FINAL SCIENTIFIC REPORT

Synthesis, structural and thermodynamic characterization of nanohybrid systems at solid-liquid interfaces (K 116323)

Within the framework of the research project (K 116323) in the period of 2015-2018 (40 months) the design, synthesis and structural characterization of different type of nanohybrid systems have been successfully carried out; the synthesized systems have been efficiently used for the development of optical biosensors and nanosized targeted drug delivery as well as controlled drug release systems. The main results of the researches have been published in 20 reputed journals (*e.g. J. Phys. Chem B., Coll. Surf. B, Carbohydrate Polymers, ChemComm, Talanta, Int. J. Biol. Macrom., Eur. J. Pharm. Sci. etc.)* and a patent (P1500356) is also submitted. The planned research work was summarized in 6 different work packages. The experimental results confirmed by the publications are presented in accordance with the work packages.

1. Thermodynamic characterization of molecular and supramolecular interactions by using two-dimensional sensor techniques and microcalorimetric investigations

Main research concept of our project was to optimize the syntheses of different type of drug-containing nanocomposites by studying the interaction between the potential carrier and the drug molecules as well as to investigate the interactions between model receptors and their potential ligands by using two-dimensional techniques, like surface plasmon resonance (SPR), quartz crystal microbalance (QCM) or optical waveguide lightmode spectroscopy (OWLS) and microcalorimetric (ITC) studies. We characterized in detail the bovine serum albumin (BSA)-based kynurenic acid- (***Eur. J. Pharm. Sci, 2016***) and ibuprofen-containing (***Coll. Surf. A, 504/2016***) nanocomposites, where the type of the interactions as well the thermodynamic binding constant, the state functions and also the stoichiometry of the interaction were determined. Moreover, the micellization of a binary nonionic surfactant system at different compositions and temperatures in aqueous medium were also studied by ITC. The results may contribute to the development of micelle-based drug delivery systems (***J. Surf. and Deterg., 2017*).** In addition to nanocomposites, for design of gold-containing nanohybrid systems the binding ability as well as the orientation of the applied stabilizing biomolecules on gold surface was also studied in detail by SPR and QCM (***Coll. Surf. A, 511/2016; Optical Mat. Exp., 2017***). In collaboration with the research group of Mária Csete (University of Szeged, Department of Optics and Quantum Electronics) numerical calculations were also performed to reproduce the measured SPR results originating from different bio-coverings. For the investigation of relevant receptor(protein)-ligand systems the reversible bindings of neuroprotective kynurenic acid on human glutamate receptor polypeptide- and serum protein-modified gold surfaces have been studied at various temperatures under physiological conditions. The studied polypeptides were synthesized in the group of Prof. Gábor Tóth (University of Szeged, Department of Medical Chemistry). The registered SPR sensorgrams were fitted by using different kinetic models without application of any commercial softwares. Relevant kinetic and thermodynamic data of the above mentioned biomolecular systems were presented in three publications (***J. Phys. Chem. B, 2016***; ***Period. Polytech., 2017***; ***Microchem. Journal, 2019***, ***under review after minor revision***).

2. Characterization of molecular and supramolecular interactions at solid-liquid interfaces by using RIfS and PRIfS self-developed technique.

The self-developed measuring apparatus was built during the project, but we were only able to study the adsorption of different gases and vapors on the surface of self-assembled metal oxide –polymer nanohybrid surfaces. The prepared films on glass and silicon wafer support in flow liquid medium have stability problem. Despite many attempts, only the development of a gas sensor was successful (Sebők Dániel, PD 116224 (2015-2019)).

3. Preparation and characterization of core-shell type nanocomposites for delivery of drug agents across the BBB (protein-based composites).

We successfully fabricated core-shell type nanoparticles (CSNPs) for controlled drug release as well as for delivery of drug agents across the blood-brain barrier (BBB). In order to execute the controlled and targeted drug delivery several inorganic and organic-based nanosized composites were synthesized in the research group. By changing the experimental conditions of the syntheses, the formation of core-shell composites was carried out at room temperature via the formation of simple electrostatic interactions between the components. Biocompatible and biodegradable BSA protein was used as carrier for the encapsulation of ibuprofen and kynurenic acid. According to our development, a BSA-based one-layered CSNPs containing kynurenic acid was prepared, which is able to penetrate the drug across the BBB confirmed by *in vitro* BBB model membrane test as well as by *in vivo* animal tests (***Eur. J. Pharm. Sci, 2016)***. The research work is under patent. The syntheses of human serum albumin-based analogous composites have also been started, where one- and two polyelectrolyte or polymer (PEI, PSS, chitosan, PAH) shells are applied. The size, the size distribution as well as the structure of the different particles were characterized by TEM, DLS, CD and FT-IR studies; the systems are currently studied by *in vitro* model BBB tests. In case of BSA/ibuprofen system the CSNPs were prepared at pH 3.0 for pH-induced controlled drug release and kinetics of the drug release process at pH 7.4 was studied in *in vitro* dissolution experiments. The size and the structure of the nanosized particles was confirmed by SAXS as well (***Coll. Surf. A, 504/2016)***.

