Bioelectrochemistry 76 (2009) 135–140

Contents lists available at ScienceDirect

Bioelectrochemistry

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

A new modified conducting carbon composite electrode as sensor for ascorbate and biosensor for glucose

Madalina M. Barsan, Christopher M.A. Brett *

Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal

ARTICLE INFO

Article history:
Received 22 December 2008
Received in revised form 9 March 2009
Accepted 12 March 2009
Available online 19 March 2009

Keywords:
Cellulose acetate
Graphite composite
Electrochemical sensor
Enzyme biosensor

ABSTRACT

A new carbon-based conducting composite has been developed as electrochemical sensor and biosensor for the amperometric detection of ascorbate and glucose. Electrocatalytic oxidation of ascorbate has been done successfully at unmodified cellulose acetate-graphite composite electrodes, the sensor being highly sensitive, selective and with a low detection limit at 0.0 V vs. SCE and was successfully applied for ascorbate determination in commercial fruit juice samples. An interference free glucose biosensor has also been developed, based on the immobilisation of glucose oxidase by cross-linking with glutaraldehyde on poly (neutral red) modified composite electrodes. The biosensor exhibits a higher sensitivity of 31.5 ± 1.7 μA cm^{-2} mM^{-1} than other carbon-composite-based glucose biosensors, a detection limit of 20.3 μM and a very short response time.

1. Introduction

There is strong interest in preparing new electrode materials for electrochemical sensors and biosensors with favourable analytical and electrochemical properties in order to improve their performances and lower the costs associated with sensing and biosensing devices. Due to their simplicity of construction, relatively low cost and outstanding electrochemical, physical and mechanical properties [1,2], composite electrodes have been the focus of various researchers in the past decade. Incorporating conducting carbon particles in desirable polymeric matrixes, new carbon composite electrodes have been developed and extensively used in electroanalytical measurements [3] for trace metal detection [4], for determination of antioxidants [5], and for dopamine, ascorbic and uric acid [6]. Due to their renewable surfaces, composite-based amperometric biosensors have been constructed mainly by incorporating, together with the conducting phase, polymer precursor and redox mediator or co-factor, and/or the desired biorecognition element, e.g. glucose oxidase [7–10], or glucose dehydrogenase [11].

Cellulose acetate (CA) has been mainly used in biosensor construction in the form of membranes containing either the biomolecules, which also act as a permselective membrane or size exclusion membrane [12–14], or mediators [15]. For the construction of ascorbate sensors, CA was employed as a casting membrane for redox compounds such as methylene blue [16] or 2,6-dichlorophenolindophenol [17,18]. Boujtita et al. reported the fabrication of screen-printed electrodes using graphite-CA ink for the detection of nitric oxide [19] or for the determination of hydrogen peroxide and L-lactate, when the ink also contained horseradish peroxidase (HRP) [20] or both HRP and lactate oxidase [21].

Recently we have reported on a new flexible graphite composite material prepared using cellulose acetate (CA) as polymer matrix for the entrapment of micro-metre sized graphite particles [22]. The composite is easily prepared in desired sizes by simple cutting and showed high robustness and very good electroanalytical properties making it useful for diverse applications in electrochemical sensors and biosensors.

The present work describes the preparation and evaluation of an ascorbate sensor and a glucose oxidase biosensor using these new graphite-CA composite electrode substrates. A direct and selective detection of ascorbate at conventional or metal electrodes is difficult due to its large overpotential and electrode fouling by oxidation products [23]. Many attempts have been reported to reduce the overpotential by using active mediators for the catalytic electrooxidation of ascorbate, e.g. copper (II) phosphate [24], copper hexacyanoferrate [25], Prussian Blue [26], polyaniline/poly(N-methylaniline) [27,28] or even by using ascorbate oxidase [29]. We here report an improved interference-free ascorbate amperometric sensor, based on unmodified CA-graphite composite electrodes which exhibits a very good sensitivity and low detection limit at an applied potential of 0.0 V vs. saturated calomel electrode (SCE).

For the amperometric detection of glucose, a biosensor assembly was prepared by immobilizing the enzyme by cross-linking with glutaraldehyde in the presence of bovine serum albumin on the top of poly(neutral red) (PNR) modified CA-graphite composite electrodes. The phenazine dye neutral red (NR) has been extensively used by us for the preparation of electropolymerised poly(neutral red) films on

* Corresponding author. Tel./fax: +351 239 835295.
E-mail address: brett@ci.uc.pt (C.M.A. Brett).

1567-5394/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2009.03.004

carbon film substrates, and has been shown to be an excellent redox mediator in various biosensor assemblies [30,31].

2. Experimental

2.1. Reagents and buffer electrolyte solutions

All reagents were of analytical grade and were used without further purification. Ascorbic acid, bovine serum albumin (BSA), cellulose acetate (≈40% acetyl content), fructose, α-D(+)-glucose, glutaraldehyde (GA) 25% (v/v) and sodium phosphate monobasic monohydrate were from Sigma-Aldrich (Steinheim, Germany). Acetone, cyclohexanone, glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 24 units/mg), neutral red (NR), 65% dye content, oxalic acid dihydrate and potassium nitrate were obtained from Fluka (Buchs, Switzerland). Potassium hydrogen phosphate, di-sodium hydrogen phosphate 2-hydrate and sodium chloride were from Riedel-deHaën, (Seelze, Germany), potassium hydrogen phosphate 3-hydrate from Panreac, (Barcelona, Spain), tartaric acid from PAHI, Lisbon, Portugal and citric acid monohydrate was from Merck (Darmstadt, Germany).

