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Evaluation of different mediator-modified screen-printed electrodes used in a flow system as amperometric sensors for NADH

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Abstract

This work presents a comparative study between two different methods for the preparation of mediator-modified screen-printed electrodes, to be used as detectors in a reliable flow injection system for the determination of the nicotinamide adenine dinucleotide (NADH) coenzyme. The best strategy was selected for the final development of compact biosensors based on dehydrogenase enzymes. For the first immobilisation strategy, different redox mediators were electropolymerised onto the SPE surface. The second immobilisation strategy was carried out using polysulfone–graphite composites, which were deposited by screen-printing technology onto the screen-printed electrode (SPE) surface. Both methods achieved an effective and reliable incorporation of redox mediators to the SPE configuration. Finally, a flow system for ammonium determination was developed using a glutamate dehydrogenase (GlDH)-Meldola's Blue (MB)-polysulfone-composite film-based biosensor.

The stability of the redox mediators inside the composite films as well as the negligible fouling effect observed on the electrode surface improve the repeatability and reproducibility of the sensors, important features for continuous analysis in flow systems. Furthermore, the optimised bio/sensors, incorporated in a flow injection system, showed good sensitivities and short response times. Such a good analytical performance together with the simple and fast sensor construction are interesting characteristics to consider the polysulfone-composite films as attractive electrochemical transducer materials for the development of new dehydrogenase-based SPEs.

Keywords: SPE; Flow injection analysis; Polysulfone; Redox mediators

1. Introduction

Despite the wide range of available dehydrogenase enzymes, dehydrogenase-based biosensors have not been as widely developed as might be expected. The reason can be found in the fact that they relay on the amperometric detection of the NADH cofactor, process that involves some drawbacks. Actually, the development of a reliable system for the amperometric detection of NADH has been during years one of the main research subjects in electrochemical sensors. The problems related to the electrochemical oxidation of NADH involve high overpotentials [1] and the subsequent formation of byproducts that foul the electrode surface (i.e. dimerisation of $NAD^{\bullet}$ radicals and other oxidation products) [2,3]. As a result, low selectivity and stability are obtained. Much work has been related to the use of redox mediators [4]. These compounds allow the construction of more sensitive, selective and stable biosensors, since they permit to lower the overpotential and so prevent the electrode fouling [5]. However, the soluble, or partially soluble, mediating species may diffuse away from the electrode surface towards the bulk solution, especially when the sensor is used in multiple analyses, e.g. in a continuous flow system. In fact, sensors used as detectors in FIA systems are exposed to flowing buffer and, consequently, special attention must be given to the mediator immobilisation. If the mediator leaks from the sensor, significant current loss will occur and therefore the lifetime of the sensor will be considerably reduced. Different strategies to incorporate redox mediators into the electrochemical system involve either adding the mediator to the solution [6,7] or immobilising it within or on the electrode, producing compact chemically modified sensors [8,9]. The latter is achieved following different methodologies, such as the dispersion of the mediator in the bulk of a composite electrode [10,11] or its immobilisation on the electrode surface by physical adsorption [12,13], covalent attachment [14,15],

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electropolymerisation [16,17], gel entrapment [18] or cross-linking [19]. Other immobilisation techniques involve the use of a polymeric film, where the mediator is covalently attached [20–23] or physically entrapped [24–26]. The chosen strategy will depend on the specific characteristics of the working system and the sensor performance under them, as the activity of the immobilised molecules depends on the immobilisation method, as well as, in the case of using an immobilising matrix [27], on its surface area, porosity and hydrophilic character.

In previous studies [28,29], the behaviour of different redox mediators, incorporated to the system by five different strategies was compared: in solution, incorporated inside a composite matrix, adsorbed or electropolymerised onto the electrode surface and incorporated inside polysulfone-composite films deposited on the electrode surface. This work was carried out with cylindrical-configuration electrodes based on epoxy–graphite composites. These new polysulfone-composite membranes were prepared by mixing polysulfone with graphite powder.