4. Preparation and structural characterization of core-shell type nanocarrier composite systems for controlled drug release (polymer-based composites).

Beyond the proteins, the possible utilization of biocompatible and biodegradable polymers and inorganic materials as potential drug carriers has also been investigated. On one hand, the preparation, structural characterization, and the kinetics of the drug release of modified hyaluronic acid (HyA)-based colloidal drug release systems which contain hydrophobic ketoprofen (KP) as model molecule was published (***Carbohydrate Polymers, 2018***). Due to modifications the coherent structure of HyA changed into an incoherent colloidal system that were verified by rheological investigations. We provided important information on the structure-dependent drug dissolution profiles. It has been verified that the modified HyA may be a potential candidate for controlled drug release of hydrophobic KP molecules. In case of polymer-based composites the effect of copolymerization of Polylacticacid (PLA) with glycolic acid on the encapsulation efficiency (EE (%)) of drugs with considerably different hydrophilicity were also studied (***Coll. Surf. B, 2019***). PLA and Poly(lactide-co-glycolide) (PLGA)co-polymers having 65% (PLGA65) and 75% (PLGA75%) PLA content have been

synthesized and characterized. We confirmed that our polymers have narrower weight distribution than the commercially available which is important in solubility properties to obtain nanoparticles with better size distribution. The polymer NPs were prepared by nanoprecipitation using Pluronic F127, CTAB and PVA stabilizing agents. It was established that the applied solvent and stabilizing agent play a decisive role in the size distribution and stability of the drug carrier NPs. We have clearly pointed out that by systematically altering the hydrophilicity of the drug carrier NPs, the EE (%) could be remarkably controlled. Moreover, the intercalation of kynurenic acid into biocompatible Mg/Al LDH lamellaeas also been carried out with simply ion- exchange reaction ***(Appl. Clay Sci., 2018)***. Structural studied have demonstrated that the kynurenic acid molecules prefer creating a paraffin type monolayer arrangement. According to the experimental results, the drug-loading capacity was about 120 mg KYNA/ g LDH. Our experimental data confirm that the anti- ulcerant drug can be safely loaded and stored into LDH's layers forming a new bio-active hybrid material. According to the dissolution studies, 18% of the loaded drug were released during 6 h. Partly related to the research project a new type of organic conducting polymer-based film was developed which exhibits both superhydrophobicity and visible light photoactivity (***ChemComm, 2018***), but a polymer-based photoreactive composite coating with composition dependent wetting features was also fabricated (***Exp. Polymer Lett., 2018***).

5. Preparation and characterization of functionalized metal/metal oxide nanoparticles and gold nanoclusters for labelling of nanocomposites

In this work package the synthesis and structural characterization of amino acid-, peptide- and protein-stabilized new type of gold nanostructures have been carried out. We applied lysozyme (***Coll. and Polymer Sci., 2016***), thiol-containing *L*-cysteine, *L*-cysteinyl-tryptophan, *L*-glutathione (***Coll. Surf. A., 511/2016***), *N*-donor side chain-containing *L*-histidine (His) and *L*-tryptophan (***Coll. Surf. A., 2017***), nucleotide (***Coll. Surf. B, 2017***) and gamma-globulin (***Sensors and Act. B, 2019***, ***under review after minor revision***) as stabilizing and reducing agents for preparation of gold nanostructured materials at mild conditions. The dominant role of $AuCl_4^-$/biomolecule molar ratios as well as the pH on the formation of final gold nano-objects was confirmed by numerous spectroscopic techniques. The blue-emitting gold/His nanohybrid system as potential bioimaging agent was successfully used to visualize fluorescently albumin- and chitosan-based carrier composite particles (***Coll. Surf. A., 2017***). We demonstrated a one-step synthesis of fluorescent AMP-Au nanoclusters (NCs) which have been utilized to develop a selective sensor for the detection of Fe^{3+} ions in aqueous medium based on fluorescence quenching. Based on the determined limit of detection (LOD = 2.0 μM) our system is capable of detecting Fe^{3+} ions in drinking water. (***Coll. Surf. B, 2017***). We first presented a simple "green" preparation procedure of red-emitting gold NCs using only γ-globulin (γG). The γG-Au NCs having high kinetic stability at physiological conditions are potent candidate for rapid detection of *L*-kynurenine (Kyn) which is a dominant molecule of the kynurenine pathway. Sensing of Kyn has been carried out in phosphate buffer solution and in artificial cerebrospinal fluid with the calculated LOD of 15 and 22 μM. Moreover, a paper-based sensor technology was also successfully developed for rapid detection of Kyn with LOD of 5 μM (***Sensors and Act. B, 2019 under review after minor revision).*** Furthermore, we successfully used the single particle ICP-MS method in order to determine the size and the

