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One-step screen-printed electrode modified in its bulk with HRP based on direct electron transfer for hydrogen peroxide detection in flow injection mode

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Abstract

The preparation and performances of screen-printed carbon electrodes modified in their bulk with HRP (HRP-SPCE) is reported. The resulting modified HRP-SPCE was prepared in a one-step procedure, and then was optimised as an amperometric biosensor operating at [0–100] mV versus Ag/AgCl in flow injection mode for hydrogen peroxide. The amperometric response was due to direct electron transfer (DET) between HRP and SPCE surface. Factors such as chemical modification of the enzyme or the nature and rate of the binder were investigated regards to their influence on the sensitivity, linear range and operational stability. The best performing HRP-SPCE in terms of sensitivity and operational stability was obtained when graphite powder was modified with HRP previously oxidised by periodate ion (IO_4^-).

Keywords: Biosensor; Screen-printed electrode; Horseradish peroxidase; Direct electron transfer

1. Introduction

Over last decade increasing interest has been reported about the application of ready-to-use biosensors in clinical, environmental and food fields (Hart et al., 2004). Screen-printing seems to be one of the most promising technologies allowing simple, rapid and inexpensive biosensors production. Different configurations for the design of screen-printed enzymatic biosensors including different techniques for enzyme immobilisation have been reviewed (Albareda-Sirvent et al., 2000). First configurations were based on manual multilayer deposition onto the screen-printed transducer (Wang et al., 1995; Collier et al., 1998; Boujtita et al., 2000; Albareda-Sirvent et al., 2001a). Then, the second one combined composite inks with printed enzyme immobilisation (Nagata et al., 1995; Albareda-Sirvent et al., 2001b). Finally, biosensors might be obtained in an one-step procedure based on the deposition of a layer of conductive biocomposite ink. In this configuration, enzymes and other materials such as graphite, stabilizers and polymeric binder are mixed together forming the biocomposite ink, which is screen-printed onto the substrate. Despite the rapidity and simplicity in the manufacturing process, only few biosensors have already been developed by this procedure (Koopal et al., 1994; Wright et al., 1995; Keay and McNeil, 1998; Crouch et al., in press; Ogonczyk et al., 2005). It requires an accurate optimisation of the ink composition regarding to rheological properties, electrochemical behaviour and electroenzymatic activity.

This work presents a simple way for preparing a SPCE modified with HRP (HRP-SPCE). HRP-modified amperometric biosensors have been widely studied and developed, not only because hydrogen and organic peroxides are important analytes but also because of the key role of hydrogen peroxide detection in coupled enzyme system, in which hydrogen peroxide is formed as the product of reactions catalysed by oxidases. In a preliminary work, we examined the voltammetric behaviour of an SPCE modified in its bulk with

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HRP. This latter was proposed as a biosensor operating in flow injection analysis mode for hydrogen peroxide detection. Then the optimisation of the analytical performances of the resulting modified HRP-SPCE was investigated. Factors such as the rate and kind of binder, solvent, graphite, enzyme loading and periodation of HRP were investigated in regards to the effect on both the sensitivity and operational stability. Finally, the optimal SPCE-HRP was characterised for flow injection analysis mode.

2. Material and methods

2.1. Reagents

Horseradish peroxidase (HRP) (EC. 1.11.1.7) (No. HRP4) was from Biozyme (Blaenavon, Gwent, UK). Hydrogen peroxide (30%, w/w) (No. H-1009), cellulose acetate (No. C-3782) were obtained from Sigma Chemical. Cyclohexanone (No. C10, 218-0) were purchased from Aldrich and graphite powder (No. 50870) from Fluka. Alumina ceramic substrates for screen-printed electrodes were from The Laser Cutting Company Ltd. (Sheffield, UK). Silver-based ink was from GEM-Gwent (Pontypool, UK). Aqueous solutions were prepared using 0.1 M phosphate buffer (pH 7.2) containing 0.1 M KCl as support electrolyte.