Composite Type 1 electrodes were prepared with graphite powder of particle diameter approximately 30 μm, obtained from spectroscopic electrode graphite bars type SW.104 produced by "Kablo Bratislava", National Corporation ("Electrocarbon Topolcany" Factory, Slovakia). Composite Type 2 electrodes were made with graphite powder (grade #38) from Fisher Scientific Corporation (Pittsburgh, PA, USA).

For electrochemical experiments, the supporting electrolyte was sodium phosphate buffer saline (NaPBS) (0.1 M phosphate buffer + 0.05 M NaCl, pH = 7.0), prepared from sodium di-hydrogenphosphate, di-sodium hydrogenphosphate and sodium chloride. Polymerisation of neutral red was carried out in an electrolyte composed of 0.025 M potassium phosphate buffer solution and 0.1 M KNO_3 (pH 5.5).

A stock solution of 0.1 M glucose was prepared in supporting electrolyte at least one day before use, to permit equilibration of α and β anomers of D-glucose; it was kept in the refrigerator and used within one week.

The samples used for ascorbate determination were commercial Cecrisina® vitamin C effervescent tablets (1.0 g of Vitamin C per tablet) (Janssen-Cilag, Queluz de Baixo, Portugal).

Millipore Milli-Q nanopure water (resistivity ≥ 18 MΩ cm) and analytical reagents were used for the preparation of all solutions. Experiments were performed at room temperature (25 ± 1 °C).

2.2. Apparatus

For batch experiments, a three-electrode electrochemical cell of volume 10 cm^3 was used, containing the composite electrode as working electrode, a platinum foil counter electrode and a saturated calomel electrode (SCE) as reference.

All electrochemical measurements were performed using a computer-controlled μ-Autolab Type II potentiostat–galvanostat running with GPES (General Purpose Electrochemical System) for Windows version 4.9 software (EcoChemie, Utrecht, Netherlands).

The pH-measurements were carried out with a CRISON 2001 micro pH-meter (Crison Instruments SA, Barcelona, Spain) at room temperature.

2.3. Composite electrode/mediator film preparation and enzyme immobilisation

A cellulose acetate gel was prepared by dissolving cellulose acetate 15% (w/v) in a mixture of solvents containing 55% (v/v) acetone and 45% (v/v) cyclohexanone. Graphite-embedded composite was then prepared by adding graphite powder 15% (w/v) to the obtained CA gel, mixing continuously. The homogenous graphite suspension obtained was allowed to dry for at least 24 h in a Petri dish and removed as a thick, flexible foil of thickness ~0.7 mm, which was then cut into square pieces of 5 mm × 5 mm. External electrical contact was made on the rear surface by a copper wire glued with silver paint; the whole rear face was then covered with insulating epoxy resin. These composite electrodes can be stored in the dry state and have not been observed to suffer any degradation after one year's storage.

Poly(neutral red) (PNR) phenazine polymer was prepared as a film on the CA-graphite composite electrode substrate by cyclic voltammetry in the buffer solution described in Section 2.1, containing 1 mM monomer, at a scan rate of 50 mV s^{-1}. The potential was cycled between −1.0 and +1.0 V vs. SCE for 15 cycles, according to procedures previously optimised in [30].

Glucose oxidase was immobilised by cross-linking with glutaraldehyde (GA). A 1% w/v GOx and 4% w/v BSA enzyme solution was prepared, by mixing the enzyme together with BSA in 0.1 M NaPBS, pH = 7.0. A volume of 10 μl of enzyme solution was then mixed with 5 μl GA (2.5% v/v diluted in water). Of this mixture, 10 μl was dropped onto the electrode surface and left to dry at room temperature during at least 4 h.

2.4. Electrode pre-treatment

Composite electrodes were polished on abrasive paper followed by smooth paper, then electrochemically pre-treated by applying a fixed potential of +0.9 V vs. SCE for 240 s in electrolyte. Before each series of measurements, they were subjected to potential cycling in the range from −1.5 to 1.5 V vs. SCE at a scan rate of 100 mV s^{-1} in the supporting electrolyte to be employed until a reproducible voltammetric curve was recorded (usually after 5 cycles).

3. Results and discussion

As is well known, electrodes used for application in electrochemical sensors and biosensors require electrochemical properties such as large potential window, low background currents, reproducibility, fast electron transfer kinetics etc. A preliminary voltammetric evaluation of these CA-graphite composite electrodes showed that both polishing and electrochemical pre-treatment improved the electrochemical properties of the composite electrode, electrodes exhibiting in this way a large potential window, high electroactive surface area and fast electron transfer kinetics, examined in redox systems such as Ru^{III}/Ru^{II} and Fe^{III}/Fe^{II} [22]. The species gave a close-to-ideal reversible behaviour, under linear-diffusion control. Cyclic voltammograms recorded at three different composite electrodes demonstrate very reproducible behaviour, with a large potential window in 0.1 M NaPBS pH 7.0. It was observed (see Fig. 1), that when composite type 2 is used

