuum (UHV) system for the molecular beam epitaxy (MBE) deposition and characterization of epitaxial films were finished.

In the modified UHV system, combination of “flag-style”(FS) and PREVAC-style (PTS) sample holders and a sophisticated two-station 4-axis manipulator allows an easy transfer of the substrate between the station proper of a given preparation step (cleaning, deposition, annealing). The dual sample holder configuration allows not only versatility of the sample environment during film growth and characterization, but also possibility of applying the external fields, in particular magnetic, at different preparation stages. The PTS sample holders are specialized for different functions and can be operated with substrates fixed directly to the holders or as adapters accepting the FS holders carrying substrates. The range of the PTS adapters includes among others the holders transmitting the magnetic field from samarium-cobalt permanent magnets to a sample mounted on the FS holder, both in-plane (up to 100 mT), and out of plane (up to 200 mT). During MF-assisted deposition the sample temperature can be as high as 500^{o}C using resistive heating, without degrading the Sm-Co magnets thanks to a water or liquid nitrogen magnet cooling system. At the preparation stages requiring even higher temperatures, the FS holders can be heated by electron bombardment in the FS holder station or in a specialized PTS holder. After each preparation step the sample structure can be *in situ* controlled using Low Energy Electron Diffraction (LEED), whereas magnetic properties can be measured using magneto-optic Kerr effect (MOKE) and Conversion Electron Mössbauer Spectroscopy (CEMS).

For testing the constructed system, the role of the magnetic field applied during epitaxial growth was investigated for Fe(001) films epitaxially grown on single crystalline MgO(001) substrates. Whereas we were not able to induce expected uniaxial anisotropy by magnetic field assisted MBE, we showed that coercive field (dependent on the type of the magnetization reversal) is very sensitive to external magnetic field during deposition applied along the easy [100] direction. On the other hand, a pilot in-field growth experiment for magnetite films revealed an enhanced perpendicular anisotropy for the in-plane magnetic field.

New generation teranostatic nanocarriers for detection, diagnosis and neuroprotective treatment of ischemic stroke brain damage

"Opus" research project NCN 2020/39/B/NZ7/01913 [2021-2025]
(Project leader: Professor Piotr Warszyński)

Ischemic stroke is one of the major causes of death or long-term disabilities worldwide; thus, detection and treatment of ischemic-related damage, being still largely unresolved problems of contemporary medicine, require new technologies for diagnostics and therapeutics. Moreover, referring to the present pandemic, there is mounting evidence that patients affected by COVID-19 may develop clinically significant complications, including ischemic stroke. The main limitation of current ischemia/reperfusion injury treatment is an inefficient delivery of neuroprotective drugs to the affected part of the brain due to the blood-brain barrier (BBB) being permeable only by small, lipophilic molecules. Additionally, some neuroprotective drugs may influence the body, causing peripheral toxicity and numerous adverse reactions. The term “theranostic” describes the integration of therapeutic and diagnostic functions in one modality. The theranostic drug carriers can deliver their therapeutic cargo to the desired organ. Simultaneously, the delivery can be monitored by some imaging techniques for optimizing targeting and dosage. The diagnostic agents used for imaging are magnetic resonance imaging (MRI) or computed tomography contrast agents. Theranostic drug carriers combine the ability to load lipophilic drugs with high efficiency with their detectability with some imaging techniques. Moreover, they should transport the drug to the targeted place in the organism without its loss and release it in the area of action without imparting the therapeutic effect.

The main project objective, realized by the consortium of four research groups from Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences, Jerzy Maj Institute of Pharmacology PAS, Henryk Niewodniczański Institute of Nuclear Physics PAS and Collegium Medicum Jagiellonian University is to develop the new strategy of delivery of selected neuroprotectants by theranostic nanocarriers that can cross BBB without side effects on its normal function. We will concentrate on the methodology of encapsulation of neuroprotectants and fluorescent or MRI contrast agents in nanocarriers with a size below 150 nm and surfaces functionalized for targeted delivery.

In 2022, we focused on the encapsulation of selected neuroprotective drugs, cyclosporine A (CsA) and tacrolimus (FK506), into polymeric or nanoemulsion core polyelectrolyte shell nanocarriers (core@shell nanocarriers). Moreover, Gadolinium complexes were used as MRI contrast agents. As the encapsulation method, the sequential deposition of charged nanoobjects (polyelectrolytes or nanoparticles) was used. The following polyelectrolytes poly-L-lysine (PLL), gadolinium labeled poly-L-lysine (PLL-Gd), poly-L-glutamic acid (PGA), and pegylated-PGA (PGA-g-PEG) were utilized for multilayer shell formation. The size of prepared nanocarriers ranged between 80-150 nm.

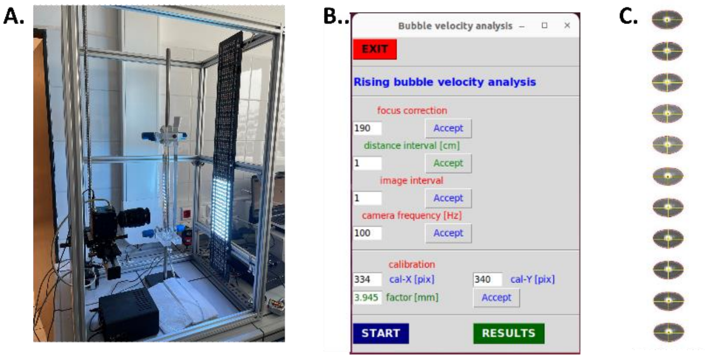
At the project's following stages, the neuroprotectants' effects in the theranostic formulations on the viability of the human neuronal-like SH-SY5Y cell line and on the organotypic hippocampal slice culture exposed to oxygen-glucose deprivation (OGD) will be examined. Moreover, the impact of theranostic formulations on the harmful factors of OGD will be measured. In vivo experiments will determine the biodistribution of nanocarriers and the efficiency of transport to the brain. At the final stage of the project, the effect of the nanocarriers containing neuroprotectants will be tested in the middle cerebral artery occlusion (MCAO) animal ischemic stroke model.

Elaboration of fast and simple physicochemical method of detection of water pollution based on monitoring of dynamic properties of liquid/gas interface

"Opus" research project NCN 2021/43/B/ST8/00053 [2022-2025]
(Project leader: Associate professor Jan Zawała)

The project in aimed to elaborate the fundamentals of simple physicochemical method of detection of surface-active contaminants (SAC) of waters, based on monitoring of changes of dynamic properties of liquid/gas interfaces. In practice, for SAC concentration determination in aqueous systems, the analysis of parameters of a single gas bubbles motion will be used, due to the fact that liquid/gas interface properties under dynamic conditions are extremely sensitive for even traces of organic substances in solution (of order of ppm and below).

In the reporting period the existing manual experimental set-up for precise determination of a single rising bubble motion parameters was rebuild and significantly updated. A new fully automated experimental set-up (Fig. 1A) was develped. It consists of: (i) high-speed camera mounted at the automated stand with stepper motor, (ii) LED diode matrix, (iii) a “Bubble-on-Demand” generator elaborated in our laboratory and (iv) square glass (borosilicate) column with thick-walled glass capillary of inner diameter 75 μm sealed at the bottom. During laboratory work the control panel of the experimental set-up was built (with the use of ARDUINO microcontroller) and the controlling software was elaborated in order to precisely control the up/down camera movement by adjusted distance interval. The camera position was correlated with programmable LED diode matrix, which illuminated only the region of interest of the column (i.e. the area where the observations of a single bubble motion were carried out). Subsequent sections of the LED matrix can be automatically switched on and off, what is associated with camera movement. Such approach ensures very good light conditions and eliminations of accidental and unwanted flashes, which can cause the loss of quality of images acquiring by the camera. Moreover, the algorithm allowing for very fast and automatic analysis of a single bubble motion parameters (velocity, size, shape deformations) on the basis of recorded movies was elaborated and the Python 3.0 script (with image analysis libraries) with user-friendly graphical user interface (GUI - see Fig. 1B). Thanks to this application it is possible to analyze hundreds of recorded photos without need of time-consuming manual analysis. The software can find the bubble outlines and geometrical center of subsequent bubble positions (see Fig. 1C) and easy calculations of bubble velocity evolution as a function of distance covered after detachment from the capillary orifice.



Rising bubble velocity analysis

focus correction: 190
distance interval [cm]: 1
image interval: 1
camera frequency [Hz]: 100
calibration
cal-X [pix]: 334
cal-Y [pix]: 340
factor [mm]: 3.945

Fig. 1. Photo of elaborated experimental set-up for automated bubble motion parameters monitoring (A) screenshot of GUI of the elaborated software for analysis of the acquired images (B) and example of sequence illustrating bubble subsequent positions analyzed by the elaborated software (C).

Excited states under magnifying glass – adaptation of approaches based on density analysis for investigation of electronically excited molecular states

"Opus" research project NCN 2021/43/B/ST4/02969 [2022-2026]
(leader: UW, IKiFP PAN partner: Associate professor Dorota Rutkowska-Żbik)

In this project, which has been launched in July, 2022, we propose a systematic examination of the QCT applicability and especially its extension with new tools, devised specifically for excited states of various types (like valence, charge-transfer, core, or Rydberg). A possibility to perform such an analysis of spatial 3D properties and their changes upon excitation will deepen our understanding of excited states to the same extent as it is now possible for the ground states. With these tools the calculations of excited electronic states will go far beyond the usual spectra simulation and the character analysis of the main excitations in the configuration expansion, providing a detailed picture of the excited molecule and increasing the predictive power of such computations. Among the tested and modified descriptors of QCT will be those from the quantum-theory of atoms in molecules (QTAIM) including extended QTAIM, electron localization function (ELF), interacting quantum atoms (IQA), and other, from which the most suitable quantities will be selected and new ones - derived and implemented. The dependence of QCT on the various methods for the computation of excited states will be also examined, since also for the ground state case some features of density or related quantities are dependent on the quality of the electron correlation description.

In 2022, a literature search was performed focusing on the methods used within Time Dependent Functional Theory (TD-DFT) to describe excited states of transition metal complexes containing tetrapyrrolic units (mostly porphyrins) and Ru(III) compounds. Test calculations for cobalt porphyrins were also executed.

Structure and Function of Protein Corona at the Nanoparticles Interface

"Opus" research project NCN 2021/41/B/ST5/02233 [2022-2026]
(Project leader: Professor Barbara Jachimska)

It is not the nanoparticles themselves, but the nanoparticle-protein system that represents the true identity of the carrier and the therapeutic responses when they enter the body. Therefore, knowledge of the mechanisms of formation of the nanoparticle-protein complex is essential to predict and manage the in vivo nanoparticle pathway, including its biodistribution, bioavailability, and toxicity. Consequently, the existing models of correlation of biological response and therapeutic effect, taking into account the effect of the protein crown of nanoparticles, are much more accurate than models in which the input factors are mainly the physicochemical properties of the nanoparticles used.

An efficient method for obtaining stable complexes of dendrimer particles with a protein corona was developed. Electrophoretic mobility and circular dichroism (CD) techniques were used to determine the protein corona coverage and secondary structure. It was also found that the zeta potential of the particles is closely correlated with the values of the white zeta potential and the corona coverage. The stability of the protein corona on the surface of the dendrimers was monitored by measuring the adsorption kinetics in dynamic conditions using the QCM-D method and SPR spectroscopy. Protein molecules effectively adsorb on negatively and positively charged dendrimer molecules in a wide range of pH. However, significant changes in the secondary structure of the protein are observed depending on the type of carrier. The orientation of a protein determines both its flexibility and the availability of optimal binding sites and protein function. The target orientation of the protein part and the level of changes in protein structures and their partial dysfunction depend on the interactive region in the protein structure. Appropriate selection of topography and surface composition and detailed information about the spatial structure of proteins allow controlling both their orientation and the properties of adsorbed proteins on the nanoparticle surface.

