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A new osmium-polymer modified screen-printed graphene electrode for fructose detection

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ABSTRACT

This paper describes the development and performance of the first fructose biosensor based on a commercial screen-printed graphene electrode (SPGE). The electrode was modified with an osmium-polymer, which allowed the efficient wiring of the enzyme fructose dehydrogenase (FDH). The immobilization of both osmium-polymer and FDH was realized in an easy way. Aliquots of 10 μL Os-polymer and 10 μL FDH were thoroughly mixed with poly(ethylene glycol) (400) diglycidyl ether (PEDGE) and deposited on the electrode surface and left there to dry overnight. The biosensor exhibits a detection limit of 0.8 μM, a linear range between 0.1 and 8 mM, high sensitivity to fructose (2.15 μA cm^{-2}/mM), good reproducibility (RSD = 1.9%), fast response time (3 s) and a stability of 2 months when stored in the freezer.

The proposed fructose biosensor was tested in real food samples and validated with a commercial spectrophotometric enzymatic kit. No significant interference was observed with the proposed biosensor.

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1. Introduction

Fructose is an insulin-independent monosaccharide with a sweetening ability higher than that of glucose or sucrose. It is used in the preparation of a variety of food, drinks, and as a diet sweetener [1,2]. Determination of fructose in food [1] and in biological fluids [3,4] is therefore of great importance. Several conventional analytical methods for the determination of D-fructose have been largely described in literature, such as gas-chromatography [5], liquid chromatography [6], fluorimetric [7], near infrared spectroscopy [8] and electrochemistry [9]. Unfortunately, these methods do not allow an easy and rapid monitoring because they require expensive instrumentation, well trained operators and often long time of analysis.

The food industry needs rapid, affordable and selective methods to determine sugars such as glucose and fructose. Biosensors offer an interesting alternative: besides their good selectivity and low cost, they can be used to develop simple, portable and cheap equipment allowing fast in situ monitoring [10–12]. Many glucose biosensors have been reported in the literature [13–17] but very few reports have been published related to fructose biosensors. This is presumably due to a common way to make fructose biosensor based on the necessity of using three types of enzymes (hexokinase, EC 2.7.1.1, phosphoglucose isomerase, EC 5.1.3.9 and glucose-6-phosphate dehydrogenase, EC 1.1.1.49) adding as well two soluble coenzymes (ATP and NAD^+), thus increasing the complexity of the analysis [1,2,13,14].

D-Fructose dehydrogenase (FDH; E.C. 1.1.99.11), first described by Ameyama and Adachi (1982), catalyses the oxidation of fructose to 5-keto-D-fructose with the concomitant reduction of the bound cofactor flavin adenine dinucleotide (FAD) [18,19]. It represents an ideal enzyme for development of fructose biosensors because no addition of any other enzyme or cofactors is required. Several biosensors, based on carbon paste [20–22], gold [23,24], Pt [25–27], graphite [28] and glassy carbon electrodes [29] have been modified with FDH. More recently a biosensor for fructose detection based on the modification of a CNTP electrode with an osmium redox polymer has been presented [30–32]. However, up today few articles about fructose biosensor using the screen printing technique have been reported [25,28].

Screen-printed electrodes (SPEs) offer a number of advantages versus conventional electrodes as they are suitable for working with microvolumes and for decentralized assays and allow the development of mass produced portable, accurate and reproducible sensors [33].

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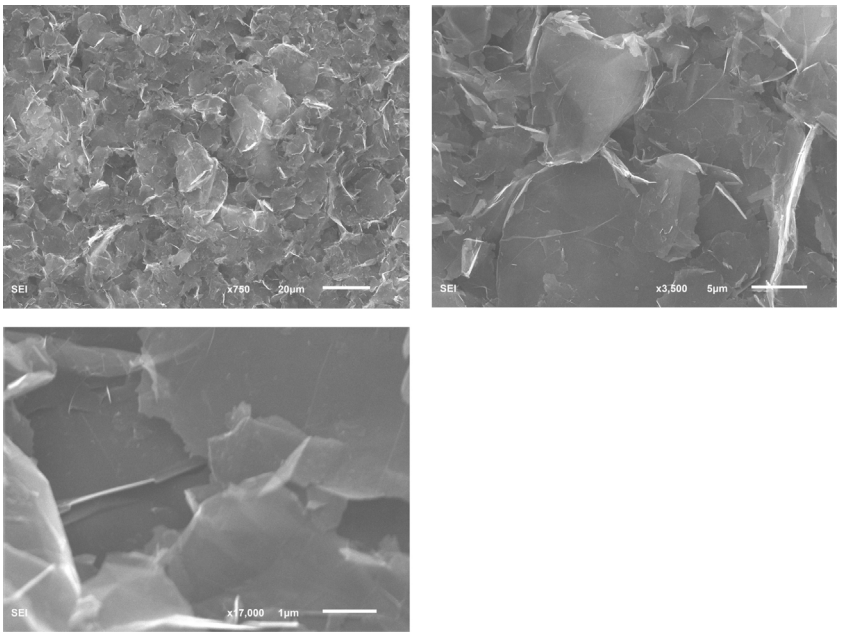

Fig. 1. SEM images of the screen-printed graphene electrode (DropSens, Oviedo, Spain).

