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A glucose biosensor based on surface active maghemite nanoparticles

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ABSTRACT

A simple carbon paste (CP) electrode, modified with novel maghemite (γ-Fe_2O_3) nanoparticles, called SAMNs (suface active maghemite nanoparticles) and characterized by a mean diameter of about 10 nm, has been developed. The electrode catalyzes the electro-reduction of hydrogen peroxide at low applied potentials (-0.1 V vs SCE). In order to improve the electrocatalytic properties of the modified electrode an ionic liquid, namely 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM-PF6), was introduced. At -0.1 V, the sensitivity of the SAMN–BMIM-PF6–CP electrode was 206.51 nA μM^{-1} cm^{-2}, with a detection limit (S/N=3) of 0.8 μM, in the 0–1.5 mM H_2O_2 concentration range. Furthermore, glucose oxidase was immobilized on the surface of maghemite nanoparticles as a monomolecular layer, by a bridge constituted of rhodamine B isothiocyanate, leading to a fluorescent, magnetic drivable nanocatalyst, containing 10 ± 2 enzyme molecules per nanoparticle. The resulting enzyme electrode presents a linear calibration curve toward glucose in solution in the concentration range of 0–1.5 mM glucose, characterized by a sensitivity of 45.85 nA μM^{-1} cm^{-2} and a detection limit (S/N=3) of 0.9 μM. The storage stability of the system was evaluated and a half-life of 2 months was calculated, if the electrode is stored at 4 °C in buffer. The present work demonstrates the feasibility of these surface active maghemite nanoparticles as efficient hydrogen peroxide electro-catalyst, which can be easily coupled to hydrogen peroxide producing enzymes in order to develop oxidase based reagentless biosensor devices.

1. Introduction

The determination of hydrogen peroxide (H_2O_2) is important in many areas, from clinical studies to industrial productions. H_2O_2 is used as an oxidizing agent in chemical and food industries and is an essential mediator in food, pharmaceutical, clinical, and environmental analysis (Sanderson, 2000). A number of methods, such as spectrophotometry (Sunil and Narayana, 2008), chemiluminescence (Chen et al., 2009), and electrochemical techniques (Huang et al., 2011; Zhao et al., 2009; Zhang et al., 2011; Mohammadi et al., 2009) have been used to detect H_2O_2. Electroanalytical methods are the most convenient, owing to their operational simplicity, low cost, and suitability for real-time detection. Recently, electrochemical approaches have gained increasing attentions for the determination of H_2O_2 in vivo and in vitro because of the high selectivity and sensitivity (Zanardi et al., 2010; Xuan et al., 2010; Chouvy, 2010; Li et al., 2010a, 2010b; Rui et al., 2010; Hung et al., 2010; Haghighi et al., 2010; Vianello et al., 2007). However, most sensors are based on enzymes or proteins and may result in limited lifetime and stability, and complicated fabrication process. Thus, the development of enzyme-free H_2O_2 sensors with low detection limit and wide responding range has become a challenge.

Nanomaterials have been used to develop enzyme-free H_2O_2 sensors, some of them exhibit high electrocatalytic activity for H_2O_2 reduction at the low potential (Ricci and Palleschi, 2005; Iost et al., 2011; Ramgir et al., 2010).

Up to now, only a few reports have been found on the non-enzymatic sensors based on nanocomposite iron oxides (Li et al., 2010; Hrbac et al., 2007), mainly because the reactivity of iron oxide nanoparticles increases with the decrease of particle size, and they may undergo rapid degradation upon direct expose to certain environments (Salgueiriño-Maceira et al., 2006).

When developing a synthetic method for generating nanostructures, the most important issue that one needs to address is the simultaneous control over dimensions, morphology (or shape), and size distribution. Another crucial point, in particular

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for electrochemistry, is to obtain a nanomaterial with a controlled crystallinity because the conductance of metal oxide nanoparticles depends on their crystal structure (Hermanek et al., 2007).

Various methods have been developed for the preparation of magnetic nanoparticles (Mornet et al., 2004; Laurent et al., 2008), generally leading to magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) structures. In most cases, in order to prevent particle aggregation during synthesis, to optimize dimension homogeneity, and to permit bioelement immobilization, a water-in-oil reverse micelle suspension is used, with the aid of a surfactant molecule (Capek, 2004). Polymers, such as dextran, polyvinyl alcohol, and diethylaminoethyl-starch, are generally added to coat the particles to permit colloidal stability, before or after the formation of iron oxide particles (Lee et al., 1996; Bergemann et al., 1999). Otherwise, magnetic nanoparticles can be coated with silica and the hydrolyzed silica surface contains a high coverage of silanol groups, which can easily be anchored with defined and generic surface chemistries (Laurent et al., 2008).

The immobilization of enzymes for sensitive bioelectronic devices development on nanostructured iron oxide is not so widespread and only the crystalline form of magnetite was seriously taken for consideration. Glucose sensing applications of SPIONs have been reported in literature (Wang, 2008; Baby and Ramaprabhu, 2010; Liu et al., 2008b).

