Tandem mass spectrometry as a bioanalytical platform for characterization of medicinally promising superparamagnetic nanoparticles

Cancer is one of the biggest challenges in public health care. Success in cancer control depends on effective diagnostics and efficiency of tumor treatment. Therefore, it is evident that special attention should be paid on approaches that allow us to simultaneously detect and cure the tumor, preferably using the same chemical probe. Such an approach exists within the realm of nanomedicine that is rested on the application of nanotechnology to diagnose and treat different diseases. Indeed, nanoparticles, i.e. the entities with the size of 1–100 nm, are attractive as imaging and therapeutic agents in oncology due to their unique physical and/or chemical properties.

Superparamagnetic iron oxide nanoparticles (SPIONs) are a class of magnetic nanomaterials that have attracted a great deal of research interest owing to their stability, low cytotoxicity, biocompatibility and biodegradability. SPIONs can be effectively used for organ imaging that provided at least a few of them the status of approved (by the Food and Drug Administration) contrast agents for magnetic resonance imaging (MRI). Nowadays, the objective is the new generation of SPIONs which possess multifunctional characteristics for combined cancer diagnostic and therapeutic applications, for example: MRI-guided anticancer drug delivery, gene delivery, photothermal therapy, photodynamic therapy or magnetic hyperthermia. Medicinal applications of SPIONs can be governed by their design. Both, physical and chemical properties of particles are to be rigorously controlled during each step of synthesis to fit various applications. An integral part of a SPIONs synthesis is surface modification (coating) and functionalization (attachment of ligands/biomolecules).

The research project is focused on emerging analytical methodologies for elucidation of preclinical pipeline for the discovery and development of SPIONs of medicinal importance. Novel customized SPION compositions will be constructed and their distribution between serum proteins and resulting speciation in human serum will be studied, with a focus on the specificity of their binding toward transferrin (serum protein responsible for the intensified iron transportation into cancer cells).

SPIONs functionalized with transferrin (synthesized under laboratory conditions) have been accomplished to further evaluate effectiveness of using this protein to target cancer cells. Despite the soundness of the idea of more selective and effective trafficking into cancer cells of SPIONs functionalized with transferrin, the obtained results have not met the expectations. This can be explained taking into account the intravenous interactions of transferrin–SPIONs with other serum proteins. These interactions have much to do with the formation of protein corona (protein–SPIONs conjugates), the phenomenon exerting a critical impact on the biological identity of nanomaterial. It has been gradually recognized that the dynamic nature of corona can facilitate the exchange between the initially bound proteins and other, free proteins in the biological environment. In the frame of the project new, changed, philosophy of investigation of SPIONs potentially selective towards transferrin under *in vivo* conditions will be proposed. Instead of functionalization of nanomaterials conjugated with transferrin under laboratory conditions we suggest so called "*in situ* functionalization of SPIONs with transferrin"- in serum. This approach, thanks to careful analytical characterization of SPIONs behavior in such environment will allow to select the most promising from them.

To develop a bioanalytical platform for the characterization of interactions of SPIONs *in vivo*, separation modules will be coupled to high-sensitivity mass spectrometry detectors (hyphenated techniques). Firstly, the methodology dedicated for monitoring commercial SPIONs in human serum will be elaborated. The species under scrutiny will be separated by capillary electrophoresis and size exclusion chromatography in accordance with their charge-to-size ratio and size, respectively, and detected by isotopically specific spectrometer. Next, a complementary source of speciation information will be exploited for identifying of the serum proteins bound to commercial SPIONs. Then, the novel custom designed SPIONs will be synthesized, carefully characterized and tested by the developed methods in the point of their selective functionalization with transferrin *in situ* after mixing with human serum. Finally, the SPIONs will be categorized with regard to degree of their transformation into the protein-bound form and recommendations will be given on designing more cell-selective SPIONs.

The methodologies, systematically optimized in the course of project execution, will greatly contribute into the field of analytical chemistry, mainly due to extending application of hyphenated techniques to the speciation analysis of novel type of analytes, SPIONs. The fulfillment of the project goals will likewise improve the general knowledge how to design novel nanomaterials with potential targeting of transferrin in human serum, due to better understanding of mechanisms by which they reach cancer cell.