Graphene, which is a recently discovered single-atom-thick planar sheet of carbon atoms perfectly arranged in a honeycomb lattice, has great potential in biosensing owing to its extraordinary electrical, physical, and optical properties [34–37]. These unique properties (fast electron transport rate, high termal conductivity, excellent mechanical flexibility and good biocompatibility) give it a large applicability in electrochemical biosensors [38–41].

The present work describes the development of the first fructose biosensor based on a commercial available screen-printed graphene electrode (SPGE) modified with a redox active osmium-polymer and FDH. It is known in literature that the efficient electron shuttling properties of the osmium redox polymer allowed its utilization for electrical wiring of cells and enzymes [42,43] and for biosensor and biofuel cell construction [44–46].

Here we successfully used the osmium-polymer as it simultaneously performs the function of both mediator and support: it is able to shuttle the electrons between FDH and the electrode allowing at the same time direct wiring of FDH itself onto the electrode surface by using poly(ethylene glycol) diglycidyl ether (PEDGE) as cross-linking agent.

Experimental parameters such as applied potential, enzyme concentration, type of material constituting the screen-printed electrode, pH and temperature have been studied and optimized. Finally, the applicability of the developed fructose biosensor was tested for fructose analysis in real samples and the results obtained were in good agreement with those determined with the standard spectrophotometric method.

2. Experimental

2.1. Reagents

Fructose dehydrogenase (FDH) (E.C. 1.1.1.47) from *Gluconobacter* sp. and D-fructose were purchased from Sigma (St. Louis, MO, USA). Poly(ethylene glycol) (400) diglycidyl ether (PEDGE) was obtained from Polyscience (Warrington, PA, USA). Poly(1-vinylimidazole)$_{12}$-[osmium(4,4′-dimethyl-2,2′-dipyridyl)$_2$Cl$_2$]$^{2+/+}$ (osmium redox polymer) was generously provided as a gift from ThereSense Inc. (Alameda, CA, USA). All other chemicals were from Carlo Erba (Milan, Italy). All solutions were prepared with high purity water produced by a Milli-Q System (Millipore, Bedford; MA, USA).

2.2. Apparatus and measurements

Cyclic voltammetry experiments were performed using an Autolab electrochemical system (Eco Chemie, Utrecht, The Netherlands) equipped with PGSTAT-12 and GPES software (Eco Chemie, Utrecht, The Netherlands). Screen-printed graphene electrodes (ref. 110GPH), single-walled (ref. 110SWCNT) and multi-walled (ref. 110MWCNT) carbon nanotubes modified screen-printed electrodes and an edge connector (ref. DRP-DSC) were purchased from DropSens (Oviedo, Spain) The SEM images of the screen-printed graphene electrode are reported in Fig. 1. The electrochemical cell consists in a graphene working electrode (4 mm diameter), and a carbon auxiliary and a silver pseudo reference electrodes. The electrochemical cell contained 10 mL of 0.1 M phosphate buffer at various pHs. All experiments were carried out at room temperature. Cyclic voltammetry experiments were performed at a scan rate of 10 mV/s over the chosen potential range using 0.1 M phosphate buffer (pH 7.0). Spectrophotometric measurements were carried out with an Amel analyser, model 433 manufactured by Amel (Milan, Italy), equipped with a printer and interfaced with a PC.

2.3. Construction of the Os-polymer modified screen-printed graphene fructose biosensor

The fructose biosensor was assembled through wiring of FDH onto the Os-polymer hydrogel. The chemical structure of the Os-polymer is shown in Fig. 2. This method involved a thorough mixing of 10 μL of a solution of the Os-polymer (10 mg/ml) in Milli-Q water, 1 μL of an aqueous solution of PEDGE (2.5 mg/mL) and 10 μL of a solution of FDH (10 U). Successively, a 10 μL aliquot of this solution was deposited on the SPGE surface and left to dry overnight at room temperature [28]. The modified electrode containing 5 U of FDH was rinsed carefully with 0.1 mol/L phosphate buffer at pH 7.0 before use. The procedure by using instead 20 μL or 40 μL of the FDH solution was used to construct biosensors with 10 or 20 U, respectively.