Biosensors and Bioelectronics 45 (2013) 13–18

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A glucose biosensor based on surface active maghemite nanoparticles

Davide Baratella [a], Massimiliano Magro [a], Giulietta Sinigaglia [a], Radek Zboril [b], Gabriella Salviulo [c], Fabio Vianello [a,b,*]

[a] Department of Comparative Biomedicine and Food Science, University of Padua, Italy
[b] Regional Center of Advanced Technologies and Materials, Department of Physical Chemistry, Palacky University in Olomouc, 17 Listopadu 12, 771 46 Olomouc, Czech Republic
[c] Department of Geosciences, University of Padua, Via Gradenigo 6, 35131 Padua, Italy

ARTICLE INFO

Article history:
Received 20 February 2012
Received in revised form
4 December 2012
Accepted 23 January 2013
Available online 30 January 2013

Keywords:
Maghemite nanoparticles
γ-Fe_2O_3
Nanomaterial electrocatalysis
Superparamagnetic nanoparticles
Hydrogen peroxide detection
Glucose biosensor

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

* Correspondence to: Department of Comparative Biomedicine and Food Safety, University of Padova, Agripolis-Viale dell'Università 16, 35020 Legnaro, PD, Italy. Tel.: +39 49 8276863; fax: +39 49 8073310.
E-mail address: fabio.vianello@unipd.it (F. Vianello).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.01.043

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

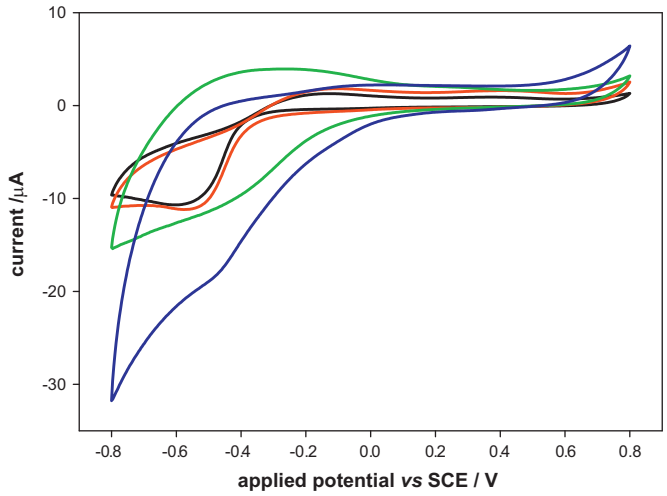

Fig. 1. Cyclic voltammogramms of carbon paste electrode and carbon paste electrode modified with 15% SAMN. Measurements were carried out in 0.05 M sodium acetate buffer, pH 5.1, equilibrated in air, in the presence and in the absence of 3 mM hydrogen peroxide. (black) CPE; (red) CPE in the presence of 3 mM H_2O_2; (green) CPE containing 15% SAMN; (blue) CPE containing 15% SAMN, in the presence of 3 mM H_2O_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

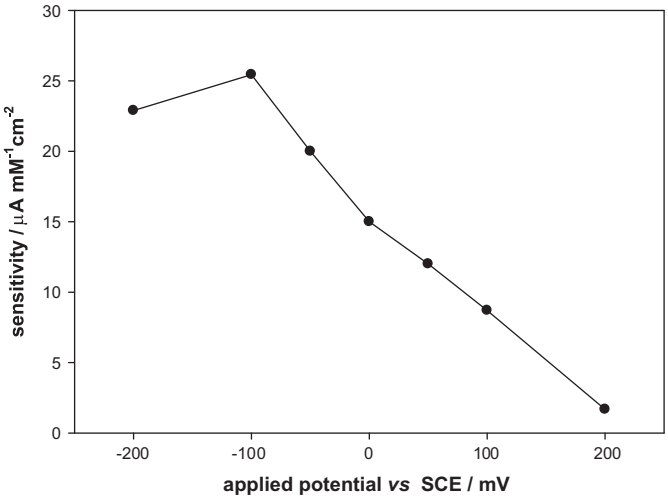

Fig. 2. Sensitivity of carbon paste electrode modified with 15% SAMN as a function of applied potential (vs SCE). Measurements were carried out in 0.05 M sodium acetate buffer, pH 5.1, equilibrated in air. SAMN–CPE sensitivity, at each applied potential, was determined by measuring electrode current response in the presence of increasing concentrations of hydrogen peroxide, in the range 10 μM–1 mM.

