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A sensitive and selective on-line amperometric sulfite biosensor using sulfite oxidase immobilized on a magnetite-gold-folate nanocomposite modified carbon-paste electrode

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ABSTRACT

We describe a novel amperometric sulfite biosensor, comprising a carbon-paste electrode (Fe_3O_4@Au-Cys-FA/CPE) modified with immobilized sulfite oxidase (SOx) on a gold-coated magnetite nanoparticle core, encased within a conjugated folic acid (FA) cysteine (Cys) shell. The biosensor electrode was fabricated using a polydimethylsiloxane (PDMS) and mineral oil mixture as binder, which also enhances the physical stability and sensitivity of the electrode. The developed biosensor displays good electrocatalytic activity toward oxidation of H_2O_2, which occurs by an enzymatic reaction between SOx and sulfite. The Fe_3O_4@Au-Cys-FA electrode exhibits good electrocatalytic activity, and has good retention of chemisorbed SOx on the electrode because of its large surface area. Sulfite was quantified using amperometric measurements from the Fe_3O_4@Au-Cys-FA/CPE biosensor, and using an in-house assembled flow cell at +0.35 V (vs. Ag/AgCl), with a phosphate buffer carrier (0.10 M, pH 7.0) at a flow rate of 0.8 mL min^{-1}. The system detects sulfite over the range 0.1–200 mg L^{-1} (r^2=0.998), with a detection limit of 10 μg L^{-1} (3σ of blank). The system exhibits acceptable precision (%R.S.D.=3.1%), rapid sample throughput (109 samples h^{-1}), and good stability (2 w). The developed biosensor shows satisfactory tolerance to potential interferences, such as sugars, anions, ascorbic acid, and ethanol. We applied the developed method to the determination of sulfite content in wines and pickled food extracts, and our results are in good agreement with those obtained by the standard iodometric method.

1. Introduction

Sulfite ion (SO_3^{2-}) has wide application as a food preservative and as an antioxidant in food and beverages to inhibit enzymatic and non-enzymatic activities that cause browning, and to suppress the growth of microorganisms during storage [1–4]. However, sulfite is potentially toxic and may cause adverse reactions in sulfite-sensitive, asthmatic individuals [1–3]. The US Food and Drug Administration (FDA), recommends warning labels on any food that contains sulfite at concentrations greater than 10 mg kg^{-1}, and on beverage and wine products containing in excess of 10 mg L^{-1} sulfite [1–5]. To accurately control the quality of manufactured products, a simple, sensitive, and accurate analytical method for the determination of sulfite is required.

Various techniques for quantitative analysis of sulfite are available, including fluorescence spectroscopy [6], high-performance liquid chromatography in combination with ultraviolet spectrophotometry [7], and chemiluminescence spectroscopy [1,8]. However, these techniques are time-consuming, need sample pre-treatment and reagent preparation, and may suffer from lack of sensitivity or precision, and so, the development of more sensitive and selective methods is crucially important for routine monitoring. Electrochemical measurements, using enzymatic modification at metallic electrodes, carbon/graphite electrodes, or chemically modified electrodes, offer several benefits over traditional methods, including greater selectivity, sensitivity, and reliability, and offer the possibility of on-line applications [9]. Fabrication of ch7emically modified electrodes allows electrocatalysis of sulfite oxidation reactions and reduction of the sulfite oxidation potential [10–12], although the reported selectivity, sensitivity, and reliability are poor. Thus, it is of considerable interest to develop an enzyme-based biosensor for the detection of sulfite ion; this would provide high selectivity, sensitivity, and accuracy in the

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quantification of sulfite, without interference from sample constituents or fouling of the electrode. A high degree of selectivity toward sulfite is achievable by using sulfite oxidase (SOx) enzyme to convert sulfite to sulfate and H_2O_2 [9, 13–15]. There are several recent reports of amperometric sulfite-biosensors using SOx immobilized on various electrodes [13–15]. These biosensors use a SOx catalyzed reaction for sulfite detection; molybdenum active sites in SOx undergo both oxidation and reduction during oxidation of sulfite to sulfate [15]. Sulfite determination can be performed by detecting the SOx-electrode signal directly, or indirectly by monitoring the depletion of oxygen or formation of H_2O_2 [9,14,15]. The simplest approach might be to measure the generation of peroxide. However, the high operating potential of such a biosensor, can result in oxidation of other components within the sample matrix, which will generate nonspecific signals. Electron-transfer mediators such as tetrathiafulvalene (TTF) [15], cytochrome c (Cyt c) [13,14], and ferro/ferricyanide [16] provide greater selectivity for sulfite determination by H_2O_2 detection. Minimization of the biosensor operating potential is based on either regeneration of the mediator, or on catalysis of H_2O_2 formation, or both [9,15].

