Electrochemical sensing of varial nanostructures' impact to biomolecules

The evolution of nanotechnology has created plenty of applications for research and commercial use in the industry of medical implants, drug delivery and cosmetics. Nevertheless, lately research result draw attention to the use of nanothecnology products with regard to the effects on organisms and biological systems, mainly for medical applications and care products. It is a necessacity to measure and predic the mentioned possible dangers, moreover the development of impact assessment methods. A widespread marker of nanostructure effects in organisms is the generated Reactive Oxygen Species (ROS), which are distinguished to radicals of superoxide anion ·O2-, hydroxyl radicals OH●, hypochlorite acid HClO and more. Extreme generation of ROS effects vital functions of cells by attacking to biomolecules as sugars, amino acids, phospholipid, DNA bases and organic acids, and effect the balance of antioxidants ending with oxidative stress.

Two types of nanostructures with excellent physicochemical properties are CdS quantum dots (qds) and the Au nanoparticles (nps). The quantum dots are inorganic semiconductor nanocrystals with a size smaller than Bohr barrier length and therefore with electron-hole pairs limited to zero dimensions. Due to their size, qds have optical properties with bright and stable fluorescence and photoluminescence as a result of more energy band gaps that are able to absorb photons of lower energy. Fluorescence stimulation events of quantum dots can, however, generate ROS, as an electron is emitted to the nearby media's environment and reacting with the molecular oxygen (triple state), as well as forming ROS the very reactive single state oxygen and the superoxide anion. Moreover noble metal nps as Au, are nanostructures that perform optical properties related to Plasmonic Effect, when in conduction band free d-electrons resonant oscillate results high response at visible spectrum. In this case bio-impacts and ROS generation differs as mostly connect with Au ions exposure. In this Master Thesis the described nanostructures synthesised and characterized for two sizes respectively by Dynamic Light Scattering. It has been used CdS qds 3.7nm and 10.5nm and Au nps 19nm and 34nm as it confirmed also from Scanning Electron Microscopy images. It took place comparative study for sensing the generation of ROS superoxide anions and hydroxyl radicals and by UV-Vis spectroscopy and the scavenger of 7 chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD Cl) and 1,1-diphenyl-2-picrylhydrazyl (DPPH), under the influence of photoactivated nanostructures by UV-C radiation. Results proved the size and optical properties depended ROS generation of superoxide anions for the case of CdS qds, but reduced in the case of Au nps.

The interactions of nanostructures with real biological conditions are complex enough so it is required studying these interactions with specific biological subsystems. One of the factors of these systems is the not well-known Protein Corona effect which is the formation of proteins from biological media covering nanostructures' surface as a shell. This shell seems to affects physicochemical properties and biological impacts of nanostructures. This research tries an approach to investigate both types of impacts. It was observed the increased of Plasmonic Response of Au nps, as a result of the monodispersion after the formation of protein corona. Using the scavenger of NBD-Cl it was observed ROS generation decrease under the influence of the formatted Protein Corona for both CdS qds and Au nps. Identifying and understanding nanoparticles-protein corona interactions is high importance topic for drug delivery and bioapplications, the notice of decreased ROS cannot be considered as positive or

negative but as a condition that needs to be managed for the properties of nanostructures, their impacts as well for cellular uptake, accumulation and opsonization.

Electrochemical biosensors used to quantify survival dsDNA after the treatment with both nanostructures photoactivated by UV-C radiation 253.7nm. Despite the lack of superoxide anions generation Au nps destroyed dsDNA, especially the bigger size, due to the Au ions exposure. CdS qds observed opposed thus the smaller sized qds destroyed dsDNA more than the bigger sized, following the generation of superoxide anions respectively. The mention result is connected with the fluorescence intensity of the qds and the generation mechanism of ROS, once for small sized qds observed sized order higher intensity that the bigger one, that produce also more superoxide anions. In all cases of nanoparticles and quantum dots, the presence of protein shells reduced the detection of ROS as well as the destruction of dsDNA, due to the dual role of the shell in the the absorption of ROS and the affinity of the tissues interacting. Further research for identification of the proteins and formation kinetics analysis is considered as important.

In the present work it was created a hybrid optical-electrochemical biosensing system for impacts evaluation. Electrochemical measurements for survival dsDNA sensing took place by using Differential Pulse Voltammetry with carbon paste electrode from Graphite Oxide (B5). This carbonaceous material selected as the best after characterization of a line of promising carbonaceous materials candidates for alternates of the conventional Graphite. These materials are presented and characterized graphite oxides (GO, B5), activated carbon (B) and graphite oxide with impurities of Ag nps (Bax-Ag). The characterization took place by Cyclic Voltammetry of the redox system K3[Fe(CN)6] and surface area calculation of a line of promising carbonaceous materials, also from Differential Pulse Voltammetry for the characteristic oxidation peak.