H_2O_2 reduction current of SAMN concentration in CPE, in the range 7.5–30% w/w, was studied. The chronoamperometric response, acquired at −0.1 V, increased with the percentage of SAMN in CPE, and as a function of hydrogen peroxide additions, in the concentration range 10 μM–1 mM ($R > 0.997$). In spite of the increase of electrode sensitivity with the amount of SAMN in CPE (see Fig. 3, inset), a concomitant increase of the noise was registered during the chronoamperometric measurements (see Fig. 3). The best signal to noise ratio (calculated as the concentration of analyte producing a signal three times greater than the background noise, S/N=3) was observed with electrodes prepared with 15% SAMN and we considered these modified CPE (15% SAMN/CPE) as the most feasible for oxidase-based biosensor applications. In the case of 15% SAMN/CPE, the detection limit (S/N=3) was about 1.6 μM, and the sensitivity was 25.44 μA mM^{-1} cm^{-2}. In contrast, the sensitivity of the unmodified CPE, at −0.1 V, was seven times lower (see Fig. 3).

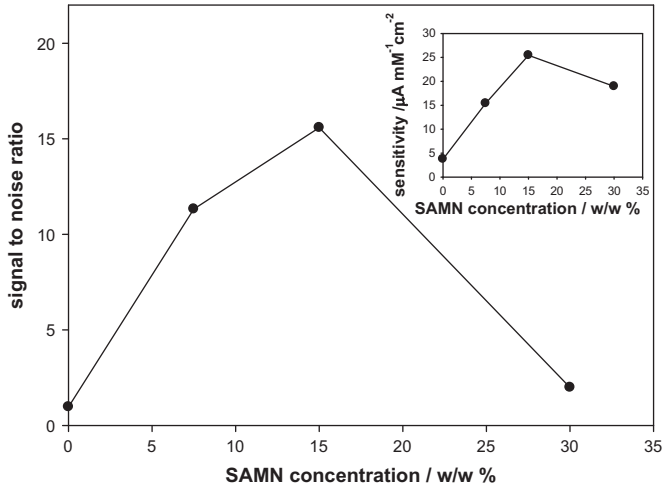

Fig. 3. Sensitivity of carbon paste electrode as a function of SAMN content. Measurements were carried out in 0.05 M sodium acetate buffer, pH 5.1, equilibrated in air, at 0.1 V (vs SCE). SAMN–CPE sensitivity was determined by measuring electrode current response in the presence of increasing concentrations of hydrogen peroxide, in the range 10 μM–1 mM.

The peculiar electrocatalytic properties of SAMNs were evidenced by performing control experiments, using a CPE modified with 15% commercial amorphous iron oxide (Fe_2O_3) nanoparticles (Sigma, cat. 544844), less than 50 nm nominal diameter. Electrode sensitivity toward hydrogen peroxide additions, at −0.1 V, resulted five times lower than that observed with SAMN modified CPE, demonstrating that reduction catalysis of H_2O_2 depends on the peculiar crystalline structure of developed SAMNs.

Ionic liquids are well known additives used to decrease overpotentials and increase electrode currents (Opallo and Lesniewski, 2011). In order to improve electrode characteristics, an ionic liquid, namely 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM-PF6) was added at the SAMN modified CPE (BMIM-PF6–SAMN/CPE). The introduction of the ionic liquid in the CPE led to a current increase, as shown in Fig. 4, where the voltammogramm of 15% SAMN/CPE without BMIM-PF6 is reported for comparison.

In agreement with what already observed with SAMN–CPE, the best signal to noise ratio of BMIM-PF6–SAMN/CPE was observed at −0.1 V vs SCE, and in the present work, this potential was applied for H_2O_2 chronoamperometric determinations.

