Highly Sensitive and Selective Glucose Biosensing at Carbon Paste Electrodes Modified with Electrogenerated Magnetite Nanoparticles and Glucose Oxidase

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Abstract
This work reports the advantages of carbon paste electrodes modified with electrogenerated magnetite nanoparticles. The nanoparticles present catalytic activity towards hydrogen peroxide reduction. The incorporation of glucose oxidase (GOx) and magnetite in a carbon paste matrix have made possible the development of an efficient glucose biosensor. The effect of the amount of GOx and magnetite present in the composite on the response of the biosensor was critically evaluated. The biosensors demonstrated to be highly selective, with negligible interference of ascorbic acid and uric acid. The proposed biosensor was challenged with human blood serum demonstrating an excellent correlation with the spectrophotometric method.

Keywords: Magnetite, Nanoparticles, Carbon paste electrode, Hydrogen peroxide, Glucose oxidase, Amperometric glucose biosensor, Biosensors

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1. Introduction

Glucose biosensing is very important in different areas, especially in Clinical Chemistry/Medicine where the concentration of glucose is used as clinical indicator for Diabetes. At present, this disease represents a serious worldwide public health problem due to the multiple and severe damages that it produces at different levels, mainly kidneys, heart, and vision. Therefore, methodologies that ensure efficient glucose quantification are highly required for early diagnosis of Diabetes as well as for its management control.

Since the pioneering work of Clark and Lyons in 1962 [1] different strategies have been proposed for the development of glucose biosensors. In this sense, electrochemical biosensors have been widely used for glucose monitoring due to their high accuracy, low cost, fast response, simplicity and better detection limits [2, 3]. The enzyme glucose oxidase (GOx) has been the most widely used biorecognition element in glucose biosensors [4]. GOx catalyzes the oxidation of glucose to gluconolactone and, in turn, reduces the natural mediator, oxygen, to hydrogen peroxide. The amperometric determination of this compound or some artificial mediator like ferrocene derivatives and osmium complexes (in case of working in anaerobic conditions) has been the most commonly used electrochemical transduction mode [3].

As it is widely known, high overvoltages are required for the oxidation and reduction of hydrogen peroxide at carbon electrodes. Under these conditions, endogenous reducing compounds such as ascorbic acid (AA) and uric acid (UA) can also be oxidized, compromising the selectivity and, hence, the overall accuracy of the assay [2–5]. Therefore, the main challenge when designing electrochemical glucose biosensors is the development of strategies that allow an important decrease of these overvoltages and, consequently, more selective hydrogen peroxide quantification.

Several alternatives have been proposed to diminish the electroactive interferences. Among them, the electrocatalytic detection of hydrogen peroxide at potentials where the interferents can not be oxidized has demonstrated to be highly successful [6–15]. Prussian blue, called "artificial peroxidase" due to the excellent electrocatalytic activity towards hydrogen peroxide reduction, has made possible the development of highly selective glucose biosensing at very low potentials [6, 7]. The modification of carbon electrodes with metals like rhodium, copper, platinum, gold or combination of them [8–18], metal oxides such as copper oxide, manganese oxide [19–22], and perovskites [23] has also demonstrated to be extremely useful for the electrocatalytic detection of hydrogen peroxide at potentials that permit the highly selective glucose quantification.

In this work we report for the first time the use of electrochemically synthesized magnetite nanoparticles for the development of electrochemical glucose biosensors. The goal of using these nanoparticles in this direction was to exploit their catalytic properties towards hydrogen peroxide reduction.

Magnetite is a common magnetic iron oxide that belongs to the spinel ferrite materials group. It is a spin-polarized, Fe (II)-Fe (III) mixed valence metal where Fe (III) and Fe (II) coexist [24, 25]. Magnetite nanoparticles have received great attention in the last years due to their unique properties and, consequently, they have been successfully used in several fields. The conventional method to obtain magnetite nanoparticles is by coprecipitation. However, this methodology presents the inconveniences of size polydispersity and aggregation of the resulting nanoparticles. On the other hand, the wastewater obtained during the chemical synthesis requires a treatment before eliminating due to its highly basic nature. Electrosynthesis of magnetite nanoparticles has made possible to obtain monodisperse nanoparticles just by controlling the applied current or potential. At the same time, the aggregation was avoided by incorporating surfactants in the electrolytic solution [26].

