AMPEROMETRIC SENSORS FOR ENVIRONMENTAL APPLICATIONS BASED ON MODIFIED ELECTRODES

This habilitation thesis presents the results obtained by the author regarding the obtaining and characterization of new amperometric sensors for environmental applications based on modified electrodes. These sensors were used for detection of three important analytes: the reduced form of β nicotinamide adenine dinucleotide (NADH), hydrogen peroxide (H_2O_2) and nitrites. The modified electrodes were obtained by using adsorption or electropolymerization of different mediators (new synthesized or commercially available) on solid electrodes (graphite or glassy carbon), or by incorporation of mediators in carbon paste, using adsorption on zeolites or clays.

Because over 300 dehydrogenases require nicotinamide coenzymes as cofactors, the electrocatalytic oxidation of β-nicotinamide adenine dinucleotide (NADH) has been of particular interest. It is a coenzyme naturally present in all living cells, being necessary for cell development and energy production at cell level. Additionally, the development of such electrocatalysts could be coupled to biosensor design for the determination of numerous species. Hydrogen peroxide (H_2O_2) detection is also of great importance in various fields including clinic, food, pharmaceutical and environmental analyses, because H_2O_2 is a chemical threat to the environment and is involved in enzymatic reactions. A large number of NAD^+-dependent dehydrogenases and hydrogen peroxide generating oxidases have been useful for realization of amperometric biosensors for environment monitoring. This is the reason for that obtaining of performing NADH and H_2O_2 amperometric sensors is useful for environmental applications.

Nitrite (NO_2^-) is an important source of nitrogen in green plants and its complete reduction is achieved in nature by nitrite reductase enzyme. It is used as an additive in some types of food and its occurrence in soils, waters, foods and physiological systems is prevalent. The presence of excess nitrite in vegetables, drinking water and food products is a serious threat to human health. Nitrite promotes the irreversible oxidation of hemoglobin and reduces the blood capacity to transport oxygen. Also it may interact in the stomach with amines and amides forming some carcinogenic N-nitrosamine. Therefore, nitrites determination is of great significance for environment security and public health. Even there are many analytical methods used for nitrite detection, the electrochemical methods based on amperometric sensors are often been employed, owing to their rapid response, cheap, safe, and simple use.

In the first chapter of the thesis are presented amperometric sensors for NADH detection, based on six types of modified electrodes: (1) glassy carbon electrode modified with an electropolymerized film of a new phenothiazine derivative (bis-phenothiazin-3-yl methane; BPhM); (2) glassy carbon electrode modified with an electropolymerized film of poly-hematoxylin; (3) graphite electrode modified with a new phenothiazine derivative, polyphenothiazine formaldehyde (PPF); (4) graphite electrodes modified with two new phenothiazine derivatives, 3,7-di(m-aminophenyl)-10-ethyl-phenothiazine (DAEP) and 3,7-di(m-hydroxyphenyl)-10-ethyl-phenothiazine (DHEP); (5) carbon paste electrodes based on a synthetic zeolite (13X type) and a natural mineral clay (bentonite), both impregnated with the new phenothiazine derivative DAEP; (6) carbon paste electrodes modified with a phenoxazine derivative, Meldola blue, and a phenothiazine derivative, Methylene Green, both adsorbed on a synthetic zeolite and using glassy carbon powder (Sigradur K) or single-walled carbon nanotubes as conductive electrode material.

It was presented the electrochemical behavior of all obtained modified electrodes in different experimental conditions (pH values and scan rates) and the heterogeneous electron transfer rate constant, k_s, was calculated. Some of the obtained k_s constants were very high (50 s^{-1}), being one of the highest values presented in literature.

Also, all obtained modified electrodes presented electrocatalytic effect toward NADH oxidation. The electrocatalytic rate constants for NADH oxidation, $k_{obs,[NADH]=0}$, estimated from rotating disk electrode measurements, had high values for some modified electrodes, around 10^5 M^{-1} s^{-1}, being one of the highest values presented in literature and giving the possibility to use these performing mediators for obtaining biosensors for detection of a large variety of compounds in environment. These electrodes were used as amperometric sensors for NADH detection, with good detection limits and sensitivities. The photoelectrocatalytic effect toward NADH oxidation was studied for some modified electrodes and was obtained a significant increase of electrocatalytic effect in the presence of light.

In the second chapter of thesis is presented another research interest focused on obtaining modified electrodes with good electrocatalytic effect toward H_2O_2 reduction by using zeolites as adsorbents for the phenothiazine derivative, Methylene Green, or two iron-enriched and one cooper-enriched natural zeolitic volcanic tuffs, and further incorporation in carbon paste. These electrodes were characterized from physical-chemical and electrochemical point of view and were used as amperometric sensors for H_2O_2 detection, with good electroanalytical characteristics.

The third chapter of thesis presents the obtaining of glassy carbon electrodes modified with poly(Toluidine Blue O) (GC/poly-TBO) and single-walled carbon nanotubes (SWCNT), for the electrocatalytic oxidation of nitrite. GC/poly-TBO were prepared by electropolymerization and used as such or after immobilizing SWCNT on the polymeric film to give a composite GC/poly-TBO-SWCNT electrode. The electrochemical and catalytic behavior of both electrodes was studied comparatively. It was observed that the presence of SWCNT contributed to enhance the electrocatalytic response for nitrite oxidation, as measured by amperometry at +0.92 V *vs.* Ag|AgCl/KCl_{sat} and pH 7. The response was linear with respect to the nitrite concentration in the 0.001 – 4 mM range, with a detection limit of 0.37 μM (based on signal to noise ratio of 3) for GC/poly-TBO-SWCNT. The proposed method was also applied to the determination of nitrite in a wastewater sample.

Based on my scientific experience that I accumulated last years and taking into account the important progresses recorded in obtaining of more performant amperometric sensors/biosensors for environmental applications, my scientific career development strategy will be focused on obtaining and characterization of new modified electrodes, which I intend to use as amperometric sensors/biosensors for detection of different analytes found in environment, as nitrites, nitrates, sulfides, ammonium, metals, etc. I intend to use different electrode materials based on (i) new synthetisez organic compounds, nanoparticles or polymeric materials; (ii) different adsorbent materials. All these sensors/biosensors will be used for monitoring of different samples of water, soil, vegetation. Additionally, I propose to obtain a system for on-line monitoring of different toxic compounds.