New Robust Redox and Conducting Polymer Modified Electrodes for Ascorbate Sensing and Glucose Biosensing

Somayeh Kakhki,[a, b] *Madalina M. Barsan*,[a] *Esmaeil Shams*,[b] *Christopher M. A. Brett**[a]

[a] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004–535 Coimbra, Portugal
tel: +351-239854470; fax: +351-239827703
[b] Chemistry Department, University of Isfahan, 81746-73441 Isfahan, Iran
*e-mail: brett@ci.uc.pt

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Abstract
A novel modified electrode with a conducting film containing poly(3,4-ethylenedioxythiophene) (PEDOT) plus poly(methylene blue) (PMB) on a glassy carbon electrode (GCE) (PEDOT/PMB/GCE) has been developed, and application illustrated as electrochemical sensor for ascorbate and biosensor for glucose. Electrocatalytic oxidation at 0.0 V vs. SCE was successfully used for the determination of ascorbate in real samples. Glucose biosensors containing glucose oxidase (GOx) immobilized on PMB/PEDOT electrodes exhibit enhanced sensitivity relative to PEDOT ones. The new robust biosensor architecture shows a far superior operational and storage stability relative to PMB alone, enabling excellent preservation of enzyme activity during more than one month.

Keywords: Electrochemical sensors, Poly(3,4-ethylenedioxythiophene), Poly(methylene blue), Ascorbic acid, Glucose

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1 Introduction

The development of novel sensors and biosensors which enable a fast, sensitive and selective determination of important analytes has been receiving considerable attention [1]. Ascorbic acid (AA), or vitamin C, is an important antioxidant involved in the prevention of cellular damage, which is the common pathway for cancer, aging, and a variety of diseases [2], and, consequently, AA is extensively used as an antioxidant in food, animal feed, beverages, pharmaceutical formulations and cosmetics [3]. Ascorbic acid is one of the electroactive analytes which can be detected easily by using electrochemical sensors, with high sensitivity and simplicity. In order to reduce the high overpotential of AA oxidation, which leads to interferences in real sample analysis using electrochemical sensors, a number of modified electrodes have been developed using appropriate redox mediators, e.g. copper hexacyanoferrate [4], dodecylbenzene sulfonic acid doped with polyaniline nanoparticles [5] or copolymers of aniline with *N*-(3-propane sulfonic acid) aniline [6] or tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCNQ) organic salt [7]. Recently, an unmodified conducting carbon composite electrode has been reported, which is able to detect ascorbate with high sensitivity at 0.0 V vs. SCE [8]. Attempts are still being made to improve the robustness of the sensor, its long term stability and decrease the detection limit.

Conducting polymers are suitable materials to be employed as electrocatalysts in sensors and biosensors [9], among them poly(3,4-ethylenedioxythiophene) (PEDOT) having some advantages, such as high chemical stability in aqueous solutions, biocompatibility with biological media and low redox potential [10,11], so that it has been proposed as an alternative to traditional polymers in sensor and biosensor construction [12–14]. PEDOT has been used in various electrode architectures for ascorbate detection, e.g. PEDOT-modified gold, platinum and glassy carbon electrodes [15–17], PEDOT/nickel hexacyanoferrate hybrid films [18] or in films containing MWCNTs together with PEDOT [19]. Other PEDOT-based sensors were developed for the detection of NADH [20], H_2O_2 [21] and cysteine [22]. Only a few applications in the biosensor area have been reported, in which PEDOT serves to construct hollow microtubes loaded afterwards with glucose oxidase [23], to load Pd nanoparticles on top of which GOx was immobilized [24], to entrap polyethyleneglycol modified GOx [25] or to be used in a biosensor architecture together with Prussian Blue and MWCNTs [26].

This paper reports the use of a newly developed PEDOT/poly(methylene blue) (PMB) modified electrode [27] as ascorbate sensor and for a glucose oxidase-based biosensor. PMB was used due to its very good performance in mediating electron transfer between the electrode substrate and the enzyme catalytic centre, as demonstrated in [28–34]. Although the stability of PMB-

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modified glassy carbon electrodes is poor due to the high solubility of PMB films, it can be improved by protecting the film with a hydrophobic PEDOT electropolymerised film [27]. Glucose oxidase has been extensively used as a model enzyme due to its good solubility in aqueous media, high stability and specificity to glucose and most importantly due to its low unit cost [35]. Therefore, GOx was chosen in this work to evaluate the performance of the PEDOT/PMB/GCE modified electrode as substrate for the construction of oxidase-based biosensors. Four cross-linking agents were tested for the immobilization of the enzyme, namely glutaraldehyde (GA), glyoxal (GO), epichlorohydrin (ECH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-*N*-hydroxysuccinimide (EDC-NHS), in order to choose the more appropriate one for the construction of biosensors using the newly-developed PEDOT/PMB/GCE modified electrodes.