Exploring metal ion cooperation in binuclear transition metal sites

"Opus LAP" research project NCN 2020/39/I/ST4/02559 [2021-2024]
(Project leader: Associate Professor Dorota Rutkowska-Żbik)

In the current project we aim at the exploration of the reactivity of the binuclear divalent transition metal ion (TMI) sites (TMI = Fe, Co, Mn, Ni) stabilized into the zeolitic matrices as models of the binuclear sites in enzymes. Last year studies were focused on a) determination the properties and reactivity of systems containing binuclear iron centers introduced into zeolitic matrix of *BEA topology (experimental studies performed with Czech partner); b) determination of reactivity of the model $[TMI(porphyrin)]_2$ systems for the O-O bond activation in O_2 and formation of the reactive oxygen species responsible for selective oxidation in model systems (theoretical studies performed mostly at ICSC PAS).

ad a) Redox properties of distant binuclear iron centers in Al-rich *BEA zeolite in O_2 splitting and CH_4 oxidation were characterized. Al-rich *BEA was obtained via a template-free synthesis procedure guaranteeing low-defected structure and a high fraction of Al-pairs enabling stabilization of binuclear iron centers. By employment of a multi-spectroscopic in-situ approach (Mössbauer and X-ray absorption), the formation of active oxygen over binuclear iron centers in Al-rich *BEA was confirmed, and subsequent CH_4 oxidation was studied. Spontaneous release of the reaction products to the gas stream, representing a significant advantage of the studied system, was proved by the results of in-situ FTIR and mass spectrometry. This is the first experimental proof of the formation of fully functioned binuclear iron centers (able to split O_2, stabilize active oxygen forms, and subsequently oxidize CH_4) in zeolite of *BEA topology.

ad b) Theoretical models of $[TMI(porphyrin)]_2$ systems were proposed in which metal ions (of +II oxidation states) were places at varying distances one from another ranging from 10 to 4 Å (with step of 0,5 Å) representing different special arrangement of TMIs. It was found that the mutual rotation of the porphyrin rings does not influence the obtained results. For each of the studied metal – metal distance a dioxygen molecule was placed in between two TMIs. In case of the Ni-Ni pair, the Ni-O-O-Ni bridges were formed when metal ions were at the distance between 4 – 4.5 Å, but no O-O bond rupture was observed. Similar results were found for the $[Co(porphyrin)]_2$ system, but in that case the Co-O-O-Co bridge was formed also for the structure with cobalt ions separated by Å. The O-O bond was elongated (from 1.22 to 1.30 Å) indicating its activation. Very similar observations were made for the manganese dimers. In the $[Fe(porphyrin)]_2$ systems in which Fe ions were separated by 4 Å, a μ-peroxo bridge linking two metallic centers was found. In these systems, the O-O bond is elongated to 1.45 Å indicating the strong activation of the dioxygen molecule. When Fe-Fe distance is increased till 5 Å the Fe-O-O-Fe bridge is formed in which the O-O bond is also weakened.

Preventive Conservation Strategies for Poly(vinyl chloride) Objects

“Opus LAP” research project NCN 2020/39/I/HS2/00911 [2022-2025]
(Project leader: Professor Łukasz Bratasz)

This project’s aim is to develop a preventive conservation strategy that will determine the most convenient economic and ecological conditions for the storage of cultural heritage objects made of poly(vinyl chloride) – PVC. Research within this project is mainly focused on following the process of degradation under mild conditions (maximum temperature 80 ^{o}C) via observation of chemical changes in PVC and their correlation with the mechanical changes of the material.

In the beginning, high-resolution images of historical PVC objects were recorded with a Hirox microscope in order to document cracking present on the mannequins from plasticized PVC prepared in the late 80’ stored in Cricoteka. The scans will be used in the later stages of the project as real examples supporting the modelling of the mechanical stress in the objects (verification of computer model).

Other investigated objects of cultural heritage are Joseph Beuys’ objects made of plasticized PVC at the beginning of 70’. Due to the presence of viscous liquid on the surface of these objects, both liquid phase and bulk material were analyzed using infrared spectroscopy (FTIR) for initial chemical identification. FTIR analysis revealed that the liquid phase is not the plasticizer (phthalic acid ester), hence other analyses of the chemical composition of the liquid phase have been planned (size exclusion chromatography, gas chromatography coupled with the mass spectrometry). Further examination of Joseph Beuys’ objects, such as mechanical testing (DMA oscillation tests), thermal analysis (thermal stability – TGA, glass transition temperature – DSC), as well as imaging of the surface with the scanning electron microscope (SEM-EDS) with elements mapping (carbon, oxygen, and chlorine) was conducted. SEM-EDS analysis was used to determine the plasticizer concentration profile (as the profile of oxygen concentration) deep into the material.

Moreover, samples of PVC granules and PVC films for accelerated ageing were purchased and thoroughly characterized using thermal analysis (TGA, DSC), size exclusion chromatography, infrared spectroscopy, as well as mechanical analyses: tensile tests and oscillatory tests (DMA). Master curves predicting the long-term behaviour of the materials have been created for all new PVCs on the basis of DMA results.

Furthermore, experimental data analysis has been undertaken in order to evaluate the applicability of such an approach (changes in plasticizer content and sample mass with time) for the determination of diffusion and evaporation coefficients of plasticizers and an alternative method (solid-state NMR diffusometry) has been proposed for diffusion coefficient determination, which is a crucial parameter for further modelling.

Accelerated ageing tests under mild thermal conditions (80 ^{o}C) for selected PVC samples with different plasticizer content have been started in cooperation with the Faculty of Chemistry (Jagiellonian University). These experiments are intended to correlate structural changes of the PVCs with mechanical changes which will be a subject of research carried out during the second year of the project.

"Sonatina" Research Projects of the National Science Centre

Multifunctional chitosan-based composite coatings for biodegradable Mg alloys

"Sonatina" research project NCN 2021/40/C/ST5/00266 [2021-2024]
(Project leader: Dzmitry Kharytonau PhD)

Magnesium alloys can be used to develop a new class of so-called biodegradable materials. Their use as implants allow to realize a new type of materials, called biodegreadable implants, which are expected to corrode gradually *in vivo* and being dissolved completely upon tissue healing with no implant residues in the human body. In this regard, the major drawback of magnesium alloys as implants is their exceptionally high corrosion rate in the presence of water, which always occurs with hydrogen gas evolution. Chitosan is a natural polymer, which is promising for biomedical applications due to its nontoxicity, biocompatibility, biodegradability, film-forming ability, mechanical properties, and low cost.

The overall goal of the project is to develop a new type of multifunctional chitosan-based coatings on the surface of magnesium alloys with enhanced corrosion resistance and antibacterial functionality.

In the reporting period, the first specific objective of the project was examined:

1. To fabricate and characterize chitosan coatings on the surface of Mg alloys using new modified electrophoretic deposition (EPD) approaches and optimize process parameters.

Three types of chitosan powders with high, medium, and low molecular weight were selected for experiments. Their physico-mechanical properties were characterized by thermogravimetric analysis (TGA), X-ray diffraction (XRD) analysis, and scanning electron microscopy (SEM). The composition of the initial powders was evaluated by FT-infrared spectroscopy and Raman spectroscopy.

As substrates, Mg alloys AZ31 (Mg-Al-Mn) and WE43 (Mg-Y-Nd-Zr) were selected. The microstructure of the alloys was analyzed by SEM and EDX, phase composition was analyzed by XRD. Different types of surface pretreatment (mechanical grinding and polishing) were utilized to evaluate the effect of the surface parameters on the coatings quality. Solutions for coatings deposition were prepared by dissolving all chitosan types in concentrations 2–10 g/L in water with 1 vol.% of 99.9% acetic acid. Chitosan coatings of the best quality were obtained by EPD process from the solution containing 60 or 90 vol.% of 99.9% ethanol at 30 V for 3–5 min.

The process of the cathodic coordinated deposition, including phosphates, silicates, and molybdates as modification additives was examined. The best results were achieved for phosphates and molybdates, while silicates did now allow to obtain stable chitosan suspensions. The parameters of the deposition process were optimized. The best coatings were obtained at potentials below 30 V and a deposition time 5–10 min. Examination of the surface by SEM revealed that obtained coatings are non-porous and cross-cut images showed good adhesion of the coating to the substrate.

Preliminary results of the corrosion properties of AZ31 and WE43 alloys in Hank's solution were performed. Several types of natural amino acids (glycine, tyrosine, cysteine, methionine, tryptophan, and phenylalanine) were tested as corrosion inhibitors, which are planned to be further added into chitosan suspensions. The highest inhibition efficiency of ca. 75% was obtained for methionine, and phenylalanine, which was selected for further tests.

"Preludium" Research Projects
of the National Science Centre

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

“Preludium” research project NCN 2017/27/N/ST4/02676 [2018-2023]
(Project leader: Agnieszka Winiarska MSc; supervisor: Professor Maciej Szaleniec)

Tungsten aldehyde oxidoreductase (AOR) enzymes catalyze reversible oxidation of aldehydes to the corresponding carboxylic acids in the presence of ferredoxin, and because of their broad substrate spectrum, they are assigned a role in detoxifying harmful aldehyde metabolites in the fermentative degradation of amino acids. The tungsten aldehyde oxidoreductase from *Aromatoleum aromaticum* (AOR_{Aa}) was chosen for the study because of its high oxygen resistance and lower temperature optimum than known AORs from hyperthermophilic archea and bacteria.

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

In previous years of the project, it was discovered that AOR_{Aa} is able to catalyse the reduction of carboxylic acids and NAD^+ cofactor to NADH in the presence of hydrogen as a sole electron donor. In 2022 conducted kinetic assays proved that AOR_{Aa} can simultaneously catalyze both above reactions, however, without the use of electron bifurcation. In addition, it has been shown that both reactions also occur at neutral pH, which makes it possible to use AORAa as a bioreductant in whole-cell systems.

Comparison of the structure of AOR_{Aa} solved in the project (with a resolution of 3.3 Å) with that of the AOR from *Pyrococcus furiosus* (AOR_{Pf}, with a resolution of 2.3 Å) showed that the active site of both enzymes is identical. The AOR_{Pf} structure was the basis for the study of tungsten cofactor geometry by theoretical methods (by optimizing the geometry of cluster and QM:MM models). The aim of the study was to identify the two missing ligands at the tungsten coordination sphere and to prepare the structure for the study of the mechanism. Three cofactor models selected from the cluster models were optimized by the QM:MM method and the obtained geometries were analyzed and compared to experimental data. Two of the models that met the criteria formulated on the basis of spectroscopic results were matched to the electron density from the AOR_{Pf} structure, and thus differential density maps were obtained. The obtained maps indicated a good fit of the cofactor model in the oxidized form W(VI) with two oxo ligands.

Moreover, in cooperation with prof. Graham George of the University of Saskatchewan in Canada, the first coordination sphere of tungsten in AOR_{Aa} was investigated by EXAFS X-ray absorption spectroscopy for the L-edge of tungsten. The obtained results for the reduced form of the cofactor were compared with the obtained QM:MM models and indicated a geometry with W(IV) and one oxo and one hydroxyl ligand.

