mgr Elżbieta Gumieniczek-Chłopek **Kraków, 18.07.2022**
Faculty of Physics and Applied Computer Science of the AGH UST
Department of Solid State Physics

Abstract of the doctoral thesis
Polymer nanocapsules with a magnetic core for biomedical applications

The aim of this work was to obtain and characterize magnetically controllable polymeric carriers in the form of capsules with oil cores stabilized by modified polymers of natural origin. According to the research hypothesis, these carriers are designed to introduce the encapsulated substance into the cell and then enabling its release. The entire process should be magnetically controlled. Two types of carriers were developed, with a negative and with a positive surface charge, which contained encapsulated magnetic nanoparticles inside. The investigated structures are dedicated for biomedical applications as systems being capable of targeted and controlled transport and release of hydrophobic biologically active substances. The therapies which provide carriers that will enable precise and targeted therapeutics delivery consist an innovative and very promising approach, especially in the treatment of neoplastic diseases. The main advantages of this form of treatment include the ability to maintain an appropriate concentration of the active substance at the target site leading to the death of diseased cells, without damaging healthy cells, i.e. without causing additional side effects of the therapy. The first stage of the research was to obtain and study the substances needed to create biopolymer capsules. Due to the dedicated biomedical use of the carrier, syntheses were carried out involving the modification of a natural origin polymer and reactions leading to the production of magnetic iron oxide nanoparticles.

A two-stage modification of chitosan was performed, involving the addition of cationic groups and hydrophobic aliphatic chains. The compound obtained, in the subsequent stages of the work, was utilized to stabilize the oil capsule cores through the phenomenon of micellar solubilization. The degree of substitution of the chitosan derivative with cationic groups, determined on the basis of conductometric titration, was 63%, while the degree of substitution with hydrophobic groups, determined on the basis of NMR spectroscopy, was 3%. Then an anionic modification of the chitosan derivative was performed.

The reaction product obtained was used to develop a second type of capsules with a negative surface charge. Proper modification was confirmed by measurement using FT-IR spectroscopy, degree of substitution of the chitosan derivative with anionic groups, determined on the basis of elemental analysis, was 53%. The last part of the work in the performed syntheses part consisted of a high-temperature reaction leading to the

obtaining of magnetic iron oxide nanoparticles coated with a hydrophobic substance, which are to be responsible for the magnetic feature of the entire carrier. STEM imaging confirmed the spherical shape of nanoparticles with an average size of 15 nm. In order to confirm the correct deposition of the hydrophobic substance layer on the surface of nanoparticles, FT-IR spectroscopy and thermal analysis TGA-DSC were performed. Based on the results of XRD measurements, it was indicated that the nanoparticles obtained had a core-shell structure, with the core made of wustite (22%), and the coating made of a different phase of iron oxide (77%). Measurements carried out with Mössbauer spectroscopy at various temperatures, showed that this second phase is maghemite, and the structures examined show superparamagnetic properties. The superparamagnetic nature of nanoparticles at temperatures above 275 K was confirmed by VSM measurements. The second stage of the research was the optimization of the capsules preparation procedure and the physicochemical analysis of two types of magnetic carriers, with a negative and with a positive surface charge. They were obtained by utilization of the compounds and structures synthesized at an earlier stage of the work. Capsules with a positive surface charge were produced by self-assembly of an amphiphilic chitosan derivative, modified with cationic groups and with grafted alkyl chains, on the surface of oil droplets containing dispersed magnetic nanoparticles. The additional application of an anionic chitosan layer on the capsule surface by the LbL technique led to the second type of carrier (with a negative surface charge). Based on the DLS measurements, changes in the mean size of the hydrodynamic diameters measured during 48 weeks were determined - the values obtained are in the range of 140-170 nm for cationic capsules and 140-230 nm for anionic capsules. The zeta potential values measured, also determined during 48 weeks, oscillated in the range of 30-40 mV for capsules with a positive charge and -35 to -45 mV for capsules with a negative charge. The values indicate the high stability of the produced carriers. Images taken with the use of cryo-TEM confirm the presence of magnetic nanoparticles in the core of the carrier and its size and spherical shape. The possibility of encapsulating hydrophobic substances was confirmed by confocal microscopy imaging of capsules which additionally contain a probe in the form of a hydrophobic fluorescent compound in the oil phase. The superparamagnetic nature of the carrier was confirmed by VSM measurements. The systems developed were subjected to cellular tests, which constituted the third stage of the experimental part of this work. The research conducted was aimed to verify the application potential of the capsules in the context of magnetically controlled carriers capable of targeted and controlled transport and release of the encapsulated hydrophobic substance. All assays were performed against a mouse mammary gland tissue derived breast cancer cell line (4T1). The cytotoxicity of the capsules was checked using the XTT test, and the obtained results determined the appropriate concentration of anionic and cationic capsules, which do not show toxicity to cells. Then, with the use of a constant magnetic field, an experiments based on the controllable introduction of the capsules, containing the model hydrophobic fluorescent dye, inside the tumour cells were performed.

As a verification of the influence of the experiments on the condition of the cells, proper confocal microscope images were collected. In the initial phase of the tests, the possibile negative influence of the constant magnetic field on the cells of the 4T1 line was excluded. The further part of the experiments showed the possibility of spontaneous penetration of capsules with a positive surface charge and that the use of an external permanent magnetic field increases the effectiveness of the phenomenon studied, contributing mainly to the accumulation of capsules in the vicinity of cancer cells. A similar experiment was carried out with the use of capsules with a negative surface charge. The images taken indicated an increased tendency for spontaneous penetration of capsules inside the cell (with reference to cationic capsules), and the use of a constant magnetic field increases the efficiency of this process. The last stage of cell research was verification the possibility of a controlled release of the substance encapsulated in a carrier that had previously been introduced into the cell by a constant magnetic field. For this purpose, a properly prepared sample was exposed to an external alternating field of various parameters. Similarly to the experiments related to the constant magnetic field, the harmful effect of the field itself on the cells was excluded. Images obtained after experiments with positive surface charge capsules indicate the phenomenon of increased penetration of capsules into the cell, but without the release of the transported dye inside the cell. In contrast to that, the results of experiments carried out for capsules with a negative surface charge confirm the possibility of releasing the encapsulated substance inside the cell through the use of an appropriate alternating magnetic field.