2 Experimental

2.1 Reagents

All chemicals were analytical reagent grade and used as received. The monomers 2,3-dihydrothieno[3,4-b]-1,4-dioxin (EDOT) and methylene blue (MB), together with ascorbic acid, bovine serum albumin (BSA), fructose, α-D(+)-glucose, glutaraldehyde (GA) 25% (v/v solution), glyoxal (GO) 40% (v/v solution), epichlorohydrin (ECH) 99% (v/v solution), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), monobasic sodium phosphate monohydrate, sodium poly(styrene sulfonate) (NaPSS), dopamine hydrochloride and uric acid were from Sigma-Aldrich (Steinheim, Germany). Glucose oxidase (GOx, EC 1.1.3.4, from *Aspergillus niger*, 24 units/mg), oxalic acid dihydrate and *N*-hydroxysuccinimide (NHS) were obtained from Fluka (Buchs, Switzerland). Di-sodium hydrogen phosphate 2-hydrate and sodium chloride were from Riedel-de-Haën (Seelze, Germany), potassium chloride, from Panreac (Barcelona, Spain), tartaric acid from PAHI (Lisbon, Portugal) and sodium tetraborate, sodium sulphate and citric acid monohydrate was from Merck (Darmstadt, Germany).

The solutions used for the polymerisation of methylene blue (MB) and EDOT were the same as reported in [27,36], namely 1 mM MB dissolved in 0.025 M $Na_2B_4O_7$ + 0.1 M Na_2SO_4 pH 9.2 and 0.01 M EDOT in 0.1 M NaPSS, respectively.

For the amperometric detection of ascorbate and glucose, the supporting electrolyte was 0.1 M sodium phosphate buffer saline (NaPBS) pH 7.0, prepared by mixing sodium di-hydrogenphosphate and di-sodium hydrogenphosphate with 0.05 M sodium chloride.

A stock solution of 0.1 M glucose was prepared in 0.1 M NaPBS pH 7.0 at least one day before use, to permit equilibration of α and β anomers of D-glucose; when not in use, the solution 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) and commercially available natural orange juice and mixture of orange with passion fruit juice.

Millipore Milli-Q nanopure water (resistivity $\geq$ 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

Electrochemical experiments were performed with a computer-controlled μ-Autolab type I potentiostat-galvanostat with GPES software (Metrohm-Autolab, Utrecht, Netherlands). A conventional three electrode cell was used, containing a modified glassy carbon electrode as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as reference electrode.

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

2.3 Sensor and Biosensor Preparation

Before use, the GC electrode was polished to a mirror finish using 6 then 3 micron diamond spray (Kemet, UK) followed by a thorough rinse with Millipore Milli-Q nanopure water. The electrode was pretreated by cycling the potential between −1.0 to +1.0 V vs. SCE at 100 mV s^{-1}, until a stable voltammogram was recorded. The preparation of PEDOT/PMB/GCE was done by following the procedure described in [27]. The ascorbate sensor consisted in the PEDOT/PMB/GCE, without any further modification.

The biosensor was prepared by immobilizing GOx on top of the PEDOT/PMB/GCE electrode, by cross-linking with one of four different cross-linking agents: glutaraldehyde (GA), glyoxal (GO), epichlorohydrin (ECH) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide together with *N*-hydroxysuccinimide (EDC-NHS). 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 cross-linking agent. Of this mixture, 10 μL was dropped on to the electrode surface and left to dry at room temperature during at least 4 h.

3 Results and Discussion

3.1 Electrode Modification by Electropolymerisation of MB and EDOT

The modification of PMB-modified electrodes with the inert, hydrophobic polymer PEDOT, immobilized on top of PMB in order to decrease its solubility in aqueous media, was the aim of previous work [27]. As observed in Figure 1a, the polymerisation of MB occurs with a de-

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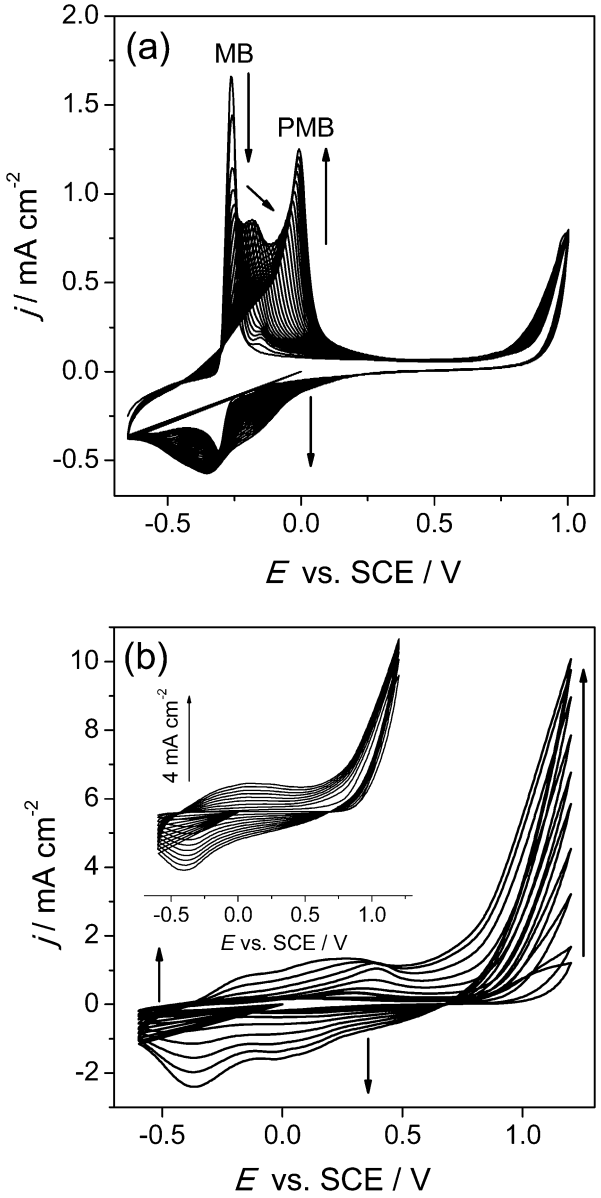