Regarding the use of magnetite nanoparticles for the development of sensors, Chen et al. [27] have proposed a glucose biosensor by dispersing a carbon nanotubes/nanomagnetite composite (obtained by coprecipitation of Fe (II) and Fe (III)), in a GOx solution, followed by a cross-linking step with glutaraldehyde. However, the authors assigned to carbon nanotubes the catalytic activity towards hydrogen peroxide reduction. Chumming and Xiangqin [28] have reported the catalytic activity of a glassy carbon electrode modified with magnetite-Prussian blue-core shell nanoparticles on the reduction of hydrogen peroxide. The authors claim that magnetite nanoparticles do not catalyze neither the oxidation nor the reduction of hydrogen peroxide and that the catalytic activity is due to Prussian blue. Lin and Le [29] have described the catalytic activity towards hydrogen peroxide of chemically synthesized magnetite immobilized at glassy carbon electrodes by drop-coating of Fe_3O_4/chitosan and final cross-linking with glutaraldehyde. Hrbac et al. [30] have presented an interesting comparison of the electrocatalytic activity of carbon paste electrodes modified with different iron (III) oxides nanoparticles (hematite, maghemite, amorphous Fe_2O_3, β-Fe_2O_3, magnetite, and ferrihydrite) towards the reduction of hydrogen peroxide at pH 3.0. They have demonstrated that, among these oxides, the best catalyst is the amorphous ferric oxide which at neutral pH, is even better than Prussian blue. It is important to mention that to the best of our knowledge, there are no reports about glucose biosensors where the analytical signal is due just to the catalytic activity of electrochemically synthesized magnetite.

In this work we present the advantages of electrosynthesized magnetite nanoparticles incorporated within a carbon paste electrode. The platform was used for the development of a glucose biosensor by dispersing GOx together with the electrogenerated magnetite nanoparticles within the composite carbon electrode. The influence of the amount of magnetite and GOx in the composite, the length of the surfactant used during the synthesis of magnetite nanoparticles and the interference of easily oxidizable compounds present in blood like ascorbic acid (AA) and uric acid (UA) on the analytical performance of the biosensor are reported in the following sections. The successful application of the proposed biosensor for glucose biosensing in blood human serum is also described.

2. Experimental

2.1. Reagents

Hydrogen peroxide (30% v/v aqueous solution) was purchased from Baker. Uric acid (UA), tetraethyl ammonium chloride, hexadecyl ammonium bromide, and glucose were from Merck and ascorbic acid (AA) was from Fluka. Glucose oxidase (GOx) (Type X-S, *Aspergillus niger*, EC 1.1.3.4, 157,500 Units per gram of solid, Catalog number G-7141) was obtained from Sigma. Graphite powder was purchased from Fisher (grade 38) while the mineral oil was acquired from Aldrich. All the chemicals were reagent grade and used without further purification.

Ultrapure water ($\rho = 18\ M\Omega$ cm) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution pH 7.40 was employed as supporting electrolyte.

Magnetite nanoparticles were electrochemically synthesized [26] using a sacrificial iron anode (Goodfellow purity 99.5%), 1 cm $\times$ 1 cm dimension, 0.2 mm thick) and a iron cathode (1 cm $\times$ 4 cm) in a 0.040 M tetramethyl ammonium chloride or hexadecyl trimethyl ammonium bromide aqueous solutions. A current of 100 mA/cm^{-2} was applied for 1800 s at a temperature of 60 °C (kept with a thermostatic bath). The obtained product was washed with distilled water and dried at 60 °C for 24 h before use.

2.2. Apparatus

The electrosynthesis of magnetite nanoparticles was performed with a potentiostat/galvanostat VersaStatTM EG&G Instruments Princeton Applied Research.

The rest of the electrochemical measurements were performed with a TEQ_02 potentiostat. The electrodes were inserted into the cell (BAS, Model MF-1084) through holes in its Teflon cover. A platinum wire and Ag/AgCl, 3 M KCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to the latter. A magnetic stirrer provided the convective transport during the amperometric measurements.

