Sensors and Actuators B 149 (2010) 306–309

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Short communication

Glucose biosensing at carbon paste electrodes containing iron nanoparticles

Fausto N. Comba[a], María D. Rubianes[a], Pilar Herrasti[b], Gustavo A. Rivas[a,*]

[a] INFIQC, Departamento de Físico Química, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina
[b] Departamento de Química Física Aplicada, Facultad de Ciencias, Universidad Autónoma de Madrid, SSIC, 28049, Cantoblanco, Madrid, España

ARTICLE INFO

Article history:
Received 13 May 2010
Accepted 8 June 2010
Available online 15 June 2010

Keywords:
Iron
Nanoparticles
Carbon paste electrode
Hydrogen peroxide
Glucose oxidase
Amperometric glucose biosensor

ABSTRACT

This work reports the advantages of incorporating a metal as common as iron in a carbon paste electrode containing glucose oxidase to develop highly selective, stable and sensitive amperometric glucose biosensors. The excellent catalytic activity of iron nanoparticles towards the reduction of hydrogen peroxide has made possible the quantification of glucose at very low potentials (−0.100 V) avoiding, in this way, the interference of easily oxidizable compounds like ascorbic acid and uric acid without needing of redox mediators or permselective membranes. Linear relationship between current and glucose concentration was obtained up to 0.020 M (3.6 g/L), with a detection limit of 0.2 mM, no interference of AA and UA, and very good correlation with the spectrophotometric methods when determining glucose in human blood serum.

1. Introduction

Glucose biosensing is very important for early diagnostic and management of diabetes, pathology widely distributed around the world, with estimations of 300 million sufferers by 2045 [1]. Amperometric biosensors based on the use of glucose oxidase (GOx) as biorecognition element have demonstrated to be highly successful [2–5]. GOx catalyzes the oxidation of glucose to gluconolactone in the presence of oxygen, which is converted into hydrogen peroxide during the regeneration cycle.

Carbon transducers containing GOx and metals that catalyze the oxidation/reduction of hydrogen peroxide have received considerable attention in the last 20 years. Ir [6–8], Ru [9,10], Rh [11,12], Cu [13], Au [14], mixtures of metals [15,16], metal oxides [17–21], perovskites [22], and magnetite electrochemically synthesized [23] as well as Prussian blue [24] have demonstrated to be highly successful for this task.

In this work we present for the first time the advantages of incorporating iron nanoparticles in carbon paste electrodes containing GOx for the highly selective and sensitive glucose biosensing based on the catalytic activity of iron towards hydrogen peroxide reduction. To the best of our knowledge, the use of this common metal for developing oxidases-based biosensors has not been reported. Just few works mentioned the catalytic activity of iron towards hydrogen peroxide reduction when discussing the effect of carbon nanotubes oxidation [25,26].

2. Experimental

2.1. Reagents

Hydrogen peroxide (30%, v/v aqueous solution) was purchased from Baker. Uric acid (UA) 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). The mineral oil, $FeSO_4 \cdot 7H_2O$ and sodium carbonate were acquired from Aldrich. Other 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.

Acicular Fe nanoparticles were obtained from thermal reduction of acicular goethite (α-FeOOH). Goethithe nanoparticles were obtained by mixing aqueous solutions of $FeSO_4$ (0.60 M) and Na_2CO_3 (0.90 M), followed by oxidation at 40 °C for 6 h by bubbling air at a constant flow rate of 2 dm^3/min. The precipitates were then cooled, washed several times with deionized water and dried at 50 °C. The powders were dehydroxylated in the presence of N_2 for 4 h at 500 °C and the resulting hematite particles were thermally reduced with hydrogen at 400 °C [27].

2.2. Apparatus

The electrochemical measurements were performed with TEQ_02 and BAS CV-37 potentiostats. A platinum wire and Ag/AgCl, 3 M KCl were used as counter and reference electrodes, respec-

* Corresponding author. Tel.: +54 351 4334169/80; fax: +54 351 4334188.
E-mail address: grivas@mail.fcq.unc.edu.ar (G.A. Rivas).