Fig. 1. CVs recorded during the electropolymerisation of a) MB on GCE and b) EDOT on PMB/GCE (inset shows EDOT on GCE) from the corresponding polymerisation solutions.

crease of the monomer oxidation current, which then shifts toward more positive potentials, where polymer oxidation occurs. During the last 10 cycles, the polymer oxidation current increases, indicating the deposition of PMB. Finally, EDOT was polymerised on top of PMB modified electrodes, and as observed by comparing Figure 1b with its inset, EDOT polymerises better on PMB/GCE than on GCE, more radical cations being formed at 1.2 V vs. SCE on the increased surface area. Nevertheless, the very similar voltammetric profile suggests that there is no chemical interaction between the two polymers. In fact, PEDOT/PMB/GCE exhibits a far superior stability compared with PMB/GCE, and so PEDOT/PMB/GCE modified electrodes were employed here as sensors for ascorbate (Section 3.2) and for glucose biosensor construction (Section 3.3).

3.2 Ascorbate Detection

3.2.1 Electrooxidation of Ascorbate at PEDOT/PMB/GCE

The determination of ascorbate at PEDOT/PMB/GCE was performed using fixed potential amperometry in neutral solution (0.1 M NaPBS pH 7.0). The effect of applied potential on the amperometric response of the PEDOT/PMB/GCE modified electrode was evaluated by comparing the change in current following the addition by injection of 0.4 mM ascorbate, at potentials from 0.0 to 0.5 V vs. SCE. The results are presented in Figure 2, and, as observed, the change in current on injection of ascorbate becomes larger at more positive potentials, closer to the oxidation potential of ascorbate (0.240 V vs. SCE at pH 7.0). Since the sensor response was already sufficiently sensitive at 0.0 V vs. SCE, this was chosen in further experiments, in order to minimize interferences from other electroactive compounds.

Figure 3a shows a typical chronoamperogram with the corresponding calibration curve presented in Figure 3b, recorded at PEDOT/PMB/GCE electrode. The newly developed sensor has a sensitivity of 45.5 ± 2.9 μA cm^{-2} mM^{-1} ($RSD = 6.4\%$, $n = 5$) with a detection limit of 12.5 ± 1.3 μM ($RSD = 10.4\%$, $n = 5$), the very high reproducibility evidencing the consistency in fabrication of the newly-developed sensors. The linear response of the sensor is maintained up to at least 7 mM, this being the highest concentration which was tested. The sensitivity is higher than in previously-reported ascorbate sensors, operating at potentials close to or at 0.0 V, e.g. 11.6 [4], 10.75 [5], 27.6 [6] or 18.3 μA cm^{-2} mM^{-1} [8].

Increasing the potential does lead to higher sensitivities but increases the likelihood of interferences e.g. at 0.15

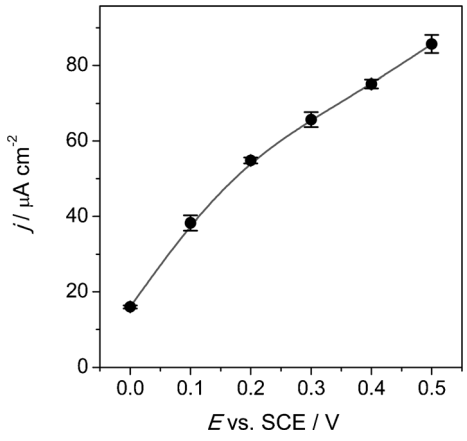

Fig. 2. Change in current recorded at PEDOT/PMB/GCE electrode, corresponding to the injection of 0.4 mM ascorbate in 0.1 M NaPBS pH 7.0, at different potentials from 0.0 to 0.5 V vs. SCE.