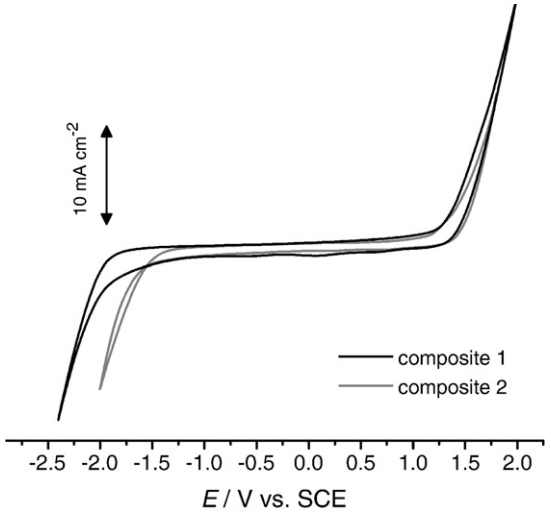

Fig. 1. Background currents recorded at both types of composite electrode in 0.1 M NaPBS pH 7.0, scan rate 100 mV s^{-1}.

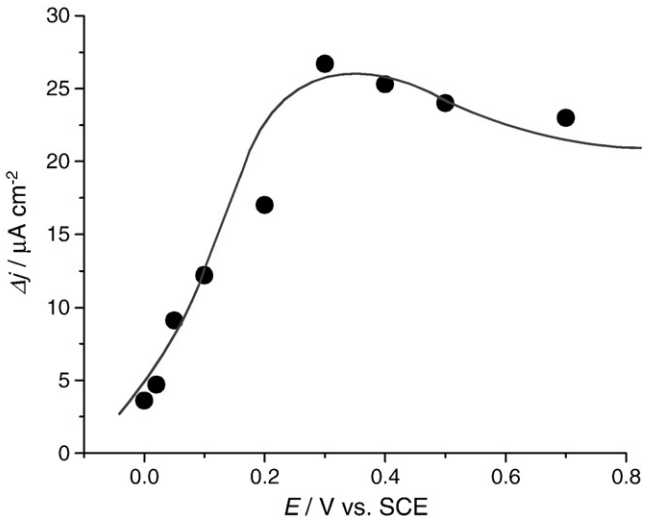

Fig. 2. Dependence of ascorbate sensor current response, Δj, on applied potential following injection of ascorbate, final concentration 0.4 mM, in 0.1 M NaPBS, pH 7.0.

as electrodes, hydrogen evolution starts at less negative potentials, the potential window decreasing slightly in this case.

3.1. Determination of ascorbate at CA-graphite composite electrodes

The newly-developed composite electrodes were applied for the determination of ascorbate in amperometric mode in neutral media (0.1 M NaPBS pH 7.0). First, the response of the sensor to 0.4 mM of ascorbate at applied potentials between 0.0 and 0.7 V vs. SCE was examined. The results are illustrated in Fig. 2, which shows that at +0.3 V vs. SCE the sensor response was the highest, being near to the oxidation potential of the ascorbate. Even at 0.0 V vs. SCE the sensor gave a very good response, and since it was desired to apply the sensor for the determination of ascorbate in juice, in order to minimize interference from other electroactive compounds, this potential was used in further experiments. The amperometric response was recorded under continuous stirring over narrow and wide concentration ranges and typical calibration curves obtained are shown in Fig. 3, with corresponding analytical parameters presented in Table 1. A very low detection limit of 3 μM was obtained with linear range up to at least 1.8 mM, much lower than the 50 μM reported in [27] or those reported in [16,17,24], in the range 10 to 15 μM, and comparable to amperometric sensors which detect ascorbate at more positive potentials with detection limits between 1.5 and 5 μM [25,28,29,32]. The sensitivity had the same value of 18.3 μA cm^{-2} mM^{-1} for both ascorbate concentration ranges, much higher compared to other ascorbate sensors for ascorbate determination using potentials close to 0.0 V vs. SCE or Ag/AgCl which exhibit sensitivities between 0.4 and 7.1 μA cm^{-2} mM^{-1} [16,17,25,32]. The sensor also exhibits a greater linear range than most already reported [17,27,29,32] and has a very short response time, below 3 s.

3.1.1. Accuracy/precision of the sensor

In order to evaluate the accuracy and precision of the sensor, current-time curves were recorded with the addition of 10 μl of either 0.1 M ascorbate standard solution or of 0.1 M ascorbate solution, obtained by dissolving a tablet of vitamin C Cecrisina®, and it was observed that the sensor presents the same sensitivity for both ascorbate solutions used for calibration. The calculated recovery was 99.3 ± 0.2% (3 measurements).

These data show that measurements at CA-graphite composite electrodes are precise and this sensor can be used for ascorbate quantification in juice or wines.

Table 1
Analytical parameters from ascorbate calibration curves recorded at CA-graphite composite electrodes for small and high concentration range.

Concentration range/mM	Sensitivity/μA cm^{-2} mM^{-1}	Intercept/μA	LOD/μM	Correlation coefficient (R^2)
0.0–0.4	18.3 ± 0.2	−0.03 ± 0.10	3.3	0.99993
0.0–1.8	18.3 ± 0.2	−0.21 ± 0.07	16.7	0.99995

3.1.2. Interferences

Besides long-term stability, another important analytical property is the ability to discriminate the interfering species having electroactivity similar to the target analyte. A study of interferences usually present in drinks was performed and it was observed that the main sugars (glucose, fructose), acids (tartaric, acetic, citric acid) and catechol did not interfere at 0.00 V operating potential in neutral phosphate buffer saline.