0925-4005/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2010.06.020

tively. All potentials are referred to the latter. A magnetic stirrer provided the convective transport during the amperometric measurements.

Carbon paste electrode (CPE) containing iron was prepared by mixing first the iron nanoparticles with mineral oil for 10 min in an agate mortar, followed by the incorporation of the graphite powder and mixing for additional 30 min. In the case of the enzymatic electrode, GOx and iron nanoparticles were first mixed with the mineral oil for 10 min before incorporating the graphite powder. 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 onto a weighing paper before starting every new experiment.

Iron nanoparticles were observed using transmission electronic microscopy (TEM) model JEOL 2000 FXII (200 kV) with a microanalyse X-EDS.

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

Fig. 1 illustrates a TEM image of iron nanoparticles. They consisted of acicular iron with an average size of 100 nm with a polydispersity degree of 25% and an axial ratio of 5 with a polydispersity degree of 23%.

Fig. 2 displays hydrodynamic voltammograms for 0.050 M hydrogen peroxide obtained at CPE (empty circles) and at CPE containing 5.0% w/w of iron nanoparticles (CPE-Fe (5.0% w/w) full circles). In the presence of iron, the reduction of hydrogen peroxide at CPE-Fe starts at 0.100 V. At −0.100 V, the current

Fig. 1. TEM image of iron acicular nanoparticles.

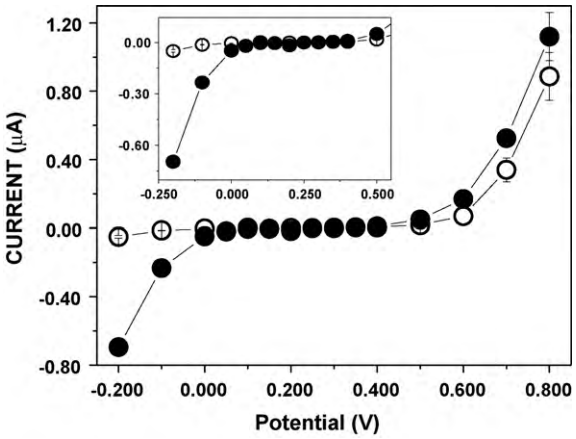

Fig. 2. Hydrodynamic voltammograms for 0.050 M hydrogen peroxide at bare carbon paste electrode (CPE) (empty circles) and at carbon paste electrode modified with 5.0% w/w iron nanoparticles (full circles). The inset shows the hydrodynamic voltammograms in a more restricted potential range. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

enhances in a factor of 17.7 (compared to CPE), indicating that iron nanoparticles have a strong catalytic activity towards the reduction of hydrogen peroxide. A less pronounced catalytic effect is observed on the hydrogen peroxide oxidation. Similarly to the behavior of CPE modified with iron oxides [28], the iron would