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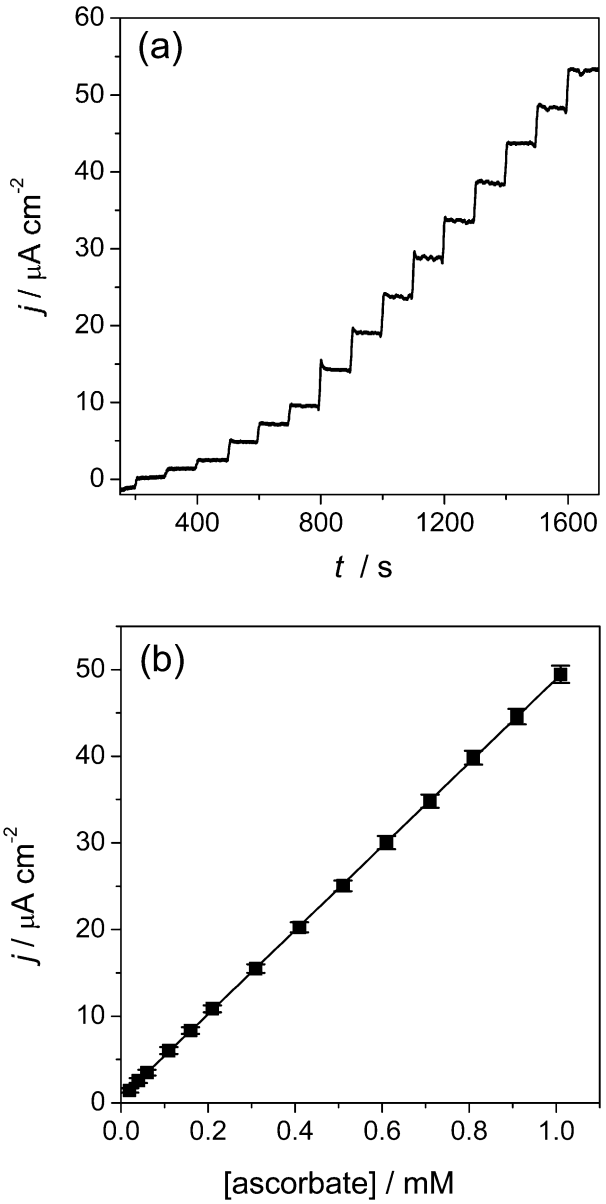

Fig. 3. a) Chronoamperogram for successive additions of ascorbate in 0.1 M NaPBS pH 7.0 recorded at PEDOT/PMB/GCE at 0.0 V vs. SCE and b) corresponding calibration curve.

and 0.4 V vs. SCE sensitivities of 65 and 448 μA cm^{-2} mM^{-1} are obtained, respectively [7,37], comparable with the ones achieved by PEDOT/PMB/GCE sensor at these potentials. Other PEDOT or MB based ascorbate sensors reported lower sensitivities of 28.5 μA cm^{-2} mM^{-1}, at a biosensor based on PEDOT, SWCNTs and ascorbate oxidase [38] and 36.2 μA cm^{-2} mM^{-1}, exhibited by a MB containing sensor [39], both operating at higher potentials of 0.4 V vs. SCE and 0.3 V vs. Ag/AgCl.

By using an unmediated PEDOT/GCE sensor, the sensitivity of only 14.8 μA cm^{-2} mM^{-1} was three times lower, underlining the advantages of using PMB films for AA detection at potentials close to 0.0 V, where the redox polymer is electroactive. PEDOT also plays a crucial role in the performance of the PEDOT/PMB/GCE sensor, improving the amperometric response of ascorbate, due to the electrostatic interaction between the ascorbate anions and the cationic-fixed sites of the polymer film [40,41], beside the fact that PEDOT films increase the stability of PEDOT/PMB/GCE, which was verified in previous work [27]. Bare GCE was unable to detect AA at 0.0 V applied potential.

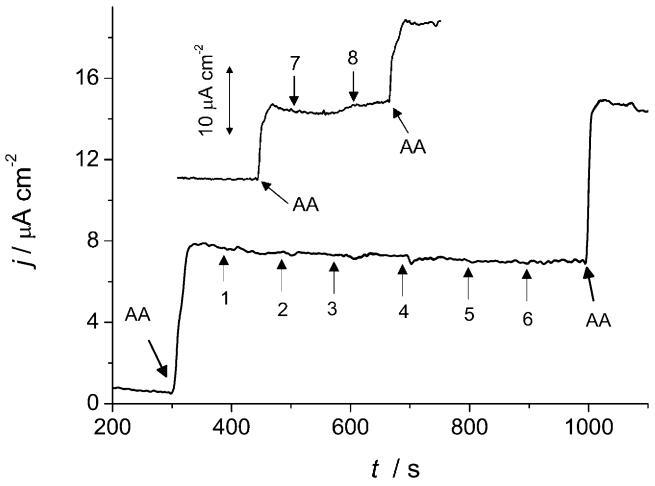

Fig. 4. Influence of electroactive interfering compounds on the response to ascorbate at PEDOT/PMB/GCE sensor; 1) glucose, 2) fructose, 3) tartaric acid, 4) citric acid, 5) acetic acid, 6) catechol, 7) dopamine and 8) uric acid; applied potential 0.0 V vs. SCE.

