World Academy of Science, Engineering and Technology
International Journal of Materials and Metallurgical Engineering
Vol:8, No:04, 2014

# Functionalization of Carbon-Coated Iron Nanoparticles with Fluorescent Protein

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**Abstract :** Invention of magnetic-fluorescent nanocomposites is a rapidly developing area of research. The magnetic-fluorescent nanocomposite attractiveness is connected with the ability of simultaneous management and control of such nanocomposites by two independent methods based on different physical principles. These nanocomposites are applied for the solution of various essential scientific and experimental biomedical problems. The aim of this research is development of principle approach to nanobiohybrid structures with magnetic and fluorescent properties design. The surface of carbon-coated iron nanoparticles (Fe@C) were covalently modified by 4-carboxy benzenediazonium tosylate. Recombinant fluorescent protein TagGFP2 (Eurogen) was obtained in E. coli (Rosetta DE3) by standard laboratory techniques. Immobilization of TagGFP2 on the nanoparticles surface was provided by the carbodiimide activation. The amount of COOH-groups on the nanoparticle surface was estimated by elemental analysis (Elementar Vario Macro) and TGA-analysis (SDT Q600, TA Instruments. Obtained nanocomposites were analyzed by FTIR spectroscopy (Nicolet Thermo 5700) and fluorescence microscopy (AxioImager M1, Carl Zeiss). Amount of the protein immobilized on the modified nanoparticle surface was determined by fluorimetry (Cary Eclipse) and spectrophotometry (Unico 2800) with the help of preliminary obtained calibration plots. In the FTIR spectra of modified nanoparticles the adsorption band of –COOH group around 1700 cm-1 and bands in the region of 450-850 cm-1 caused by bending vibrations of benzene ring were observed. The calculated quantity of active groups on the surface was equal to 0,1 mmol/g of material. The carbodiimide activation of COOH-groups on nanoparticles surface results to covalent immobilization of TagGFP2 fluorescent protein (0.2 nmol/mg). The success of immobilization was proved by FTIR spectroscopy. Protein characteristic adsorption bands in the region of 1500-1600 cm-1 (amide I) were presented in the FTIR spectrum of nanocomposite. The fluorescence microscopy analysis shows that Fe@C-TagGFP2 nanocomposite possesses fluorescence properties. This fact confirms that TagGFP2 protein retains its conformation due to immobilization on nanoparticles surface. Magnetic-fluorescent nanocomposite was obtained as a result of unique design solution implementation – the fluorescent protein molecules were fixed to the surface of superparamagnetic carbon-coated iron nanoparticles using original diazonium salts.

**Keywords :** carbon-coated iron nanoparticles, diazonium salts, fluorescent protein, immobilization
**Conference Title :** ICNN 2014 : International Conference on Nanoscience and Nanotechnology
**Conference Location :** Paris, France
**Conference Dates :** April 28-29, 2014