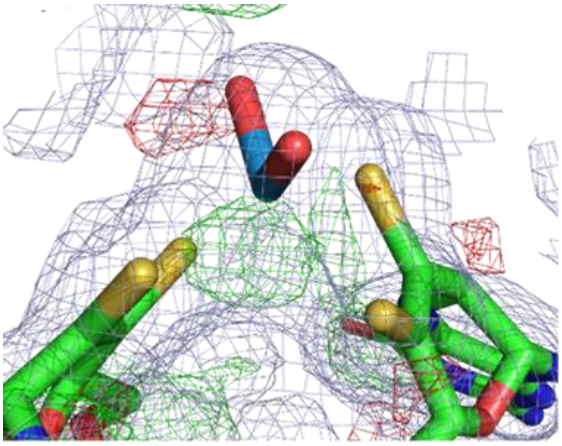


Figure. Difference map of AOR_{Pf} calculated from crystallographic data and oxidized W (VI) with two oxo ligands model prepared by QM:MM modelling. The excess electron density of the model is shown in red, the defficient electron density of the model is shown in green.

The analysis of the influence of selected factors on changes in α-synuclein structure

“Preludium” research project NCN 2020/37/N/ST4/02132 [2021-2023]
(Project leader: Paulina Komorek MSc, supervisor: Professor Barbara Jachimska)

Research on the development of Parkinson's disease has indicated that the hallmark of the disease is the appearance of Lewy bodies containing mainly α-synuclein. In Levy bodies, α-synuclein occurs in a misfolded form and may cause neuronal death. Although quite a lot of research has already been performed on the structure of α-synuclein, there are still many questions concerning changes in the secondary structure of this protein and the source of its toxicity. An important but poorly understood effect of protein misfolding is adsorption, which causes changes in conformation due to the protein’s interactions with the surface. Therefore, studies aimed at the structural analysis of the adsorbed protein were carried out using several physicochemical methods, including the characteristics of the protein in solution and on the adsorption surface. Using circular dichroism (CD), infrared spectroscopy (IR), and atomic force microscopy (AFM), it was confirmed that the interaction of the protein with the gold induces changes in its conformation, the direction, and intensity of structural changes, which depend on the pH of the environment in which adsorption was carried out. Adsorbed molecules are characterized by an increase in the content of disordered structures and β-turns with a simultaneous decrease in the content of α-helices compared to the structure in the solution. MD simulations showed that at pH = 3.5, more cysteines are exposed outside the molecule, which may be responsible for the adsorption to the gold, and thus may cause an increased change in the structure of adsorbed molecules. Changes in the secondary structure, and thus the surface topography of the formed films, were revealed by changes in the contact angle (CA) of the gold. Complementary experiments using MP-SPR and QCM-D showed that the adsorption conditions and the structure of molecules have a stronger effect on the hydration of formed films than the degree of surface coverage..

Development of evidence-based environmental specifications for short religious, cultural and commercial events in historic buildings

“Preludium” research project NCN 2020/37/N/HS2/01727 [2021-2023]
(Project leader: Magdalena Soboń PhD Eng., supervisor: Professor Łukasz Bratasz)

As part of the project, work was continued on the development of rational, evidence-based guidelines for climate control in historic interiors, which will allow curators and owners of similar buildings to make decisions to protect the cultural heritage remaining in their custody, mainly constituted by the massive wooden objects, i.e., sculptures and decorative elements. In particular, the project aims to define a safe strategy for heating historic interiors for religious, cultural, and commercial short-term events.

A database of lime wood’s mechanical properties was expanded and updated for the purposes of the two-dimensional model of a massive wooden element, allowing to analyze the crack propagation process under the influence of climate fluctuations. The model, created in the COMSOL Multiphysics program, allows solving physical problems using the finite element method and requires a set of mechanical parameters of the simulated material.

In the analysis of crack development in massive objects made of lime wood, the energy release rate (G) was adopted as a parameter determining the risk of damage. The critical value, G_{Ic}, of lime wood was considered a risk criterion, the exceeding of which causes an increase in the depth of the crack in the sculpture. Laboratory measurements were carried out using the so-called *wedge-splitting method* on lime wood samples conditioned in four different conditions of relative humidity (approx. 38%, 58%, 80% and 98%). This method allows to determine the energy needed to propagate crack from the notch initially made in the sample along the desired direction in the wood and therefore estimate G_{Ic}. For each relative humidity (RH) 5-7 measurements were performed on samples from 3 different lime wood trunks to consider the dispersion of material properties within the wood species. A dependence of G_{Ic} on RH was observed: G_{Ic} increased with increasing RH.

Wedge-splitting measurements were combined with acoustic emission method and data was recorded throughout the whole experiment. Acoustic emission allows to calculate the amount of energy released in the cracking process and correlate it with the increase in crack length, which will be analyzed in the next stage of the project.

From the same lime wood trunks, samples in a dog-bone shape were obtained for measuring Young's modulus and Poisson's ratio. Static tensile tests of samples stabilized in four different RH conditions (approx. 38%, 58%, 80% and 98%) were carried out on the universal testing machine. For each RH, 10-15 measurements were performed on samples from 3 different trunks. The mechanical properties of the wood were determined from a combination of the optical and mechanical methods used. Young's modulus in the radial direction and tangential direction of wood increased with decreasing RH, while Poisson's ratios for combinations of these directions showed no significant dependence on RH.

"Beethoven Life" Research Projects of the National Science Centre

Structure and function of fumarate-adding glycyl radical enzymes: biochemistry, modeling and application

"Beethoven Life" research project NCN 2018/31/F/NZ1/01856 [2020-2023]
(Polish leader: Professor Maciej Szaleniec, German leader: Professor Johann Heider)

The project aim is the investigation of the biochemistry and reaction mechanisms of glycyl radical enzymes which catalyze the addition of fumarate (fumarate-adding enzymes, FAE). The prototype representative enzyme of the FAE class is benzylsuccinate synthase (BSS) from *Thaurea aromatica,* which catalyses radical addition of toluene to fumarate yielding (*R*)-bezylsuccinate.

During the third year of the project, we developed LC-MS/MS analytical methods for analysis of reaction mixture with benzylsuccinate substituted with CH_3 or OH in the phenyl ring. Furthermore a GC-MS analytical method for detection of benzylsuccinate without derivatization was developed. The activity of T197S and T197P BSS mutants was confirmed with xylene and crexol substrates as well as for R508P with acetylacrylate. The pilot scale fermentation of *Aromatoleum evansii* with p.ASG3'-BSS plasmid was run at the 5 L scale as a preparation for biotechnological application. Several other expression hosts (*Mixta theicola*, *Paracoccus denitrificans*, *Shimwella blattae)* were genetically transformed and tested for expression of BSS.

The modeling of FAE enzyme reactivity encompassed study of mutation on substrate dynamics in the active site of homology models wild type BSS from strain K172 and EbN1 as well as their R504K, Y193S mutants. The MD simulations were conducted with various organic acids i.e., fumarate, crotonate, trans-glutaconate, 2-entenoate, and 3-acetylacrylate as well as tolune and its derivatives (p-,m-,o-xylenes and crezols). Based on statistical analysis of stable production phase of simulation the probability of finding the substrate in the near acttack conformation was established. The results showed that mutation R504K increases the chance of reaction between toluene and crotonate and 2-pentenoate and to lesser extent for trans-glutaconate but reduced the probability of reaction with 3-acetylacrylate. The Y193 mutation is not significantelly influencing enzyme reactivity with o- and m-cresol but increases the probability of finding m-xylene in NAC.

Furhtermore, the modelling of EPR spectra for BSS-benzyl alcohol-fumarate complex was EasySpin was conducted (see Fig. 1). This part of research was conducted in a cooperation wth professor A. Pierik from Technische Universität Kaiserslautern. The aim of simulations was unequivocal interpretation of geometry for radical adduct of fumarate and benzyl alcohol. Two alternative hypothesis are considered. The first one assumes formation of radical hydroxy-benzylsuccinate and the other dehydrated radical trans-phenylitaconate. The modelling was supported with QM:MM modelling of the inhibited BSS enzyme containing radical intermediate product.

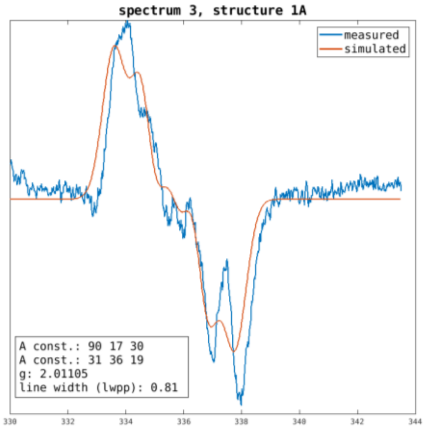


Fig. 1. Resul of simulation for radical intermediate product

"Solar-Driven Chemistry" Research Projects of the National Science Centre

SolarMethaChem: Solar light-driven photochemical processes for methane chemical conversion to valuable product

„Solar-Driven Chemistry" research project NCN nr 2019/01/Y/ST4/00024 [2020-2023]
(Project leader: Associate Professor Dorota Rutkowska-Żbik)

The major goals of this project are *i)* the nanoengineering of new efficient materials for efficient oxidative coupling of methane under light, *ii)* identification of the reaction mechanisms of methane coupling and *iii)* optimization of the photochemical reactor and operating conditions. The team from the ICSC PAS is responsible for theoretical studies in the project, in particular to propose models for the photocatalytic systems and elucidation of their electronic structure as well as the determination of the reaction mechanism.

In 2021 the following research tasks were undertaken:

1) Geometry and electronic structures of the studied catalysts for methane coupling were determined, i.e., structures of M_{13}/TiO_2 (M = Cu, Ag, Au) and HPA/TiO_2 (HPA = HPW, HPMo), where as TiO_2 model a cluster cut from anatase (101) surface was taken. The DFT:PBE+D3/def2-TZVP method within cluster model was used. It was found that the structure of the metallic cluster depends on the type of the metal. Copper creates spherical shaped species, whereas gold and silver clusters are flattened on the surface maximizing metal – support interactions (see Figures 1 a, b, c). Heteropolyacids retain their structure after deposition on the surface.

2) For the most active methane coupling catalyst (Au_6/TiO_2 – here a smaller model than described above was used due to the computational complexity) the reaction mechanism was proposed. The reaction profile of the first step, i.e. C-H bond activation and dissoecition in methane, which is considered as a rate determining step is depicted in Figure 1 d. The energy barrier accompanying this process is equal to 146 kJ/mol, when it occurs on the $TiO_2^{\delta+}$ system (which represents photo-induced titanium dioxide). Once the gold cluster is deposited on TiO_2, this activation energy is lowered to 67 kJ/mol. The performer calculations explain an experimentally found difference between activity of Au/TiO_2 towards TiO_2.

