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New Carbosilane Polymers with Interacting Ferrocenes as Support and Bioelectrocatalysts of Oxidases to Develop Versatile and Specific Amperometric Biodevices

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Abstract In this work, the bioelectrocatalytical properties and kinetic characteristics of new oxidase amperometric biosensors based on two different ferrocene functionalized carbosilane polymers, polydiallylmethylsilane (PDAMS) and polymethyldiundecenylsilane (PMDUS) are described. In the development of these biodevices, glucose oxidase has been used as example of oxidase enzyme, and two different immobilization procedures have been studied. The polymer-modified electrodes act as efficient transducers for glucose sensing in anodic and cathodic aerobic conditions and also in anodic anaerobic conditions, and this fact turns them into useful devices for a wide field of applications. PMDUS has shown to be the bioelectrocatalyst with best kinetic and analytical properties in aerobic media while PDAMS was better in anaerobic conditions. The best aerobic biosensor developed displayed a strictly linear range from 0 to 3.0 mM, a detection limit of 7.8 μM and a response time less than 2 s in an ascorbate interference free work potential interval. The apparent Michaelis–Menten constant was calculated to be 1.36 mM according to the Lineweaver–Burk equation.

Keywords Bioelectrocatalysis · Glucose oxidase · Biosensors · Carbosilane polymers · Interacting ferrocenes · Electron transfer

Introduction

Macromolecular systems containing multiple ferrocenyl units are currently being investigated by several groups because of their synthetic accessibility, the ideal redox properties of this metallocene and their numerous applications. Within this context, the development of dendrimers [1, 2] and polymers [3, 4] bearing pendent ferrocenyl units is a burgeoning field

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of study because they can be deposited onto several electrode surfaces with good mechanical stability. Moreover, highly branched structures together with a large area make them useful materials for enzyme immobilization and biosensors fabrication [5, 6]. We have long researched the chemistry of ferrocene-containing macromolecular structures [7–10] and have demonstrated that dendritic macromolecules containing a controlled number of redox-active organometallic units at the periphery of the structure are good catalytic and biocatalytic systems. We have reported the synthesis, redox behaviour and electrocatalytic properties of several dendritic building backbones functionalized with interacting ferrocenyl moieties [11–13]. These works show that the short distance between adjacent ferrocenyl moieties within the polymer film allows simultaneous or quasi-simultaneous charge transfers to the enzymatic redox sites, decreasing the high overpotentials required for the electrochemical oxidation of the enzymes. Consequently, the electrocatalytic activity of these organometallic compounds is favoured by the suitable distance between metallic ferrocene centres and the electronic communication. Specially, polymers containing electronically communicated ferrocenyl groups have shown to be efficient electrocatalysts for the electro-oxidation and electro-reduction of the hydrogen peroxide enzymatically generated and good direct bioelectrocatalysts of oxidase enzymes as glucose oxidase (GOx) or lactate oxidase [7, 11, 12, 14] and nicotinamide adenine dinucleotide (NADH) [13].

Amperometric enzyme electrodes combine the specificity of biological molecules with the direct transduction into current response and are powerful tools in the field of analysis and biotechnology. Oxidase-based enzyme sensors for determination of glucose are one of the most popular and well-known applications of biosensors [15]. GOx is a relatively cheap enzyme with reasonable life time and applications in blood glucose clinical analysis. In addition, glucose determination has increasing relevance in other areas such as fermentation control and food analysis [16, 17]; therefore, the glucose biosensors are in continuous improvement.

The current developments are still limited by the enzyme stabilities and the loss of activity in physiological environments or reactors. This fact has a special relevance in bioprocess control when continuous measurements are required. For continuous measurements, the activity of the enzymes should be regenerated after the catalytic reaction. In aerobic media, the natural electron acceptor, oxygen, is present and the enzyme electrodes based on monitoring the reduction or oxidation of the H_2O_2 generated by the enzymatic reaction are often used. The major limitation of these techniques is the high operating potential required for detecting H_2O_2, which makes these devices susceptible to interfering substances. The modification of the electrode surface with catalysts which are able to decrease the overvoltage constitutes one of the more studied ways to minimize the interferences and improve the sensitivity of aerobic oxidase-based sensors [13, 14]. The incorporation of nano-sized materials [18–21] into the sensing device greatly increases biosensor sensitivity and response speed due to their properties.

We have recently demonstrated that dendrimers functionalized with interacting ferrocenes allow developing aerobic oxidase biosensors which exhibit analytical characteristics competitive and comparable with or even better than those of other recently reported biosensors with more complex preparation procedures ([2, 22] and cites therein). If anaerobic working conditions are required, the natural mediator oxygen must be replaced by other electron acceptors. In this case, it has been widely demonstrated [23, 24] that ferrocene is a good direct mediator for oxidases and peroxidases. In this article, we report the preparation and electrochemical characterization of enzyme electrodes based on glucose oxidase immobilized on Pt electrodes, previously modified with two carbosilane polymers with different length of branches, and their use for the determination of glucose in both aerobic (anodic and cathodic modes) and anaerobic conditions.