2.2. Screen-printed electrodes preparation

DEK Albany model 245 screen printer machine and stainless screens with a 200 mesh and variable thickness (13, 23 or 36 μm) are used to prepare the three-electrodes system (Fig. 1) in four printing steps: (1) printing of Ag/AgCl electrode (13 μm) using the commercially available ink Ag/AgCl (GEM-Gwent), the resulting printed Ag/AgCl electrode presents a stable half-cell potential 0.276 V versus NHE, (2) printing of both the counter electrode and the conducting tracks of working electrode using graphite-AC ink, (3) printing of the activated surface using the graphite-binder (AC or PVC) ink modified with HRP (the diameter of the activated surface is 2 mm) and (4) printing of non-conductive dielectric layer to define the working area surface. For each printing step a group of four electrodes was simultaneously printed on alumina ceramic substrate (1.5 cm × 1.5 cm). All printed layers were cured at room temperature overnight.

2.3. Preparation of graphite-binder inks

The counter electrode and the conducting track of working electrode were prepared from the graphite-CA ink. This latter was prepared by mixing an appropriate amount of graphite powder (1.4 g) in CA binder already dissolved in cyclohexanone (3.4 g of solution at 7%, w/w).

HRP-SPCE-A was printed from ink obtained by mixing 1 g of the previous graphite-CA ink with 5.8 mg of freeze-dried HRP.

HRP-SPCE-B was prepared using previously activated graphite powder mixed with CA binder. The active graphite powder (Charpentier and El Murr, 1995; Rajendran et al., 1998; Zaydan et al., 2004) was prepared by dissolving 5.8 mg of commercial HRP in phosphate buffer (pH 8) before the addition of graphite (1 g). The mixture was stirred for 1 h and freeze-dried. The resulting graphite powder modified with HRP was then mixed with the binder (CA 7%, w/w, in cyclohexanone) to obtain the graphite-HRP ink (HRP-SPCE-B).

HRP-SPCE-C was made by dissolving 5.8 mg of HRP in sodium periodate ($NaIO_4$) solution (1.6 mg mL^{-1}). The solution was then filtrated through a membrane (Amicon) with 30,000 Da molecular weight cut-off in order to remove the salts. After filtration, the HRP was dissolved in 0.05 M phosphate buffer (pH 8). The graphite powder (1 g) was then added and the resulting mixture was stirred for 1 h. After freeze-drying, the HRP graphite powder was then mixed with binder (CA or PVC in cyclohexanone) to obtain the ink used to prepare HRP-SPCE-C.

2.4. Spectroscopic procedures

Absorbance spectra of HRP between 300 and 700 nm were determined with 0.1 mg mL^{-1} enzyme in aqueous buffer (phosphate 0.1 M, pH 7.2) solution. Measurements were carried out at 25 °C on a Spectronic Genesis (Milton Roy) spectrophotometer.

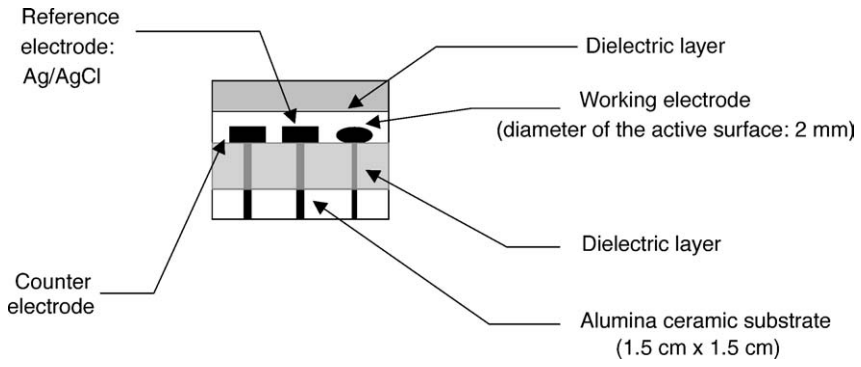

Fig. 1. Schematic representation of the screen-printed electrodes used in this work.