3.1.3. Determination of ascorbate in juices

Taking into account the very good accuracy and precision of the sensor as well as the absence of interference from the possible electroactive compounds present in juice, the sensor was applied for the quantification of ascorbate in natural orange and passion fruit juices using the standard addition method (see Fig. 4). The calculated concentrations of ascorbate were 17.1 ± 0.3 μM and 13.0 ± 0.2 μM (3 measurements) in orange and passion fruit juice respectively, in good agreement with the values declared by the producer: 16.8 μM and 12.8 μM respectively.

3.2. PNR/GOx biosensor

The selectivity and overall accuracy of a biosensor can be improved by replacing oxygen with synthetic electron acceptors, able to shuttle electrons from the redox active centre of the enzyme to the electrode surface. Even if this is a difficult achievement, due to the protein shell surrounding the FAD redox centre, we have noted that PNR films can communicate with FAD when the GOx is immobilised by cross linking with glutaraldehyde. In previous work [22], we observed that NR polymerises better on CA-graphite composite electrodes, than on carbon film electrodes, that have commonly been used by us as substrates in biosensor construction. For these two reasons, PNR in the form of a film was also chosen to be a redox mediator in this biosensor assembly.

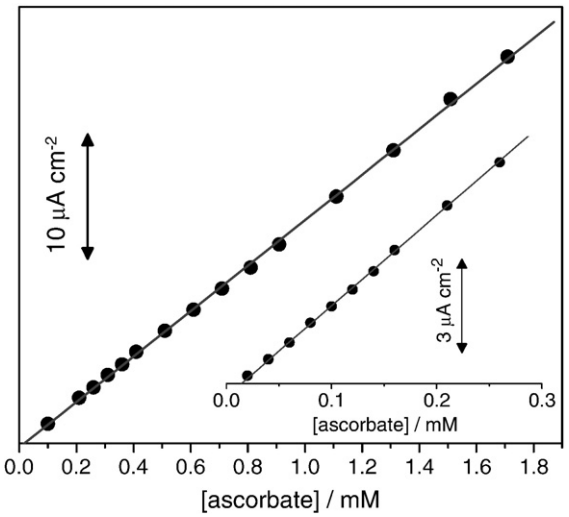

Fig. 3. Calibration curves for ascorbate at CA-graphite composite electrodes in 0.1 M NaPBS pH 7.0 at 0.0 mV vs. SCE operating potential; in the inset calibration curve in the small concentration range.

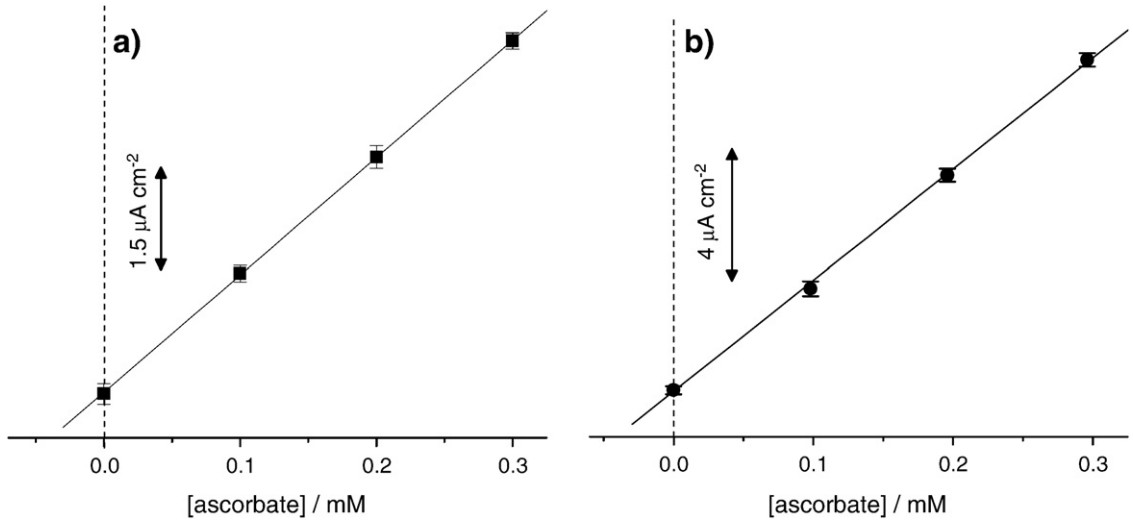

Fig. 4. Standard addition method used for the determination of ascorbate in a) orange juice and b) passion fruit juice at CA-graphite electrode in 0.1 M NaPBS pH 7.0; applied potential 0.0 V vs. SCE.