The carbon paste electrode (CPE) was prepared in a regular way by mechanically mixing graphite powder (70.0% w/w) and mineral oil (30.0% w/w) in an agate mortar for 30 min. CPEs containing magnetite were pre-

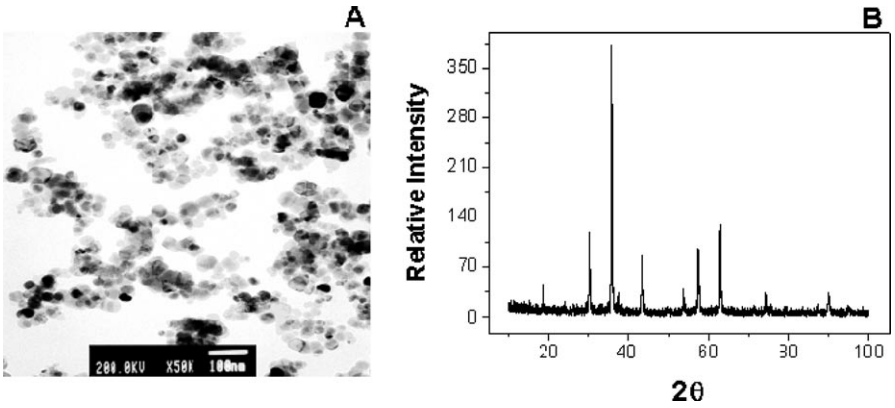

Fig. 1. a) TEM micrographs of magnetite nanoparticles obtained at 100 mA cm^{-2}. Electrolysis time 1800 s, $T = 60\,^{\circ}C$; b) X-ray diffractogram of magnetite sample.

pared in a similar way, mixing first the magnetite nanoparticles with mineral oil for 1 min, followed by the incorporation of the graphite powder and mixing for additional 30 min. In the case of the enzymatic electrode, GOx and magnetite nanoparticles were first mixed with the mineral oil for 10 min before incorporating the graphite powder and then mixed for additional 30 min. A portion of the given paste was packed firmly into a Teflon tube cavity (3 mm diameter). The electric contact was established through a stainless steel screw. The surface was smoothed on a weighing paper before starting every new experiment.

The structural characterization of magnetite nanoparticles was carried out by X-ray powder diffraction using an X′ Pet PRO Panalytical diffractometer, with a $\theta - 2\theta$ geometry, equipped with a primary and secondary monochromators and an ultrafast X'Celerator detector with a CuK radiation.

Morphological observations of the nanoparticles were performed by transmission electronic microscopy (TEM) with a JEOL-2000 FXII electron microscope operated at 200 KeV.

2.3. Procedure

Amperometric measurements were conducted in a stirred 0.050 M phosphate buffer solution pH 7.40 by applying the desired working potential and allowing the transient currents to decay to a steady-state value prior to the addition of the analyte and subsequent current monitoring. All measurements were performed at room temperature.

3. Results and Discussion

Figure 1A illustrates a TEM micrograph of the electrogenerated magnetite nanoparticles. They present a quasi-spherical shape with a mean size of (20 ± 4) nm. Figure 1B displays the X-ray diffraction (XDR) pattern of magnetite nanoparticles. All peaks have been indexed as the corresponding ones to magnetite (reference code: JCPDS 01-088-0315), demonstrating the absence of impurities. The crystal size calculated from the broadening of the (311) reflection of the spinel structure was similar to the one determined by TEM.

Figure 2A compares cyclic voltammograms for 0.050 M hydrogen peroxide obtained at CPE (dotted line) and at CPE containing 5.0% w/w of magnetite nanoparticles electrosynthesized in the presence of tetramethylammonium chloride (CPE-Fe_3O_4 (5.0% w/w), solid line). The corresponding hydrodynamic voltammograms for 0.050 M hydrogen peroxide obtained at CPE (empty circles) and at CPE-Fe_3O_4 (5.0% w/w) (full circles) are displayed in Figure 2B. Both figures show that in the presence of magnetite nanoparticles there is a very important decrease in the overvoltage for the reduction of hydrogen peroxide