In the work reported here, two previously optimised immobilisation strategies have been used to prepare mediator-modified SPEs. Results show that electropolimerised mediators and mediator-modified polysulfone-composite films overcome one of the most critical points in the manufacture of thick-film sensors, such as the adhesion of the sensing layer to the transducer layer. The excellent stability of electron mediators immobilised inside polysulfone-composite materials together with the fact that they can be easily incorporated to the sensors through an additional layer in the screen-printing process, allow a massive production of disposable mediator-modified electrodes. Furthermore, the leakage absence and the negligible surface fouling ensure the feasibility to consider SPEs based on polysulfone-composite films as electrochemical detectors for flow injection systems. Finally, it has been demonstrated the usefulness of these sensors for the development of dehydrogenase-based biosensors to be used in FIA systems, showing as an example the performance of GlDH-mediator-polysulfone SPEs.

2. Experimental

2.1. Reagents

Polysulfone Ultrason nature 3120 was obtained from BASF. NADH, Meldola's Blue (MB), *o*-phenylenediamine (*o*-PDA), glutamate dehydrogenase (GlDH, EC 1.4.1.3 from bovine liver, 42 units/mg prot.) and α-ketoglutarate were purchased to Sigma. 3,4-dihydroxybenzaldehyde (3,4-DHB), *p*-benzoquinone (*p*-BQ) and *N*,*N*-dimethylformamide (DMF) were bought to Aldrich and dichlorophenolindophenol (DCPIP) to Fluka. Ammonium chloride, sodium dihydrogen phosphate and potassium chloride were purchased from Panreac. A platinum sheet (Ref. PT000251, Goodfelow, England) with 99.95% purity and 0.125-mm thick, a fibreglass support (Ariston), silver conductive resin 410E and the proper hardener (Epoxy Technology, Billerica, MA, USA) and epoxy diacrilate (Ebecril 600, UCB Chemicals) have been used for the construction of a planar-configuration platinum electrode. Clear polyester sheets, 0.5-mm thick, were used as support for the SPEs. SPEs have been prepared by successive layer printing with inks supplied by Acheson: silver ink (Electrodag 418 SS), graphite ink (Electrodag 423 SS) and insulating ink (Electrodag 451 SS).

All solutions were prepared using deionized water obtained from a Milli-Q purification system, and they were de-aerated prior to their use.

2.2. Electrochemical measurements

Cyclic voltammetry was carried out using a multipotentiostat, AUTOLAB model PGSTAT10 (Eco Chemie) in a conventional electrochemical cell of 10-mL volume. Amperometrical studies were performed on a LC-4C potentiostat (BAS, USA) in a flow injection system. All experiments were carried out at room temperature, in a conventional three-electrode system with a planar-configuration platinum electrode or a SPE as working electrode. The auxiliary electrode was an epoxy–graphite composite electrode in a tubular configuration, and the reference electrode was a double-junction Ag/AgCl electrode (Orion 92-02-00), with a commercial inner filling solution (Thermo Orion 900002) and 0.1 M KCl as outer filling solution. The three electrodes were disposed along the flow system using methacrylate supports. The flow injection system consisted of a Gilson (Minipuls 3) peristaltic pump, interconnecting Teflon tubing (0.8 mm inner diameter) and a sample injection valve.

2.3. Electrode preparation

Glass fibre with photolithographed cooper tracks was used as conductive support for the construction of the planar-configuration platinum electrode. For this electrode, with an active surface area of 24 mm^2, the electric contact between the cooper and the platinum was achieved using a conductive silver resin, which allows the welding of both materials and, finally, the electrode was encapsulated with an epoxy resin.

A DEK 248 screen-printing system (DEK, UK) was used to fabricate the SPEs (with a geometrical area of 24 mm^2). The electropolymerisation process used for the preparation of the mediator-modified SPEs was previously described for conventional electrodes [28]. Briefly, the composite SPE surface was pre-treated by dipping it in phosphate buffer solution and performing cyclic voltammograms between −0.2 and +0.1 V for 10 min at 0.05 V s^{-1} [16,30]. The aim of this pre-treatment was to enhance the reproducibility of the electrode surface characteristics. The second step was the electropolymerisation of the mediator on the SPE surface, by applying a constant potential to the electrode immersed in a redox mediator solution. The applied potential, as well as the mediator concentration and the electropolymerisation time, varied depending on the used mediator. Finally, the electrode surface was activated by recording 10 cyclic voltammograms at 0.05 V s^{-1}, using potential windows that depended on the mediator. On the other hand, for the preparation of the polysulfone–graphite composite-modified SPEs, a 7.5 wt% polysulfone solution was prepared in dry DMF. Once the solution was homogenised, 150 μL of this solution were mixed with 30 mg of graphite and 1.5 mg of redox mediator