3.2.1. Effect of applied potential on the amperometric response

The effect of applied potential on the amperometric response of the PNR/GOx biosensor using composite 1 as substrate electrode as a function of glucose concentration was studied. Amperometric measurements were performed in stirred 0.1 M NaPBS pH 7.0 solution by injecting 40 µl of 0.1 M glucose solution after baseline stabilisation at each applied potential. The results obtained are presented in Fig. 5, Δi corresponding to an anodic change in current over the whole range of applied potentials tested, from −0.45 to −0.10 V vs SCE, due to oxidation processes which occur at the electrode surface following glucose injection. The same dependence of the amperometric biosensor response with applied potential was observed for other monoenzymatic biosensor assemblies already studied in our group, which use the same PNR mediator [30,33,34]. We discussed the possibility of a direct electronic communication between PNR and the enzyme cofactor, flavine adenine dinucleotide (FAD) [31] and experiments, not shown here, demonstrated that the GOx biosensors which use PNR as mediator exhibit almost the same sensitivity in the absence of O_2 or in the presence of catalase in the enzymatic layer. So the mechanism involves the reoxidation by the PNR of $FADH_2$, formed during the enzymatic oxidation, the oxidised form of which is regenerated at the electrode itself, in this way giving an anodic change in current with substrate injection. As observed in Fig. 5, going to more negative potentials, closer to the formal potential of the FAD/$FADH_2$ couple ($E^{0'}_{FAD/FADH2} \cong -0.45$ V vs. SCE), the biosensor response is higher when compared with that recorded at less negative applied potentials. With the aim of minimising possible interferences a potential of −0.35 V vs. SCE was chosen for further amperometric experiments.

3.2.2. Analytical properties of the PNR/GOx biosensor

3.2.2.1. Reproducibility and analytical parameters. The reproducibility and analytical properties of the new PNR/GOx composite biosensor assembly was determined by the response of three different biosensors to successive injections of glucose at 0.35 V vs. SCE. The calibration curves recorded at both types of PNR/GOx assembly using composite 1 and 2 as electrode substrates are shown in Fig. 6 and the respective analytical parameters are given in Table 2. The first biosensor assembly exhibits a higher sensitivity of 31.5 ± 1.7 µA cm^{-2} mM^{-1} (RSD = 5.4%) and a slightly higher detection limit (signal-to-noise-ratio = 3) of 22.7 µM compared with the second type with a corresponding sensitivity of 20.5 ± 0.9 (RSD = 4.6) and a detection limit of 20.3 µM. For both biosensor

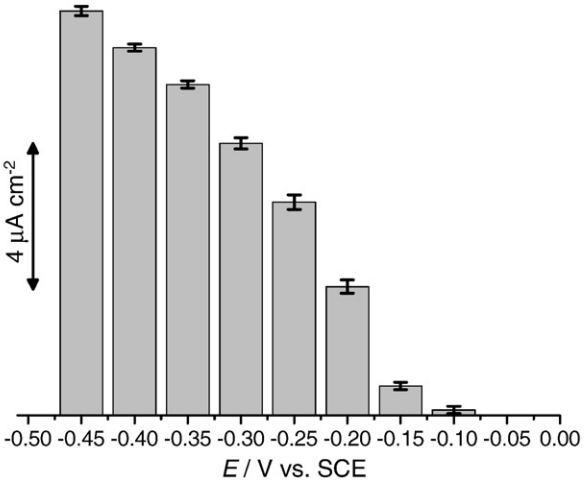

Fig. 5. Dependence of PNR/GOx biosensor current response on applied potential, Δj, following injection of glucose, final concentration 0.4 mM, in 0.1 M NaPBS, pH 7.0.

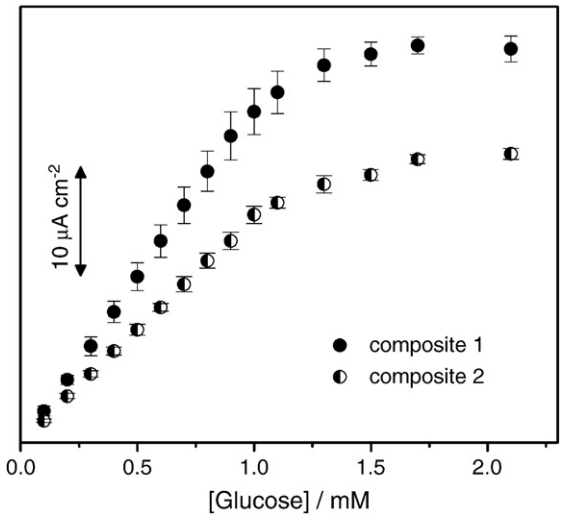

Fig. 6. Calibration curves at PNR/GOx biosensors using as substrate electrode both type of composite, recorded in 0.1 M NaPBS pH 7.0 at −0.35 V vs. SCE.

Table 2
Analytical parameters from calibration curves recorded at GOx/PNR composite biosensors for both type of composites.

GOx biosensor	Sensitivity/μA cm^{-2} mM^{-1}	Intercept/μA	LOD/μM	Correlation coefficient (R^2)
Composite 1	31.5 ± 1.7	-0.39 ± 0.19	22.7 ± 2.5	0.9997
Composite 2	20.3 ± 0.9	-0.11 ± 0.08	20.3 ± 3.5	0.9998

assemblies the linear range was up to 1.0 mM with a linear regression coefficient (R^2) of 0.9998 ± 0.0001.