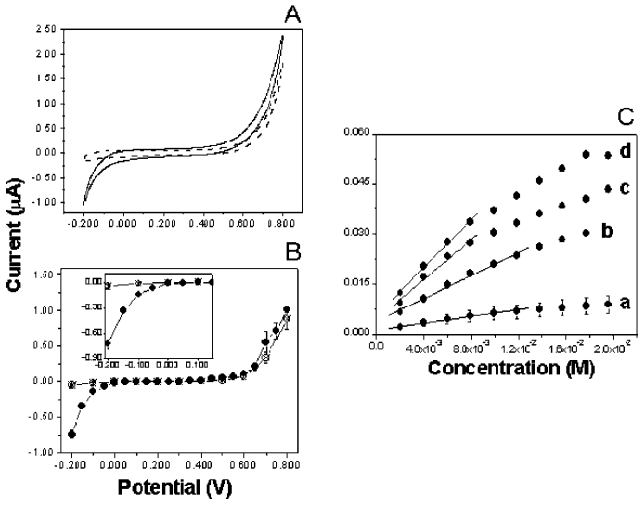

Fig. 2. (A) Cyclic voltammograms for 0.050 M hydrogen peroxide at bare carbon paste electrode (CPE) (dotted line) and at CPE modified with 5.0% w/w magnetite (solid line). Scan rate: 0.100 V s^{-1}. (B) Hydrodynamic voltammograms for 0.050 M hydrogen peroxide at CPE (empty circles) and at magnetite (5.0% w/w)-CPE (full circles). (C) Calibration plots obtained from amperometric experiments performed at -0.100 V at CPE containing different percentages of magnetite: 0.0 (a), 2.5 (b), 5.0 (c), and 7.5 (d) % w/w: Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

and a significant enhancement in the associated currents, allowing in this way, a very convenient low-potential amperometric detection of this important analyte. In fact, the reduction of hydrogen peroxide at CPE-Fe_3O_4 starts at potentials 300 mV less positive than at bare CPE, indicating that the electrogenerated magnetite nanoparticles have a strong catalytic activity towards the reduction of hydrogen peroxide. Just a small catalytic effect is observed on the hydrogen peroxide oxidation. As it was already proposed by Lin and Leu [29], the catalytic center is the Fe (II) of magnetite, which reacts with hydrogen peroxide generating Fe (III), that at the working potential is reduced to Fe (II), giving place to the reduction current.

Figure 2C depicts calibration plots obtained from amperometric experiments performed at -0.100 V for successive additions of hydrogen peroxide at CPE containing different percentages of Fe_3O_4, 0.0 (a), 2.5 (b), 5.0 (c), and 7.5 (d)% w/w. The corresponding sensitivities are the following: (0.5 ± 0.2), (1.71 ± 0.08), (3.0 ± 0.3), and (3.6 ± 0.2) $\mu A\ M^{-1}$ for CPE containing 0.0; 2.5; 5.0; and 7.5% w/w magnetite, respectively. Amperometric experiments performed at CPE modified with 50.0% w/w Fe_3O_4 gave a sensitivity of (32 ± 2) $\mu A\ M^{-1}$ (not shown). However, cyclic voltammograms obtained for CPE containing increasing amounts of magnetite (from 5.0 to 50.0% w/w) showed that the increment in the reduction currents for hydrogen peroxide is accompanied by a large increase in the capacitive currents and by a highly resistive behavior due to a poor agglutination of the carbon matrix and the nonconductive nature of magnetite. These results clearly demonstrate the incidence of the amount of magnetite nanoparticles present in the composite on the overall performance of the resulting modified electrode. Based on these results, a 5.0% w/w magnetite represents the best choice for a highly sensitive response.

Thus, we have demonstrated for the first time that the electrosynthesized magnetite nanoparticles are very efficient for the electrocatalytic reduction of hydrogen peroxide. Compared to carbon paste modified with amorphous iron (III) oxide [30], although the sensitivity of the bioelectrode reported here at neutral pH is smaller, the reproducibility is better and the long term stability is considerably higher. It is important to remark that we use smaller amount of magnetite (5.0% w/w); and perform the amperometric determination of hydrogen peroxide at -0.100 V, potential at which the base line stabilization if faster due to the lower interference of oxygen reduction. The sensitivity obtained with the magnetite-modified glassy carbon electrode [29] is similar to the one we report here, although as in the case of ref. 30, the working potential is -0.200 V.

