Abstract

A large-surface carbon film electrode (ls-CFE) represents a suitable alternative to the commercially available disposable screen-printed carbon working electrodes. Its simple, fast and inexpensive preparation, simple mechanical renewal of the electrode surface (by wiping off the old film with filter paper and forming a new one), good reproducibility of measurements, elimination of problems connected with "electrode history" and simple chemical modification are its main advantages.

In this Bachelor Thesis, the utilization of ls-CFE for the preparation of a simple electrochemical DNA biosensor for the detection of carcinogenic environmental pollutant, 2-aminofluorene (2-AF), is presented. The composition of carbon ink suspension was optimized and tested using a Fe(II)/Fe(III) redox system by cyclic voltammetry (CV). A large-surface solid electrode, covered by a carbon film of the optimum composition, was then used as a transducer for the preparation of double stranded DNA (dsDNA) based electrochemical DNA biosensor (dsDNA/ls-CFE).

The electrochemical behavior of dsDNA (i) present in the measured supporting electrolyte (0.1 mol.L⁻¹ acetate buffer of pH 4.7) or (ii) attached at the ls-CFE surface (by covering with dsDNA stock solution and leaving to dry) was investigated using square wave voltammetry (SWV). On the basis of oxidative voltammetric responses of guanine and adenine moieties, both above mentioned approaches were tested. Much better results were obtained using a dsDNA attached at the ls-CFE surface and, therefore, thus prepared DNA biosensors were then used for the investigation of interaction of dsDNA with genotoxic 2-AF.

The interaction of dsDNA with 2-AF was investigated using both SWV and CV at dsDNA/ls-CFE. Using SWV at dsDNA/ls-CFE, the decrease of peak currents of guanine and adenine moieties was observed (after interaction of dsDNA with 2-AF), indicating a well known intercalation of the analyte into the dsDNA structure causing dsDNA strand breaks. Moreover, a strong binding interaction of 2-AF with dsDNA was confirmed by observation of enhanced SWV response of 2-AF accumulated into the dsDNA; this phenomenon will be used in further investigations to increase the sensitivity of the determination of 2-AF at dsDNA/ls-CFE in contrast to unmodified ls-CFE.

An indirect detection of DNA damage caused by 2-AF was investigated using Fe(II)/Fe(III) redox system by CV. The obtained results confirmed that the interaction of dsDNA with 2 AF caused a dsDNA damage, leading to the formation of strand breaks and desorption of DNA fragments from the electrode surface.