These results are very promising, since the sensitivity of the sensor made with both types of graphite powder is much higher than other biosensors for glucose based on composite materials. For example, when GOx was immobilised together with carbon nanotubes (MWCNT) in a sol–gel matrix, the biosensor obtained exhibited a sensitivity of only 0.98 μA cm^{-2} mM^{-1}, and a much higher detection limit, of 50 μM [35]. S. Alegret et al. [10] also developed biosensors for glucose, by incorporating the enzyme in the composite, and depending on the polymer used for the non-conductive matrix preparation, the sensitivity of the sensor was between 0.22 and 0.27 μA cm^{-2} mM^{-1} in the case of epoxy resin, silicone and polyester, and 1.1 μA mM^{-1} cm^{-2} for methacrylate, and they observed much lower values of sensitivity. Finally, when GOx and CNT were employed for the construction of carbon paste electrodes (CNTPE), the sensitivity was only 0.03 μA cm^{-2} mM^{-1} with a very high detection limit of 0.13 mM, the properties improved slightly with the use of ordered mesoporous carbon (OMCPE), the sensitivity achieved being 0.15 μA cm^{-2} mM^{-1} with a detection limit of 72 μM [9]. So, as can be seen, the analytical properties of this newly developed PNR/GOx-CA-graphite composite biosensor are significantly better than those of other previously-reported composite-based electrochemical biosensors.

The response dynamics was calculated by measuring the time taken by the transducer to reach 95% of the final chronoamperometric response when the glucose concentration in solution was increased by 0.1 mM. The recorded times were between 3–4 s, very similar for all tested biosensors. The low values suggest the absence of enzyme layer diffusion barriers and little influence on enzyme kinetics.

3.2.2.2. Stability of the sensor. Long term stability is one of the most important features required for the satisfactory application of a biosensor. In order to evaluate the storage stability, the sensor was tested after 6 weeks of storage in 0.1 M NaPBS buffer pH 7.0, at 4 °C. There is a slight decrease in sensitivity of the sensor of about 6% from the initial value, revealing a very good preservation of the bioactivity

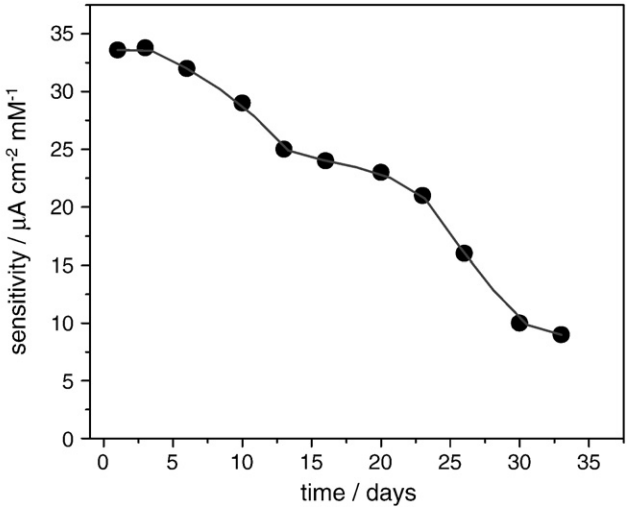

Fig. 7. Operational stability of PNR/GOx biosensors using composite 1 as electrode substrate.

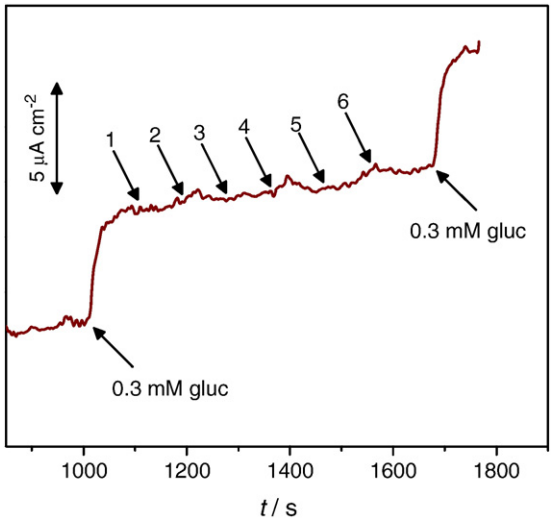

Fig. 8. Influence of electroactive interfering compounds at the applied potential −0.35 V vs. SCE at PNR/GOx composite biosensor; arrows indicate the addition in 0.1 M NaPBS pH 7.0 of: 1 – catechol, 2 – tartaric acid, 3 – citric acid, 4 – fructose, 5 – ascorbic acid and 6 – oxalic acid; concentration ratio of interfering compound to glucose is 2:1.

of the enzyme as well as a good adherence of the enzyme layer to the PNR-modified composite electrode.

The long-term operational stability of the enzyme electrode was also investigated by recording the biosensor response to successive additions of glucose three times per week. The PNR/GOx biosensor was stored in 0.1 M NaPBS buffer pH 7.0, at 4 °C when not in use. Results are shown in Fig. 7. As noted, the sensitivity of the sensor slightly increased after 3 days of storage in buffer solution, probably due to conformational changes of the immobilised enzyme which may lead to a better accessibility of the substrate to the catalytic site of the enzyme. After 1 week of use, biosensor sensitivity decreases by only 4.8%. During a period of 7 weeks of use, recording a 12-point calibration curve every 3 days, the final sensitivity is ~27% of the initial sensitivity. The sensitivity, making three measurements each time is ~70% after this period, which compares well with other biosensor assemblies of this kind under the same conditions [7,36,37].

