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Improvement of the detection limit for biosensors: Advances on the optimization of biocomposite composition

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ABSTRACT

In this work the application of advanced characterization techniques in the development of amperometric biosensors based on biocomposites is described. The optimization of the conductive particle distribution and the amount of the biological material inside the biomaterial have allowed an improvement of the electrochemical properties, regarding the electroanalytical properties such as signal stability and limit of detection. The high signal-to-noise ratio obtained in the electrochemical transduction has allowed enhancing the limit of detection of the biosensor. In the present study, it has been demonstrated the feasibility of electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) for the characterization and optimization of biosensors based on graphite–epoxy–enzyme, using an enzyme model. The optimum biocomposite proportion based on graphite–epoxy which incorporates the enzyme glucose oxidase (GOD) on the matrix ranges between 16% and 17% of graphite using 1% and 2% of enzyme. This range provides the optimal electroanalytical properties. Low limit of detection and good sensitivity have been achieved. Furthermore, confocal laser scanning microscopy was used to visualize the enzyme distribution onto the surface electrode.

1. Introduction

The need of detecting small amounts of different kinds of compounds, which are usually present in a complex matrices, has led to the development of new sensitive, economical and robust (bio)sensor devices based on composite materials that allow to perform in situ and real analysis [1,2]. Moreover, the use of conductive materials as transducers or conductive phase based on carbon materials (i.e. graphite, carbon nanotubes, etc.) and dispersed on a polymeric matrix (i.e. epoxy, methacrylate, Teflon, etc.) has been opened a new generation of rigid conducting composites that have been applied in the realization of (bio)sensors. Electrodes obtained by using a mixture of particulate conductive carbon phase and an insulating matrix represent an attractive approach for the fabrication of electrochemical (bio) sensors, whose surfaces can be renewed by polishing [3–6]. Biocompatibility and capability to incorporate chemical species without their loss in operating medium are of utmost importance [7]. A biosensor based on a biocomposite is defined as a rigid material made by combining two or more materials of different nature (phases) where at least one of them has a biological origin [8]. An important aspect of the development of biosensors is the method of immobilization of the enzyme [9]. The biological component of biosensors has traditionally been placed on the surface of the transducer, either by direct adsorption [10], cross-linking [11,12] or covalent attachment [13,14] or immobilized previously on a membrane [15]. Another alternative strategy proposed in the development of biocomposites is the immobilization of the biological component inside the matrix of the composite by physical entrapment [16–20]. This immobilization that allows forming a rigid and renewable sensing surface showed excellent performance for enzymatic determinations.

An important feature of composite electrodes is that their overall analytical performance is strongly influenced by the carbon loading within polymeric matrix. It is due to carbon loading that influences directly on the electrochemical surface and inner structure (bulk resistance) of the composite electrode [21,22]. Both parameters strongly affect on the overall electroanalytical performance of such composite electrodes [23]. The characterization and optimization of composites based graphite–epoxy have been widely studied using different strategies based on several techniques as well as percolation theory [24–26], atomic force microscopy (AFM) [3,25] or chronoamperometry [3,24]. Up to now the principle applied to the optimization of the composite proportions has been done using the percolation theory, under the criteria of maximizing the conductive particle loading, without losing its physical and mechanical stability, but without taking care if the composite provides the best electroanalytical characteristics of response [24,27–29]. Recently, it has established new alternative strategies of characterization which demonstrates that if the composite proportions are optimized the response of the electrode is improved [30] in terms of the signal-to-noise ratio which has a direct relationship with the limit of detection.

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These techniques are electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). EIS measurements provide, in an easy way, information about the electron-transfer rate, double-layer capacitance, contact resistance and resistance of the solution [31,32]. The electrochemical properties required by a transducer are high electron-transfer rate, the lowest double-layer capacitance and ohmic resistance in order to guarantee the optimal electroanalytical characteristics of the electrode response as high sensitivity, a high signal/noise ratio, and low detection limits. Consequently, by EIS technique it is possible to determine the optimal composite composition that exhibits these improved electroanalytical properties. These results can also be complemented with voltammetric measurements. This is the first time that these strategies are applied in the characterization and optimization of biocomposite electrodes in order to develop more efficient biosensors for determining low analyte concentrations in a final analytical application.

The study of the electrochemical properties of the biocomposites as a function of the conductive particle and biological charge material present on the transducer matrix has been performed. In the development of amperometric biosensors based on biocomposites, the incorporation of a biological compound produces a modification in the spatial separation and inner distribution of the conductive particles. The electrochemical response of a biosensor based on a biocomposite depends on the electron-transfer rate and also the active area of the electrode. Moreover, the electronic-transfer on the surface electrode depends on the conductive particle loading and hence on their distribution [33,34]. The improvement of the electrochemical properties of the biosensor is due to an appropriate distribution of the conductive particles inside the biocomposite and, in consequence, in the biosensor surface.