Experimental

Reagents

The polymers were synthesized by hydrosilylation reaction of the enyl groups in the polydiallylmethylsilane (PDAMS) and polymethyldiundecenylsilane (PMDUS) with bis (ferrocenylmethyl)silane (Losada, manuscript sent for publication). GOx from *Aspergillus niger* (type VII, 185 units/mg), bovine serum albumin (BSA), glutaraldehyde (25 % (*w*/*w*) solution in water) and glucose were supplied by Sigma. A solution of Nafion (5 wt.%) in mixed alcohols and water was obtained from Aldrich. Glucose solutions were left to reach mutarotational equilibrium at room temperature for 24 h before use. All other chemicals were analytical grade and were used without further purification. Ultrapure water was used for preparation of the buffers and standards and for electrochemistry.

Apparatus

Electrochemical measurements were performed using an Ecochemie BV Autolab PGSTAT 12. The experiments were carried out in a conventional three-electrode cell at 20–21 °C with a Pt disk of 3 mm diameter as working electrode and a Pt wire as auxiliary electrode. A BAS non-aqueous Ag/AgCl reference electrode or a saturated Calomel reference electrode (SCE) were employed in organic solvents and aqueous media respectively. In amperometric steady-state measurements, an Autolab rotating-disk electrode (ω=500 rpm) was used. All amperometric measurements were performed in 0.01 M phosphate buffer pH 7.0 with 0.1 M $NaClO_4$. The solutions for anaerobic determinations were deoxygenated by bubbling high-purity nitrogen for at least 15 min. The solutions for glucose measurements in aerobic conditions were saturated with oxygen. The background current was allowed to decay to a steady value before aliquots of stock glucose solution were added.

Electrodes Preparation

Pt disk electrodes were polished using 0.1 μm alumina powder and rinsed with water in an ultrasonic bath. Each electrode surface was then conditioned by cycling the potential between the limits for hydrogen and oxygen evolution in 0.5 M H_2SO_4 solution until well-defined cyclic voltammograms were obtained and then rinsed with water.

Films of the corresponding ferrocenyl functionalized polymers PDAMS and PMDUS were deposited on the above prepared surface from the electrolyte bath containing 10^{-4}M in the redox active species (ferrocene) and 0.1 M tetrabutylammonium hexafluorophosphate in dichloromethane, which was deaerated with nitrogen prior to electro-oxidation, under potentiostatic control at a potential of +0.7 V (vs. Ag/AgCl reference electrode). The coated electrodes were rinsed with dichloromethane and water. The surface coverage of electroactive ferrocenyl sites in the films (Γ) was determined from the integrated charge of the cyclic voltammetric waves.

Enzyme Immobilization

To immobilize the enzyme by covalent cross-linking, 5 μl of pH 7.0 phosphate buffer containing 5 U of enzyme and 40 μg BSA were applied to the PDAMS or PMDUS modified electrode surface, and then the electrodes were kept for 15 min in glutaraldehyde vapour at

room temperature. The prepared enzyme electrode was allowed to dry in air and rinsed thoroughly with the buffer [12].

For the enzyme immobilization by electrostatic adsorption, each modified electrode was immersed in an electrochemical cell containing 0.1 % of GOx in acetate buffer at pH≈5 (where the enzyme exists in the anionic form GOx^{m-} with $m\approx9$), and a potential of +0.6 V vs. SCE was applied during 30 min under stirring and N_2 bubbling. The electrodes were subsequently kept for 15 min in glutaraldehyde vapour at room temperature in order to prevent the enzyme desorption. The prepared enzyme electrodes were allowed to dry in air and rinsed with phosphate buffer prior to use. All the biosensors were stored in water at 4 °C when not in use.

Results and Discussion

Electrodes Characterization

The SEM micrographs of PDAMS (Fig. 1a) and PMDUS (Fig. 1b) electrochemically deposited reveal that the morphology of both films is rather different. The film of PDAMS is rougher whereas that of PMDUS forms a compact deposit, smoother, homogeneous and denser material. This structural difference between the films suggests that both polymers will offer also different properties as support of enzymes and as bioelectrocatalyst electrode materials.

For the enzyme immobilization, two different procedures have been tried: cross-linking by means of a bifunctional group (Fig. 2a) and entrapment by electrostatic adsorption (Fig. 2b). Both immobilization methods provided different results attending to the polymer type and according to different mechanisms of operation in aerobic or anaerobic media (Fig. 2c).