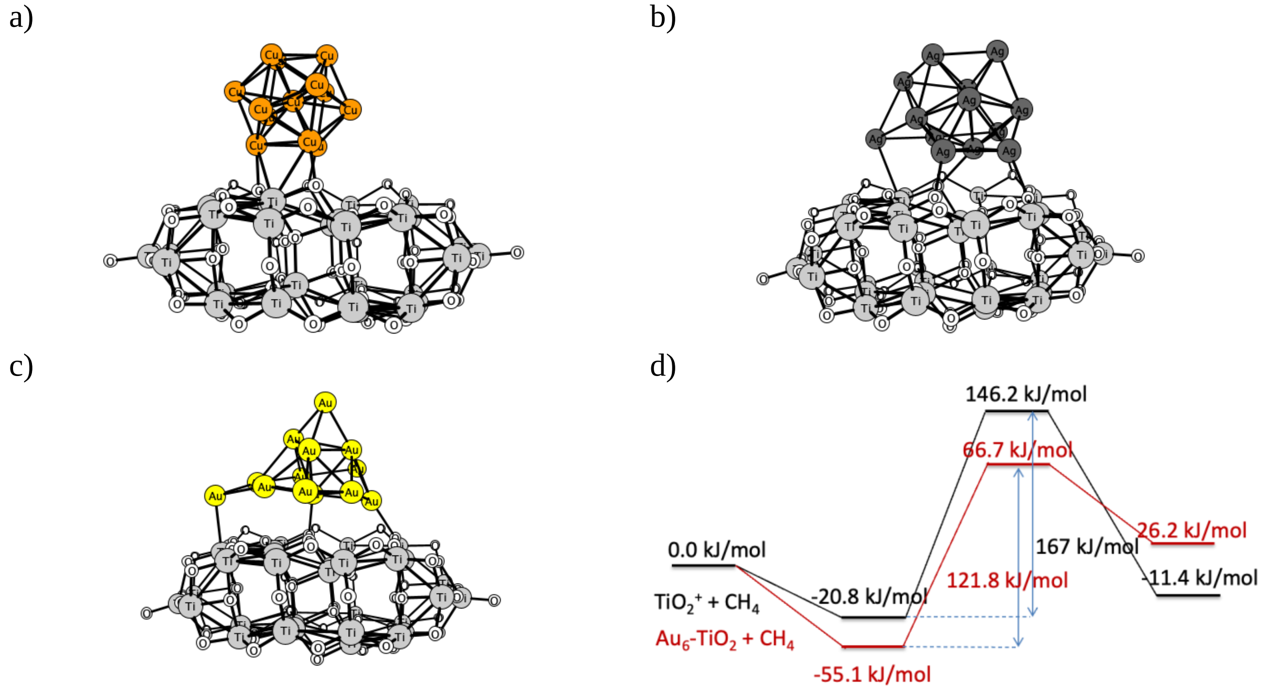


Figure 1. Geometries of a) Cu_{13}/TiO_2, Ag_{13}/TiO_2, c) Au_{13}/TiO_2 and d) energetic profile of CH_4 activation on TiO_2 and Au_6/TiO_2.

"GRIEG" Research Projects of the National Science Centre

Craquelure – Model of paintings with craquelure patterns for evidence-based environmental control in museums

"GRIEG" research project NCN 2019/34/H/HS2/00581 [2020-2023]
(Project leader: Professor Łukasz Bratasz;
co-investigator: *Marcin Bury PhD, Marcin Strojecki PhD, Arkadiusz Janas PhD, Sergii Antropov PhD, Katarzyna Poznańska MSc Eng.)*

In 2022, the research team implementing the GRIEG Craquelure project continued to develop a database of properties of historical and artistic materials used in paintings, and contemporary materials used to consolidate a paint layer. The measurements of elasticity modulus, strain at break, and swelling and shrinkage isotherms of tempera paints carried out in previous years were extended in order to verify uncertain results and to improve the measurement statistics. Mechanical tests were accompanied by tests of chemical evolution taking place in selected samples of tempera paints using FTIR spectrometry, Raman spectroscopy, contact angle measurements and electron paramagnetic resonance spectroscopy. The tests confirmed that changes in the tested samples occur only in the initial period of their natural ageing and after a period of 1-2 months they stabilize. This means that the mechanical properties determined for samples aged from 6 to 12 months reflect the properties of historical tempera paints. Based on these results and a comparison with the properties of the types of paints and ground, an important hypothesis was made that tempera paints with yolk binder are essentially animal glue-based paints and the fatty acid component is negligible in the development of mechanical properties.

Another important element of the research was the development of a three-dimensional model of the painting on canvas. This model is based on the finite element method and COMSOL Multiphysics software. The model assumes that the painting is made of a wooden stretcher, canvas glued with animal glue and a layer of glue-based ground (gesso) - a rigid material similar in mechanical properties to ageing brittle oil paints. A new element of the research was to take into account the actual mechanical parameters of the sized canvas, taking into account the directions along the weft and along the warp. In particular, directional elasticity moduli for different relative humidity and directional moisture expansion of the sized canvas were used. Extensive parametric studies were carried out to determine the sensitivity of the model to input parameters. It was found that in the corners of the painting, there may be a significant stress concentration. The area where stresses are increased is much larger than in the case of the model which does not take into account the anisotropy of the sized canvas.

The last important research component of the project implemented in 2022 was the start of work on the validation of computer models based on measurements of samples imitating real paintings on canvas and panel. In total, several dozen samples with different types of substrate, geometries, types of materials used, and humidity conditions in which the samples were produced, were prepared. A system for measuring elongation and bending was developed for samples imitating panel paintings, and a system for monitoring stresses in canvas paintings. For both types of objects, an acoustic emission system was adopted to track precisely micro damage development in the paint layer. Samples prepared in this way were subjected to initial cycles of relative humidity changes in order to determine their response time and appropriately plan the time sequence of relative humidity changes. For practical reasons, it was decided that the samples of panel paintings will be subjected to cycles of relative humidity at 50°C. Under these conditions, the response time to reach 63% total deformation of 40mm thick samples is 5 weeks. Validation measurements are currently underway and the results will be available and interpreted next year.

Theranostic nanocarriers for drug delivery in central nervous system disorders

"GRIEG" research project NCN 2019/34/H/ST5/00578 [2021-2024]
(Project leader: Professor Piotr Warszyński
co-investigator: Associate professor Krzysztof Szczepanowicz, Marta Szczęch PhD, Tomasz Kruk PhD)

Prevention and treatment of neurodegenerative diseases and stroke-related brain damage are major and unresolved problems of contemporary medicine. Despite the progress in understanding molecular mechanisms of neuronal injury and preventing them, few neuroprotective substances are used in the clinic, and their efficiency in treating stroke and neurodegenerations is unsatisfactory. One of the significant limitations to current neurodegenerative disease treatment is an inefficient delivery of neuroprotective drugs to the affected part of the brain and difficulties in the diagnosis if the drug is well addressed, i.e., if it reaches the targeted organ. Theranostics is a new medical branch that joins therapeutic and diagnostic functions into one entity. Application of nanotechnology in theranostics will allow the engineering of drug carriers simultaneously deliver therapeutic components and possess diagnostic functions.

The main objective of the project is to develop a new strategy to deliver neuroprotectants by applying theranostic nanocarriers for neuroprotective drugs, which are able to cross the blood-brain barrier without imposing side effects on its normal function and can be detected in a given part of a brain by Nuclear Resonance Imaging (MRI). Three Polish scientific institutions, Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences, Jerzy Maj Institute of Pharmacology PAS, Henryk Niewodniczański Institute of Nuclear Physics PAS and two Norwegians SINTEF Oslo and Oslo University Hospital participate in the project consortium.

In 2022, we focused on the encapsulation of carnosic acid (neuroprotective substance) together with contrasting agents: gadolinium complex (for MRI) or Rhodamine (fluorescent imaging). The nanocarriers of neuroprotective drugs and contrasting agents were formed by the sequential adsorption of charged nanoobjects method, called layer by layer (Figure 1a). The size of the developed theranostic nanocarriers was 150 nm (Figure 1b). Nanocarriers were non-toxic; moreover, they were functionalized by PEG to allow long circulating after administration. Developed theranostic nanocarriers of carnosic acid were delivered to project partners for in vitro and in vivo tests.

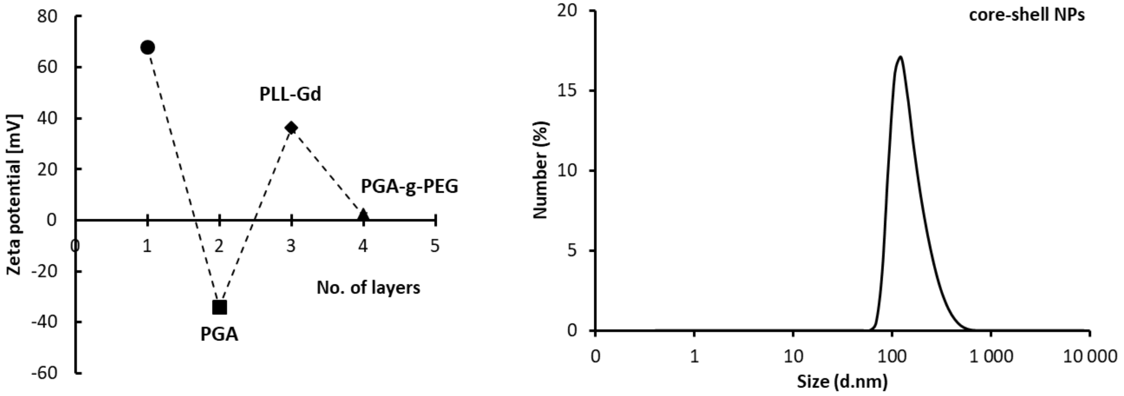


Figure 1. Nanocarriers' characterization a) zeta potential dependence with a number of layers b) size distribution of developed nanocarriers.

"Miniatura" Research Projects of the National Science Centre

Isothermal titration calorimetry (ITC) in biochemical characterization of α-ketoglutarate dependent enzymes. Internship at McGill University

"Miniatura" Research Projects NCN 2019/03/X/ST4/0163 [2019-2022]
(Project leader: Anna Miłaczewska-Kręgiel PhD)

The internship was planned for the training in a usage of Isothermal Titration Calorimetry (ITC) method for biochemical studies of enzymes dependent on α-ketoglutarate. The internship took place at McGill University, in the group held by Prof. Mittermaier. The main aims of the internship were gaining new experience in thermodynamic characterization of enzymes using ITC and to conduct a research on selected enzymes:

- kanamycin B dioxygenase (KanJ) – an enzyme on a pathway of kanamycin biosynthesis. Previous research showed that KanJ enzyme is able to catalyze deamination of various antibiotics, with the highest rate in pH~6.5, but in this condition is also the fastest deactivated
- codeine O-demethylase (CODM) and its truncated form (CODMt) – enzyme involved in morphine biosynthesis and other benzylisoquinoline alkaloids metabolism in opium poppy
- 3Cl protease from SARS-COV-2 virus – the key enzyme for virus replication.

Within 7-weeks internship I tested binding affinity of various antibiotics (kanamycin B, tobramycin and amikacin) to kanamycin B dioxygenase (KanJ). Comparison of binding in three different pH: slightly acidic 6.2, neutral 7.2 and basic 8.2 showed that neutral pH is the most favorable for ligand binding. The performed studies broadened the knowledge of aminoglycoside antibiotics synthesis in the part catalysed by KanJ.

Based on performed tests of activity using ITC, I proposed the method to study enzymes dependent on iron (II) and α-ketoglutarate. The most favorable condition for the reaction is a slightly acidic environment (pH = 6.6), as the resultant pH for the stability of iron Fe (II) and protein, in the presence of the reducing agent - sodium ascorbate. Designated method is planned to be used in kinetic studies for KanJ.