In the literature some references have been found which realize a study of the influence of the enzyme load. On one side, Pérez et al. [35] reported a study of L-lactate biosensors based on polysulfone/carbon nanotubes membranes where the criterion of optimization of the amount of enzyme presented on the biosensor is the amount that provides the wider linear range according to the analytical requirements. On the other side, Wang et al. [36] reported a study based on enzyme dispersed on carbon nanotubes for monitoring glucose where the criterion of optimizing the enzyme loading followed is the amount that provides the best electroanalytical signal. Moreover, under the same criterion, Caro-Jara et al. [37] optimize the GOD/HRP ratio in a bienzymatic amperometric biosensor. However, in any case the optimization of the transducer has been considered.

The main goal of this study is the use of alternative strategies of characterization [30], in order to characterize and optimize the biocomposite composition based on graphite–epoxy which incorporates different amounts of an enzyme model on the polymeric matrix. Enzymatic amperometric glucose biosensors have been widely studied in the last four decades [38] because of the relatively high durability of the enzyme and low cost, so glucose oxidase (GOD) has been chosen as an enzyme model for this study.

Firstly, we have constructed two series of graphite–epoxy–GOD with different graphite loadings between the percolation threshold zone and amounts of GOD. We have applied the electrochemical strategies of characterization in order to obtain the optimized biocomposite composition. The analytical response of the optimized biocomposite has been evaluated with synthetic samples of glucose. Besides the electroanalytical characterization, the surface of the biosensors has been also characterized by optical techniques such as fluorescence microscopy.

2. Experimental

2.1. Apparatus

Electrochemical impedance spectroscopy and voltammetric measurements were performed using a computer controlled Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie, Utrecht, The Netherlands) with a three-electrode configuration. A platinum-based electrode 53-671 (Crison instruments, Alella, Barcelona, Spain), an AgCl covered silver wire and the constructed graphite biocomposite electrodes were used as a counter, reference, and working electrodes, respectively.

Amperometric measurements were done using an amperimeter LC-4C (Bio analytical Systems Inc., West Lafayette, IN, USA), connected to a personal computer by data acquisition card ADC-42 Pico Technology (St. Neots, Cambridgeshire, UK) for data registering and visualization. Electroanalytical experiments were carried out in 20 mL glass cell, at room temperature (25 °C), using three-electrode configuration. A single junction reference electrode Ag/AgCl Orion 900100 (Thermo Electron Corporation, Beverly, MA, USA) and platinum-based electrode were used as reference and auxiliary, respectively. The graphite biocomposite electrodes were used as working electrode. A magnetic stirrer provided the convective transport during the amperometric measurements.

Confocal laser scanning microscopy microphotographs were taken with a LEICA TCS SP2 microscope.

2.2. Chemical reagents

Graphite powder (particle size 50 μm) was received from Merck (Merck Millipore, Darmstadt, Germany). Epoxy resin Epotek H77A and hardener Epotek H77B were obtained from Epoxy Technology (Epoxy Technology, Billerica, MA, USA). Potassium ferricyanide/ferrocyanide (99.8%), potassium chloride (99.5%), potassium phosphate monobasic (99.5%), nitric acid (65%), potassium dibasic-anhydrous (98%), D-(+)-glucose (99.5%) and glucose oxidase type VII from *Aspergillus niger* (174,400 units/g) were supplied from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. EZ-Link Sulfo-NHS-LC-Biotin was purchased from Thermo Scientific (Rockford, USA). All the dissolutions were prepared using deionized water from Milli-Q system (Millipore, Billerica, MA, USA).

2.3. Fabrication of the electrodes

2.3.1. Working electrodes

Handmade graphite–epoxy composites were prepared following the conventional methodology previously established in the research group [39]. A resin Epotek H77 and their corresponding hardener compound were mixed in the ratio 20:3 (w/w). The graphite composite was prepared by loading different amounts of graphite (13, 14, 15, 16, 17, 18, 19 and 20% (w/w)) into the epoxy resin before hardening. The composite was homogenized for 30 min. After the homogenization time, the glucose oxidase amount (1% and 2% for each series, respectively) was introduced to the composite paste and homogenized for 15 min more. The final biocomposite paste electrode was allowed to harden during 5 days at 40 °C and when not in use it was stored at 4 °C [16]. Finally the electrode surface was polished with different sandpapers of decreasing grain size. The final electrode dimensions were 28 mm^2 and 3 mm for its geometric area and thickness, respectively.

2.3.2. Graphite–epoxy–GOD electrodes for fluorescence measurements

In order to perform the fluorescence measurements, graphite–epoxy–GOD biocomposite was prepared as discussed in Section 2.3.1, but it was introduced into a special support. Hand-made biocomposite was placed into a PVC disk with an external diameter of 35 mm, internal diameter of 15 mm, and thickness of 3 mm. The process of hardening was 5 days at 40 °C and when not in use it was stored at 4 °C. The electrode surface was polished using different sandpapers of decreasing grain size.

2.4. Procedure

2.4.1. Electrochemical characterization

EIS and voltammetric measurements were made in a 0.1 M potassium chloride solution containing 0.01 M potassium ferricyanide/ferrocyanide under quiescent condition. Amperometric detection was made under force convection by stirring the solution with magnetic stirrer.