3.2.2 Interference Study

One of the most important problems to solve in the practical application of amperometric sensors is to minimize the effect of interfering species that may be present in real samples. Possible compounds that may interfere in the amperometric determination of ascorbate, such as uric, acetic, citric, oxalic and tartaric acids, dopamine, catechol and fructose were investigated. Ascorbate was injected before and after the injection of the above mentioned compounds, so that its final concentration (0.4 mM in solution) was a factor of two lower than that of the interferents. As observed in Figure 4, none of these compounds oxidises or reduces at 0.0 V, and the sensor recovery was 100 %.

3.2.3 Ascorbate Detection in Real Samples

To evaluate possible practical application of the PEDOT/PMB/GCE ascorbate sensors, they were employed to determine ascorbate in effervescent tablets of vitamin C and in fruit juices using the standard addition method, results being presented in Table 1.

A 0.10 M ascorbate solution was prepared by dissolving the vitamin C tablet in the right amount of water. In order to evaluate sensor accuracy, calibration curves were recorded with the addition of 10 μL of 0.1 M ascorbate standard solution or of 0.1 M vitamin C tablet solution.

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Table 1. Determination of ascorbate in vitamin C tablets and in fruit juice.

Sample	Labelled value (μM)	Detected value (μM)
Tablet of vitamin C	100.0	99.1 ± 0.6
Passion fruit juice	27.0	26.9 ± 0.5
Mix of orange and passion fruit juice	27.2	27.0 ± 0.4

Very close sensitivities were found, being less than 1% lower when vitamin C tablet solution was used for calibration. Furthermore, the standard addition method was used to determine vitamin C tablet ascorbate content, and the determined sensor accuracy was 99.1 ± 1.6%, n = 3).

Other samples tested were passion fruit juice and a mix of orange and passion fruit juice. The juices were injected without dilution. Measurements were repeated 3 times for each sample and the values obtained are very similar to those declared by the producers (see Table 1).

The high precision in determining AA in real samples with the newly-developed PEDOT/PMB/GCE electrode shows its promising applicability as a sensor in the food industry.

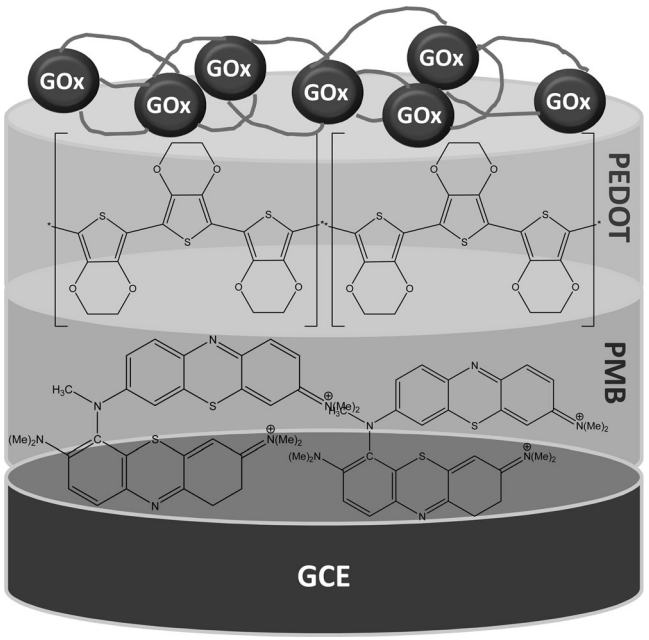
3.3 PMB/PEDOT/GOx Biosensor

3.3.1 Choice of Cross-Linker for Enzyme Immobilization

The model enzyme glucose oxidase was immobilized on the top of PEDOT/PMB/GCE electrodes using GA, GO, ECH or EDC-NHS crosslinkers, a representation of the constructed biosensors being presented in Scheme 1. Amperometric measurements were performed in 0.1 M NaPBS pH 7.0 solution by 6 successive addition of 0.1 mM glucose at −0.30 V vs. SCE. The sensitivities and detection limits exhibited by these biosensors are presented in Table 2, it being observed that the biosensor with the smallest sensitivity is the one containing EDC-NHS, the most sensitive one being that containing the GOx immobilized with GA. Thus, it can be concluded that the best cross-linking agent for GOx immobilization at PEDOT/PMB/GCE is GA and it was chosen for further biosensor construction.

3.3.2 Effect of Applied Potential on Biosensor Response

To optimize the biosensor, the effect of applied potential on the amperometric response was studied. The applied potential was changed from −0.50 to −0.10 V vs. SCE with −0.05 V increments, and the change in current corresponding to the injection of 0.4 mM glucose was measured (see Figure 5). The anodic changes in current recorded at all applied potentials suggest that at the GCE electrode surface, an oxidation process occurs. This can



Scheme 1. Schematic representation of the GOx/PEDOT/PMB/GCE biosensor.