I compared binding parameters for codeine O-demethylase and its truncated mutant. The goal was to understand how the introduced change in the protein sequence affects ligand binding using a selected alkaloid - allocryptopine - as an example. It occurred there is no distinct difference in ligand binding (similar stoichiometry and binding affinity for the both forms of codeine O-demethylase). It means that truncated fragment is not essential for ligand binding in the active niche. Therefore truncated CODMt is a good model of codeine O-demethylase for biochemical studies, including crystallography. Obtained structure with ligands is a good approximation of the full structure, while there is no possibility to obtain a structure for a full CODM protein.

I also got an opportunity to work on the 3Cl protease from SARS-COV-2 virus. 3Cl was planned to be tested with various inhibitors using automated ITC, but the current measurement conditions made it difficult to observe the inhibition effect during long experiment. I tested various buffers and optimized the method to study how inhibitors affect 3Cl protease and its mutants. The conditions, I found for testing the activity of the 3Cl protease, contain a phosphate buffer, which allowed us to obtain the highest signal. The solution contained an addition of the bovine serum albumin (BSA) and dithiothreitol - enzyme stabilizing factors, which helps to keep the enzyme in an active form for more than 48 hours. The proposed method will be used to study susceptibility of 3Cl mutants to inhibitors.

Fluorescent layered materials based on gold nanoclusters – preparation by self-assembly processes

"Miniatura" research project NCN 2021/05/X/ST5/00244 [2021-2022]
(Project leader: Julia Maciejewska-Prończuk PhD Eng.)

The main goal of the scientific activity was determination and description of the mechanism of deposition of fluorescent gold nanoclusters obtained using bovine serum albumin and lysozyme on a model silicon surface and comparison of the collected results with the data for the proteins forming the matrix. The maximum degree of coverage and the stability of gold nanocluster layers as a function of ionic strength and pH were determined.

The initial stage of research was the synthesis of gold nanoclusters with the use of bovine serum albumin and lysozyme. The obtained hydrosols of nanoclusters were purified by membrane ultrafiltration. The physicochemical characteristics of gold nanoclasters on a protein matrix included:

- Determination of their hydrodynamic diameter in a wide range of ionic strength and pH using the dynamic light scattering technique (DLS);
- Determination of their electrophoretic mobility in a wide range of ionic strength and pH using the microelectrophoresis technique;
- Registration of absorption and emission spectra using spectroscopic methods;
- Determination of the size of the metal core on the basis of image analysis from high-resolution transmission electron microscopy (HR-TEM);
- Determination of the mass concentration of gold in colloidal suspensions of nanoclusters was determined by atomic absorption spectrometry (AAS).

Physicochemical characteristics of gold nanoclusters on a protein matrix dispersed in hydrosols, provided information on the hydrodynamic diameter, the range of their stability and electrokinetic properties. Gained data was of crucial importance by selecting the conditions for the deposition of gold nanoclusters on solid surfaces in order to obtain stable, homogeneous and fluorescent layers. The most important stage of the research was to determine the kinetics of nanocluster deposition using: quartz microbalance with energy dissipation monitoring (QCM-D), flow potential measurements, surface imaging by atomic force microscopy (AFM) and scanning electron microscopy (SEM). The fluorescence properties of the deposited layers were also established by analyzing images collected with confocal microscopy. The influence of pH, ionic strength and the presence of a positively charged anchor layer (polydiallyl dimethyl ammonium chloride - PDDA) on the maximum degree of surface coverage with nanoclusters was investigated. Microscopic images (AFM, SEM) confirmed the assembly of stable, homogeneous layers. The measurements, described above, confirmed the hypothesis formulated in the conclusion that the self-assembly process of gold nanoclusters, leading to formation of layers on silicon surface, was mainly controlled by electrostatic interactions.

Effect of redox and acidobasic properties of the acitve sites on the activity of non-heme iron dependent enzymes - computational studies

"Miniatura" research project NCN 2022/06/X/ST4/00078 [2022-2023]
(Project leader: Zuzanna Wojdyła PhD)

The project covers a short post-doctoral fellowship at the J. Heyrovský Institute of Physical Chemistry in the Computational Chemistry group, under the supervision of RNDr Martin Srnec. The aim of the fellowship was to develop a computational model of the pterin-dependent enzyme, which will be in line with the recent experimental findings, and will serve to decsribe the relationship between the redox and acidobasic properties of the active site of the enzyme and its activity.

The initial stage of the studies was based on classical modelling – docking of the cofactor (4a-hydroxytetrahydrobiopterin) and the substrate within the binding cavity of the enzyme, followed by molecular dynamics (MD) simulations for the obtained complexes. In the next step, the complexes most representative for the simulations were optimised with use of QM/MM methods and benchmarked with experimental results (by comparison of results of TD-DFT calculations with experimental UV-vis spectra).

To obtain the model of the activated enzyme (i.e. containing the reactive oxoferryl moiety, responsible for substrate oxidation) the reaction with molecular oxygen was modelled using QM/MM methods. In parallel a set of auxillary simplified models for the active site was prepared with the purpose of elucidating their inherent redox and acidobasic properites (redox potentials and acidobasic constants, respectively) and the mechanisms for the hydrogen abstraction reaction (with the set of auxillary substrates). The correlation of the inherent properties and the reaction mechanism will allow to elucidate to what extent redox and acidobasic properties of the active site of the studied enzyme shape its activity.

Synthesis of new conjugates of neuroleptics belonging to phenothiazine derivatives with gold nanoparticles and evaluation of their activity in protein fibrillation processes

"Miniatura" research project NCN 2022/06/X/ST5/00350 [2022-2023]
(Project leader: Magdalena Oćwieja DSc)

Neurodegenerative diseases are incurable, hence they are in the center of interest of scientist who are looking for the methods to prevent them. Pathogenesis and etiology of neurodegenerative diseases have not been fully understood and described in the literature. Nevertheless, it was established that in the case of hereditary neurodegenerative diseases as well as in the case of these which were acquired, formation of pathological protein structures that accumulate in nervous cells and damage neurons is a major factor responsible for the degeneration of nerve system. Many literature evidences proved that undesired aggregation of the proteins to fibrils is related to etiology of neurodegenerative diseases. Moreover, it was proved that the formation of toxic fibrils can be induced by chemical substances exhibiting pro-fibrilliar properties. On the other hand, it was established that some chemicals and nanostructures are able to reduce and prevent the uncontrolled fibrillation of proteins. It was proved, that some gold nanoparticles, which are nanometric structures of sizes smaller than 100 nm, exhibit anti-fibrillar properties. It is worth mentioning that methods of preparation of gold nanoparticles (AuNPs) of controlled size, shape and surface properties are well-developed and described in the literature. Nevertheless, it is still not known how to design and synthesize AuNPs with the desired anti-fibrillar properties which will be inhibit formation of toxic structures of proteins which are responsible for the induction of neurodegenerative diseases. Hence, the aim of the undertaken research is to verify the hypothesis that the activity of AuNPs in fibrillation processes depends mainly on their surface properties, which in turn are modeled by adsorbed stabilizer molecules. The role of electrostatic interactions also seems to be important, as well as the susceptibility of AuNPs to aggregation, which can stimulate the formation of protein fibrils.

In the first stage of the work, AuNPs with controlled morphology and surface properties were prepared. Three types of quasi-spherical negatively charged AuNPs with an average size of 5, 22 and 55 nm and one type of positively charged AuNPs with an average size of 10 nm were obtained. The nanoparticles were used to synthesize chlorpromazine-AuNPs conjugates (CPZ-AuNPs). The conjugates were obtained by electrostatic interaction-controlled adsorption of drug molecules on the surface of AuNPs. A series of experimental work was carried out to determine the correlation between the electrokinetic properties of the conjugates and the concentrations of the substrates used in the syntheses. The experimental work showed that the electrokinetic properties of conjugates strictly depend on the amount of CPZ adsorbed on the surface of AuNPs. The obtained experimental results proved that it is possible to obtain conjugates characterized by negative as well as positive zeta potential. Moreover, it was shown that the surface charge of conjugates can be controlled not only by the amount of adsorbed CPZ molecules, but also by ionic strength and pH. Spectroscopic studies, conducted using UVvis electron spectroscopy, as well as surface-enhanced Raman spectroscopy (SERS), confirmed the deprotonation of the tertiary amine group of CPZ, which is responsible for the inversion of the zeta potential sign of the conjugates in alkaline media at $pH > 8.5$. In addition, based on SERS studies, it was found that the orientation of CPZ molecules on the surfaces of AuNPs and therefore the structure of the conjugates depend on the size of the nanoparticles used for synthesis.

Research Projects
of the National Centre for Research and Development

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

„EIG CONCERT-JAPAN" research project NCBiR EIG CONCERT-JAPAN/1/2019 [2019-2023]
(Principle investigator: Aleksandra Pacuła D.Sc. Eng.)

The aim of the research was to evaluate photocatalytic performance of metal-TiO_2 composites obtained via deposition of metal (Pt or Ag) on commercial TiO_2. The nominal concentration of metal deposited in the form of nanoparticles (NPs) was 0.05, 0.1 and 0.5 wt. % of Pt or 0.05 and 0.1 wt. % of Ag. Metal NPs were obtained via chemical reduction of ions (Pt^{4+} or Ag^{+}) using $NaBH_4$. Metal NPs in the aqueous suspension were electrostatically stabilized with the addition of sodium citrate, therefore the measured zeta potential (ξ) in both cases was negative. The deposition of metal NPs on the surface of TiO_2 was carried out by adsorption. It is worth noting that TiO_2 grains in aqueous suspension (with a concentration of ~ 0.3 g dm^{-3}) formed agglomerates (with a diameter of ~ 200 nm) showing a positive ξ, therefore in the case of deposition of Pt and Ag NPs characterized by negative ξ, adsorption was additionally electrostatically assisted. Metal NPs had diameters in the range of 5 – 20 nm, among them those with a diameter of ~ 11 nm were dominating for Pt NPs or ~ 9 nm were dominating for Ag NPs.

Photocatalytic properties of the composites were examined in the photocatalytic degradation reaction of dyes: methyl orange (MO) and rhodamine B (RB). Tests were carried out using a UV lamp (50 W) with radiation emission in the wavelength range: 365–367.5 nm, mixing a suspension containing 15 mg of photocatalyst and 50 ml of dye solution (with a concentration of ~ 1×10^{-5} mol dm^{-3}). Before UV irradiation, degree of decolorization was checked due to adsorption of the dye on the surface of the photocatalyst during the first 2 hours of mixing the suspension in the dark. The dye concentration was spectrophotometrically monitored by recording UV–Vis spectra in the range of 350 – 650 nm for liquid samples (after separating the liquid from the photocatalyst) at each stage of the three stages of the experiment: (a) after 2 hours of mixing in the dark, (b) after exposure lasting 1 hour and (c) after exposure lasting another hour, i.e. a total of 2 hours of exposure.