composition of spherical and rod-like monometallic Au NPs and spherical bimetallic Au/Ag NPs with less than 10% relative inaccuracy and better than 3% precision compared to the other three methods. The analysis is fast and only requires the usual standard colloids for size calibration. (***J. Anal. Atomic Spectr., 2017; Talanta, 2018).*** In cooperation with the research group of Ernő Kuzzmann and Károly Lázár ^{197}Au Mössbauer spectroscopic technique is also used for structural characterization of gold NPs. This technique is quite novel for structural studying Au NPs (***Coll. Surf A. 504/2016***). In collaboration with Matthias Kling's research team, we have visited to Munich 3 times, and the results of the joint work (study of the surface and electronic structure of gold NPs by XUV radiation) were presented at the ICEL-2017 Conference.

6. Fluorescence and CD spectroscopy investigations. Study of the adhesion between the nanocomposites and the target particles/cells.

We have acquired a new circular dichroism (CD) instrument with the financial support of the project. The binding of drug agent to the protein sometimes results in a change in the secondary structure of the protein which can be followed by CD and FT-IR as well as fluorescence studies. In the literature there is not any work which studies the conformation changes of serum proteins with these basic physical-chemical methods at wide pH range (pH 2-12) in the absence and in the presence of salt, in aqueous solution. We confirmed that the increase in the pH resulted in the increase in the size of the BSA protein. We also found that the BSA is in monomer form in the absence of salt while the dimer form ratio significantly increases in the presence of 150 mM NaCl. We confirmed that the BSA in E-form can bind more hydrophobic molecules which is coupled with higher binding affinity than in N-form (***Int. J. Biol. Macrom., 2016***). Similar to BSA the structural changes in the lysozyme for lysozyme –directed gold nanohybrid systems is also studied by FTIR, SAXS and fluorescence studies which provided important structural information on the nanohybrid structure (***Coll. and Polymer Sci., 2016)***. In case of the adhesion studies between nanocomposites and target cells the interaction of Ag NPs/TiO_2 composite with bacteria have been studied. The existence of electrostatic interactions between the negatively charged bacteria (from -0.89 to -3.19 μeq/10^9 cfu) and positively charged photocatalyst hybrid particles (in the range of +0.38 and +12.3 meq/100 g) was also proven by charge titration measurements. The surface inactivation of the bacteria and the photocatalytic degradation of the cell wall component were also confirmed by fluorescence and TEM observations (***Appl. Surf. Sci, 2016***).

In conclusion, we have successfully completed the planned research work which is confirmed by the publications. With the financial support of the project, a new CD instrument with several accessories was purchased. The scientific results were presented at several international (29th ECIS 2015, Bordeaux, France; 6th ICC 2016 Berlin, Germany; CTB2016, Brno, Czech Republic; 30th ECIS 2016 Rome, Italy; Nanomed-2016 Dubai, UAE; BITE-2017 Riva del Garda, Italy; 7th ICC 2017 Barcelona-Sitges, Spain; 31th ECIS 2017, Madrid, Spain; 9th Global Chemistry Congress 2018, Lisbon, Portugal; 17th Int. Meeting on Chemical Sensors 2018, Vienna, Austria; 11th CCC 2018, Eger, Hungary; NANOCON2018, Brno, Czech Republic) and hungarian conferences (Tavaszi Szél Konferencia 2016, 2017, 2018, MTA Komplexkémiai, MTA Kolloidkémiai Munkabizottsági Ülések 2016-2018) in the form of 18 oral presentations (10 english, 8 hungarian) and 11 posters.