Table 2. Analytical parameters obtained from the calibration curves recorded at GOx/PEDOT/PMB/GCE biosensors, containing the enzyme immobilized with different cross-linking agents.

Cross-linker	Sensitivity (μA cm^{-2} mM^{-1})	*LOD* (μM)
GA	31.0	7.2
GO	19.8	2.7
ECH	7.1	0.4
EDC-NHS	0.4	0.2

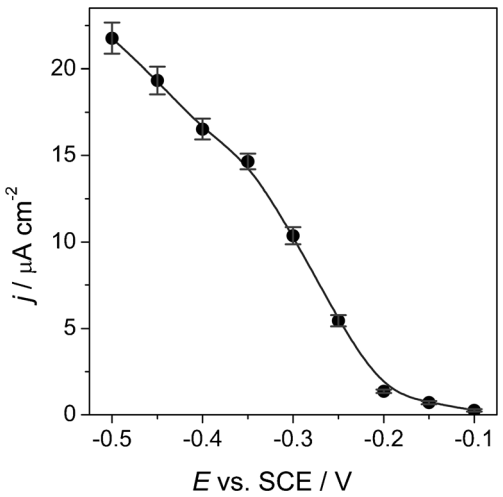


Fig. 5. Change in current recorded at GOx/PEDOT/PMB/GCE biosensor corresponding to the injection of 0.4 mM glucose in 0.1 M NaPBS pH 7.0, at potentials from −0.50 to −0.10 V vs. SCE.

be explained by direct electronic communication between PMB and the enzyme cofactor, so that PMB reduces while receiving electrons from GOx, subsequent to glu-

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cose oxidation, and then regenerates at the electrode surface, giving rise to oxidation currents. Other reported PEDOT containing biosensors are based on mechanisms involving the monitoring of the decrease in oxygen concentration at +0.7 V vs. Ag/AgCl [23] or detection of peroxide formed during the enzymatic reaction (when oxygen is the electron acceptor from $FADH_2$), either by its oxidation at +0.4 V and +0.3 vs. Ag/AgCl respectively [24,25] or reduction at −0.1 V vs. Ag/AgCl [26]. At applied potentials closer to the formal potential of FAD/$FADH_2$ cofactor ($E^{\circ\prime}_{FAD/FDH2} \approx -0.45$ V vs. SCE), the biosensor response is higher, also observed when PNR is employed as redox mediator [8]. A potential of −0.30 V was selected as working potential, being considered as the best to enable the sensitive detection of glucose whilst minimizing possible interferences.

3.3.3 Comparison of GCE, PEDOT/GCE and PEDOT/PMB/GCE GOx Biosensor Performance

The biosensors with GOx enzyme immobilized on GCE, PEDOT/GCE and PEDOT/PMB/GCE, were evaluated for glucose amperometric detection under the same experimental conditions: successive glucose injections in 0.1 M NaPBS pH 7.0, at −0.3 V vs. SCE. The amperometric responses and the corresponding calibration curves are displayed in Figure 6a and b respectively. PEDOT substantially increases the biosensor sensitivity from 1.10 ± 0.06 at GOx/GCE ($RSD = 5.5\,\%$, $n = 3$) to 20.08 ± 1.09 $\mu A\,cm^{-2}\,mM^{-1}$ ($RSD = 5.4\,\%$, $n = 3$). The best performance was achieved by the GOx/PEDOT/PMB/GCE biosensor, with a sensitivity of 31.4 ± 1.9 $\mu A\,cm^{-2}\,mM^{-1}$ ($RSD = 5.9\,\%$, $n = 3$). A PMB/GCE biosensor was also tested, and its sensitivity, 28.3 ± 1.9 $\mu A\,cm^{-2}\,mM^{-1}$ ($RSD = 6.7\,\%$, $n = 3$), is very similar to that of GOx/PEDOT/PMB/GCE. These two biosensors were subjected to a stability study, during one week, recording one amperogram per day. The results clearly indicate that PEDOT substantially improves biosensor stability, by impeding the PMB dissolution via passage through the enzyme layer. The PMB/GCE biosensor response was lost, while PEDOT/PMB/GCE maintained 95 % of its initial sensitivity after one week of use.

3.3.4 Analytical Parameters of the GOx/PEDOT/PMB/GCE Biosensor

In the current – time amperometric curve recorded at the chosen potential of −0.30 V vs. SCE, the change in current, following glucose injection, reaches 95 % of the maximum value within 4 s, which indicates a fast response. As can be seen in Figure 7, the biosensor displays a good linear range from 0.02 to 1.40 mM, with a sensitivity of 31.4 ± 1.9 $\mu A\,cm^{-2}\,mM^{-1}$ ($RSD = 5.9\,\%$, $n = 3$), significantly higher than those reported in literature for PEDOT containing biosensors, with sensitivities between 0.1 to 2.7 $\mu A\,cm^{-2}\,mM^{-1}$ [23–26], which also have more complex architectures. From the detection limit (LOD)

