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Flow-injection amperometric determination of glucose using a biosensor based on immobilization of glucose oxidase onto Au seeds decorated on core Fe_3O_4 nanoparticles

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ABSTRACT

An amperometric biosensor based on chemisorption of glucose oxidase (GOx) on Au seeds decorated on magnetic core Fe_3O_4 nanoparticles (Fe_3O_4@Au) and their immobilization on screen-printed carbon electrode bulk-modified with manganese oxide (SPCE{MnO_2}) was designed for the determination of glucose. The Fe_3O_4@Au/GOx modified SPCE{MnO_2} was used in a flow-injection analysis (FIA) arrangement. The experimental conditions were investigated in amperometric mode with the following optimized parameters: flow rate 1.7 mL min^{-1}, applied potential +0.38 V, phosphate buffer solution (PBS; 0.1 mol L^{-1}, pH 7.0) as carrier and 3.89 unit mm^{-2} enzyme glucose oxidase loading on the active surface of the SPCE. The designed biosensor in FIA arrangement yielded a linear dynamic range for glucose from 0.2 to 9.0 mmol L^{-1} with a sensitivity of 2.52 μA mM^{-1} cm^{-2}, a detection limit of 0.1 mmol L^{-1} and a quantification limit of 0.3 mmol L^{-1}. Moreover, a good repeatability of 2.8% (number of measurements $n=10$) and a sufficient reproducibility of 4.0% (number of sensors $n=3$) were achieved. It was found that the studied system Fe_3O_4@Au facilitated not only a simpler enzyme immobilization but also provided wider linear range. The practical application of the proposed biosensor for FIA quantification of glucose was tested in glucose sirup samples, honeys and energy drinks with the results in good accordance with those obtained by an optical glucose meter and with the contents declared by the producers.

1. Introduction

The development of biosensors has attracted much attention due to their possible applications in the fields of clinical monitoring and diagnosis as well as in industrial quality and process control [1–3]. Very widespread are amperometric enzyme biosensors which are often based on the oxidation of the target analyte with an appropriate oxidase, where the formation of hydrogen peroxide is monitored (first generation oxidase biosensors) [4–6]. Glucose oxidase represents one of the most exploited enzymes of the oxidase group [7]. The fabrication of glucose biosensors was achieved with immobilization of the enzyme by different methods such as physical adsorption [8,9], microencapsulation [10,11], entrapment [12,13], cross-linking [14,15], or covalent bonding [16,17]. However, some of these sensors show only fair sensitivity and stability or have the disadvantage that the immobilization procedures are long and require many steps.

Food production and quality control requires fast and easy-to-manage sensing of certain food components in order to guarantee proper processing. The quicker the analysis can be performed, the better the production process can be altered or adapted. Facile analysis and quick handling can be simply achieved by magnetic nanoparticles.

Nanoparticles of noble metals have attracted much attention recently, particularly because of their high surface area combined with improvements of the electron transfer. Thus, very often catalytic or even synergistic effects with other modifiers can be observed with corresponding electrochemical sensors.

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Recently, Fe_3O_4@Au core shell magnetic gold nanoparticles have been widely investigated as great materials for the detection of biomolecules in biosensors due to excellent biocompatibility, magnetic properties, low toxicity, and high stability [18–22]. Fe_3O_4@Au magnetic nanoparticles in biosensors feature many advantages, such as (i) rapid production of the biosensor with the modified nanoparticles under chemically mild conditions using an external magnetic field only, (ii) high surface-to-volume ratio and biocompatibility of Au resulting in increased sensitivity, (iii) less cluster aggregation when Fe_3O_4@Au magnetic nanoparticles are directly used, and (iv) simply renewable surface of the electrode with the aid of an external magnetic field. Moreover, the incorporation of some metal oxides in carbonaceous materials results in an improvement of the biosensor's sensitivity and operation potential. MnO_2 is considered as a promising candidate for many electrochemical applications because it has many user-friendly properties, such as low-cost, easy availability and good chemical stability; it is eco-friendly and can be used in green chemistry [23–27].

The growing interest of using biosensors as quantitative detectors in flow-injection analysis derives from their excellent selectivity, high sensitivity as well as good repeatability and response times in compatibility with flow-injection analysis (FIA). Screen-printed carbon electrodes (SPCE) in combination with FIA have been frequently used for the construction of simple portable devices for fast screening purposes and in-field/on-site monitoring, because of their low cost, high sample throughput and easy integration into mass-production processes [28–30]. Screen-printed electrodes are an ideal type of mass-produced sensors because they show many advantages over other heterogeneous electrodes (e.g., carbon paste), such as easy, quick and reproducible mass-production with equivalent electrode characteristics, high mechanical robustness, long shelf-lifetime and possibility of miniaturization.

This paper describes the construction, characterization and application of a glucose biosensor based on GOx/Fe_3O_4@Au/SPCE{MnO_2} using FIA in amperometric mode. The basic cross-over effects between MnO_2 and Au-NPs should be investigated focusing on easy handling of the sensor in combination with low intensity of labor involved. A synergistic effect of MnO_2 as a mediator in combination with Fe_3O_4 nanoparticles, resulting in an enhanced sensitivity, was observed. Moreover, the practical application of this biosensor in FIA arrangement was successfully manifested by quantifying the glucose content in food samples.