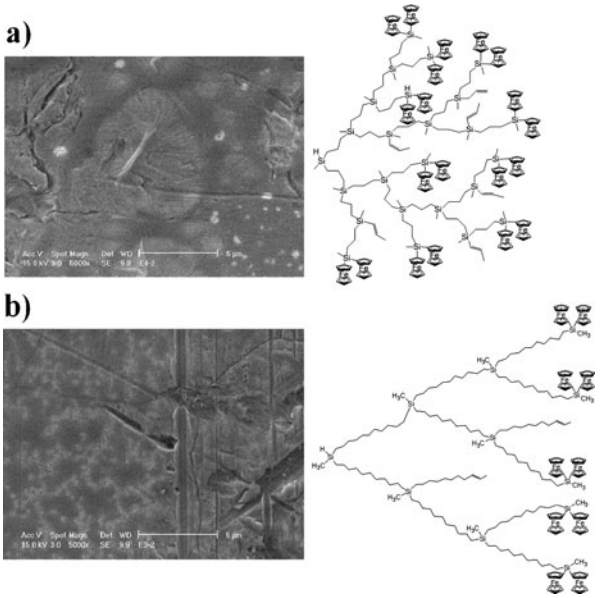

Fig. 1 Schematic representation of the polymers: **a** polydiallylmethylsilane (PDAMS) and **b** polymethyldiundecenylsilane (PMDUS) and SEM micrographs of platinum wire electrodes modified with electrodeposited films of these polymers

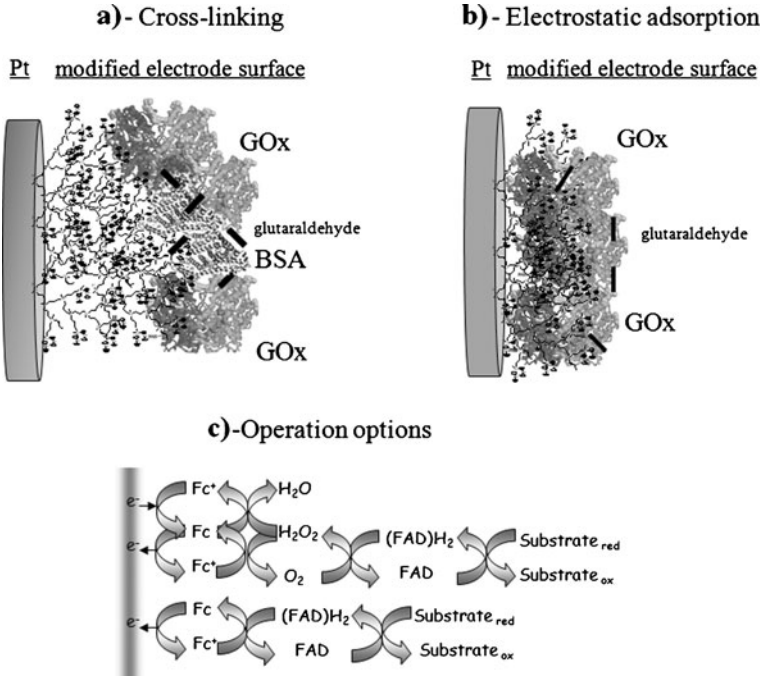

Fig. 2 Schematic illustration of the surface of biosensors prepared with GOx immobilized by **a** cross-linking and **b** electrostatic adsorption. **c** Scheme of the operation modes of the glucose biosensors

The effect of the polymer film thickness on the response signal of the sensors has also been studied. For this purpose, films with coverages from 5×10^{-11} to 1×10^{-9} mol of ferrocene sites cm^{-2} have been used in the preparation of the biosensors. From these studies, it has been found that polymer films with a coverage of about 2×10^{-10} mol Fc cm^{-2} exhibited the best response for both polymers.

Aerobic Glucose Biosensors

The ability of these modified electrodes to act as electrocatalysts for the electrochemical reduction and oxidation of hydrogen peroxide has allowed us to develop efficient anodic and cathodic amperometric sensors of H_2O_2 that can operate in a wide range of working potentials (Losada, manuscript sent for publication). As expected, the new enzyme–polymer electrodes also exhibit both anodic and cathodic activity whose mechanisms of detection are based on the reactions shown in the scheme in Fig. 2c. In the anodic operation mode, the ferrocenes are oxidized and they oxidize the hydrogen peroxide [25]:

$$H_2O_2 + 2Polymer - Fc^+ \rightarrow O_2 + 2H^+ + 2Polymer - Fc$$

And in the cathodic operation mode, the mechanism can be described as follows:

$$Polymer - Fc - H_2O_2 + 2H^+ + 2e^- \rightarrow Polymer - Fc + 2H_2O$$

The electrochemical properties of the modified electrodes are highly sensitive to the nature of the solution (Losada, manuscript sent for publication), as has been seen with other polymers and dendrimers with interacting ferrocenes studied earlier [2, 11–13, 22]. The

cyclic voltammograms in aqueous solutions display the peaks corresponding to the oxidation of the interacting ferrocenyl units practically overlapped (see insets in Fig. 3). Figure 3 shows the bioelectrocatalytical responses of GOx/PDAMS and GOx/PMDUS biosensors with the cross-linked enzyme, in the absence and presence of 8 mM glucose taken at 2 mV s^{-1} in oxygen saturated phosphate buffer pH 7.0.

