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Integration of a glucose biosensor based on an epoxy-graphite-TTF·TCNQ-GOD biocomposite into a FIA system

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Abstract

An amperometric glucose biosensor based on graphite and non-conducting epoxy resin biocomposite was constructed. Glucose oxidase (GOD) and the tetrathiafulvalene–tetracyanoquinodimethane (TTF·TCNQ) conducting organic salt were incorporated into the bulk of the composite to form a renewable biosensor. Several graphite-TTF·TCNQ ratios (w/w) were studied in order to select the best biosensor to be integrated in a FIA system for the automated detection of glucose. The optimal amount of GOD in the composite was studied as well. The selection was based on the analytical response of the electrodes. Best results were obtained by an electrode whose composition was 5% GOD, 76% polymer, 9.5% graphite and 9.5% TTF·TCNQ. An especially designed flow amperometric cell was constructed so that the biosensor could be integrated into a FIA system and glucose in beverage samples could be determined.

Keywords: Glucose; Biosensor; Graphite-epoxy biocomposite; TTF·TCNQ; FIA; Amperometric detection

1. Introduction

The search for novel materials to design electrochemical biosensors is a problem of great concern. The development of composite-based electrodes may lead to important advances in biosensor devices. These rigid conducting composites combine the electrical properties of graphite with the ease of processing of plastics, showing excellent electrochemical, physical, mechanical and economical properties [1,2]. It should also be taken into account that a great number of biological materials can be incorporated by blending them with the polymer and the graphite to form a biocomposite material [3]. In this way, the sensing material acts as a reservoir of the biological components, and the polishing of the surface allows its renewal. Rigid conducting graphite-polymer biocomposites have been extensively used in our laboratories for electrochemical sensing. Thus, enzymatic [4–7], immunologic [8,9] and genetic [10,11] sensors have been developed in the last decade.

Enzymatic amperometric glucose biosensors have been widely studied in the last four decades [12], and many approaches referring to their design, materials, membrane composition or enzyme immobilisation strategies have been described [13–15]. The first devices developed (first-generation of glucose biosensors) were based on the use of the oxygen as natural co-substrate and on the detection of the produced hydrogen peroxide. The main problem of this simple approach was that the amperometric measurement of hydrogen peroxide required the application of a potential in which other species could be oxidized (i.e. ascorbic and uric acids) limiting the selectivity of the method. Another drawback was the errors produced by the fluctuations in the oxygen tension, known as the "oxygen deficit". In the second-generation of glucose biosensors, the oxygen is replaced by another electro active species able to transfer electrons from the redox centre of the enzyme to the surface of the electrode. Ideally, these mediators should be able to react rapidly with

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the reduced enzyme, its heterogeneous kinetics should be reversible, they should have a low and pH independent overpotential for the regeneration of its oxidized form, both oxidized and reduced forms should be stable and finally, the reduced form should not react with oxygen [16]. Despite all these theoretical requirements, most of the mediators traditionally used (ferrocene and its derivatives, tetrathiafulvalene, some organic dyes, ferricyanide) are soluble in both or at least one of their redox states. This fact requires them to be used in solution or entrapped onto the electrode surface by means of membranes. Other elaborated strategies to keep mediators in contact or as close as possible to the immobilised enzyme in the biosensor are polymerisation and electrodeposition.

Organic conducting salts, such as tetrathiafulvalene–tetracyanoquinodimethane (TTF·TCNQ) allow direct electron-transfer with a number of enzymes, particularly flavoproteins (glucose oxidase) through their prosthetic group. NADH can also be oxidized onto the organic salt surface and thus, dehydrogenases can be used together with the organic salt as well. However, the electron-transfer mechanism is not clearly understood. Different approaches have been proposed: homogeneous mediation due to small amounts of TTF·TCNQ dissolved [17], heterogeneous catalysis [18] and direct electron transfer, which is the so-called Third-Generation of glucose biosensors [19].

