Abstract

Nanoparticles because of their unique properties due to their size are of great interest as matrices for attaching biomolecules. The immobilization of enzymatic proteins on the surface of nanoparticles can lead to hybrid systems showing synergistic properties, as well as improve the stability, activity or resistance of proteins to pH changes. Gold nanoparticles are an excellent candidate for creating this type of structures because of their biocompatibility and ability to form functionalized bioconjugates, as well as silver nanoparticles because of their antibacterial properties.

The purpose of this work was to produce functional nanoparticle-protein conjugates by attaching enzyme proteins, catalase and superoxide dismutase to the surface of gold and silver nanoparticles while maintaining their enzymatic activity, and to develop a method for quantitative analysis of the protein attached to the surface of nanoparticles based on polyacrylamide gel electrophoresis.

Various methods of protein attachment were used during the study. The first was non-specific physical adsorption, based on electrostatic, hydrophobic, van der Waals and hydrogen bonds. Another method was specific physical adsorption, which is based on the specific interactions between the protein polyhistidine tag and the linker present on the surface of nanoparticles containing Ni^{2+} ion. Non-specific chemical adsorption was also used, consisting in the formation of a peptide bond between the linker present on the surface of the nanoparticle and the primary amine groups present in the protein structure.

As part of the doctoral dissertation, a method of quantifying the protein attached to the surface of nanoparticles was developed. It was based on the incubation of an aqueous colloid of nanoparticles with a protein solution, followed by the detection of unbound protein by polyacrylamide gel electrophoresis.

The produced nanoparticle-protein bioconjugates were characterized by measuring diffuse light intensity change techniques, UV-Vis spectroscopy and high resolution scanning electron microscopy. In vitro and in vivo tests of the activity of immobilized enzymes were also conducted in cooperation with the Medical University of Łódź and the Medical University of Białystok.