As can be seen, the anodic electrochemical behaviour of GOx/polymer electrodes is influenced by the presence of glucose in solution. The addition of glucose leads to a substantial enhancement of the oxidation current whereas the cathodic peak disappears, as

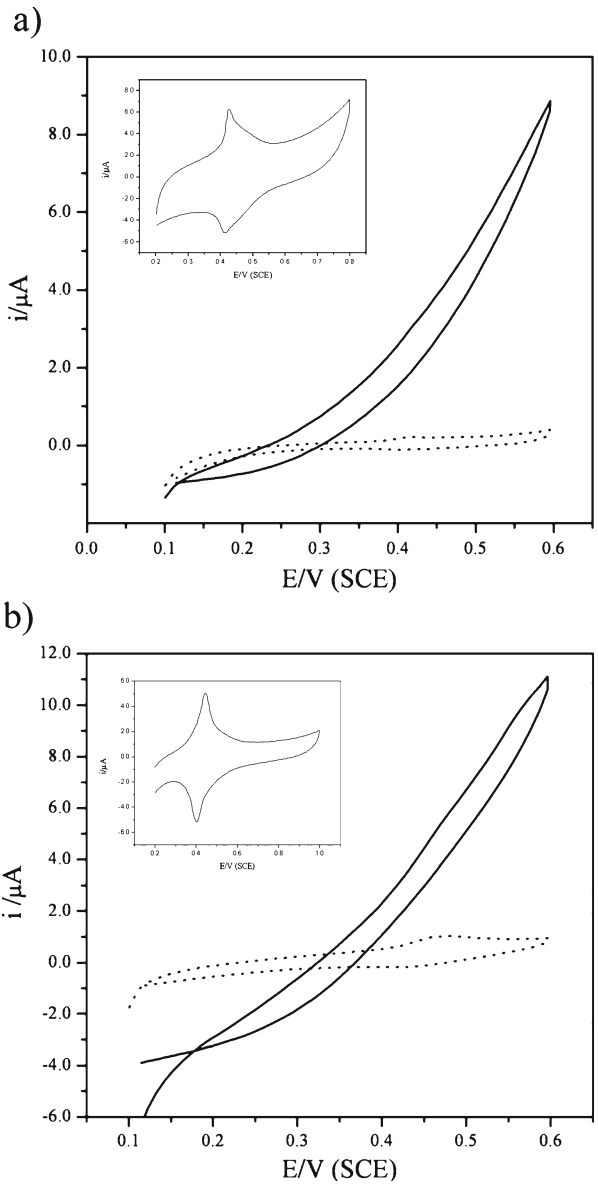

Fig. 3 Cyclic voltammograms of **a** GOx/PDAMS ($\Gamma=6.17\times10^{-10}$ molFc/cm^2 thickness film) and **b** GOx/PMDUS ($\Gamma=1.38\times10^{-10}$ molFc/cm^2) biosensors in absence (*dotted line*) and presence (*solid line*) of glucose 8.0 mM in oxygen saturated phosphate buffer (pH 7) and $NaClO_4$ 0.1 M as supporting electrolyte. Scan rate, 2 mV/s. *Insets* cyclic voltammograms of a PDAMS (**a**) modified electrode ($\Gamma=8.50\times10^{-10}$molFc/$cm^2$ thickness film) and a PMDUS (**b**) modified electrode ($\Gamma=6.50\times10^{-10}$molFc/$cm^2$ thickness film) in deaerated phosphate buffer (pH 7)/$NaClO_4$ 0.1 M. Scan rate, 100 mV/s

expected for a catalytic process. The efficiency of the bioelectrocatalytical processes, calculated as the current enhancement (relative to the signal of the biosensor in absence of glucose), observed at the anodic process at +0.50 V, is about 450 % for the GOx/PDAMS device and 560 % for the GOx/PMDUS biosensor.

Figure 3 also shows the cathodic response of the enzyme electrodes to glucose concentration depending on the operation potential. However, the cathodic reduction of the oxygen present as electron acceptor in the enzyme regeneration can interfere on the glucose measurements at potentials lower than −100 mV vs. SCE [2, 10]. Therefore, the potential value of −100 mV (vs. SCE) should be considered as the lowest potential suitable for cathodic determination of glucose. The bioelectrocatalytical efficiency of the reduction is also derived from the enhancement of the current relative to the current at the biosensors in the absence of glucose. The current at +0.2 V was enhanced by 80 % for the GOx/PDAMS electrode and 360 % for the GOx/PMDUS device.

In the devices prepared with the enzyme electrostatically adsorbed, the response of both polymer–biosensors is different. Figure 4 shows the steady-state response of GOx/PDAMS and GOx/PMDUS biosensors prepared by electrostatic adsorption, to consecutive addition of 1 mM aliquots of glucose. As can be seen, the response of GOx/PMDUS is faster than those of GOx/PDAMS, although their sensitivities are similar. The responses of both biosensors with the enzyme electrostatically immobilized were always lower than those of biosensors prepared by cross-linking. This behaviour has been previously observed [22] and is probably due to the fact that the cross-linking immobilization method affords a better substrate–enzyme contact than the adsorption one.

For thick films, the response of biosensors prepared by electrostatic adsorption decreases to render them useless. This behaviour has also been observed in previous works and is attributed to the increasing limitations for the GOx penetration into the film by electrostatic adsorption and to the difficulty in the charge propagation throughout the thicker films.