2. Experimental

2.1. Reagents and solutions

Highly pure water (Mili-Q cartridge system, 18.2 MΩ cm) was used throughout for preparing solutions. Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4, specific activity 250 kU/g, GOx) and $HAuCl_4 \cdot 3H_2O$ were purchased from Sigma Aldrich (Austria). All other chemicals were of analytical grade (Sigma Aldrich, Austria) and used without further purification. A permanent neodymium magnet (8 mm in diameter, 3 mm in depth) was purchased from Master Magnetics, Inc. (Castle Rock, China). Phosphate buffer solutions (PBS, 0.1 mol L^{-1}) with different pH values were prepared by mixing solutions of potassium dihydrogen phosphate (0.1 mol L^{-1}) and disodium hydrogen phosphate (0.1 mol L^{-1}). The glucose stock solution (5.55 mol L^{-1}) was prepared by dissolving glucose in water. Energy drinks and glucose sirup were bought in a supermarket in Thailand, honey samples were purchased from local supermarkets in Thailand and Austria. Dilution of working solutions and samples was performed just before analysis with the desired pH phosphate buffer solution.

2.2. Apparatus

The flow-injection system was constructed using a peristaltic pump (ISMATEC, REGLO Analog model MS-2/6, Switzerland), an injection valve (5020 Rheodyn, Cotati, CA, USA), and a thin-layer electrochemical detector (LC 4C, BASi, West Lafayette, Indiana, USA) with a flow through cell (spacer thickness 0.19 mm; CC-5, BASi) in combination with an electrochemical workstation (Auto-Lab, PGSTAT12, Metrohm). The working electrode (screen printed carbon electrode bulk-modified with MnO_2) was placed into the groove of a corresponding Teflon plate which was fixed directly via the spacer to the back plate of the thin layer cell. A cylindrically shaped neodymium magnet was placed into a drilling on the outer side of the Teflon plate, positioned in the center just behind the working electrode. Silver conductive paint (Electrolube Ltd., Wargrave Berkshire, UK) was applied on one end of the SPCE, to which a crocodile clamp was attached for electrical contact; an Ag/AgCl-electrode (3 mol L^{-1} KCl) served as the reference. The steel back plate of the thin layer cell acted as the auxiliary electrode. The pH of solutions was measured with a pH-meter from Sartorius (PP-50, Germany). A glucose meter (FreeStyle Lite Test Strips, Abbott Diabetes Care, USA) was used as a comparative method for analysis of the samples.

2.3. Synthesis of Fe_3O_4@Au seeds nanoparticles

The nanoparticles were prepared as described elsewhere [31–34]. In short, all glassware used for the synthesis was cleaned with aqua regia. Fe_3O_4 nanoparticles (0.0150 g, diameter < 50 nm, Sigma Aldrich) were suspended in 200 mL of water and sonicated for 10–15 min at 0 °C in an ultrasonic bath (Transsonic 700/H, Elma). Au seeds on the Fe_3O_4 cores were prepared by adding $HAuCl_4$ (0.8 mole per mole of Fe_3O_4) and subsequently 0.04 mole $NaHB_4$ to the cooled suspension which was sonicated for further 10–15 min and kept in a refrigerator until use.

2.4. Preparation of the glucose biosensor for flow-injection analysis

Screen-printed electrodes were prepared by mixing manually MnO_2 (0.05 g) and carbon ink (1 g; Electrodag 421 SS, Acheson) for 10–30 min and subsequent sonication for 30 min in the ultrasonic bath. Five percent of MnO_2 is the optimum concentration of the modifier in the ink according to previous studies [23,24,35]. The resulting mixture was printed onto an inert laser pre-etched ceramic support ($113 \times 166 \times 0.635$ mm^3, CLS 641000396R, CoorsTek, Glenrothes, Scotland) by a semi-automatic screen-printing device (SP-200, MPM, Franklin, MA, USA). Thick layers of the modified carbon ink were formed by brushing the ink through an etched stencil (thickness 100 μm, electrode printing area 105 mm^2). The screen printed plates were dried at room temperature overnight before use.

The Fe_3O_4@Au seeds nanoparticles were loaded with the enzyme through adsorption by adding 20 μL of the GOx solution (25 U mL^{-1}) in PBS to 200 μL of the nanoparticle suspension in a vial (1.5 mL micro-centrifuge tube, 616.201, Greiner Labor Technic), mixing with a vortex mixer for 15 min and then left overnight in a refrigerator at 4 °C until use; with exposure times of 8 h or more maximum signal was obtained. The resulting mixture was directly applied onto the active area of the SPCE (≈ 14 mm^2) prior to launching the measurements. The particles were attached to the electrode surface by the magnet of the electrode holder (Fig. 1); the sensor surface was rinsed with water before assembling the thin layer cell.