Decrease in the concentration of dye in solution due to its adsorption on the surface of photocatalysts ranged from 15 to 30%. Degree of decolorization of dye solutions in the presence of TiO_2 was: 43 or 71% after 1 or 2 h of UV irradiation for MO, 53 or 85% after 1 or 2 h of UV irradiation for RB. Degree of decolorization of MO solution with the addition of Pt-TiO_2 composites containing 0.05, 0.1 and 0.5 wt. % of Pt, after 1 h of UV exposure was 49, 50 and 44%, respectively, and after 2 h of UV exposure it was 78, 82 and 72%, respectively. Degree of decolorization of RB solution with the addition of Pt-TiO_2 composites containing 0.05, 0.1 and 0.5 wt. % of Pt, after 1 h of UV irradiation was 68, 68 and 42%, respectively, and after 2 h of UV irradiation it was 96, 97 and 71%, respectively. Degree of decolorization of MO solution with the addition of Ag-TiO_2 composites containing 0.05 and 0.1 wt. % of Ag, after 1 h of UV exposure was 43 and 32%, respectively, and after 2 h of UV exposure it was 74 and 63%, respectively. Degree of decolorization of RB solution with the addition of Ag-TiO_2 containing 0.05 and 0.1 wt. % of Ag, after 1 h of UV exposure was 57 and 56%, respectively, and after 2 h of UV exposure it was 87 and 86%, respectively.

Deposition of Pt onto TiO_2 increases the efficiency of the degradation process compared to pure TiO_2. The content of metal NPs on the surface of TiO_2 is important. The recommended concentration of Pt per TiO_2 is 0.1 wt. %. Spreading Ag on TiO_2 (0.1 wt. %) does not significantly increase (in the case of RB) but even decreases (in the case of MO) the efficiency of the degradation process compared to pure TiO_2.

Technology of biorefining vegetable oils for the production of advanced composite materials

„TechMatStrateg” research project NCBiR TECHMATSTRATEG2/407507/1/NCBR/2019
[2019-2022]
(Project leader: Associate professor Maciej Guzik)

During the current reporting period, construction of the hall housing the biorefinery prototype line on the Institute's premises was completed. The new prototype enclosure consists of a laboratory and office area, as well as a technological area (Fig. 1.).

Fig. 1 The demonstration hall of the biorefinery prototype.

The project's goals of synthesizing two types of biopolymers - polyhydroxybutyrate (PHB) and medium-chain-length polyhydroxyalkanoate (mcl-PHA) into a quarter-technical scale have been achieved. This enabled a steady supply of both polyhydroxyalkanoates (PHAs) to the consortium partners. A methodology for producing polymer blends and polymer-ceramic composites was developed at the Cracow University of Technology. A partner from the AGH University of Science and Technology developed a technology for coating calcium phosphate-based 3D scaffolds with thin films based on PHA blends. This enabled pilot *in vitro* studies conducted by a partner from the Warsaw Medical University on a mesynchemal cell line. Compatibility studies showed that a promising material for bone regeneration was a ceramic matrix material coated with a thin layer of PHB. This composite showed no cytotoxicity to the tested cell line, as well as enabling optimal cell proliferation and differentiation into bone cells. In parallel, the blends produced by CUT allowed the Smart Labs partner to produce medical and non-medical test objects.

CULDIGI - Integrated digitalization, filing and documentation system for cultural heritage

„Program operacyjny Inteligentny Rozwój” research project NCBiR POIR.04.01.04-00-0145/19 [2021-2023]
(Project leader: Associate professor Jakub Barbasz, Leszek Krzemień PhD)

The main goal:
The aim of this project is development of a full surface scanner, a device that scans big art artefacts like painting drawings and documents with microscopic resolution.

In 2022, the planned work was carried out.
Optimization work was carried out on the control of camera movement - in each of the three scanning directions (X,Y and Z). Work was carried out to improve the stability of the realized motion with regard to the sharpness of the obtained images.
A working prototype was realized, allowing work on the final results of the project.

Tests of working LED lighting were also carried out, along with tests of infrared LED lighting. The first test scans of real objects (images) were also realized.
Work was also carried out on the analysis of known algorithms with functionality similar to that required for the final product.

Work continued on the implementation of systems for finding so-called "control points" or "common points" for positioning sequentially collected images in two-dimensional space, and potentially also in 3D space.

Tests on an actual prototype scanner in the so-called "natural environment" were also worked on.

Development of a functional form of application of sugar esters of fatty acids based on bacterial polyhydroxyalkanoates that inhibit cancer cell growth

„TANGO" research project NCBiR TANGO-V-A/0013/2021 [2022-2023]
(Project leader: Associate professor Maciej Guzik)

In the current reporting period, the polymer polyhydroxynonanoate (PHN) was produced by bacterial fermentation in a 30L bioreactor. The bacterial biomass was lyophilised and the polymer was extracted using ethyl acetate. PHN was then subjected to acidic methanolysis to obtain methyl esters of a mixture of monomers - (*R*)-3-hydroxynonanoate and (*R*)-3-hydroxyheptanoate. The methyl esters were subjected to modification - a fluorine modification on the 3-hydroxyl group of the monomers was introduced by acting with tri-fluoroethylsulfonyl tri-fluoromethane. Both the prepared monomers and the unmodified esters were used for the biocatalytic synthesis (by TL-IM lipase) of the sugar esters of glucose, galactose and lactose. All compounds obtained were subjected to analyses (IR, MS/MS and NMR) for structural verification. Emulsions were produced from the obtained sugar esters. The pure esters and their emulsions were subjected to *in vitro* tests on 10 cell lines (colorectal cancer lines, melanomas and appropriate controls of non-cancerous lines). The fluorine-modified PHN esters of lactose and galactose, as well as the unmodified lactose esters, were the most toxic compounds in pure form against the cancer lines. The emulsions had no cytotoxic effect against the tested lines.

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

International Scholarship Exchange of PhD Candidates and Academic Staff

Project NAWA PPI/PRO/2018/1/00006/U/001 PROM [2019-2023]
(Project coordinator: Professor Tomasz Borowski)

During 2022, the following scholarship competitions under the PROM Program were conducted and resolved:

1. Competition for scholarships to finance the costs of participation in short forms of education abroad (scientific conference, science school, scientific workshops, scientific courses, scientific training) for doctoral students of ICSC PAS (3 editions, calls from: 21 April, 13 June, 7 October),
2. Competition for scholarships to finance short-term internships in foreign research units for doctoral students of ICSC PAS (3 editions, calls from: 21 April, 13 June, 7 October),
3. Competition for scholarships to finance short-term internships at ICSC PAS for doctoral students from Ukraine (a call from 21 April).

In addition, in December 2022 a competition was opened for scholarships to finance short-term internships in foreign research units for doctoral students of ICSC PAS. This competition will be resolved in January 2023.

As a result of all the above mentioned competitions that were resolved in 2022, 13 applications were qualified for funding.

During 2022 the following short forms of education were realized within the PROM Program:

- three female and one male PhD students from IKiFP PAN took part in a scientific conference abroad;
- two female PhD students from IKiFP PAN took part in a scientific conference abroad and an associated course;
- one female PhD student from IKiFP PAN took part in a scientific conference abroad and an associated seminar;
- one female and one male PhD students from IKiFP PAN completed a research internship at a foreign institution;
- one male PhD student from abroad completed a research internship at IKiFP PAN.

“Polish Returns” Programme - CRAQUELURE

“Polish Returns” Programme NAWA
PPN/PPO/2018/1/00004/U/00001 [2018-2022]
(Project leader: Professor Łukasz Bratasz; co-investigator: Marcin Bury PhD, Łukasz Berger Eng.)

The aim of the "Polish Returns" program, and thus the CRAQUELURE project, is to create a project group conducting research on the development of a physical model of paint layers in paintings based on financial resources obtained from external sources and conducting other research in the area of cultural heritage preservation. Therefore, the fourth year of the project was devoted to the further submission of applications for research funding. During this period, several applications for external financing were submitted to:

- European Commission “DAta driven MAterials science for heritAGE– Doctoral Network” call HORIZON-MSCA-2022-DN-01,
- European Commission “GOGREEN: GREEN STRATEGIES TO CONSERVE THE PAST AND PRESERVE THE FUTURE OF CULTURAL HERITAGE, ID: 101060768” call HORIZON-WIDERA-2022-ACCESS-07— Hop On Facility,
- Getty Foundation “Further development of the HERIe platform” – proposal submitted on the invitation of the Foundation.

At the beginning of 2022, Dr. Marcin Bury, a computational physicist, joined the research team, whose task was to develop a three-dimensional model of a painting on canvas. The painting model is based on the finite element method and was developed in the COMSOL Multiphysics software. The model assumes that the painting is made of a wooden stretcher, canvas glued with animal glue and a layer of glue-based ground (gesso) - a rigid material similar in mechanical properties to ageing brittle oil paints. A new element of the research was to take into account the actual mechanical parameters of the glued linen, taking into account the directions along the weft and along the warp. In particular, directional elasticity moduli for different relative humidity and directional moisture expansion of the sized canvas were used. Extensive parametric studies were carried out to determine the sensitivity of the model to input parameters. It was found that in the corners of a painting there may be a significant stress concentration, but its range is much larger than in the case of the model that does not take into account the anisotropy of the sized canvas.

The project continued the development, testing and optimization of the HERIe conservation prevention platform. As part of the work, research was undertaken to implement modules that allow the determination of colour changes in materials sensitive to light. The research was conducted in cooperation with the Canadian Conservation Institute and consisted of the synthesis of available reflectance spectra obtained for 108 samples of historically produced synthetic dyes, which were exposed to several dozen different light doses produced by a LED type source. A data visualization method has been developed so that the user can estimate the colour change of the test sample depending on the amount of colour remaining (depending on the light intensity, number of hours, number of days and years of past exposure, and the number of photos taken with the flash) and the predicted colour change for future exposure. In addition, HERIe continued to be extensively tested and optimized, and identified problems found will be fixed in the years to come.

Work has also begun on the integration of COMSOL Multiphysics finite element software with the HERIe platform, which will be continued as part of a pre-approved project with the Getty Foundation.

Structure and Function of Protein Corona at the Nanoparticles Interface

Project NAWA Canaletto PPN/BIT/2021/1/00089/U/00001 [2022]
(Project leader: Professor Barbara Jachimska; co-investigator: Magdalena Szota MSc Eng.)

Due to the hyperbranched structure of the poly(amidoamine) dendrimers (PAMAM) molecules, they form complexes with active ingredients in two ways. By binding the drug inside the structures or on their surface. The location of the drug molecules has a significant impact on the kinetics of the active substance release and the mechanism of internalization of the carrier into the cell. The study monitored the effect of the degree of ionization of the G4PAMAM dendrimer molecule and the anticancer drug 5-fluorouracil (5FU) on the efficiency of complex formation. Using the SAXS and DLS method, the size of the carrier was controlled, and a decrease in the size of the dendrimer particle radius was shown with a change in the protonation degree of the system in alkaline conditions. With the decrease in protonation, the dendrimer structures observed at high pH values are gradually closed and thus hinder the migration of 5FU molecules to the inside of the support structure, thus favoring the immobilization of the drug on the surface. 1H NMR and DOSY spectra confirm that electrostatic interactions determine the process of complex formation. The most favorable conditions for forming the G4PAMAM-5FU complex are the low degree of protonation of the dendrimer molecule with the simultaneous presence of the drug in a deprotonated form. The stability of the complex is confirmed by the fluorine components in the XPS spectra. Using MD simulations, the localization profile and the number of 5FU molecules forming a complex at the atomic scale were visualized.