The incidence of the surfactant present in the magnetite electrosynthesis solution on the electrocatalytic activity of CPE modified with the resulting nanoparticles towards the reduction of hydrogen peroxide was also evaluated. The sensitivities for hydrogen peroxide obtained from amperometric experiments performed at -0.100 V at CPE modified with 5.0% w/w magnetite nanoparticles electrogenerated in the presence of hexadecyltrimethyl ammonium bromide and tetramethylammonium chloride, were almost the same, (2.9 ± 0.4) versus (3.0 ± 0.3) $\mu A\ M^{-1}$, respectively (not shown). These results reveal that, for the evaluated surfactants, the length of the aliphatic chain of the surfactant has almost no influence on the electrocatalytic activity of the magnetite nanoparticles towards the reduction of hydrogen peroxide.

Considering that the Fe_3O_4-modified carbon paste electrode will be finally used for the development of glucose biosensors, and that the usual interferents in blood glucose determinations are AA and UA, we investigate the catalytic activity of magnetite nanoparticles towards the oxidation of these compounds. Figure 3 shows typical cyclic voltammograms obtained at 0.100 V s^{-1} for 1.0×10^{-3} M AA (A) and 1.0×10^{-3} M UA (B) at CPE (dotted line) and at CPE-Fe_3O_4 (5.0% w/w) (solid line). At the magnetite-modified electrode, there is a decrease of 50 mV in the oxidation peak potential for AA and an increase from 22.0 to 27.7 μA in the associated current. For UA, the effect of the magnetite nanoparticles is more pronounced, with a decrease of 135 mV in the oxidation peak potential and an increase in the oxidation current at the peak potential by a factor of 2.5. Therefore, although electrogenerated magnetite nanoparticles also catalyze the oxidation of AA and UA, this effect is not as important as in the case of hydrogen peroxide. In fact, amperometric experiments at -0.100 V demonstrated that CPE-Fe_3O_4 (5.0% w/w) is not responsive to successive additions of AA and UA (not shown), making possible, in this way, a highly selective amperometric detection of hydrogen peroxide.

Based on the excellent electrocatalytic activity of magnetite nanoparticles on the hydrogen peroxide reduction, we designed a glucose biosensor by dispersing GOx within CPE modified with 5.0% w/w of the electrogenerated magnetite nanoparticles. The analytical signal was the reduction of the hydrogen peroxide produced during the step of GOx regeneration.

The amount of GOx within the composite has demonstrated to be an important variable in the development of the glucose biosensor. Figure 4 illustrates the effect of the percentage of GOx (2.5, 5.0, 7.5, and 10.0% w/w) dispersed within CPE-Fe_3O_4 (5.0% w/w) on the sensitivities obtained from amperometric recordings at -0.100 V for successive additions of 5.0×10^{-3} M glucose. The sensitivity increases with the amount of GOx in the bioelectrode up to 5.0% w/w to remain constant thereafter. It is important to notice that for GOx amounts higher than 10.0% w/w, the oxygen consumption becomes more important, producing a shortening of the linear range. The best compromise between sensitivity and dynamic linear range was reached with a bioelectrode containing 5.0% w/w of GOx and 5.0% w/w magnetite nanoparticles.

Figure 5A shows the current-time recordings obtained at -0.100 V for successive additions of 1.0×10^{-3} M glucose using CPE-Fe_3O_4(5.0% w/w)-GOx(5.0% w/w). A fast and well-defined response is observed for each glucose addition. Similar amperometric experiments performed at CPE-GOx

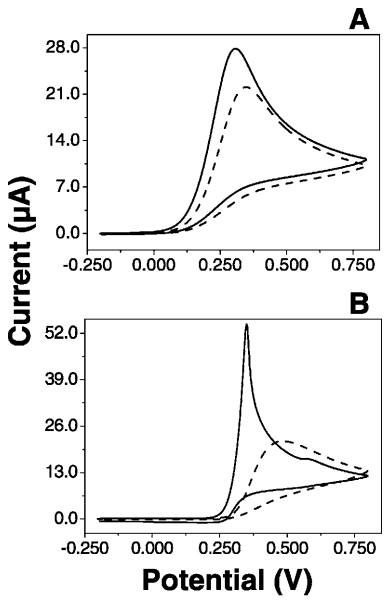

Fig. 3. Cyclic voltammograms for 1.0×10^{-3} M ascorbic acid and 1.0×10^{-3} M uric acid (B) at bare CPE (dotted line) and at CPE modified with 5.0% w/w magnetite (solid line). Scan rate: 0.100 V s^{-1}. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