In all cases, the aerobic devices, in both anodic and cathodic operation modes and for both polymers, showed the best results (best sensitivity, detection limit, linear range and time of response) when the enzyme was immobilized by cross-linking. This is probably due to the

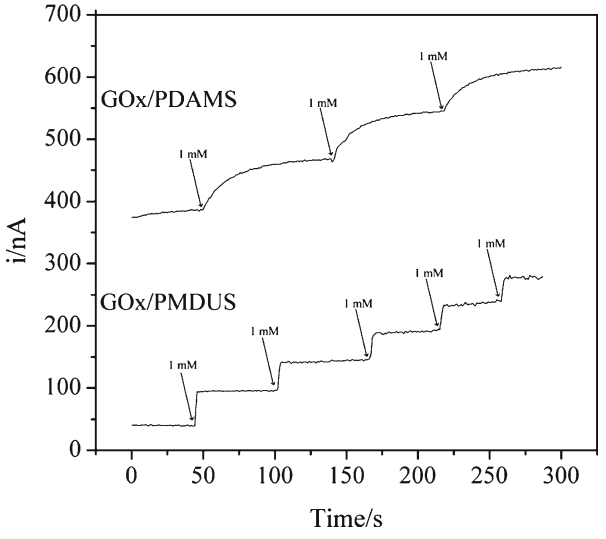

Fig. 4 Amperometric responses of the GOx/PDAMS and GOx/PMDUS biosensors with the enzyme electrostatically adsorbed to the consecutive addition of glucose aliquots of 1 mM into stirred and air saturated solution at E=+0.5 V (vs. SCE)

more difficult diffusion of glucose, through the polymer film, to the active centres of GOx electrostatically entrapped.

Figure 5 shows the glucose calibration plots at several applied potentials for biosensors with cross-linked GOx with both mediators. It can be seen that GOx/PMDUS biosensors yielded the best results. GOx/PMDUS polymer produces smoother films and has longer branches capable of going into the enzyme structure and oxidize or reduce the hydrogen peroxide as soon as it is produced, increasing the reaction rate.

As expected from the voltammetric profiles (Fig. 3), in all cases, the anodic detection of glucose is more sensitive than the cathodic one and the sensitivity increases with the increasing applied potential. Nonetheless, the cathodic mode offers an interference-free interval of working potential because most interferences occur in the anodic zone and the oxygen is reduced at potentials more negative than −0.1 V (vs. SCE). In all cases, the

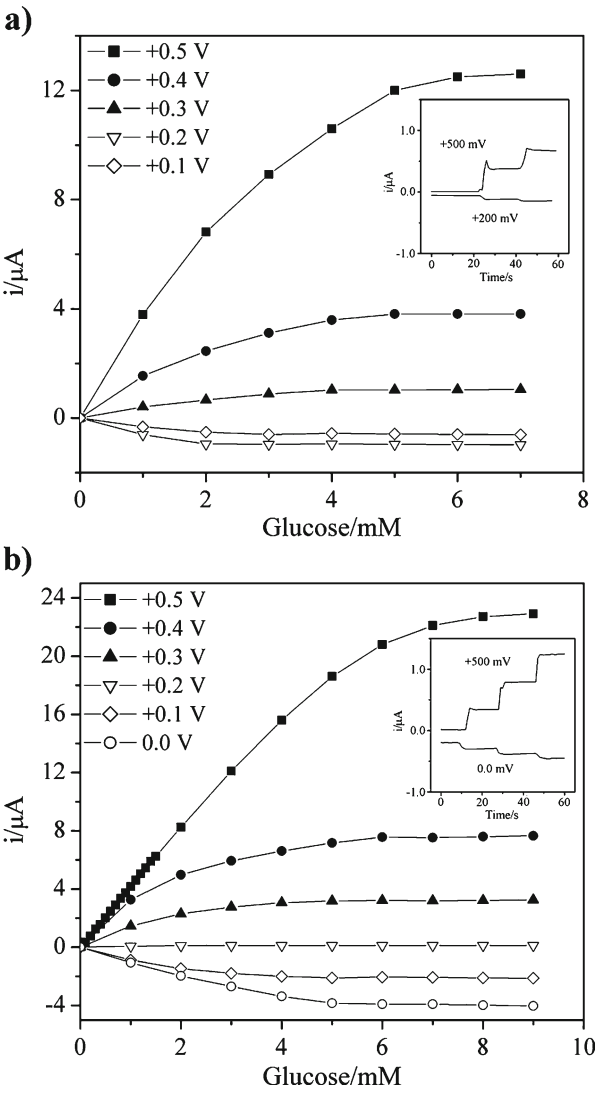

Fig. 5 Anodic and cathodic glucose calibration plots obtained at **a** GOx/PDAMS ($\Gamma \approx 4 \times 10^{-10}$ mol Fc/cm^2 thickness film) and **b** GOx/PMDUS ($\Gamma \approx 2 \times 10^{-10}$ mol Fc/cm^2) in oxygen saturated phosphate buffer. Each value is the mean result for five electrodes. The *insets* show the typical amperometric responses of both biosensors to the addition of glucose aliquots of 0.1 mM

response was fast and the time elapsed to reach the steady-state value was less than 3 s (see insets in Fig. 5). The analytical characterization data of biosensors obtained with both polymers are collected in Table 1.

