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Modified gold surfaces by poly(amidoamine) dendrimers and fructose dehydrogenase for mediated fructose sensing

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ABSTRACT

An electrochemical biosensor for detection of fructose in food samples was developed by immobilization of fructose dehydrogenase (FDH) on cysteamine and poly(amidoamine) dendrimers (PAMAM)-modified gold electrode surface. Electrochemical analysis was carried out by using hexacyanoferrate (HCF) as a mediator and the response time was 35 s at +300 mV vs. Ag/AgCl. Moreover, some parameters such as pH, enzyme loading and type of PAMAM (Generations 2, 3 and 4) were investigated. Then, the FDH biosensor was calibrated for fructose in the concentration range of 0.25–5.0 mM. To evaluate its utility, the FDH biosensor was applied for fructose analysis in real samples. Finally, obtained data were compared with those measured with HPLC as a reference method.

1. Introduction

Fructose is an important monosaccharide in food and clinical samples [1]. A selective, fast, simple and low cost method for fructose determination is desirable, for this aim various biosensors using immobilized enzymes have been applied in batch or in flow mode. To analyze fructose in food and beverages, several electrodes such as gold [2], carbon paste [3], Pt [1,4], graphite [5,6], epoxy graphite [7] and GCE [8] have been modified by using fructose dehydrogenase by means of various immobilization techniques. Fructose dehydrogenase (FDH; EC1.1.99.11) is a membrane-bound oxidoreductase, which catalysis oxidation of fructose to 5-keto-D-fructose [9–11]. FDH was first detected by Yamada et al. in *Gluconobacter cerinus* [9,12,13]. FDH has both a pyrroloquinoline/quinine (PQQ) redox site and a few heme c sites like other membrane-bound dehydrogenases of *Gluconobacter* such as alcohol dehydrogenase and aldehyde dehydrogenase [14]. Active center of FDH is based on PQQ [3]. So that use of FDH is advantageous due to the fact that oxygen is not necessary to regenerate the catalytic center of the enzyme [15]. Nowadays, nanotechnology and nanomaterials are enormous chance to develop an efficient novel tool for food and bioprocess industry [16].

As immobilization materials, dendrimers are nanomaterials with highly branched, three dimensional uniform structures. Central core, branches and surface groups are main components of their dendritic architectures [17]. The use of dendrimers has been attractive subject in some areas such as drug delivery, gene therapy, highly sensitive analytical devices because of their nanosize (in the range 2.5 to10 nm) and ease of functionalization of end groups [18–20]. The well-known dendrimers are poly(propylene imine), poly(amidoamine) and poly(benzyl ether) dendrimers [21]. Moreover, amino-terminated amphiphilic poly(amidoamine) dendrimers (PAMAM) with NH_3 core, is a mix of polyamides and amines and have ellipsoidal or spheroidal shape [19,22]. PAMAM is a specific family of dendritic polymers which can be used in biomedical applications such as carriers for the delivery of drugs and DNA or oligonucleotides because of their different terminal groups [23–25]. Also, immunoconjugates of boronated PAMAM and monoclonal antibody have been applied as an efficient anti-cancer reactive. PAMAM has been employed as magnetic resonance imaging contrast agent to improve the quality of the clinical diagnostics [26]. PAMAM has also been used as stabilizer of the gold particles [22]. On the other hand, PAMAM-modified electrodes have great promise for various biosensing applications, for instance, DNA detection methods based on PAMAM were previously reported by different groups [27–29]. Moreover, Chen et al. reported the nitrite biosensor by the immobilization of cytochrome *c* (Cyt *c*) on glassy carbon electrode via PAMAM–chitosan nanocomposite [30]. In another study, acetylcholinesterase and choline oxidase were co-immobilized onto the Au electrode by means of PAMAM dendrimer for the pesticide analysis [31]. Use of PAMAM for the immobilization of alcohol dehydrogenase (ADH) onto carbon cloth platforms and its application as bioanode in ethanol/O_2 biofuel cells was carried out by Forti et al. [32]. Additionally, Horseradish peroxidase