The 15% SAMN–CPE modified with 18% BMIM-PF6 showed improved analytical performance toward the reduction of hydrogen peroxide at −0.1 V. With respect to the un-modified 15% SAMN/CPE, the sensitivity of the BMIM-PF6 modified electrode increased by a factor of 8 (206.51 μA mM^{-1} cm^{-2}), with a detection limit (S/N=3) of 0.8 μM H_2O_2 (S/N=3) and a noise of 1.01 nA. The linear regression equation is i (μA)=1.625×10^{-3}–2.949 mM H_2O_2, with a 0.998 correlation coefficient. In contrast, a CPE electrode, without SAMN, and modified only with ionic liquid presented a sensitivity of 18.63 μA mM^{-1} cm^{-2}. The same results were obtained using phosphate buffer (0.1 M, pH 7), containing 50 mM KCl as supporting electrolyte: the sensitivity of BMIM-PF6–SAMN/CPE was seven times higher than SAMN/CPE (120.45 and 18.04 μA mM^{-1} cm^{-2} respectively), while the BMIM-PF6/CPE had a sensitivity of 20.45 μA mM^{-1} cm^{-2}. These results demonstrate that there is a cooperative interaction between ionic liquid and SAMN in CPE and suggesting that H_2O_2 could be easily detected at very low concentrations using the chronoamperometric method. Moreover, the chronoamperometric behavior of CPE, SAMN–CPE and BMIM-PF6/CPE were compared at a positive applied potential (+ 0.8 V) in order to test the electrocatalytical

properties of modified and unmodified carbon paste electrodes toward hydrogen peroxide oxidation. In this case, the sensitivity of SAMN/CPE (15.67 $\mu A\,mM^{-1}\,cm^{-2}$) resulted lower than simple CPE (27.82 $\mu A\,mM^{-1}\,cm^{-2}$) and modified BMIM-PF6/CPE (51.69 $\mu A\,mM^{-1}\,cm^{-2}$), demonstrating that SAMNs efficiently catalyze the reduction, and not the oxidation, of hydrogen peroxide, differently from other nanoparticle modified electrodes (Luo et al., 2006).

The chronoamperometric response of the BMIM-PF6–SAMN/CP electrode to H_2O_2 additions, in 50 mM sodium acetate, pH 5.1, containing 50 mM KCl, at an applied potential of −0.1 V, was linear ($R > 0.99$) in the range 0–1.5 mM H_2O_2. Finally, analytical performances of this H_2O_2 sensor, if stored at room temperature after use, resulted stable for at least 12 months.

The above presented results indicated that the BMIM-PF6–SAMN/CPE can be successfully used for the detection of hydrogen peroxide.

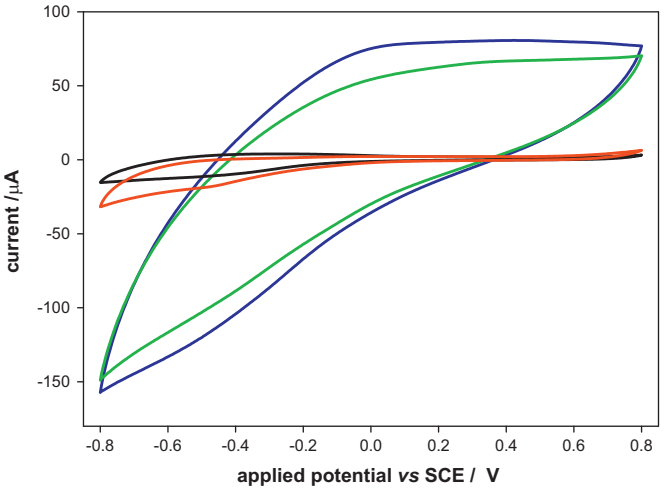

Fig. 4. Effect on ionic liquid introduction in carbon paste electrodes. Cyclic voltammogramms of carbon paste electrode containing 18% BMIM-PF6 and carbon paste electrode modified with 15% SAMN containing 18% BMIM-PF6. Measurements were carried out in 0.05 M sodium acetate buffer, pH 5.1, equilibrated with air, in the presence and in the absence of 3 mM hydrogen peroxide. (black) CPE containing 15% SAMN; (red) CPE containing 15% SAMN in the presence of 3 mM H_2O_2; (green) CPE containing 15% SAMN and 18% BMIM-PF6; (blue) CPE containing 15% SAMN and 18% BMIM-PF6, in the presence of 3 mM H_2O_2. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Biosensor development with SAMNs coated with rhodamine B isothiocyanate and glucose oxidase (SAMN@RITC–GOx)

The BMIM-PF6–SAMN/CPE was used for the preparation of a biosensor, using immobilized glucose oxidases as bioelements, which produces hydrogen peroxide during the catalytic cycle.

Amperometric biosensors based on the immobilization of enzymes within a carbon paste were extensively studied (Kalcher et al., 1995; Švancara et al., 2009). Nevertheless, we applied a nanocomposite carbon paste electrode for the determination of glucose by immobilized GOx on SAMN surface (SAMN@-RITC–GOx).