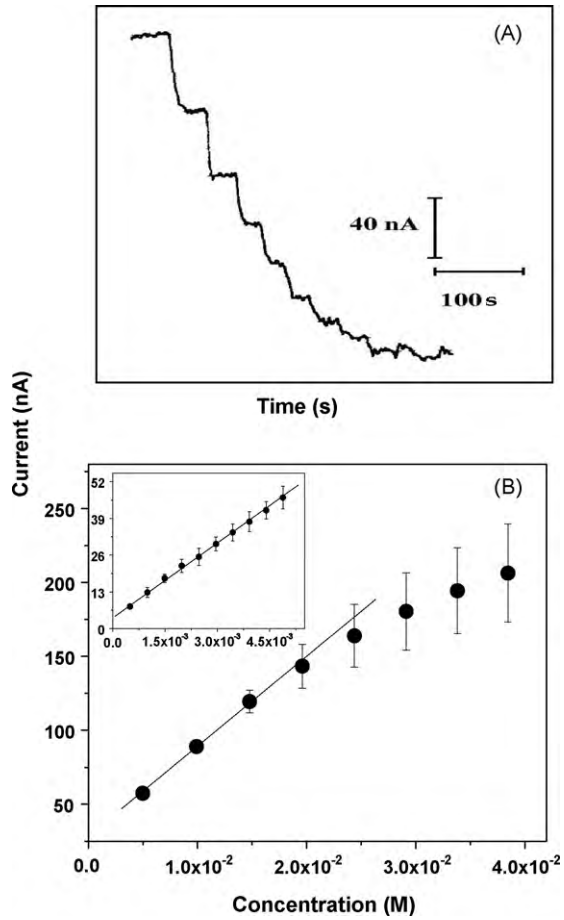

Fig. 3. (A) Amperometric recordings for additions of 5.0×10^{-3} M glucose solution using a CPE containing 5.0% w/w iron nanoparticles and 5.0% w/w GOx. (B) Calibration plots obtained from amperometric recordings shown in (A). The inset shows a calibration plot for lower of glucose concentrations range. Working potential: −0.100 V. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

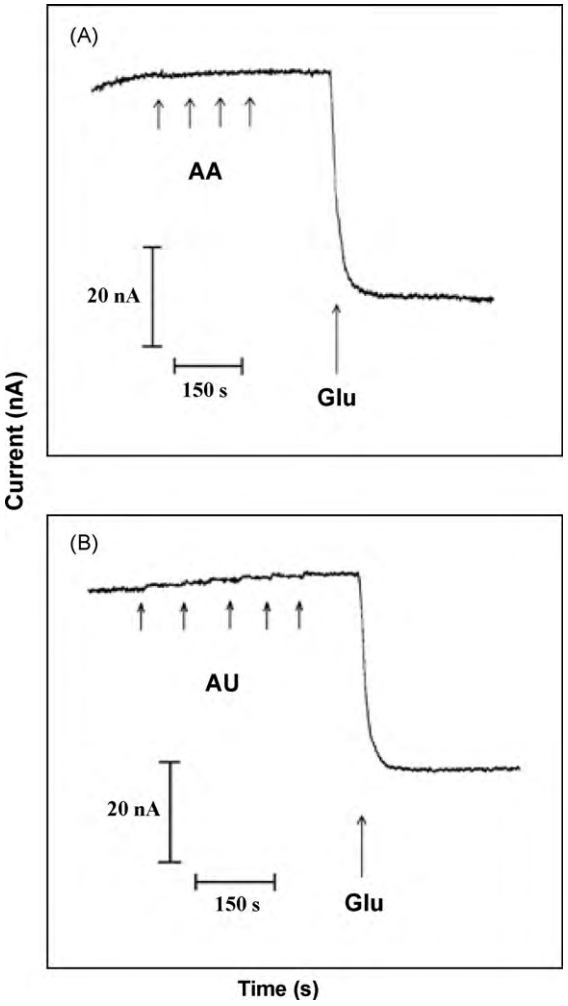

Fig. 4. Current–time profiles performed at Fe(5.0%, w/w)-GOx(5.0%, w/w)-CPE for successive additions of (A) 2.5×10^{-5} M AA and (B) 1.0×10^{-4} UA followed by one addition of 5.0×10^{-3} M glucose. Other conditions as in Fig. 3.

be oxidized by hydrogen peroxide to Fe(III) which at the working potential is easily reduced giving place to the catalytic reduction current.

Fig. 3(A) shows the current–time recordings obtained at −0.100 V for successive additions of 5.0×10^{-3} M glucose using CPE-Fe(5.0%, w/w)-GOx(5.0%, w/w). A well-defined response is observed for each glucose addition. A linear relationship between current and glucose concentration is obtained up to 2.0×10^{-2} M glucose (Fig. 3(B)). The inset of Fig. 3(B) shows a calibration plot for smaller glucose concentrations. The average sensitivity obtained at −0.100 V was $(8.8 \pm 0.5)\,\mu A\,M^{-1}$ and the detection limit, 0.2 mM.

Fig. 4 displays amperometric recordings at −0.100 V obtained after four successive additions of 2.5×10^{-5} M ascorbic acid (AA) (A) or five additions of 1.0×10^{-4} M uric acid (UA) (B) followed by one addition of 5.0×10^{-3} M glucose. No interference was observed even for the maximum physiological concentrations of AA and UA found in human blood serum clearly evidencing the advantages of the proposed biosensor not only to obtain high sensitivity but also excellent selectivity.