Dynamic nanostructures at moving liquid/gas and liquid/liquid interfaces

Project NAWA PPN/BEK/2020/1/00025 [2021-2022]
(Project leader: Associate professor Jan Zawała)

In the scholarship framework a 8-months stay at the Department of Chemical Engineering, Stanford University, USA, were performed. The main goal of the project was to use Dynamic Fluid-Film Interferometry (DFI) technique, elaborated at the Stanford University, to examine an influence of dynamic nanostructures induced at a rising air bubble surface in solutions of simple surfactants and proteins on dynamics of coalescence and stability of foam films. To fulfill planned research tasks, the original DFI set-up had been modified in order to observe dynamics of drainage of foam films formed by an air bubble freely rising in a liquid column and colliding with a solution/air interface. The experiments were aimed at qualitative determination of the bubble coalescence distributions and qualitative (direct) measurements of evolution of the foam film thickness in time. To determine an influence of motion-induced surface nanostructures at the rising bubble interface, two liquid column of different length (short and long) were used, allowing observations of foam film drainage at different distances covered by the bubble in solutions of surface-active substances (1 cm and 40 cm, respectively). It was shown that in particular surfactant concentration range, the foam film drainage was much faster in long column comparing to short, i.e. when the dynamic nanostructures (dynamic adsorption layer) were assumed to be present at the floating bubble interface. Moreover, it was proved that this phenomenon depends on values of relative humidity of the ambient atmosphere. In addition it was shown that this effect is of completely different character in solutions of proteins, where opposite trend of coalescence distributions and foam films drainage dynamics was revealed. The observed opposite effect was related to different structure of dynamic adsorption layer in protein solutions, related to formation of viscoelastic (rheological) structure at the rising bubble surface and top solution surface.

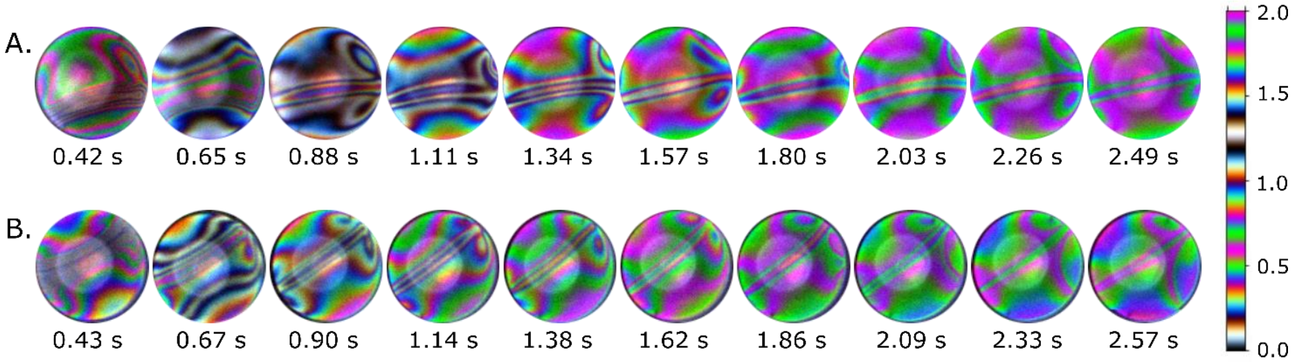


Fig. 1. Photos of single foam film formed after an air bubble collision at liquid/air interface in (A) short column and (B) long column, taken using DFI technique. Colors evolution indicates different thickness of a thin liquid layer separating two interacting liquid/gas interfaces (color scale on the right indicates the thickness given in microns)

Horizon Programme

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

Horizon 2020 Programme no. 814624 [2019-2022]
(Project leader: Professor Łukasz Bratasz; co-investigator: Arkadiusz Janas PhD, Sergii Antropov PhD, Katarzyna Poznańska MSc Eng.)

As part of the research task, research started in previous years was continued, aimed at determining the mechanical properties of several samples of 30-year-old oil paint from the reference collection of Dr. Marion Mecklenburg from the Smithsonian Institution. The measurements were made using a universal testing machine and using optical methods in climatic chambers at a temperature of 22°C and relative humidity of 50%. The aim of the research was to extend the previously conducted research, which allowed to determine the elasticity modulus, strain at break and shrinkage resulting from the chemical changes of paints. Comparison of the data obtained with the data collected by dr. Mecklenburg made it possible to determine the evolution of mechanical properties and, on this basis, formulate a hypothesis regarding the mechanism of crack formation in paintings. The results of the elasticity modulus of oil paints were separated into two groups of paints based on the development of their stiffness. The first group consists of paints which systematically increase their stiffness as a result of continuous cross-linking processes. The second are paints that are characterized by small stiffness modules during the first dozen or so years of natural ageing, and which significantly increase after this dormant period. The first group includes lead white, lead white with litharge, zinc white, verdigris and sap green. The second group includes red iron oxide, malachite and synthetic ultramarine.

Experiments in the testing machine also made it possible to determine the strain at break and to compare the obtained results with the previous one. It has been shown that chemical transformations in oil paints lead to a significant increase in the brittleness of all paints, i.e. to a decrease in their strain at break. Poisson's ratios were also independently determined for most of the tested samples.

In addition, the annealing shrinkage of oil paints was determined based on the ASTM D6911-17E standard adapted to the requirements of the experiment. It has been found that this shrinkage can vary from unmeasurable values for red iron oxide and synthetic ultramarine up to 1.7% for verdigris, and thus can significantly exceed the strain at break values.

This allowed for the formulation of a hypothesis concerning one of the mechanisms of crack formation in paintings, which is the result of chemical changes in the oil binder depending on the type of metal ion derived from the pigment used. Competing hydrolysis and cross-linking processes also lead to the formation of small molecules that evaporate from the material and thus cause shrinkage of the material. When the shrinkage value exceeds the critical values, the material cracks. This hypothesis was positively verified with images obtained for a 17th-century fragment of a Flemish panel painting using CT imaging, which showed the existence of an isotropic pattern of cracks in the paint layer on an uncracked ground layer. Thus, the cracks could not have arisen as a result of the metric response of the wooden support to changes in relative humidity.

From a broader perspective, the conducted research allowed to obtain basic data for the development of a computer model of the image and verification of its predictions.

IPERION HS - Integrating platforms for the European Research Infrastructure on heritage science

Horizon 2020 Programme no. 871034 [2020-2023]
(Project leader: Professor Łukasz Bratasz, Marcin Strojecki PhD, Marcin Bury PhD, Magdalena Soboń MSc Eng., Łukasz Berger Eng.)

The goal of the IPERION HS project is to establish and operate a distributed pan-European research infrastructure, strengthening key national research facilities of recognised excellence in heritage science. Heritage science is a young and cross-cutting scientific domain embracing a wide range of research disciplines enabling a deeper understanding of the past and improved care for the future of heritage. Since 2016, heritage science is included in the European Strategy Forum on Research Infrastructures Roadmap as one of the strategic areas in the domain of Social Sciences and Humanities, where it is represented by the European Research Infrastructure for Heritage Science. IPERION HS will provide the advanced community of heritage science a further level of pan-European integration, in view of the establishment of European Research Infrastructure for Heritage Science. The project is a further step towards a unified scientific approach to the most advanced European instruments for the analysis, interpretation, preservation, documentation and management of heritage objects in the fields of art history, conservation, archaeology and palaeontology. The IPERION HS project will be offering not only trans-national access to a wide range of high-level scientific instruments and methodologies, but for the first time, it will focus on the needs of preventive conservation in museums and other memory institutions. One of the key actions will be the development of the Preventive Conservation Digital Platform (PCDP) enabling users to model and plan future risk scenarios and adjust preventive conservation measures accordingly. The PCDP will be the first step for the heritage science community to actively support the European Open Science Cloud initiative to build a competitive data and knowledge economy in Europe.

In 2022, the project continued to thoroughly test and optimize the decision-supporting tools for practitioners, curators and object owners developed in previous years. In particular, the tests covered tools for assessing the risk of mechanical damage in panel paintings and wooden objects restrained by rigid construction. They showed the imperfection of the approach based on the Fast Fourier Transform, especially for massive objects with a thickness of 20-40 mm subjected to changes in relative humidity at low temperatures. Errors appearing in the initial and final phase of strain histories result from the necessity to make assumptions regarding the periodicity of the climatic data, i.e. knowledge of the state of the system at time 0. This makes it necessary to artificially reconstruct climatic data in the period ahead of the data uploaded by the user. The currently adopted algorithms turned out to be insufficiently universal to properly reconstruct all data uploaded by users. The work is being continued to improve the existing algorithms to increase the accuracy of the calculations.

Work has also been undertaken on integrating COMSOL Multiphysics finite element modelling software with the HERIe platform. Java communication was pre-tested with several versions of sample files generated by COMSOL's "application compiler". The work will be continued as part of the agreed project with the Getty Foundation.

NanoPaInt - Dynamics of dense nanosuspensions: a pathway to novel functional materials (nanoPaInt)

Project H2020-MSCA-ITN-2020 nr 955612 [2021-2024]
(Project leader: Professor Piotr Warszyński)

Nanoparticles serve as additives for controlling the liquid properties; used extensively in food industry and home care products. Found in ink, paint formulations and coatings, they are used for manufacturing of functional materials and are promising drug carriers. The presence of nanoparticles in air or in water can influence the Earth ecology and human health. Although dilute suspensions of nanoparticles which are close to equilibrium have been extensively studied, in many important applications, such as food industry and printing, the suspensions are dense, or strongly interacting, they experience significant external stress and are far from equilibrium. A fundamental understanding of interaction between nanoparticles in liquid bulk and at interfaces, allowing to effectively use particulate systems in these applications, has not yet been achieved. NanoPaInt network aims to understand, predict, model and apply the strongly interacting nanoparticle-laden systems out of equilibrium. The specific objectives are: understanding strong interactions between the nanoparticles and their effect on the rheological properties of suspensions; understanding the behavior of nanoparticles in complex interfacial flows under strong external forces and with wetting and dewetting; design and applications of smart capillary nanosuspensions; drying of nanosuspensions towards assembly and fabrication of functional superparticulate systems. The training aim of nanoPaInt network is to support the career development of young researchers both in academic and non-academic sectors and to train a new generation of creative, mobile, entrepreneurial and innovative early-stage researchers (ESRs) through the experience of independent and interdisciplinary research, participation at local and network-wide training activities and secondments. In the network there are 15 participants representing European research institutions and industrial laboratories.

ESR 13, based at the Institute of Catalysis and Surface Chemistry in Krakow, conducts research on core@shell nanomaterials as the main components of inks for the production of conductive tracks or coatings. In particular, he focuses on the creation of nanowires with core@shell structure for copper@silver stable against oxidation process in atmosphere. During the work carried out in 2022, the focus was on optimizing the synthesis process of this type of nanostructures with the desired physicochemical properties. In the first stage of the research, copper nanowires were obtained as the core of the "core@shell" structures, with a diameter of 50-150 nm and a length of 10-50 µm, which was confirmed using CONFOCAL and SEM microscopy (Fig. 1a) and the XPS method. A silver sheath was formed on the obtained nanowires as a result of the transmetalation reaction (Fig. 1b). Copper and copper-silver nanowires ("core@shell") were used in combination with stabilizing, dispersing and wetting agents to produce inks, which in the next year of the project will be used in the process of obtaining conductive materials.