The performances of (SAMN@RITC–GOx)/CPE toward the determination of H_2O_2 were evaluated by chronoamperometry at −0.1 V (vs SCE). The modification of SAMN surface by RITC and GOx did not alter significantly the analytical performances of SAMN/CPEs toward the H_2O_2 determination. Electrode sensitivity and detection limit for hydrogen peroxide were unaffected (sensitivity of 200.00 $\mu A\,mM^{-1}\,cm^{-2}$ and detection limit of 0.8 μM H_2O_2, S/N=3). Furthermore, the response of (SAMN@-RITC–GOx)/CPE was investigated upon glucose addition. In this case, in the 0–1.5 mM glucose concentration range, a sensitivity of 2.67 nA $\mu M^{-1}\,cm^{-2}$ and a detection limit (S/N=3) of 20 μM were calculated. The stability of the (SAMN-RITC–GOx)/CPE for detection of 100 μM glucose was also investigated and the half-life of this reagentless glucose biosensor was found to be higher than 2 months.

Despite ionic liquids are increasingly used to improve carbon electrode performances (Opallo and Lesniewski, 2011), in some cases they could affect biosensor response, because of enzyme denaturation, modification of electron transfer properties and introduction of diffusional barriers (Shiddiky and Torriero, 2011). Specifically, Wu reported on the negative effects of the introduction of an ionic liquid on electrocatalytic activity of glucose oxidase immobilized on carbon nanotubes (Wu et al., 2009). The ionic liquid, BMIM-PF6, was added at the SAMN@RITC–GOx modified CPE and the resulting (BMIM-PF6–SAMN@RITC–GOx)/CPE sensor improves its performances upon ionic liquid addition with respect to the unmodified (SAMN@RITC–GOx)/CPE. In the present case, the introduction of BMIM-PF6 showed a positive effect, increasing biosensor response by about 17 times. In particular, in the presence of 18% BMIM-PF6, the sensing system showed a sensitivity of 45.85 nA $\mu M^{-1}\,cm^{-2}$, in the 0–1.5 mM glucose concentration range, and a detection limit of 0.9 μM glucose. The regression equation was $I(nA) = 3.79 \times 10^{-3}$–0.655 μM glucose, $R = 0.998$ ($n = 8$).

Table 1
Comparison of analytical characteristics of most recent glucose detecting enzyme electrodes reported in literature.

Configuration of biosensor	Applied potential (V)	Sensitivity ($\mu A\,mM^{-1}\,cm^{-2}$)	Detection limit (μM)	Linear range (mM)	References
GOx/PdNPs/CS-GR/GCE	+0.7	31.2	0.2	0.001–1	Zeng et al. (2011)
GOx/Ptnano/SWCNT/Nafion/GCE	+0.55	30.0	0.5	0.0005–5	Hrapovic et al. (2004)
GOx/CS/CNT/AuE	+0.7	6.7	2	0.005–8	Zhou et al. (2007)
GOx/SWCNHs/GCE	+0.3	15.0	6	0–6	Liu et al. (2008)
$(GOx/AuNPs/MWCNTs)_9$/PtE	+0.35	35.8	6.7	0.1–10	Wu et al. (2007)
$(ConA/GOx)/Pt_{nano}$-CNTs-CS/GCE	+0.3	41.9	0.4	0.001–2	Li et al. (2011)
GOx/CS/BCNiNPs/GCE	−0.2	3.5	8.3	0.025–1.2	Yang et al. (2011)
$GOx/(AuNPs/MWCNT)_5$/AuE	+0.3	19.3	2.3	0.02–10	Si et al. (2011)
PB/MWNTs-GOx-CS-ICPTES/GCE	−0.1	15.2	7.5	0.025–1.3	Fu et al. (2011)
Nafion/GOx/brushite/PtE	+0.6	52.5	9	0.009–3	Lopez and Lopez-Ruiz (2011)
GOx/PANAA/PtE	+0.6	6.8	0.5	0.005–3	Shan et al. (2006)
PDDA/GOx/ZnO/MWNTs/PGE	−0.1	50.2	0.25	0.1–16	Wang et al. (2009)
GOx/MTMOS/chitosan/GCE	+0.35	3.9	10	2–14	Chen et al. (2003)
GOx/MWCNT/PtNPs/CS/SiO_2/GCE	+0.6	58.9	1	0.001–23	Zou et al. (2008)
GOx/AuNPs/CS/PB/GCE	−0.05	69.3	0.7	0.001–1.6	Xue et al. (2006)
GOx/Fe_3O_4 NPs/CS/PtE	+0.4	11.5	6	0.006–2.2	Yang et al. (2009)
GOx/FMC/AMWNTs/GCE	+0.35	10.6	3.4	0.01–4.2	Qiu et al. (2008)
BMIM-PF_6–SAMN-RITC–GOx/CPE	−0.1	45.85	0.9	0.001–1.5	Present work