TTF·TCNQ has been incorporated as part of biosensing membranes in different ways. Thus, it can be found packed with the enzyme immobilised on its surface [20,21], adsorbed [22] or grown at the surface of electroconductive films [23–25]. All these techniques are either difficult to perform by non-qualified personnel or time-consuming, and therefore, non-suitable for electrode mass production. In this work TTF·TCNQ powder is hand-mixed together with graphite, glucose oxidase and epoxy resin to easily construct a glucose biosensor based on a rigid conducting biocomposite. This novel sensing material offers attractive features to be used in batch or FIA systems for glucose determinations.

2. Materials and methods

2.1. Reagents

Graphite powder with a particle size of 50 μm (BDH Laboratory Supplies), epoxy resin Epo-Tek H77 (Epoxy Technology), TTF and TCNQ (Fluka), and GOD type VII 185 U mg^{-1} from *Aspergillus niger* (Sigma) were used to prepare the biocomposite paste.

TTF·TCNQ organic salt was prepared by mixing identical volumes of equimolar solutions (0.1 M) of each compound in hot anhydrous acetonitrile. The reaction was let to complete overnight with stirring and the black crystals formed were rinsed with cold acetonitrile and diethyl ether, and then dried under vacuum.

Glucose stock solutions were prepared with D-(+)-glucose monohydrate (Fluka) in phosphate buffer, and were left at 4 °C overnight to allow the equilibration of the anomers. A commercially available Glucose Assay Kit (Sigma) was used for comparison purposes.

Aqueous solutions of 0.1 M phosphate and 0.1 M KCl, buffered at pH 7.5 were used to perform both batch and FIA experiments. All other reagents used were of analytical grade.

2.2. Instrumentation

Current intensities were measured with an LC-4C amperometric controller (BAS Bioanalytical Systems Inc.) connected to a Labograph 517 graphic recorder (Metrohm). Cyclic voltammetries were performed with an Autolab PG-STAT20 (Eco Chemie). The reference electrode was a double junction Ag/AgCl electrode (Orion 900200) with 0.1 M phosphate and 0.1 M KCl as external reference solution. In batch experiments, a platinum electrode (Crison 52-671) was used as counter electrode. pH measurements were performed with a glass electrode (Crison 52-03) connected to a potentiometer (Crison MicropH 2002).

The flow system, shown in Fig. 1, consisted in a Miniplus 3 peristaltic pump (Gilson), a six-way injection valve (Omnifit) and a homemade methacrylate flow cell which integrated both working and counter electrodes. The flow cell volume was 140 μL. This flow cell was formed by a stainless steel counter electrode (block A) that was screwed in methacrylate block B, where the biosensor (C) was allocated.

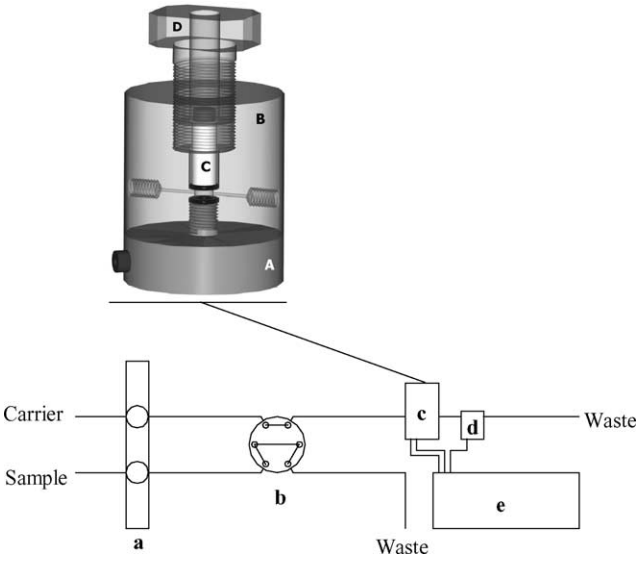

Fig. 1. Flow injection system diagram and flow-through cell detail. The cell (c) has a stain-less steel part (A) that serves as counter electrode. The methacrylate cell body (B) is attached to this lower block by screwing forming an inner compartment where the biosensor (C) is placed and fixed by means of a methacrylate sealing screw (D). The space between the counter electrode and the biosensor defines the cell volume. A standard double junction Ag/AgCl reference electrode (d) is positioned next to the flow-through cell, in the downstream flow. A peristaltic pump (a), a six-way injection valve (b) and the amperometric detection unit (e) complete the flow system.