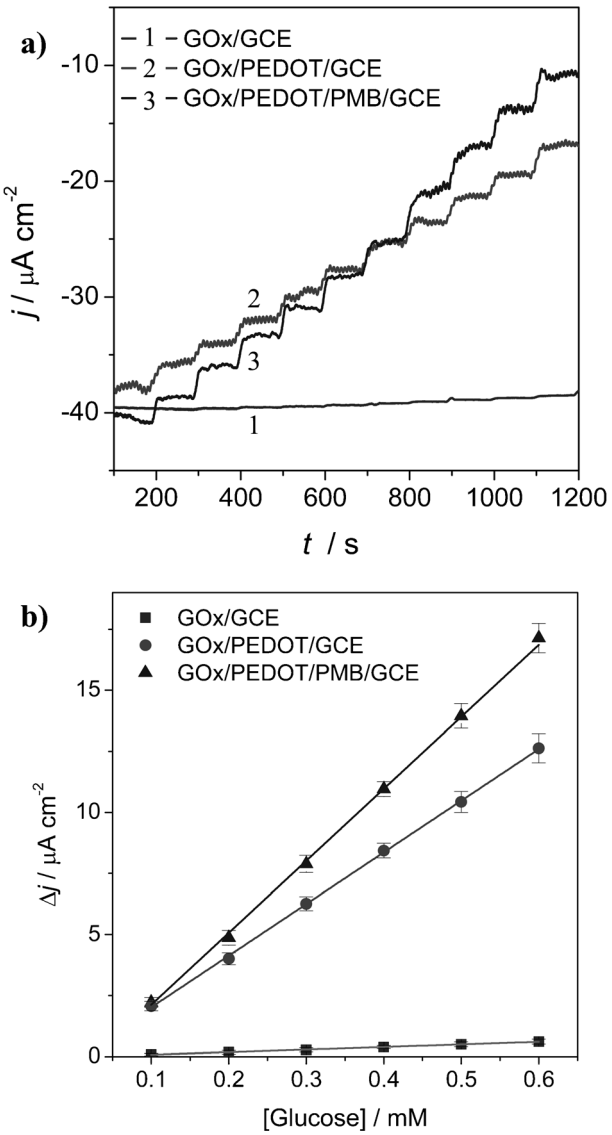

Fig. 6. a) Chronoamperograms recorded at GCE, PEDOT/GCE and PEDOT/PMB/GCE GOx biosensors in NaCl pH 7.0 at −0.3 V vs. SCE and b) corresponding calibration curves.

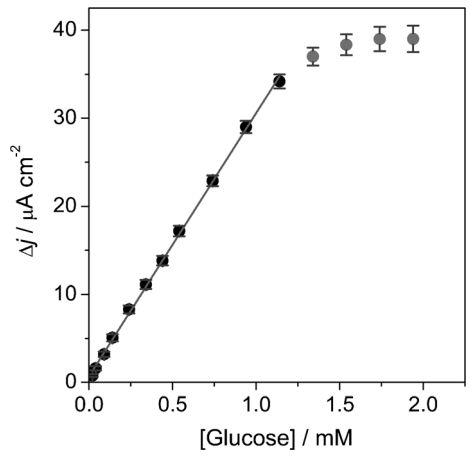

Fig. 7. Calibration curve recorded at GOx/PEDOT/PMB/GCE biosensor, for successive injections of glucose in 0.1 M NaPBS pH 7.0 at −0.30 V vs. SCE.

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point of view, the new biosensor is also far superior to other reported similar biosensors, having a *LOD* of 7.20 ± 0.35 μM ($RSD = 4.9\%$, $n = 3$), much lower than values in the literature which vary from 75 μM to 200 μM [23,24,26]. Only a few recently (2011–2012) published papers report higher sensitivities between 35.9 and 92.1 $\mu A\,cm^{-2}\,mM^{-1}$, with comparable or lower detection limits from 0.5 to10 μM [42–45]. These biosensors all have a more complex construction, containing Pt nanocubes [42], Pd nanoparticles [44], a poly(methyl-methacrylate)-BSA core shell nanoparticle on which GOx was adsorbed [43] and a bienzyme (HRP together with GOx) biosensor based on Au-Fe_2O_3@SiO_2 magnetic nanocomposite [45].

3.3.5 Operational and Storage Stability of Biosensor

Long term stability is a critical issue for practical application of the prepared glucose biosensors and was investigated by recording 10 point calibration curves at three biosensors, two times per week, during 1 month. The biosensors were kept in 0.1 M NaPBS buffer pH 7.0 at 4 °C when not in use. The variation of the biosensors sensitivity with time is shown in Figure 8a. As observed, the sensitivity increased slightly after the first use and preparation, which usually occurs with enzyme biosensors, due to conformational changes that better expose the active site of the enzyme in the enzymatic layer, when the biosensor is left in buffer solution after its first test. Afterwards, the sensitivity only decreases slightly, maintaining $89.4 \pm 3.7\%$ ($RSD = 4.1\%$, $n = 3$) of the initial value after 1 month, far superior to other PEDOT-based biosensors, which reported 19, 20 and 25% decrease of the initial sensitivities after shorter periods of use, of 7, 15 and 12 days respectively [23–25]. This clearly shows the robustness and good enzyme activity retention of the newly-developed biosensors.