Control experiments were carried out in order to demonstrate the effect of enzyme immobilization on the iron oxide nanoparticle surface on biosensor performances. A carbon paste electrode, containing 15% SAMNs (without RITC coverage to avoid GOx binding), 18% BMIM-PF6 and 1.5% GOx was prepared. The amount of GOx was comparable to the previously prepared (BMIM-PF6–SAMN@RITC–GOx)/CPE sensor. In this case the sensitivity and the detection limit toward glucose addition were 6.02 nA μM^{-1} cm^{-2} and 5.3 μM, respectively. The biosensor containing GOx simply mixed into the carbon paste matrix, in the presence of the same amount of BMIM-PF6–SAMN, showed a seven times lower sensitivity and a five times higher detection limit than the proposed (BMIM-PF6–SAMN@RITC–GOx)/CP electrode. Enzyme immobilization on SAMN surface improves biosensor characteristics, whose analytical performances result comparable to other nano-composite based biosensors was reported in literature, see Table 1.

3.3. Interference study

An interference study is an important aspect in the determination of any species by electrochemical methods. The effect of common interfering electroactive substances, such as ascorbic acid, was assessed. The chronoamperometric response of the biosensor to subsequent injection of 0.15 mM ascorbic acid, at −0.1 V, was evaluated. At the applied potential used with the proposed biosensor, the effect of these interfering species on 100 μM glucose response was negligible, indicating a high selectivity.

3.4. Applications on real samples

The developed BMIM-PF6–(SAMN-RITC–GOx)/CPE was used to determine glucose in fruit juices, by determining the chronoamperometric response following the addition of juice (2 μL) to the buffered solution in the electrochemical cell (5 mL). The glucose concentration determined by the biosensor was 0.644 ± 0.018 M ($n=3$) in apricot and 0.669 ± 0.021 M ($n=3$) in peach juice, close to the values obtained by spectrophotometry, of 0.646 ± 0.025 M ($n=3$) and 0.657 ± 0.017 M ($n=3$), respectively (see Fig. 5). The spectrophotometric method used for glucose determination is described in "Supplementary Materials".

4. Discussion

In the present paper, we developed and characterized a carbon paste electrode, in which newly synthesized iron oxide nanoparticles (called SAMNs) and a ionic liquid (namely 1-butyl-3-methylimidazolium hexafluorophosphate, BMIM-PF6) were introduced by a rapid and simple procedure to form a BMIM-PF6–SAMN/CPE nano-composite. The proposed electrode shows good electrocatalytic properties toward hydrogen peroxide reduction and it could be used for sensitive and selective determination of H_2O_2 at low applied potentials (−0.1 V vs SCE). The resulting electrode, based on nanostructured metal oxides, allows a high signal-to-noise ratio and sensitivity and, not withstanding the simple immersion probe electrodes are generally much less sensitive than FIA sensors, the BMIM-PF6–SAMN/CP electrode characteristics are comparable to those reported in literature with much more complicated and cumbersome solutions (Chen et al., 2012). This behavior can be explained considering that surface Fe(III) on SAMN surface can be easily electrochemically reduced, forming Fe(II), at the electrode, as suggested by Zhang et al. (2010). Then, a two electron–two proton reduction of H_2O_2 on Fe(II), forming back surface Fe(III) and producing H_2O, occurs.

The attractive electrochemical and structural properties of SAMNs suggested their application in the development of a biosensor. Following the suggestion of previous work (Sinigaglia et al., 2012), SAMN nanoparticles were surface derivatized with rhodamine isothiocyanate (RITC). Interestingly, the presence of RITC coverage on nanoparticle surface did not affect the electrocatalytic properties of the nanostructured electrode. Exploiting the chemical properties of the isothiocyanate functionality, glucose oxidase was covalently bound to RITC, to develop a rhodamine-based magnetic nano-catalyst. Thus it was possible to bind GOx on SAMN@RITC surface, by simple incubation in water and the resulting SAMN@RITC–GOx adduct still showed enzymatic activity demonstrating that the immobilized enzyme, as a monomolecular layer, retains its catalytic activity toward the oxidation of β-D-glucose. SAMN@RITC composite, not only provides a friendly microenvironment for immobilized GOx, allowing the maintenance of its bioactivity, but also guarantees the electron transfer occurrence between H_2O_2 and the carbon paste. The preparation of the final CP electrode, containing the SAMN@-RITC–GOx adduct, permits the control of enzyme amount and activity of the resulting biosensor, improving reproducibility.