Anaerobic Glucose Biosensors

The electrochemical behaviour of the enzyme electrodes is also influenced by the presence of glucose in solution in the absence of oxygen. When glucose was added to the electrolyte solution, a substantial increase in the oxidation current was observed at potentials more positive than +0.25 V vs. SCE, indicating that ferrocene molecules immobilized in the film are efficient electron mediators between the electrode and the redox centres of FAD/GOx. The mechanism of the reaction can be described as follows (Fig. 2c):

$$Glucose + GOx(FAD) \rightarrow Glucono - D - lactone + GOx(FADH_2)$$

$$GOx(FADH_2) + 2Fc^+ \rightarrow GOx(FAD) + 2H^+ + 2Fc$$

In these devices, the current enhancement due to the presence of glucose 8.0 mM at +0.50 V was observed to be about 6 %.

For these biosensors, the two immobilization procedures described in the experimental section have also been compared. Figure 6 shows the calibration curves for electrodes modified with both polymers obtained at +0.5 V vs. SCE as a function of the glucose concentration, for electrolyte solutions saturated with N_2.

As it can be observed in Fig. 6, when the ferrocene–polymers act as mediators, GOx/PDAMS with the enzyme electrostatically adsorbed is the best bioelectrocatalyst (Fig. 6a). This can be due to the less dense films formed by PDAMS (see Fig. 1a) that allows GOx to go deeply into the film achieving more efficient electronic transference with the active centres of the enzyme.

The anaerobic GOx/PMDUS device shows the same behaviour with the different immobilization modes than the aerobic devices. The anaerobic biosensors respond quickly to the substrate additions with a good linear response region at the working potentials. The analytical properties measured in the best conditions for these glucose anaerobic biosensors are collected in Table 1.

Table 1 Analytical and kinetic parameters

Biosensor	E (V/SCE)	Detection limit (μM)	Linear range (mM)	Response time (s)	Sensitivity ($\mu A\,mM^{-1}\,cm^{-2}$)	k_{app} ($A\,mM^{-1}$)	K'_M (mM)
GOx/PDAMS[a,b]	0.5	0.7	0–2	2	48.7	4.52×10^{-6}	5.90
	0.2	40.2	0–2	2	6. 8	2.70×10^{-6}	0.79
GOx/PMDUS[a,b]	0.5	0.4	0–4	2	60.4	4.57×10^{-6}	5.08
	0.0	7.8	0–3	2	12.8	5.12×10^{-6}	1.36
GOx/PDAMS[c,d]	0.5	51.0	0–3	6	0.6	4.48×10^{-8}	9.30
GOx/PMDUS[b,c]	0.5	88.5	0–4	3	0.2	2.77×10^{-8}	4.21

[a] Aerobic

[b] Enzyme cross-linked

[c] Anaerobic

[d] Enzyme electrostatically adsorbed

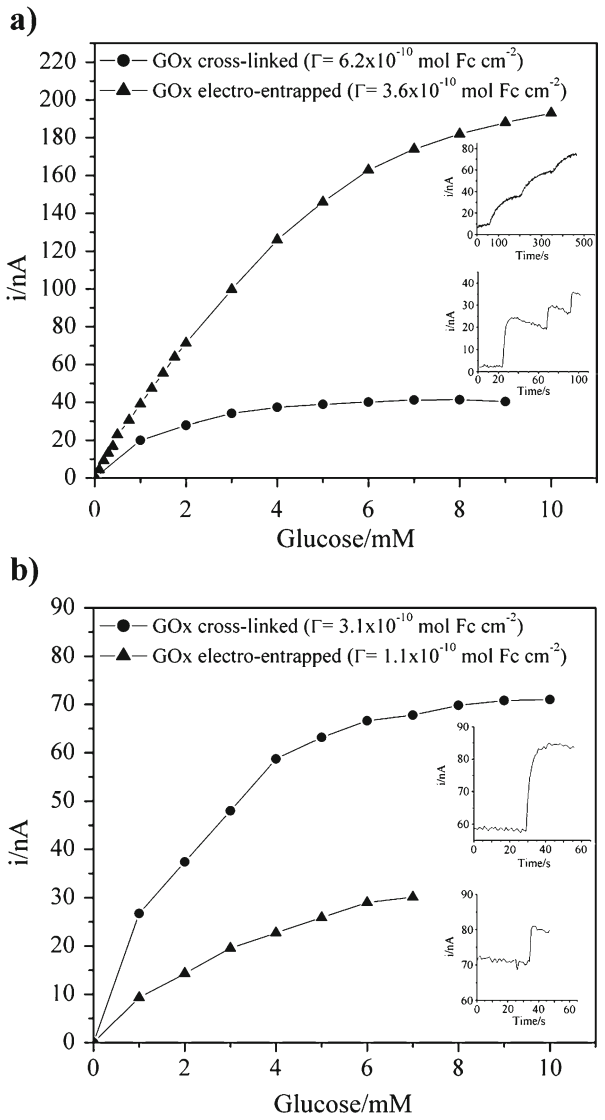

Fig. 6 Glucose calibration plots obtained at **a** GOx/PDAMS and **b** GOx/PMDUS biosensors with the enzyme immobilized by both procedures measured at +0.5 V (vs. SCE) in stirred deaerated phosphate buffer. *Insets* amperometric responses of the biosensors to the addition of glucose aliquots of 1.0 mM

