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Rapid and direct determination of fructose in food: A new osmium-polymer mediated biosensor

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ABSTRACT

This paper describes the development and performance of a new rapid amperometric biosensor for fructose monitoring in food analysis. The biosensor is based on the activity of fructose dehydrogenase (FDH) immobilised into a carbon nanotube paste electrode according to two different procedures. The direct wiring of the FDH in a highly original osmium-polymer hydrogel was found to offer a better enzyme entrapment compared to the immobilisation of the enzyme in an albumin hydrogel. The optimised biosensor required only 5 U of FDH and kept the 80% of its initial sensitivity after 4 months. During this time, the biosensor showed a detection limit for fructose of 1 μM, a large linear range between 0.1 and 5 mM, a high sensitivity (1.95 μA cm^{-2} mM), good reproducibility (RSD = 2.1%) and a fast response time (4 s).

Finally, the biosensor was applied for specific determination of fructose in honey, fruit juices, soft and energy drinks. The results indicated a very good agreement with those obtained with a commercial reference kit. No significant interference was observed with the proposed biosensor.

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1. Introduction

D-Fructose is an important sugar used as a low-cost sweetener by the food and beverage manufacturers. It is widely distributed in fruit juices, honey, soft and energy drinks and diabetic food as its sweetening ability is greater than that of glucose and sucrose. Excessive uptake of fructose is harmful and therefore it is an analyte of great interest for the food industry and clinical diagnostics (Frattali, 1982). Food quality control is essential both for consumer protection and for food industries. Conventional methods for sugar analysis use techniques such as chromatography, spectrophotometry, electrophoresis and titration (AOAC, 1995; Beutler, 1984) but these methods do not allow an easy and rapid monitoring because they require expensive instrumentation, well trained operators and often elaborate sample pretreatment with an increasing time of analysis. A great deal of research is therefore needed to develop a simple, fast and sensitive method, which could be effectively used by the food industries. Biosensors offer a promising alternative: besides their good selectivity and low cost, they can be used to develop simple and portable equipment allowing fast in situ monitoring of raw materials and food processing steps (Eggins, 2002; Tran & Cahn, 1993; Wagner & Guilbault, 1994).

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) (Ameyama & Adachi, 1982; Kamitaka, Tsujimura, Setoyama, Kajno, & Kano, 2007), thus representing an ideal enzyme for biosensor construction because no addition of the well-known $NAD(P)^+$/NAD(P)H cofactor is required. Several biosensors, based on carbon paste (Bassi, Lee, & Zhu, 1998; Garcia, Neto de Oliveira, Kubota, & Grandin, 1996; Paredes, Parellada, Fernandez, Katakis, & Domínguez, 1997; Parellada, Domínguez, & Fernandez, 1996), gold (Campuzano, Loaiza, Pedrero, Villena, & Pingarron, 2004; Damar & Demirkol, 2011), Pt (Antiochia & Palleschi, 1997; Moscone, Bernardo, Marconi, Amine, & Palleschi, 1999; Trivedi, Lakshminarayana, Kothari, Patel, & Panchal, 2009), graphite (Piermarini, Volpe, Esti, Simonetti, & Palleschi, 2011) and glassy carbon electrodes (Tkac et al., 2001) have been modified with FDH using different immobilisation techniques. In recent years, nanotechnology and nanomaterials have been revolutionising the area of biosensors. In particular carbon nanotubes have begun to attract enormous interest in electrochemistry for biosensor construction because of their small size and their good electrochemical properties (Antiochia, Lavagnini, & Magno, 2005; Antiochia, Lavagnini, Magno, Valentini, & Palleschi, 2004a; Valentini, Amine, Orlanducci, Terranova, & Palleschi, 2003). Recently, for this reason and for their easy preparation and the possibility of renewal of their surface, carbon nanotube paste (CNTP)

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electrodes have started to become popular for electrode modification (Antiochia, Lavagnini, & Magno, 2004b; Gooding, 2005; Wang, 2005).

The objective of the present work is to report a new alternative biosensor for fructose detection based on the modification of a CNTP electrode with an osmium redox polymer. The efficient electron shuttling properties of the osmium redox polymer allowed its utilisation for electrical wiring of cells and enzymes (Timur, Yigzae, & Gorton, 2006b; Timur et al., 2006a) and for biosensor construction (Antiochia & Gorton, 2007; Heller, 1992; Heller & Feldman, 2008).

In our work the osmium redox polymer was used as redox mediator to shuttle the electrons between the immobilised enzyme and the single-walled carbon nanotube paste (SWCNTP) electrode and also as a support for direct wiring of FDH itself into the paste by using poly(ethylene glycol) diglycidyl ether (PEDGE) as a cross-linking agent. As well as optimisation studies, application of the proposed biosensor for fructose analysis in real samples were carried out and the results obtained were in good agreement with those determined with the standard spectrophotometric method.