The bioelectrode was challenged with blood human serum samples (Standatrol S-E-2, Wiener Lab.) with elevated values of different bioanalytes usually present in blood. The glucose concentration obtained after 6 determinations of serum samples was $(1.8 \pm 0.3) \times 10^{-2}$ M, with a 12.5% error compared to the value obtained by the spectrophotometric method (1.6×10^{-2} M). These results indicate that our methodology can be used to determine glucose in samples as complex as human serum even with elevated concentrations of typical interferents.

The % R.S.D. for the sensitivities obtained from 10 successive calibrations plots performed with the same CPE-Fe-GOx surface was 10.9% evidencing an excellent short-term stability. The reproducibility for the same CPE-Fe-GOx composite and six different surfaces was 6.4%. After 140 days, the sensitivity remained in a 91% of the original value for the electrode stored at 4 °C or at room temperature (25 °C).

4. Conclusions

This work reports for the first time an amperometric glucose biosensor based on the excellent catalytic activity of iron nanoparticles towards the reduction of hydrogen peroxide. The biosensor allows working at low potentials, with excellent selectivity without need of redox mediators or polymeric membranes. Compared to other metallized electrodes, this one presents several advantages like wider dynamic linear range (compared to Ir [6,7], Cu [8], and Au [14] based biosensors); preferential catalytic activity towards hydrogen peroxide (compared to Cu [8], Pt [16], Pd [16]); comparable or even better long-term stability than others. A secondary, but not less important advantage is that iron offers the possibility to develop cheaper sensors compared to Ir-, Rh-, Pd- or Au-based sensors.

In summary, this interesting approach has allowed us to develop a robust and highly stable, sensitive and selective biosensor able to detect glucose not only in pure solutions but also in complex matrices like human serum, and represents a highly promising alternative for further developments of other oxidases-based biosensors.

Acknowledgements

The authors thank CONICET, SECyT-UNC, ANPCyT, Ministerio de Ciencia y Tecnología de Córdoba for the financial support. FNC acknowledges CONICET for the fellowship.

References

[1] F. Davis, S.P.J. Higswon, MicroFuel Cells (2009) 243.
[2] O.A. Sadik, A.O. Aluoch, A. Zhou, Biosens. Bioelectron. 24 (2009) 2749.
[3] T.M.-H. Lee, Sensors 8 (2009) 5535.
[4] J. Wang, Chem. Rev. 108 (2008) 814.
[5] A. Heller, B. Feldman, Chem. Rev. 108 (2008) 2482.
[6] J. Wang, G. Rivas, M. Chicharro, Electroanalysis 8 (1996) 434.
[7] M.C. Rodríguez, G.A. Rivas, Electroanalysis 11 (1999) 434.
[8] G. Rivas, M.C. Rodríguez, Electroanalysis 13 (2001) 1179.
[9] J. Wang, J. Liu, L. Chen, F. Lu, Anal. Chem. 66 (1994) 3600.
[10] J. Wang, L. Fang, D. Lopez, H. Tobias, Anal. Lett. 26 (1993) 1819.
[11] F. Wang, L. Lu, J. Angnes, H. Liu, H. Sakslund, Q. Chen, M. Pedrero, L. Chen, O. Hammerich, Anal. Chim. Acta 305 (1995) 3.
[12] S.A. Miscoria, G.D. Barrera, G.A. Rivas, Sens. Actuators B 115 (2006) 205.
[13] M.C. Rodriguez, G.A. Rivas, Electroanalysis 13 (2001) 1179.
[14] M.S. Celej, G.A. Rivas, Electroanalysis 10 (1998) 771.
[15] J. Liu, F. Lu, J. Wang, Electrochem. Commun. 1 (1999) 341.
[16] S. Miscoria, G. Barrera, G. Rivas, Electroanalysis 14 (2002) 981.
[17] L. Zhang, H. Li, Y. Ni, J. Li, K. Liao, G. Zhao, Electrochem. Commun. 11 (2009) 812.
[18] N.W. Beyene, P. Kotzian, K. Schachl, H. Alemu, E. Turkusic, A. Copra, H. Moderegger, I. Svancara, K. Vytras, K. Kalcher, Talanta 64 (2004) 1151.
[19] E. Turkusic, J. Kalcher, E. Kahrovic, N.W. Beyene, H. Moderegger, E. Sofic, S. Begie, K. Kalcher, Talanta 65 (2005) 559.
[20] G.L. Luque, M.C. Rodríguez, G.A. Rivas, Talanta 66 (2005) 467.
[21] M.S. Lin, H.-J. Leu, Electroanalysis 17 (2005) 20.
[22] G.L. Luque, N.F. Ferreyra, A.G. Leyva, G.A. Rivas, Sens. Actuators B 142 (2009) 331.
[23] F.N. Comba, M.D. Rubianes, L. Cabrera, S. Gutiérrez, P. Herrasti, G. A. Rivas, Electroanalysis (in press).
[24] X. Cui, G. Liu, Y. Lin, Nanomed. Nanobiotechnol. Biol. Med. 1 (2005) 130.
[25] K. Jurkschat, X. Ji, A. Crossley, R.G. Compton, C.E. Banks, Analyst 132 (2007) 21.
[26] M. Pumera, Langmuir 23 (2007) 6453.
[27] K. O'Grady, H. Laidled, J. Magn. Mater. 200 (1999) 616.
[28] J. Hrbac, V. Halouzka, R. Zboril, K. Papadoupoulos, T. Mantis, Electroanalysis 19 (2007) 1850.