3.2.2.3. Interferences. Compounds usually present in matrices where glucose is determined such as ascorbic, acetic, citric, oxalic and tartaric acids, catechol and fructose were tested for their ability to be potential interferents, either directly electrochemically active at the potentials used or indirectly as matrix components. Fig. 8 shows chronoamperograms recorded at a PNR/GOx composite biosensor. The response to the interfering compounds was tested in the presence of 0.3 mM glucose, which was previously added to the buffer solution. As observed, under the experimental conditions employed for glucose detection, the newly developed biosensor does not lead to any interference for the tested analytes, not even for ascorbate, which usually interferes significantly, showing in this way a very high selectivity towards glucose. Since the response to glucose in the presence of the interfering compounds remains the same, this biosensor assembly is very useful for glucose detection in complex matrices such as beverages or food samples.

4. Conclusions

The studies undertaken demonstrate that the new CA-graphite composite can be used for the fabrication of electrochemical sensors of any desired shape with attractive performance. CA-graphite composite sensors exhibit very high sensitivity and low detection limits even at an applied potential of 0.0 V vs. SCE, The performance in ascorbate determination is better than that of much more complex

sensor assemblies containing mediators or even enzymes. The GOx-modified PNR composite exhibited excellent sensitivity with a low detection limit and no interferences for the determination of glucose. Compared with other types of biosensor based on composite electrodes, the preparation of this new type of biosensor assembly is simple, cheap, fast and reproducible, being promising for the design of a wide range of new biosensors.

Acknowledgements

Financial support from Fundação para a Ciência e Tecnologia (FCT), SFRH/BD/27864/2006 (M.M. Barsan), projects PTDC/QUI/65255/2006 and PTDC/QUI/65732/2006, POCI (co-financed by the European Community Fund FEDER), and CEMUC® (Research Unit 285), is gratefully acknowledged.