2. Experimental

2.1. Chemicals

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). Single-walled carbon nanotubes Carbolex (diameter 1–2 μm) were obtained from Aldrich (Steinheim, Germany). Mineral oil was obtained from Fluka (Buchs, Switzerland). 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. Construction of the Os-polymer modified CNTP electrode

The CNTP electrodes were prepared by hand-mixing SWCNTs and graphite powder with mineral oil at a 60:40% ratio (w/w) (Valentini et al., 2003). The paste was mixed in a mortar and packed into a cavity (3 mm diameter, 0.5 mm depth) at the end of a Teflon tube and electrical contact was established via a copper wire connected to the paste. The electrode surface was gently smoothed by rubbing it on a piece of filter paper before use.

The Os-polymer CNTP electrode was prepared by depositing on the CNTP electrode surface 10 μL of a solution (10 mg/mL) of the Os-polymer in Milli-Q water and 1 μL of an aqueous solution (2.5 mg/mL) of the cross-linker agent PEDGE (Antiochia & Gorton, 2007). After the deposition, the electrodes were left to dry overnight at room temperature.

2.3. Construction of the modified CNTP fructose biosensor

The fructose biosensor was assembled according to two different procedures of FDH immobilisation. In the first procedure FDH was immobilised in an albumin hydrogel. A 5 mg aliquot of albumin (BSA) was dissolved in 40 μL of 0.1 mol/L phosphate buffer with 10 μL of a solution of FDH (10 U). A 25 μL aliquot of the FDH-matrix system was successively mixed with 3 μL of glutaraldehyde (0.25% v/v) and entrapped between two polycarbonate membranes and fixed to the surface of the Os-polymer CNTP electrode. A 0.1 M phosphate buffer solution was used to gently rinse the biosensor and remove those of the glutaraldehyde molecules, which did not react with the polymeric matrix.

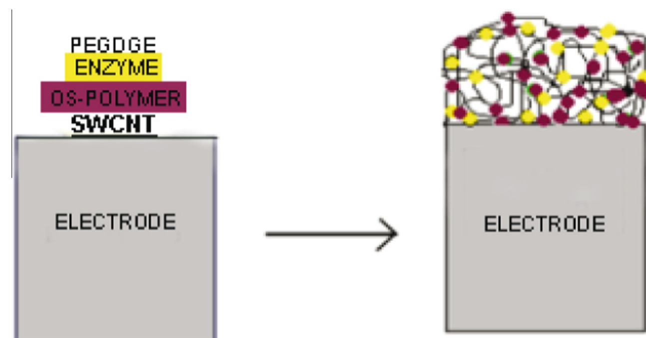

Fig. 1. Schematic representation of the FDH wiring through redox hydrogel of Os-polymer on CNTP electrode.

In the second procedure FDH was directly wired into the Os-polymer hydrogel. 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 CNTP electrode surface and left to dry overnight at room temperature (Antiochia & Gorton, 2007). The modified electrode was rinsed carefully with 0.1 mol/L phosphate buffer at pH 7.0 before use. A schematic representation of the wiring of FDH through the Os-polymer redox hydrogel on the CNTP electrode is shown in Fig. 1.

2.4. Electrochemical characterization

Electrochemical measurements were performed using an Autolab electrochemical system equipped with PGSTS-12 with GPES software (Eco Chemie, Utrecht, The Netherlands). All electrochemical experiments were carried out in a conventional three-electrode cell at room temperature with the modified CNTP electrode (3 mm diameter) as working electrode, an Ag|AgCl/KCl(sat) as the reference and a platinum wire as the counter electrode. The electrochemical cell contained 10 mL of 0.1 M phosphate buffer or 0.1 M acetate buffer at various pHs. A fixed potential, +200 mV versus Ag|AgCl, was used to make the amperometric experiments.

2.5. Biosensor response

For fructose determinations aliquots of a stock solution of D-fructose in 0.1 M acetate buffer at pH 5.0 were successively added in the electrochemical cell and the steady-state current values were recorded. The steady-state current was achieved within 20–25 s.

2.6. Analysis of food samples

The fructose biosensor was tested for the analysis of food samples like honey and some beverages, like fruit juice, soft and energy drinks, purchased from local supermarkets. Fructose concentrations in real samples were determined in five replicates on the basis of the calibration curve obtained for the standard fructose solution. The results obtained were compared with those obtained with the enzymatic spectrophotometric assay kit (Mannheim, Germany, Cat.N. 139106). The determination of fructose is related to the amount of NADPH formed and spectrophotometrically measured at 340 nm. Honey and beverage samples did not require any pretreatment. The honey samples were diluted as follows: 1 g of

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