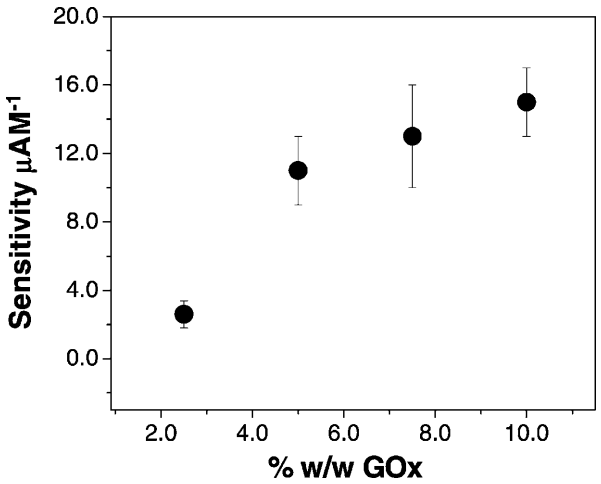

Fig. 4. Sensitivity to glucose obtained from amperometric experiments at -0.100 V at CPE modified with 5.0% w/w magnetite and GOx as a function of the amount of GOx dispersed within the composite. Supporting electrolyte: 0.050 M phosphate buffer pH 7.40.

gave no signal even after ten additions of 1.0 mM glucose due to the poor reduction of hydrogen peroxide at -0.100 V (not shown). The corresponding calibration plot is depicted in Figure 5B. A linear relationship between current and glucose concentration is obtained up to 8.0×10^{-3} M glucose. After that, the current increases non-linearly with the substrate concentration, as expected for biocatalytic reactions. The average sensitivity measured at -0.100 V was (32 ± 4) μA M^{-1} and the detection limit was 3.0×10^{-4} M. Eadie–Hofstee plots obtained from the results shown in Figure 5, gave the following kinetics parameters: $K_M^{app} = 0.018$ M and $I_{max} = 0.67$ μA. The value of the K_M is within the range reported for the K_M of GOx in solution, suggesting that the environment where GOx is dispersed is adequate.

One very important aspect to solve in the development of electrochemical glucose biosensors is the interference of easily oxidizable compounds present in blood serum such as AA and UA. Figure 6 displays amperometric recordings at -0.100 V obtained after one addition of 5.0×10^{-3} M glucose, followed by additions of 2.5×10^{-5} M AA (A) and 1.0×10^{-4} M UA (B) (up to final concentrations of 1.0×10^{-4} M AA and 4.0×10^{-4} M UA). No interference was observed for such concentrations even higher than the maximum physiological levels found in human blood serum, demonstrating the advantages of the preferential electrocatalytic detection of the enzymatically generated hydrogen peroxide not only to obtain high sensitivity but also an excellent selectivity.

To evaluate the usefulness of the bioelectrode for practical applications, the electrode was challenged with blood human serum samples (Standatrol S-E-2, Wiener Lab.). The glucose concentration obtained after 4 determinations of serum samples was $(8.5 \pm 0.1) \times 10^{-3}$ M. The concentration reported by Wienner laboratory was 8.6×10^{-3} M. Therefore, the 1.2% error with the informed value obtained by the classical spectrophotometric method is indicative of an excellent performance of the proposed biosensor.

The response to glucose was highly reproducible. The% *RSD* for the sensitivities obtained from ten successive calibrations plots performed using the same CPE-Fe_3O_4-GOx surface was 5.4%, evidencing an excellent short-term stability. The reproducibility for five different surfaces of the same CPE-Fe_3O_4-GOx composite was 6.9%. The reproducibility inter-composites was also evaluated. Five different CPE-Fe_3O_4-GOx composites gave an *RSD* of 9.5%. These % *RSD* are a clear demonstration of the good reproducibility not only in the amperometric quantification, but also in the composite and surface preparation conditions. The long-term stability of the composite stored at 4 °C was analyzed by measuring the sensitivity of calibrations plots obtained from amperometric experiments at -0.100 V. The sensitivity after 10 months remained in a 90.0% of the original value, evidencing the excellent stability of the biosensor and indicating that the environment of the composite and magnetite nanoparticles preserves the biological activity of GOx.