Biographies

Fausto N. Comba obtained his degree of Biochemist (2007) from Cordoba National University (Cordoba, Argentina). At present he is doing the Ph. D. at the same University with a fellowship from Argentine Research Council. His doctoral thesis focuses on the development and characterization of electrochemical biosensors based on the use of biomolecules, magnetic nanoparticles and carbon nanotubes

María D. Rubianes obtained her Ph. D. in Chemistry (2005) from Cordoba National University (Cordoba, Argentina). She did the postdoctoral training in the group of Polymers at the Organic Chemistry Department, Faculty of Chemical Sciences (Cordoba National University). At present, she is assistant professor at the Department of Physical Chemistry, Faculty of Chemical Sciences of Cordoba National University and Assistant Researcher at Argentine Research Council (CONICET). Dr. Rubianes is an active member of the Biosensors Group at the Physical Chemistry Department, and her research interests focus on the development and characterization of electrochemical (bio)sensors based on the use of nanostructured materials and modified polymers.

Pilar Herrasti obtained her Ph. D. in Chemistry (1987) from Autonomous Madrid University (Madrid, Spain). She did the postdoctoral training at Southampton University (Southampton, England) between 1989 and 1990. She is full professor at Autonomous Madrid University. She is secretary from Spain of the Iberoamerican Society of Electrochemistry. Her research now is focusing in the development of nanomaterials for different applications such as hyperthermia, drug delivery and electrocatalysis. She has over 70 peer-reviewed papers, one patent, and one book chapter.

Gustavo A. Rivas obtained his Ph. D. in Chemistry (1991) from Cordoba National University (Cordoba, Argentina). He did the postdoctoral training at University of Valence, Valence (Spain) in 1994 and 1995 and at New Mexico State University, Las Cruces (USA) between 1995 and 1996. At present, he is full professor at Cordoba National University and Principal Researcher at Argentine Research Council (CONICET). Since 2008 Dr. Rivas heads the Department of Physical Chemistry at the Faculty of Chemical Sciences. He was President of the Argentinean Society of Analytical Chemists between 2005 and 2007. Professor Rivas is the recipient of the Ranwell Caputto Award from National Academy of Sciences of Argentina (2001) and Rafael Labriola Award from Argentinean Society of Chemistry (2004). His research interests focus on the design and characterization of electrochemical (bio)sensors based on nanostructured materials, the development of new bioanalytical platforms for biosensing in batch and flow systems, the study of DNA damage, and the design of new strategies for nanomaterials and polymers modification. He has over 100 peer-reviewed papers and two book chapters. Prof. Rivas belongs to the Editorial Board of Sensors and Actuators B, Analytical Letters, Electroanalysis, and Journal of Biomedical Sciences.