In most of the other nanocomposite electrodes (see Table 1), in which enzyme immobilization was carried out by simple dropping enzyme solution on electrode surface, it is difficult to control enzyme concentration, distribution, and reproducibility of the resulting system. No control of the amount neither of the catalytic parameters of the immobilized enzyme are generally observed.

In the proposed biosensor, the kinetic characterization of the immobilized enzyme, the evaluation of the number of enzyme molecules on each nanoparticle surface (and thus the enzyme amount) and the intimate contact between the enzyme and the electro-catalytic nanoparticle, homogenously dispersed in the carbon paste matrix, were guaranteed.

SAMN@RITC–GOx system was used to prepare an amperometric biosensor (BMIM-PF6–SAMN@RITC–GOx)/CPE) for the determination of glucose, showing interesting analytical characteristics (0–1.5 mM linear range and a 0.9 μM detection limit).

The peculiar analytical performances were due to the high enzyme loading on the final electrode, the enzyme friendly environment of SAMN@RITC surface and to the good electrochemical properties of the highly crystalline SAMN structure toward hydrogen peroxide reduction.

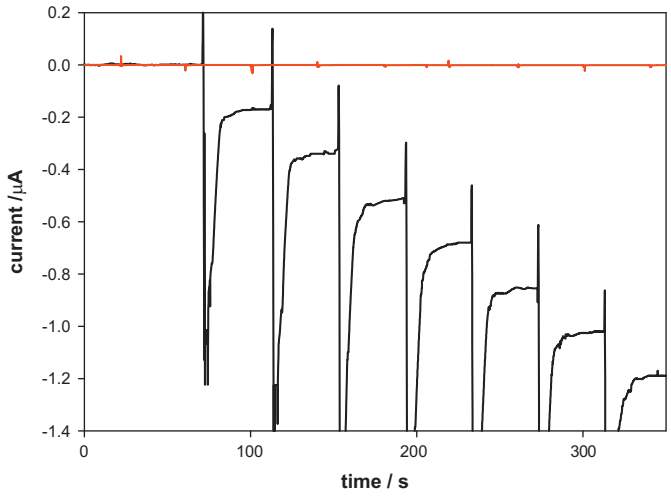

Fig. 5. Chronoamperometric response of the BMIM-PF6-(SAMN@RITC–GOx)/CPE biosensor with real samples. Apricot juice aliquots (2 μL) were added to the buffered solution in the electrochemical cell (5 mL). (red) BMIM-PF6–(SAMN-RITC–GOx)/CPE response upon buffer additions; (black) BMIM-PF6–(SAMN-RITC–GOx)/CPE response upon additions of apricot juice aliquots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

5. Conclusions

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, containing an ionic liquid, namely BMIM-PF6, and characterized by a sensitivity of 206.51 $\mu A\ mM^{-1}\ cm^{-2}$, a detection limit of 0.8 μM H_2O_2 (S/N = 3) and a noise of 1.01 nA. Furthermore, these metal oxide nanoparticles were used to form stable conjugates with rhodamine isothiocyanate, acting as a bridge, permitting the covalent binding of glucose oxidase. The resulting bio-conjugate was used to develop a nanocomposite, carbon paste-BMIM-PF6 based, biosensor, characterized by a sensitivity of 45.85 $nA\ \mu M^{-1}\ cm^{-2}$, in the 0–1.5 mM glucose concentration range, and a detection limit of 0.9 μM glucose. The system was tested on real samples without any sample preparation procedure and results suggest that BMIM-PF6–(SAMN@RITC–GOx)/CPE biosensor could be a promising, low cost, option for the development of GOx based biosensors for glucose determination.

We are planning to use SAMN@RITC conjugate for covalent binding of other hydrogen peroxide producing enzymes in order to expand the field of application of this convenient system.