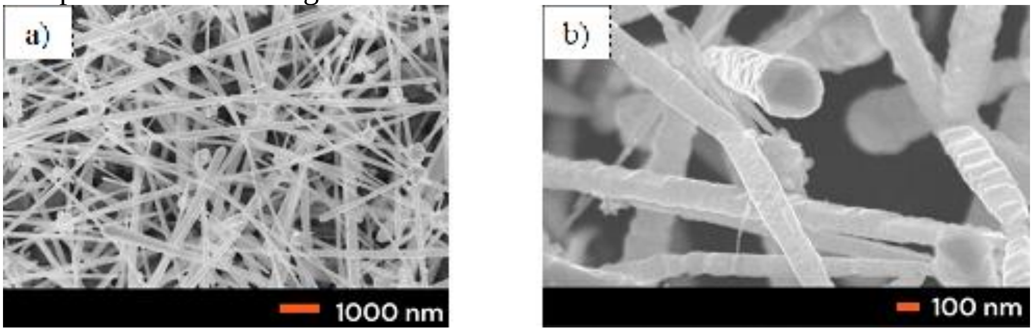


Figure 1. SEM images: a) copper nanowires, b) copper@silver nanowires

NewCat - Teaching Lytic Polysaccharide Monooxygenases to do Cytochrome P450 Catalysis

Horizon Europe EIC-PATHFINDER nr 101046815 [2022-2025]
(Project leader: Professor Tomasz Borowski, Anna Miłaczewska-Kręgiel PhD, Anna Kluza PhD, Zuzanna Wojdyła PhD)

EU Green Deal strategy and Climate Mitigation mission require the application of radically new technologies non existing yet on the market. The EIC Pathfinder call goes beyond what is already known and welcomes the high-risk/high gain and interdisciplinary cutting-edge science collaborations that underpin technological breakthroughs.

The NewCat project was inspired by nature and aims to provide a universal platform facilitating organic synthesis via clean, organic solvent-free and low energy consumption processes. The required knowledge is granted by a combination of the state-of-the-art experimental and computational methods in the area of protein engineering, enzyme catalysis, nanomaterials and large-scale protein production delivered by partners of the consortium.

The project is realized by a consotrium of four partners: Norwegian University of Life Science, Norway, a coordinator, Technical University Graz, Austria, ICSC PAS, Poland and Silesian University of Technology, Poland. The team at ICSC PAS contributes to the project its expertise in the field of computational studies on metalloenzymes as well as protein crystallography.

EU COST Actions

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

EU COST Action no 18234 [2019-2023]
(country coordinator: Professor Małgorzata Witko)

This COST Action intends to focus on bridging the knowledge gaps between different theoretical methods (DFT, molecular mechanics and molecular dynamics, Monte-Carlo dynamics, microkinetic modelling) and computer codes in order to facilitate the modelling of all processes which accompany water splitting, in a longer term enabling the discovery of novel electrocatalytic materials.

During the last year our work was focused on the quantum-chemical description of processes occurring in vanadium batteries. They comprise of the system in which the reversible water splitting process occurs on the cathode:

$$VO^{2+} + H_2O - e^- \rightleftarrows VO_2^+ + 2H^+$$

whereas vanadium(III) ions are reduced on the anode:

$$V^{3+} + e^- \rightleftarrows V^{2+}$$

The process was modelled employing Density Functional Theory (DFT) method, with PBE functional and def2-TZVP basis set. The optimum geometries of the vanadium complexes were determined which can be identified during the abovementioned electrode processes. Two cases were considered: with chloride ions and with sulfate ions (of different protonation states). The electronic structures of the complexes were also described.

Projects of EU Structural Funds Programmes

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

EU Structural Funds project NCBR POWR.03.02.00-00.I013/16 [2017-2023]
(project leader: Associate Professor Renata Tokarz-Sobieraj)

The aim of the project is to carry out 45 interdisciplinary doctoral dissertations (hard indicator of the project), in topics located on the border of exact sciences and medical sciences: (1) multidisciplinary - each doctoral student works under the supervision of two supervisors representing different disciplines, (2) transdisciplinary - obligatory and common for all doctoral students, seminars and lectures are a place to search for new, innovative methodological solutions, combining and complementing specialized research methods of various disciplines of science; (3) cross-disciplinarity - problems of one field are solved using methods typical for another field, which leads to synergy effects.

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

In 2022, 43 doctoral students continued their doctoral studies. The education program was completed, the doctoral students of the InterDokMed Environmental Doctoral Studies carried out only scientific research, under the supervision of two tutors, from two different partner units, representing different scientific disciplines.

In the academic year 2021/2022, InterDokMed doctoral students completed 17 scientific internships, participated in 22 courses/workshops, thematically related to the work carried out. They were co-authors of 47 publications from the JCR list, 2 chapters in books, 2 popular science articles. In addition, they co-authored 29 oral presentations and 37 posters presented at national and international conferences. InterDokMed PhD students won three awards for the best conference presentations, four people received additional scholarships, two people became laureates of scientific competitions.

22 people graduated after the fifth year. In 2022, 18 people defended their doctoral dissertation, 9 doctoral dissertations were awarded.

In total, by the end of 2022, out of 50 candidates admitted to doctoral studies, 28 people completed the education program, 24 people obtained a doctoral degree, 14 doctoral dissertations were awarded.

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

Fundusze Europejskie
Wiedza Edukacja Rozwój

Unia Europejska
Europejski Fundusz Społeczny

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

Project of Operational Program Knowledge Education Development (POWER) for interdisciplinary doctoral study programs POWR.03.02.00-00-I004/16 [2017-2023]
(deputy project manager: Anna Micek-Ilnicka DSc. Eng.)

The project is financed by the Foundation for the Development of the Education System and is a result of a competition announced by The National Centre for Research and Development (NCBiR) in 2017 for interdisciplinary doctoral studies programs. By the decision of NCBiR, the FCB project was extended until October 31, 2023. This project is coordinated by the Faculty of Physics and Applied Computer Science (WFiIS) at the AGH University of Science and Technology (AGH) with the participation of four institutions; the Faculty of Materials Science and Ceramics (WIMiC) at the AGH, the Faculty of Chemistry at Jagiellonian University (WCh UJ), The Henryk Niewodniczański Institute of Nuclear Physics (IFJ) of the Polish Academy of Sciences and Jerzy Haber Institute of Catalysis and Surface Chemistry of the Polish Academy of Sciences (IKiFP).

FCB doctoral studies are interdisciplinary. The interdisciplinary nature of FCB doctoral studies results from the participation of doctoral students in lectures and seminars in natural sciences in the discipline of chemical sciences and physical sciences. The assumptions of this project envisaged the education of 75 PhD students, currently 72 PhD students are studying under the project, including 3 students studying at IKiFP PAN.

Until September 2022, 11 defenses of FCB PhD students took place (2 defenses from WFiIS AGH, 4 defenses from WIMiC AGH and 4 from WCh UJ and 1 defense from IFJ AGH). The promotion proceedings for the award of a doctoral degree to 3 FCB PhD students from the IKiFP PAN have been initiated. The planned deadline for submitting their doctoral dissertations is the first quarter of 2023.

The scientific achievements of FCB PhD students from IKiFP PAN (3 people) in the academic year 2021/2022 include: 4 publications in the journals: *ACS Catalysis, Molecules, Applied Sciences, Nanomaterials*, 1 patent application EP22164459 and 4 oral presentations.

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Other International Research Projects

Biocompatible foams and emulsions stabilized by natural surfactants and particles for bio-medical application

Joint research project of the Polish Academy of Sciences and CNR National Research Council of Italy [2020-2022]
(project leaders: Marcel Krzan PhD)

During the project, we developed technology for the generation of stable foam and emulsions formulated from mixtures of hypoallergenic, non-toxic biological polymers, mainly "green surfactants". Such surface thin film could soon become the basis of the cosmetic and dermatological composition for multiple-use products. The possibility of introducing other biologically active compounds, such as chitosan, to the interfacial layer was also thoroughly investigated. A similar multicomponent film layer would be the first line of the skin body's defense against various pathogens. The skin will be simultaneously protected, lubricated, and regenerated thanks to them. Similar thin surface film (created from our foams or emulsions) could also be used as natural preservatives as a pathogen barrier in the food industry. The same technology of introducing additional ingredients into the surface layer can be used in agricultural production in natural green pesticides, in flotation processes and during the removal of waste nanoparticles from the aquatic environment.

During the last year of the project, we studied the mutual interaction between saponin and chitosan. It has been proven, using Sum Frequency Generation IR experimental technique and measurements of surface activity and elasticity, that saponin and chitosan interact with each other in solution, creating complex systems, thanks to which the inactive surface chitosan adsorbs to a significant excess in the surface layer, dramatically changing its flexibility and surface stability.

Moreover, we studied the model system of hydrophilic negatively charged silica (Levasil) nanoparticles. In this case, we have developed a method of changing their surface potential and hydrophobization using a biodegradable and safe surfactant lauroyl ethyl arginate, and biopolymer/polycations chitosan. Changing the surface charge and hydrophobization were necessary to remove particles from the water phase in the foaming process. We have proved that in this way, it is possible to safely clean the aquatic environment from impurities in the form of hydrophilic nanoparticles.

Project funds also allowed for two one-week research internships in Genoa for Dr. Marcel Krzan and Dr. Ewelina Jarek.

Biocompatible particle-stabilized foams and emulsions for biomedical applications

Joint research project of the Polish Academy of Sciences and the Bulgarian Academy of Sciences cooperation [2018-2021]
(project leader: Marcel Krzan PhD)

The project aims to develop a technology for producing and applying stable liquid foams and emulsions based on mixtures of hypoallergenic, non-toxic biological polymers, mainly the so-called biosurfactants. Furthermore, we want to enrich the developed formulations with additional nanoparticles, e.g. metallic silver or other nanoparticles of natural origin (e.g. chitosan, silk fibroin or liposomes). Surface films based on such naturally bioactive compositions would be the first line of defence against various pathogens.

During the project, measurements of dynamic and equilibrium surface activity and surface elasticity in solutions of one of the so-called "Green surfactants," i.e. n-dodecyl-ethyl-arginate enriched with colloidal silica nanoparticles (with different particle sizes - 6, 9 and 30nm). Based on the obtained results, the concentration ranges of the tested substances and mixtures necessary for forming a stable foam film were determined, which was verified by tests in an apparatus for measuring foam stability.

It was also found that the presence of particles influences the stability of surface layers made from n-dodecyl-ethyl-arginate much more than for films generated from BSA or methylcellulose. Particles with a diameter below 10 nm significantly increased the durability and height of the LAE foam. Moreover, the obtained results describing the initial heights of the foams were in good agreement with the data obtained from measurements of changes in dynamic surface tension. The obtained foam-forming and foam-stability tests showed that the foam-formation is closely correlated with the surface adsorption processes of the tested bio-surfactants and hybrid systems containing biosurfactants and solid particles.

The results obtained in the project were presented during scientific conferences and will be reported in a joint publication in the ISI / JCR journal in 2022. It was also used to prepare one review chapter in a scientific book.

Project “Learning mobility of individuals”

EU Erasmus+ Programme [2014-2022]
(coordinator of the project: Aleksandra Pacuła DSc Eng.)

In 2022, the following learning mobilities took place:

a) students for traineeships

- Valerii Lutsyk – The Biomedical Research Foundation of the Academy of Athens (BRFAA) (Greece)
- Patrycja Gnacek – University of Szeged (Hungary)

b) staff members for training

- Maciej Nowak – University of Belgrade (Serbia)
- Magdalena Oćwieja – University of Malaga (Spain)
- Magdalena Soboń – TU/e Eindhoven University of Technology (Holland)
- Mateusz Tataruch – Slovak University of Technology in Bratislava (Slovakia)

The participation in the mobilities resulted in the collection of research data for PhD thesis, articles in scientific journals of international scope and for conference contributions.

The project will continue in 2023 and another application for funding will be submitted for the 2023/2024 academic year.

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