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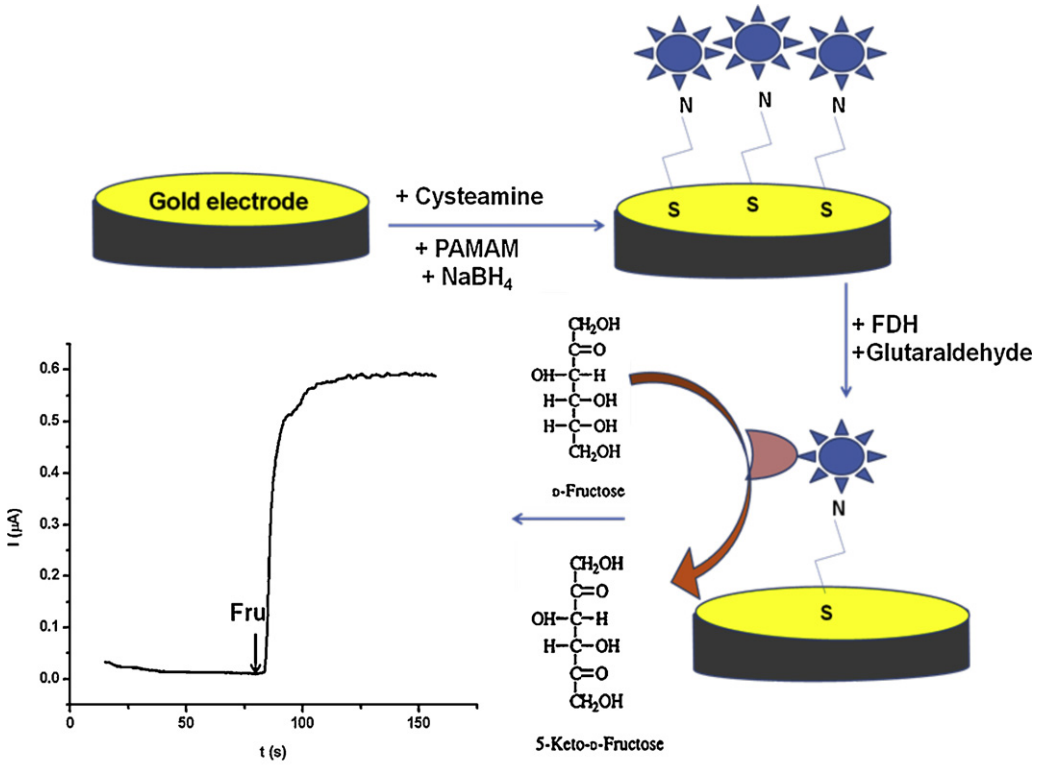

Scheme 1. Schematic representation of FDH/PAMAM biosensor construction including biosensor response current after addition of fructose in reaction medium.

(HRP), glucose oxidase (GOx), pyranose oxidase (PyOx) and alcohol oxidase (AOx) were immobilized on to Au gold surface modified by PAMAM/cysteamine [33–36].

In this study, a mediated fructose biosensor was prepared. FDH was immobilized on Au electrode after PAMAM modification. As well as characterization studies, optimization and application of FDH/PAMAM biosensor for fructose analysis in the real samples were carried out.

2. Materials and methods

2.1. Materials

Fructose dehydrogenase (FDH from *Gluconobacter* sp., 1.9 mg solid, 131 U/mg), fructose, cysteamine hydrochloride, and sodium borohydride were from Fluka (Steinheim, Germany), Poly(amidoamine) dendrimers (PAMAM, 75% amino and 25% [*N*-(2-hydroxydodecyl)] surface groups; 25% C12 Generation 4.0 dendrimer, 10 wt.% in methanol; 25% C12 Generation 3.0 dendrimer, 20 wt.% in methanol and 25% C12 Generation 2.0 dendrimer, 20 wt.% solution in methanol) and glutaraldehyde solution (25%, v/v) were purchased from Sigma–Aldrich (Dorset, UK).

2.2. Instruments

Cyclic voltammetric and amperometric measurements were carried out on PalmSens Electrochemical Measurement System. A three-electrode cell (10 mL) was used with the modified gold electrode (Au; BASI, USA) as a working electrode, a silver chloride (Ag/AgCl; Metrohm) as a reference electrode, and a platinum electrode (Metrohm) as a counter-electrode. Cyclic voltammetric experiments were performed in unstirred acetate buffer (pH 4.5; 50 mM).

2.3. Fabrication of FDH/PAMAM biosensor

The gold surface was initially polished with alumina polishing suspension (Baikowski International Corporation, 0.05, 0.1, 0.3, 1.0 and 3.0 μm). Then, it was cleaned in 0.5 M H_2SO_4 solution by cyclic voltammetry between 0 and +1.5 V until a reproducible response was obtained. The polished gold electrode was subsequently immersed in 0.1 M cysteamine for 30 min, glutaraldehyde solution (5.0% in sodium phosphate buffer; pH 7.0, 50 mM) for 30 min, PAMAM dendrimer (1.0%; in 50 mM sodium phosphate buffer; pH 7.0) for 1 h and $NaBH_4$ solution (5.0 mM) for 30 min, respectively [34–36]. After each step, the electrode surface was fully rinsed with distilled water. Finally, glutaraldehyde (10 μL; 1.0% in 50 mM pH 7.0 phosphate buffer) and FDH (1.0 μL; 12.4 U) solutions were dropped on the PAMAM modified gold electrode surface. In this case, the cross-linkages between amino groups of PAMAM and aldehyde groups of glutaraldehyde and then the cross-linkage between another aldehyde group of glutaraldehyde and amino group of the enzyme were occurred. After the surface was dried at room temperature (1 h), the FDH/PAMAM biosensor was washed with water to remove unbound enzyme and excess of glutaraldehyde. The schematic representation of FDH/PAMAM biosensor fabrication was shown in Scheme 1. The FDH/PAMAM biosensors were stored in acetate buffer (pH 4.5; 50 mM) at 4 °C when not in use. Daily prepared electrodes were used during the experiments.

2.4. Measurements

All measurements were performed at room temperature in an open vessel filled with the vigorously stirred 5.0 mM acetate buffer solution, pH 4.5. Increasing concentrations of fructose were adjusted by adding definite volumes of the stock solution of 1.0 M fructose (in pH 4.5, 50 mM acetate buffer). The biosensor response were evaluated via the following steps: Firstly, the FDH/PAMAM