Kinetic Studies

The apparent Michaelis–Menten constants, K'_{M}, and the apparent rate constants k_{app} were determined from the double reciprocal Lineweaver–Burk equation:

$$\frac{1}{i} = \frac{1}{[Substrate]} \times \frac{1}{k_{app}} + \frac{1}{k_{app} \times K'_M}$$

In a previous paper [10], we have reported an adaptation of the Savinell's model [26] including a new term relative to the presence of oxygen as natural mediator for GOx. The adapted model allows to obtain the same diagnostics and to identify the rate-limiting step in

the aerobic operation. In order to apply this mathematical model to the new sensors, the Lineweaver–Burk plots for the steady-state response for sensors built with the two polymers were plotted. Figure 7 shows the obtained anodic Lineweaver–Burk plots for the best aerobic and anaerobic biosensors.

In aerobic media (Fig. 7a), the resulting plots at an applied potential of +500 mV (vs. SCE) are rather different for each polymer. The double reciprocal plot for the GOx/PDAMS device was in agreement with the case II of the Savinell's model, when the rate of electrolysis is not fast enough to be neglected (e.g. the applied potential is not high enough) and the substrate diffusion does not control the overall reaction rate. In the region where l/i is large, the plot is essentially a straight line, but it exhibits an upward concave curve in the region of small l/i. In this case, the apparent Michaelis constant depends on the rate of electrolysis and is intrinsically smaller than the Michaelis constant in homogeneous enzyme kinetics. However, the Lineweaver–Burk plot of GOx/PMDUS device at the same potential

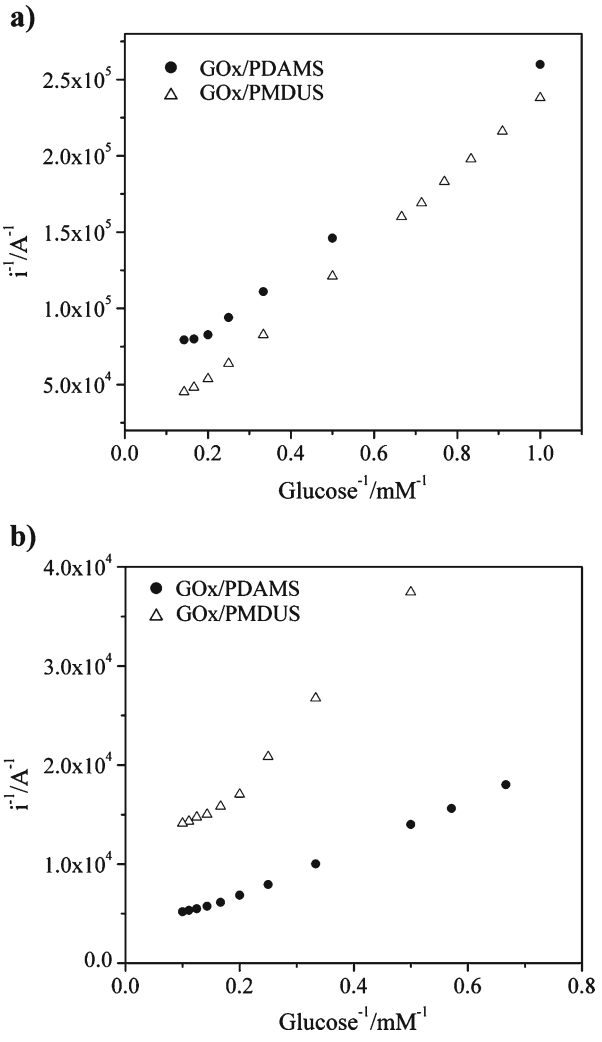

Fig. 7 Lineweaver–Burk plots at +0.5 V/SCE, **a** for GOx/PDAMS ($\Gamma \approx 4.0\times10^{-10}$ molFc/cm^2) and GOx/PMDUS ($\Gamma \approx 2\times10^{-10}$ mol Fc/cm^2) biosensors in oxygen saturated phosphate buffer, in anodic operation mode, and **b** for GOx/PDAMS ($\Gamma \approx 4.0\times10^{-10}$ mol Fc/cm^2) and GOx/PMDUS ($\Gamma \approx 3\times10^{-10}$ molFc/cm^2) biosensors in deaerated phosphate buffer. Each value is the mean result for five electrodes

is practically linear, in best agreement with the case I of the Savinell's model, when neither the diffusion nor the electrolysis process controls the overall rate of the enzyme electrode and a combination of enzyme catalysis and electron mediation reactions limits the overall reaction. This behaviour differences must be related to the different structure of the PDAMS and PMDUS films. As it has been described, PMDUS films are more compact and the ferrocene groups bonded to longer branches are closer, and the charge transport becomes easier.