References

[1] F. Céspedes, E. Martínes-Fábregas, S. Alegret, New materials for electrochemical sensing. 1. Rigid conducting composites, Trends Anal. Chem. 15 (1996) 296–304.
[2] S. Ramirez-Garcia, S. Alegret, F. Cespedes, R.J. Forster, Carbon composite electrodes: surface and electrochemical properties, Analyst 127 (2002) 1512–1519.
[3] K. Kalcher, J.M. Kauffmann, J. Wang, I. Svankara, K. Vytras, C. Neuhold, Z. Yang, Sensors based on carbon-paste in electrochemical analysis – a review with particular emphasis on the period 1990–1993, Electroanalysis 7 (1995) 5–22.
[4] F. Albertús, A. Llerena, J. Alpízar, V. Cerdá, M. Luque, A. Ríos, M. Valcárcel, A PVC-graphite composite electrode for electroanalytical use. Preparation and some applications, Anal. Chim. Acta 355 (1997) 23–32.
[5] M. Luque, A. Ríos, M. Valcárcel, A poly(vinyl chloride) graphite composite electrode for flow-injection amperometric determination of antioxidants, Anal. Chim. Acta 395 (1999) 217–223.
[6] R. Aguilar, M.M. Dávila, M.P. Elizalde, M. Mattusch, R. Wennrich, Capability of a carbon-polyvinylchloride composite electrode for the detection of dopamine, ascorbic acid and uric acid, Electrochim. Acta 49 (2004) 851–859.
[7] M. Cano, J.L. Ávila, M. Mayén, M.L. Mena, J. Pingarrón, R. Rodrígez-Amaro, A new, third generation, PVC/TTF-TCNQ composite amperometric biosensor for glucose determination, J. Electroanal. Chem. 615 (2008) 69–74.
[8] A. Guzman-Vázquez de Prada, N. Peña, C. Parrado, A.J. Reviejo, J.M. Pingarrón, Amperometric multidetection with composite enzyme electrodes, Talanta 62 (2006) 896–903.
[9] L. Zhu, C. Tian, D. Zhu, R. Yang, Ordered mesoporous carbon paste electrodes for electrochemical sensing and biosensing, Electroanalysis 20 (2008) 1128–1134.
[10] S. Alegret, F. Céspedes, E. Martínez-Fàbregas, D. Martorell, A. Morales, Carbon-polymer biocomposites for amperometric sensing, Biosens. Bioelectron. 11 (1996) 35–44.
[11] A. Morales, F. Céspedes, S. Alegret, Graphite-methacrylate biocomposite material with renewable sensing surface for reagentless amperometric biosensors based on glucose dehydrogenase, Mater. Sci. Eng. C7 (2000) 99–104.
[12] Minakshi, C.S. Pundir, Construction of an amperometric sensor for triglyceride determination, Sens. Actuators, B, Chem. 133 (2008) 251–255.
[13] J.P. Hart, S.A. Wring, Recent developments in the design and application of screen-printed electrochemical sensors for biomedical, environmental and industrial analysis, Trends Anal. Chem. 16 (1997) 89–103.
[14] M.I. Prodromidis, M.I. Karayannis, Enzyme based amperometric biosensors for food analysis, Electroanalysis 14 (2002) 241–261.
[15] J. Tkáč, I. Voštiar, E. Šturdík, P. Gemeiner, V. Mastihuba, J. Annus, Fructose biosensor based on d-fructose dehydrogenase immobilised on a ferrocene-embedded cellulose acetate membrane, Anal. Chim. Acta 439 (2001) 39–46.
[16] A.A. Hoffman, S.L.P. Dias, J.R. Rodrigues, Methylene blue immobilized on cellulose acetate with titanium dioxide, an application as sensor for ascorbic acid, J. Braz. Chem. Soc. 19 (2008) 943–949.
[17] A.B. Florou, M.I. Prodromidis, M.I. Karayannis, S.M. Tzouwara-Karayanni, Flow electrochemical determination of ascorbic acid in real samples using a glassy carbon electrode modified with a cellulose acetate film bearing 2,6-dichlorophenolindophenol, Anal. Chim. Acta 409 (2000) 113–121.
[18] A.B. Florou, M.I. Prodromidis, M.I. Karayannis, S.M. Tzouwara-Karayanni, Fabrication and voltammetric study of lanthanum 2,6-dichlorophenolindophenol chemically modified screen printed electrodes. Application for the determination of ascorbic acid, Anal. Chim. Acta 423 (2000) 107–114.
[19] S. Miserere, S. Ledru, N. Ruillé, S. Griveau, M. Boujtita, F. Bedioui, Biocompatible carbon-based screen-printed electrodes for the electrochemical detection of nitric oxide, Electrochem. Commun. 8 (2006) 238–244.
[20] S. Ledru, N. Rouillé, M. Boujtita, One step screen-printed electrode modified in its bulk with HRP based on direct electron transfer for hydrogen peroxide detection in flow injection mode, Biosens. Bioelectron. 21 (2006) 1591–1598.
[21] F. Ghamouss, S. Ledru, N. Rouillé, F. Lantier, M. Boujtita, Bulk modified screen-printing carbon electrodes with both lactate oxidase (LOD) and horseradish peroxidase (HRP) for the determination of L-lactate in flow injection analysis mode, Anal. Chim. Acta 570 (2006) 158–164.
[22] M.M. Barsan, E.M. Pinto, M. Florescu, C.M.A. Brett, Development and characterization of a new conducting carbon composite electrode, Anal. Chim. Acta 635 (2009) 71–78.
[23] S.A. Wring, J.P. Hart, Voltammetric behaviour of ascorbic-acid at a graphite epoxy composite electrode chemically modified with cobalt phthalocyanine and its amperometric determination in multivitamin preparation, Anal. Chim. Acta 229 (1990) 63–70.
[24] M.F.S. Teixeira, L.A. Ramos, O. Fatibello-Filho, E.T.G. Cavalheiro, Carbon paste electrode modified with copper(II) phosphate immobilized in a polyester resin for voltammetric determination of L-ascorbic acid in pharmaceutical formulations, Anal. Bioanal. Chem. 376 (2003) 214–219.
[25] R. Pauliukaite, M.E. Ghica, C.M.A. Brett, A new, improved sensor for ascorbate determination at copper hexacyanoferrate modified carbon film electrodes, Anal. Bioanal. Chem. 381 (2005) 972–978.
[26] I.L. de Mattos, L. Gorton, Metal-hexacyanoferrate films: a tool in analytical chemistry, Quim. Nova 24 (2001) 200–205.
[27] I. Jurevičiūtė, K. Brazdžiuvienė, L. Bernotaitė, B. Šalkus, Polyaniline-modified electrode as an amperometric ascorbate sensor, Sens. Actuators, B 107 (2005) 716–721.
[28] K. Brazdžiuvienė, I. Jurevičiūtė, A. Malinauskas, Amperometric ascorbate sensor based on conducting polymer: poly(N-methylaniline) versus polyaniline, Electrochim. Acta 53 (2007) 785–791.
[29] X. Wang, H. Watanabe, S. Uchiyama, Amperometric L-ascorbic acid biosensors equipped with enzyme micelle membrane, Talanta 74 (2008) 1681–1685.
[30] M.E. Ghica, C.M.A. Brett, Development of novel glucose and pyruvate biosensors at poly(neutral red) modified carbon film electrodes. Application to natural samples, Electroanalysis 18 (2006) 748–756.
[31] R. Pauliukaite, M.E. Ghica, M. Barsan, Characterisation of poly(neutral red) modified carbon film electrodes; application as a redox mediator for biosensors, J. Solid State Electrochem. 11 (2007) 899–908.
[32] J.Y. Heras, A.F.F. Giacobone, F. Battaglini, Ascorbate amperometric determination using conducting copolymers from aniline and N-(3-propane sulfonic acid) aniline, Talanta 71 (2007) 1684–1689.
[33] M.E. Ghica, C.M.A. Brett, Development and applications of a bienzymatic amperometric glycerol biosensor based on a poly(neutral red) modified carbon film electrode, Anal. Lett. 39 (2006) 1527–1542.
[34] M.M. Barsan, C.M.A. Brett, An alcohol oxidase biosensor using PNR redox mediator at carbon film electrodes, Talanta 74 (2008) 1505–1510.
[35] A. Salimi, R.G. Compton, R. Hallaj, Glucose biosensor prepared by glucose oxidase encapsulated sol–gel and carbon-nanotube-modified basal plane pyrolytic graphite electrode, Anal. Biochem. 333 (2004) 49–56.
[36] H.N. Choi, J.H. Han, J.A. Park, J.M. Lee, W.Y. Lee, Amperometric glucose biosensor based on glucose oxidase encapsulated in carbon nanotube-titania-nafion composite film on platinized glassy carbon electrode, Electroanalysis 19 (2007) 1757–1763.
[37] J. Park, H.K. Kim, Y. Son, Glucose biosensor constructed from capped conducting microtubules of PEDOT, Sens. Actuators, B 133 (2008) 244–250.