Acknowledgments

This work was funded in part by Progetti di Ateneo (A.0ED00.0487PRAT08), by the University of Padua. The work was supported by the Operational Program Research and Development for Innovations-European Social Fund (CZ.1.05/2.1.00/03.0058) and by the Academy of Sciences of the Czech Republic (KAN115600801).

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.01.043.

References

Baby, T.T., Ramaprabhu, S., 2010. Talanta 80, 2016–2022.
Bergemann, C., Muller-Schulte, D., Oster, J., Brassard, L.A., Lubbe, A.S., 1999. Journal of Magnetism and Magnetic Materials 194, 45–52.
Capek, I., 2004. Advances in Colloid and Interface Science 110, 49–74.
Chen, W., Li, B., Xu, C., Wang, L., 2009. Biosensors and Bioelectronics 24, 2534–2540.
Chen, X., Jia, J., Dong, S., 2003. Electroanalysis 15, 608–612.
Chen, W., Cai, S., Ren, Q-Q., Wen, W., Zhao, Y-D., 2012. Analyst 137, 49–58.
Chouvy, C.D., 2010. Biosensors and Bioelectronics 25, 2454–2457.
Fu, G., Yue, X., Dai, Z., 2011. Biosensors and Bioelectronics 26, 3973–3976.
Haghighi, B., Hamidi, H., Gorton, L., 2010. Sensors and Actuators B: Chemical 147, 270–276.
Hermanek, M., Zboril, R., Medrik, I., Pechousek, J., Gregor, C., 2007. The Journal of the American Chemical Society 129, 10929–10936.
Hrapovic, S., Liu, Y., Male, K.B., Luong, J.H.T., 2004. Analytical Chemistry 76, 1083–1088.
Hrbac, J., Halouzka, V., Zboril, R., Papadopoulos, K., Triantis, T., 2007. Electroanalysis 19, 1850–1854.
Huang, K.J., Sun, J.Y., Jin, C.X., Jing, Q.S., Zhou, T., 2011. Thin Solid Films 519, 3925–3930.
Hung, C.C., Wen, T.C., Wei, Y., 2010. Materials Chemistry and Physics 122, 392–396.
Iost, R.M., da Silva, W.C., Madurro, J.M., Madurro, A.G., Ferreira, L.F., Crespilho, F.N., 2011. Frontiers in Bioscience 3, 663–689.
Kalcher, K., Kauffmann, J.M., Wang, J., Švancara, I., Vytřas, K., Neuhold, C., Yang, Z.P., 1995. Electroanalysis 7, 5–22.
Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Elst, L.V., Muller, R.N., 2008. Chemical Reviews 108, 2064–2110.
Lee, J., Isobe, T., Senna, M., 1996. Journal of Colloid and Interface Science 177, 490–494.
Li, S., Zhang, L., Liu, H.S., Pan, M., Zan, L., Zhang, J.J., 2010a. Electrochimica Acta 55, 4403–4411.
Li, W., Yuan, R., Chai, Y., Zhong, H., Wang, Y., 2011. Electrochimica Acta 56, 4203–4208.
Li, Y., Zhang, J.J., Xuan, J., Jiang, L.P., Zhu, J.J., 2010b. Electrochemistry Communications 12, 777–780.
Li, Z., Ni, Y., Wang, X., Zhao, G., 2010c. Talanta 82, 196–201.
Liu, X., Shi, L., Niu, W., Li, H., Xu, G., 2008a. Biosensors and Bioelectronics 23, 1887–1890.
Liu, Z., Wang, J., Xie, D., Chen, G., 2008b. Small 4, 462–466.
Lopez, M.S.-P., Lopez-Ruiz, B., 2011. Electroanalysis 23, 280–286.
Luo, X., Morrin, A., Killard, A.J., Smith, M.R., 2006. Electroanalysis 18, 319–326.
Magro, M., Sinigaglia, G., Nodari, L., Tucek, J., Polakova, K., Marusak, Z., Cardillo, S., Salviulo, G., Russo, U., Stevanato, R., Vianello, F., Zboril, R., 2012. Acta 8, 2068–2076.
Magro, M., Valle, G. Russo, U., Nodari, L., Vianello, F., 2010. Patent no. PCT/EP2010/060486.