In summary, compared to other methodologies based on the use of carbon electrode modified with metals or metal oxides, the biosensor proposed here has demonstrated a similar or even better sensitivity for the detection of glucose from the reduction of the enzymatically generated hydrogen peroxide. The selectivity is better than the one obtained at carbon paste electrodes containing Pt, Pd or Cu microparticles [16], and similar to that obtained at carbon paste electrode modified with Rh [10], Ir [11], Au [12], Ru [16] or Prussian blue [17]. Although it has been already demon-

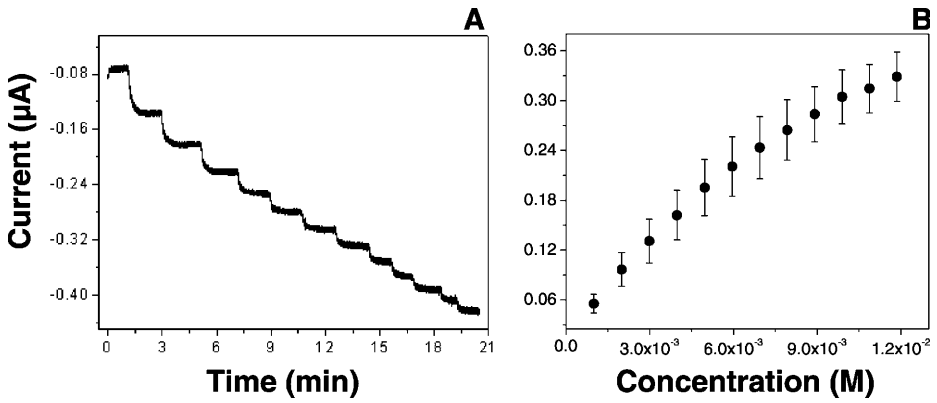

Fig. 5. (A) Amperometric recordings for additions of 1.0×10^{-3} M glucose solution using a CPE containing 5.0% w/w Fe_3O_4 and 5.0% w/w GOx. (B) Calibration plots obtained from the amperometric recordings shown in (A). Working potential: -0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

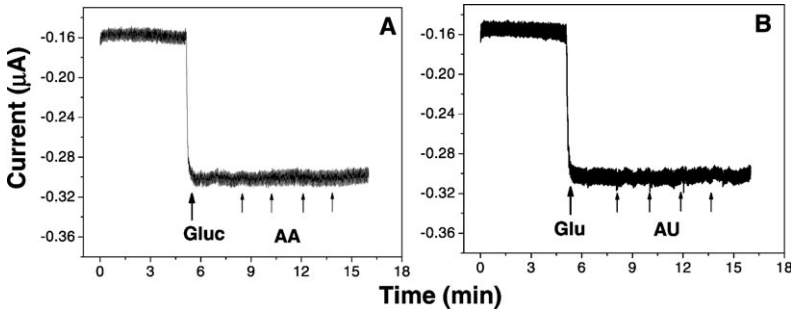

Fig. 6. Current – time profiles performed at Fe_3O_4(5.0% w/w)-GOx(5.0% w/w)-CPE for one addition of 5.0×10^{-3} M glucose and successive additions of (A) 2.5×10^{-5} M AA (up to a final concentration of 1.0×10^{-4} M) and (B) 1.0×10^{-4} UA (up to a final concentration of 4.0×10^{-4} M). Other conditions as in Figure 5.

strated that the metallized carbon composites constitute an adequate environment for GOx, the proposed bioelectrode presents an even higher stability than the ones obtained at other metalized electrodes.

4. Conclusions

We have presented for the first time the use of electrochemically synthesized magnetite nanoparticles for the development of electrochemical biosensors. The electrogenerated nanoparticles have demonstrated an excellent catalytic activity towards hydrogen peroxide reduction, at variance with previous works where the magnetite nanoparticles, obtained by the classical chemical synthesis, have been used mainly as support for glucose oxidase or catalysts [27, 28].

The electrocatalytic detection of hydrogen peroxide has made possible the development of a highly sensitive and selective amperometric glucose biosensor even in the presence of large excess of easily oxidizable compounds or complex matrices. The resulting bioelectrode demonstrated a very good sensitivity, reproducibility and selectivity comparable and even better than those observed for other metallized glucose biosensors. The simplicity of the preparation, the high sensitivity and selectivity, the excellent reproducibility of the proposed biosensor, and the excellent correlation of the results for blood human serum with those obtained using the spectrophotometric method, convert the proposed biosensor in a very good promise for practical applications in real biological systems.