Recently, we have developed a novel wet synthesis pathway for producing a new type of superparamagnetic nanoparticles of maghemite, γ-Fe_2O_3, called SAMNs, revealing peculiar surface characteristics, excellent colloidal stability, reversible direct binding of organic molecules without the necessity of any additional organic modification, unique spectroscopic properties and well-defined crystalline stoichiometric structure (Magro et al. 2010).

In the present paper we firstly demonstrated the peculiar electro-catalytic behavior of SAMNs by developing a cheap carbon paste electrode aimed to hydrogen peroxide detection. Furthermore, these metal oxide nanoparticles are able to form stable conjugates with some important biomolecules (e.g., rhodamine isothiocyanate) and act as a bridge permitting the covalent binding of redox enzymes, or electrical labels, for biorecognition events. In the present case glucose oxidase was immobilized on the surface of rhodamine modified magnetic nanoparticles, generating a fluorescent, magnetically drivable, enzymatically active, nanomaterial, that was used to develop a carbon paste based, glucose biosensor. Fluorescence and superparamagnetism allow the easy detectability and controllability of the nanomaterial, also in the case of very small amount of nanoparticles. This was used for the control of all preparation and purification steps of SAMN@RITC–GO*x* and in principle could be used for large scale production. Furthermore, the proposed biosensor is reagentless, and it can be used without the addition of any commercial reagent or substance.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased at the commercially available purity and were used without further treatment. Iron(III) chloride hexahydrate (97%), sodium borohydride ($NaBH_4$), rhodamine B isothiocyanate (RITC), tetramethylammonium hydroxide (TMA), 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM-PF6) and ammonia solution (35% in water) were obtained from Aldrich (Sigma-Aldrich, Italy). Glucose oxidase, type X–S, from *Aspergillus niger* (GOx), cat. G7141 (285 units mg^{-1} solid specific activity), was from Sigma (Sigma-Aldrich, Germany).

The synthesis of SAMNs was presented in Supplementary Materials (Magro et al., 2012). In "Supplementary Materials" the detailed preparation and characterization of SAMN complexes with RITC and GOx was also described, in which an estimate of 10 ± 2 enzyme molecules per nanoparticle was reported.

2.2. Instrumentation

Voltammetric experiments were carried out by a computer-controlled electrochemical system (PGSTAT 10, EcoChemie, The Netherlands). The standard three-electrode arrangement consisted of a SCE reference electrode (Amel, Italy), a Pt counter electrode (Amel, Italy) and carbon paste electrode (CPE) as a working electrode, in a 5 mL electrochemical cell.

Measurements were carried out at constant temperature (22.0 ± 0.2 °C). All experiments were repeated at least five times.

Stock solutions of *D*-glucose (0.1 M) were prepared with double distilled water and allowed to mutarotate at room temperature for 24 h before measurements. Working solutions were freshly prepared before use by diluting the stock solution with double distilled water.

2.3. Electrode preparation

Carbon paste electrodes (CPEs) were prepared by mixing 70:30 graphite powder to silicon grease weight-to-weight ratio. The preparation of modified CPEs with SAMNs, functionalized SAMNs, ionic liquid and free GOx molecules was performed by simple mixing the proper amount of the different compounds to CP. The resulting CPEs were inserted into the cavity of glass electrode holders (1.35 mm diameter). A copper wire had been inserted into the paste through the opposite side of the glass capillary to create the electrical contact with the potentiostat. Finally, the electrode surface was carefully smoothed on a weighting paper and rinsed with double distilled water before each experiment.

3. Results

3.1. Electrode characterization

Amperometric biosensors based on the immobilization of nanostructures on electrode surface have gained considerable attention (Ramgir et al., 2010; Iost et al., 2011).

In order to characterize the electrocatalytic properties of SAMNs aimed to the determination of hydrogen peroxide, we prepared SAMN modified CPEs and we checked their behavior by cyclic voltammetry, in the range from -0.8 to $+0.8$ V. With the aim to develop a glucose biosensor, we used the buffer in which the enzyme showed the best catalytic activity, namely 50 mM sodium acetate, pH 5.1, containing 50 mM KCl as supporting electrolyte. Tentatively, a SAMN/CPE containing 55% w/w graphite, 30% w/w silicone grease and 15% w/w iron oxide nanoparticles, was prepared.

In contrast to bare CPE, which, in the presence of H_2O_2, showed a small increase of the cathodic and anodic currents at potentials more negative than -0.6 V and more positive than $+0.6$ V, in the case of SAMN–CPE, a current increase is observed at a potential below 0 V, leading to an undefined peak at -0.5 V (Fig. 1). The cathodic current increased linearly with hydrogen peroxide concentration. SAMN-CP electrode sensitivity was studied as a function of applied potential (vs SCE) and results showed that the highest current response at increasing H_2O_2 concentration was observed at -0.1 V, see Fig. 2.

From our preliminary experiments, electrode response current was related to the amount of SAMN in CPE, therefore, the effect on