Mohammadi, A., Moghaddam, A.B., Kazemzad, M., Dinarvand, R., Badraghi, J., 2009. Materials Science and Engineering C 29, 1752–1758.
Mornet, S., Vasseur, S., Grasset, F., Duguet, E., 2004. Journal of Materials Chemistry 14, 2161–2175.
Opallo, M., Lesniewski, A., 2011. Journal of Electroanalytical Chemistry 656, 2–16.
Qiu, J.-D., Deng, M.-Q., Liang, R.-P., Xiong, M., 2008. Sensors and Actuators B: Chemical 135, 181–187.
Ramgir, N.S., Yang, Y., Zacharias, M., 2010. Small 6, 1705–1722.
Ricci, F., Palleschi, G., 2005. Biosensors and Bioelectronics 21, 389–407.
Rui, Q., Komori, K., Tian, Y., Liu, H.Q., Luo, Y.P., Sakai, Y., 2010. Analytica Chimica Acta 670, 57–62.
Salgueiriño-Maceira, V., Correa-Duarte, M.A., Spasova, M., Liz-Marzán, L.M., Farle, M., 2006. Advanced Functional Materials 16, 509–514.
Sanderson, W.R., 2000. Pure and Applied Chemistry 72, 1289–1304.
Shan, D., He, Y., Wang, S., Xue, H., Zheng, H., 2006. Analytical Biochemistry 356, 215–221.
Shiddiky, M.J.A., Torriero, A.A.J., 2011. Biosensors and Bioelectronics 26, 1775–1787.
Si, P., Kannan, P., Guo, L., Son, H., Kim, D.-H., 2011. Biosensors and Bioelectronics 26, 3845–3851.
Sinigaglia, G., Magro, M., Miotto, G., Cardillo, S., Agostinelli, E., Zboril, R., Bidollari, E. and Vianello, F., International Journal of Nanomedicine, in press.
Sunil, K., Narayana, B., 2008. Bulletin of Environmental Contamination and Toxicology 81, 422–426.
Švancara, I., Walcarius, A., Kalcher, K., Vytřas, K., 2009. Central European Journal of Chemistry 7, 598–656.
Vianello, F., Zennaro, L., Rigo, A., 2007. Biosensors and Bioelectronics 22, 2694–2699.
Wang, Y.T., Yu, L., Zhu, Z.Q., Zhang, J., Zhu, J.Z., Fan, C., 2009. Sensors and Actuators, B: Chemical 136, 332–337.
Wang, J., 2008. Chemical Reviews 108, 814–825.
Wu, B.Y., Hou, S.H., Yin, F., Zhao, Z.X., Wang, Y.Y., Wang, X.S., Chen, Q., 2007. Biosensors and Bioelectronics 22, 2854–2860.
Wu, X., Zhao, B., Wu, P., Zhang, H., Cai, C., 2009. Journal of Physical Chemistry B 113, 13365–13373.
Xuan, J., Jiang, L.P., Zhu, J.J., Chin, J., 2010. Analytical Chemistry 38, 513–515.
Xue, M.H., Xu, Q., Zhou, M., Zhu, J.J., 2006. Electrochemistry Communications 8, 1468–1474.
Yang, L., Ren, X., Tang, F., Zhang, L., 2009. Biosensors and Bioelectronics 25, 889–895.
Yang, L., Xiong, H., Zhang, X., Wang, S., Zhang, X., 2011. Biosensors and Bioelectronics 26, 3801–3805.
Zanardi, C., Terzi, F., Zanfrognini, B., Pigani, L., Seeber, R., Lukkari, J., Ääritalo, T., 2010. Sensors and Actuators, B: Chemical 144, 92–96.
Zeng, Q., Cheng, J.-S., Liu, X.-F., Bai, H.-T., Jianf, J.-H., 2011. Biosensors and Bioelectronics 26, 3456–3463.
Zhang, L., Ni, Y., Wang, X., Zhao, G., 2010. Talanta 82, 196–201.
Zhang, Y., Luo, H.Q., Li, N.B., 2011. Bioprocess and Biosystems Engineering 34, 215–221.
Zhao, W., Wang, H.C., Qin, X., Wang, X.S., Zhao, Z.X., Miao, Z.Y., Chen, L.L., Shan, M.M., Fang, Y.X., Chen, Q., 2009. Talanta 80, 1029–1033.
Zhou, Q., Xie, Q., Fu, Y., Su, Z., Jia, X., Yao, S., 2007. Journal of Physical Chemistry B 111, 11276–11284.
Zou, Y., Xiang, C., Sun, L.X., Xu, F., 2008. Biosensors and Bioelectronics 28, 1010–1016.