Storage stability was also tested, the biosensors maintaining $96.4 \pm 1.8\%$ ($RSD = 1.9\%$, $n = 3$) of the initial sensitivity after 5 weeks of storage in 0.1 M NaPBS buffer pH 7.0 at 4 °C, also superior when compared to a similar biosensor architecture containing Prussian Blue together with PEDOT, which exhibited a 18% decrease in sensitivity after one month of storage [26].

3.3.6 Interferences

Possible interferences from electroactive compounds, such as ascorbic, uric, acetic, citric, oxalic and tartaric acids, dopamine, catechol and fructose, commonly found in samples where glucose is present, were tested and results are displayed in Figure 8b. No responses were observed after the additions of 0.8 mM interfering compound solutions to 0.1 M buffer solution pH 7.0, containing 0.4 mM glucose, and demonstrate high selectivity for glucose determination at the GOx/PEDOT/PMB/GCE biosensor. Moreover, the biosensor presented a recovery of 99.3%, calculated by measuring the response to 0.4 mM of glucose after the injection of the interfering compounds. All the above indicate that the biosensor is a good candidate for glucose detection in complex matrix samples.

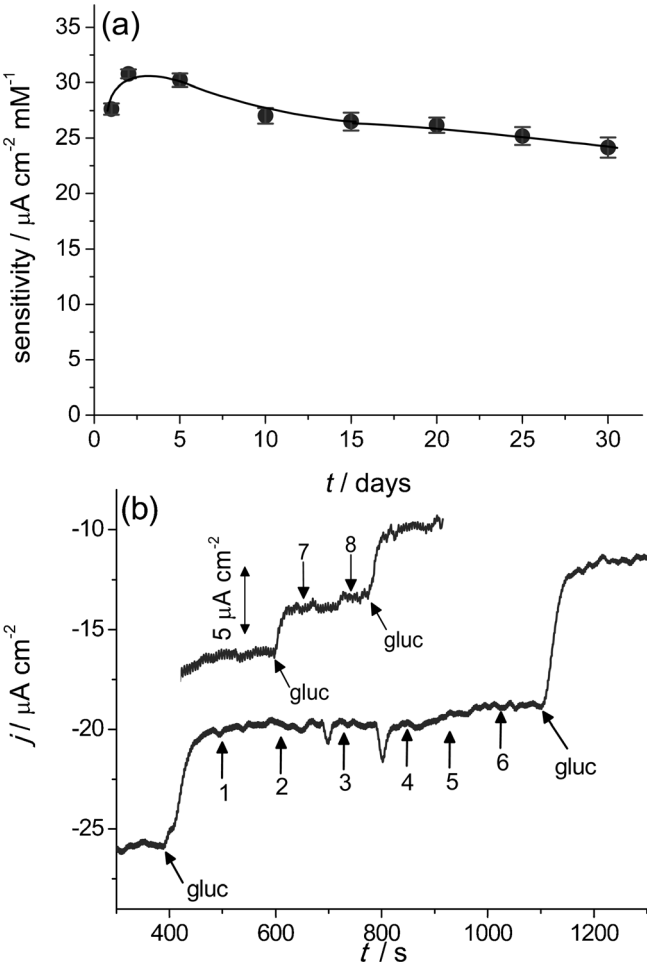

Fig. 8. (a) Operational stability and (b) influence of electroactive interfering compounds on the response to glucose of GOx/PEDOT/PMB/GCE biosensor; 1) ascorbic acid, 2) oxalic acid, 3) tartaric acid, 4) citric acid, 5) catechol, 6) fructose, 7) dopamine and 8) uric acid; applied potential −0.3 V vs. SCE.

4 Conclusions

The study carried out in the present paper indicates that PEDOT/PMB/GCE exhibits electrocatalytic activity towards ascorbic acid oxidation. Advantages in using both PMB and PEDOT are clearly shown, PMB substantially increasing the sensitivity of the sensor while positively charged PEDOT attracts ascorbate anions due to electrostatic interaction. Ascorbate is accurately measured in fruit juices and the recovery of the sensor is 100%. This, together with very good stability, makes this sensor very attractive for ascorbate detection in real samples. The PEDOT/PMB/GCE electrode was successfully employed for a glucose biosensor by adding a GOx enzyme layer,

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and among various cross linking agents, GA led to the construction of a biosensor with the best analytical characteristics. It was shown that both PEDOT and PMB increase biosensor sensitivity, PEDOT also ensuring physical stability. The new biosensor did not shown any interferences from other co-existing electroactive species and it is very robust, underlined by the good operational stability of the biosensor, the sensitivity of which decreased only with 10 % after a period of 1 month of use. These indicate the applicability of the PEDOT/PMB/GCE electrode in the development of new sensitive and very stable oxidase enzyme biosensor architectures.

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