Magnetite (Fe_3O_4) nanoparticles have useful physical and chemical properties including being superparamagnetic, having a large surface area, strong adsorption characteristics, good mechanical stability and electrical conductivity, and superb electrocatalytic activity [17–19]. There are many approaches available for the functionalization or modification of Fe_3O_4 surfaces for biomedical applications. Methods include using metals, oxides, organic monolayers, and polymers [17]. One of the most promising modifications is to use gold-coated magnetite (Fe_3O_4@Au). This material is simple to prepare, offers the possibility of bioconjugation, and has good biocompatibility for drug delivery applications [17,18]. Samphao et al.[20] and Li et al. [21] reported the application of Fe_3O_4@Au to improve electrocatalytic activity in enzyme-based sensors for the analysis of glucose [20] and *Escherichia coli* [21]. Fe_3O_4@Au nanoparticles were integrated into composite materials to immobilize glucose oxidase [20] and horseradish peroxidase [21] via chemisorption or physisorption, and then used to modify screen printed electrodes [20] and glassy carbon electrodes [21]. Recently, Karamipour et al. [22] reported the synthesis of folic acid (FA)-cysteine (Cys)-conjugated gold-coated magnetite nanoparticles (Fe_3O_4@Au-Cys-FA) using L-cysteine as a bi-functional linker for attachment to the gold surface via its thiol group. The nanomaterial was characterized and designed its utilization to drug delivery applications. However, there are no reports of catalyst or biosensor applications using Fe_3O_4@Au-Cys-FA nanocomposite materials.

In this work, we report the synthesis of Fe_3O_4@Au-Cys-FA nanoparticles and fabrication of a sensitive and selective sulfite biosensor. The biosensor was constructed by immobilizing sulfite oxidase on a Fe_3O_4@Au-Cys-FA nanocomposite-modified carbon paste electrode via direct chemisorption. To our knowledge, this is the first report of using Fe_3O_4@Au-Cys-FA to enhance the electrocatalytic activity and enzyme immobilizing performance of a sulfite biosensor. The Fe_3O_4@Au-Cys-FA/CPE provides significant improvement in biosensor performance. The large surface area and unique nanostructure of Fe_3O_4@Au-Cys-FA provide good electrocatalytic activity and facilitate retention of SOx on the electrode, resulting in high sensitivity and selectivity by the biosensor. When applied to on-line amperometric detection of sulfite in a flow injection (FI) system, the sensor successfully quantified sulfite in pickled food extracts and wine samples. The biosensor exhibits excellent sensitivity, selectivity, and stability with a sample throughput of 109 samples per hour.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical reagent grade. Deionized-distilled water (Water Pro-PS, USA) was used for preparation of standards and reagents. Chicken liver sulfite oxidase (SOx, 30–70 U mg^{-1}) was purchased from ProNique Scientific, Inc. (Castle Rock, USA). Sodium sulfite (Na_2SO_3) and horse heart cytochrome c (Cyt c, 90%) were purchased from Sigma-Aldrich (St. Louis, USA). Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$), 99.99%), N, N′-dicyclohexylcarbodimide (DCC), folic acid ($C_{19}H_{19}N_7O_6$, 97% purity), L-Cysteine ($C_3H_7NO_2S$), tri-sodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), $NaH_2PO_4 \cdot H_2O$, Na_2HPO_4, ferric nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), ferrous sulfate heptahydrate ($Fe(SO_4)_2 \cdot 7H_2O$), and graphite powder were purchased from Acros Organic (Geel, Belgium). Poly(dimethylsiloxane) (Sylgard® 184) were purchased from Dow Corning (Wiesbaden, Germany).

2.2. Apparatus

2.2.1. Cyclic voltammetry

Cyclic voltammetry (CV) was performed in batches using an eDAQ potentiostat (model EA161, Australia) equipped with e-corder (model 210), and e-Chem software v2.0.13. For CV measurements, we used a self-assembled three-electrode cell, comprising a Fe_3O_4@Au-Cys-FA-SOx carbon-paste working electrode, an Ag/AgCl (sat.) reference electrode, and a platinum wire counter electrode. The carbon paste electrode (CPE) active surface area was approximately 0.031 cm^2 (inner diameter 0.2 cm). Electrochemical measurements were performed in phosphate buffered solution (PBS) (0.1 M, pH 7).

For the cyclic voltammetry study, we assembled electrodes by packing Fe_3O_4@Au-Cys-FA-SOx paste inside a glass tube (inner diameter 0.2 cm, length 7.5 cm) and fixing a copper wire in place using epoxy resin. The Fe_3O_4@Au-Cys-FA electrode surface was polished to a smooth surface with weighing paper. The electrode was encapsulated by drop casting 0.01% Nafion® solution in water (10 μL) onto the polished electrode surface. Finally, the electrode was dried at room temperature, and then stored at 4 °C in a refrigerator until required for use.

2.2.2. On-line amperometric detection using a simple flow injection system

The flow injection system used for amperometric detection of sulfite [23] comprised a Shimadzu pump (model LC-10CE, Japan), a Rheodyne injector (model 7725, USA) fitted with a 20 μL sample loop, and an electrochemical detector (ECD). For the measurements, we used an eDAQ potentiostat (EA161), equipped with an e-corder 210, Chart software v5.5.11, and our in-house three-electrode flow cell. The Fe_3O_4@Au-Cys-FA carbon-paste biosensor served as the working electrode, Ag/AgCl as the reference electrode, and a stainless steel tube as the counter electrode. The electrode area in the flow cell was approximately 0.060 cm^2.

The Fe_3O_4@Au-Cys-FA/CPE electrodes were assembled by packing composite paste inside the working electrode block of the thin-layer flow cell (Fig. 1B). The surface of the electrode was covered with a 0.01% Nafion® film (40 μL), formed by drop casting and drying at room temperature. In the ECD, a silicone rubber gasket (flow channel = 0.1×0.6 cm^2) provided a spacer between the base of the cell and the working electrode. The analyte solution passes through an inlet passage in the base, and along a channel in the gasket contacting the biosensor, then to the outlet.