Based on the catalytic activity of electrogenerated magnetite nanoparticles and their know possibilities of functionalization, is expected that the derivatization of the electrosynthesized magnetite nanoparticles with biomolecules will allow to have very efficient biosensors.

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References

[1] L. Clark, Jr., C. Lyons, *Ann. NY Acad. Sci.* **1962**, *102*, 29.
[2] J. Wang, *Chem. Rev.***2008**, *108*, 814.

[3] O. A. Sadik, A. O. Aluoch, A. Zhou, *Biosens. Bioelectron.* **2009**, *24*, 2749.
[4] T. M.-H., Lee, *Sensors* **2009**, *8*, 5535.
[5] A. Heller, B. Feldman, *Chem. Rev.* **2008**, *108*, 2482.
[6] A. Karyakin, E. E. Karyakina, *Sens. Actuators B* **1999**, *57*, 268.
[7] A. A. Karyakin, E. A. Puganova, I. A. Budashov, I. N. Kurochkin, E. E. Karyakina, V. A. Levchenko, V. N. Matveyenko, S. D. Vartfolomeyev, *Anal. Chem.* **2004**, *76*, 474.
[8] M. Somasundrum, K. Kirtikara, M. Tanticharoen, *Anal. Chim. Acta* **1996**, *319*, 59.
[9] H. Sakslund, J. Wang, O. Hammerich, *J. Electroanal. Chem.* **1996**, *402*, 149.
[10] J. Wang, J. Liu, L. Chen, F. Lu, *Anal. Chem.* **1994**, *66*, 3600.
[11] J. Wang, G. Rivas, M. Chicharro, *Electroanalysis* **1996**, *8*, 434.
[12] M. S. Celej, G. A. Rivas, *Electroanalysis* **1998**, *10*, 771.
[13] G. Rivas, M. C. Rodríguez, *Electroanalysis* **2001**, *13*, 1179.
[14] J. Liu, F. Lu, J. Wang, *Electrochem. Commun.* **1999**, *1*, 341.
[15] M. C. Rodríguez, G. A. Rivas, *Anal. Lett.* **2001**, *34*, 1829.
[16] S. Miscoria, G. Barrera, G. Rivas, *Electroanalysis* **2002**, *14*, 981.
[17] S. A. Miscoria, G. D. Barrera, G. A. Rivas, *Electroanalysis* **2005**, *17*, 1578.
[18] S. A. Miscoria, G. D. Barrera, G. A. Rivas, *Sens. Actuators B* **2006**, *115*, 205.
[19] G. L. Luque, M. C. Rodríguez, G. A. Rivas, *Talanta* **2005**, *66*, 467.
[20] L. Zhang, H. Li, Y. Ni, J. Li, K. Liao, G. Zhao, *Electrochem. Commun.* **2009**, *11*, 812.
[21] N. W. Beyene, P. Kotzian, K. Schachl, H. Alemu, E. Turkusic, A. Copra, H. Moderegger, I. Svancara, K. Vytras, K. Kalcher, *Talanta* **2004**, *64*, 1151.
[22] E. Turkusic, J. Kalcher, E. Kahrovic, N. W. Beyene, H. Moderegger, E. Sofic, S. Begie, K. Kalcher, *Talanta* **2005**, *65*, 559.
[23] G. L. Luque, N. F. Ferreyra, G. Leyva, G. A. Rivas, *Sens. Actuators B* **2009**, *142*, 331.
[24] M.-P. Pileni, *Adv. Funct. Mater.* **2001**, *11*, 323.
[25] R. J. Harrison, A. Putins, *Am. Miner.* **1995**, *80*, 213.
[26] L. Cabrera, S. Gutierrez, N. Menendez, M. P. Morales, P. Herrasti., *Electrochim. Acta* **2008**, *53*, 3436.
[27] S. Qu, J. Wang, J. Kong, P. Yang, G. Chen, *Talanta* **2007**, *71*, 1096.
[28] J. Chumming, L. Xiangqin, *J. Solid State Electrochem.* **2009** *13*, 1273.
[29] M. S. Lin, H.-J. Leu, *Electroanalysis* **2005**, *17*, 2068.
[30] J. Hrbac, V. Halouzka, R. Zboril, K. Papadopoulos, T. Triantis, *Electroanalysis* **2007**, *19*, 1850.