Although these apparent constants cannot be compared directly with literature values, the obtained equivalent Michaelis constant is of the order of magnitude of reported constants [24, 26]. These kinetic parameters are shown in Table 1.

In relation with the cathodic mode, both polymers showed the same kinetic behaviour corresponding to case II described above (see supplementary material). This fact is explained on the basis of the low working potentials used in order to avoid interferences. However, the GOx/PMDUS biosensors apparent rate constant is similar to that obtained in the anodic mode, while those of the GOx/PDAMS devices become smaller (Table 1), confirming the best bioelectrocatalytical properties of PMDUS for oxidase aerobic biosensors.

The Lineweaver–Burk plots for the optimal anaerobic GOx/PDAMS and GOx/PDMUS biosensors (Fig. 7b) respond to the same kinetic model [26] as those of the aerobic devices (Fig. 7a), when the rate of electrolysis controls the overall reaction rate. However, the slopes and the intercepts of Lineweaver–Burk plots for both polymer–biosensors are quite different and demonstrate, according to the obtained apparent constants (k_{app}, K'_M), that the GOx/PDAMS anaerobic device is more efficient according to the sensitivity shown by the calibrate plots. Table 1 contains the kinetic data of the anaerobic sensors.

Interferences

A major concern in the development of biosensors is the elimination of signals arising from electrochemically interfering compounds present in real matrices. The electro-oxidation of principal interfering agents in biological samples, ascorbic acid (AA), uric acid (UA) and *p*-acetaminophenol (ACE) occurs at potentials more positive than +0.2 V [17], producing a substantial increase in the steady-state anodic current response of sensors. The response of the biosensors reported in this work was unaffected by the presence of these species in physiological concentrations (0.1 mM). In fact, the signal due to the addition of 0.1 mM AA, UA and ACE to a 1-mM glucose solution was unappreciable for operating potential values between +0.1 and −0.05 V (vs. SCE). Unfortunately, the GOx/PDMAS and GOX/PDMUS biosensors suffer these interferences when working potential values more positive than +0.10 V (vs. SCE) need to be used. In these cases, the AA, UA and ACE interferences can be avoided by using an additional Nafion layer [27]. The response of Nafion/GOx/polymer in comparison with uncovered sensors shows a linear range twice extended because the Nafion/GOx/polymer sensor is affected by the substrate diffusion through the additional Nafion layer, but the amperometric signal decreases by ca. 40 %. Despite these restrictions, the proposed devices present detection limits and a linear behaviour in a range enough to provide the suitable conditions to quantify glucose in real samples. In any case, if necessary, the sensitivity of Nafion–GOx/polymer biosensors could be improved (by about 80 %) increasing the applied potential. The oxygen interference does not constitute a problem for the chosen working potentials as quoted already above.

Reproducibility and Stability

The reproducibility of the biosensors was examined in a 2.0-mM glucose solution (air saturated), and the relative standard deviation was 1.3 % (n=5). The electrode-to-electrode reproducibility was also examined for five different electrodes in the above solution, and the relative standard deviation was calculated to be 3.8 %. The stability of the biosensors was evaluated by intermittent measurements in presence of glucose 2.0 mM, and their response remained unchanged during a period of 6 days. A 35 % decrease of the initial glucose response was observed for an electrode stored at 4 °C in pH 7.0 phosphate buffer for 7 weeks.

Conclusions

The redox polymers PDAMS and PMDUS functionalized with interacting ferrocenes, co-immobilized with GOx on platinum electrodes, have been successfully used as versatile amperometric biosensors for glucose determinations. The results obtained indicate that both anodic and cathodic operation modes may be used for the determinations, since the electrodes modified with these polymers act as electrocatalysts in the oxidation or reduction of hydrogen peroxide enzymatically generated in aerobic media. The sensor behaviour is affected by the structural characteristics of the polymers and by the enzyme immobilization procedure. In general, the PMDUS polymer, with longer branches, shows the best electrocatalytical properties for the oxidation and reduction of hydrogen peroxide in aerobic conditions, and the cross-linking was the best immobilization method for this purpose. These carbosilane-modified electrodes can act as interference-free biosensors of oxidase substrates in the working potential interval between +0.10 and −0.05 V vs. SCE.

Moreover, electrodes modified with these polymers have demonstrated to be effective mediators in the electrochemical oxidative regeneration of GOx under anaerobic conditions. In this case, PDAMS polymer with the enzyme electrostatically adsorbed is the best bioelectrocatalyst despite its slower response.

The biosensors developed in this work respond quickly to the substrate with a good linear response region and exhibit analytical characteristics competitive and comparable with or even better than those of other recent reported biosensors with more complex preparation procedures [22]. In the light of the versatility that offers these new biodevices to be used with several oxidases and in a wide range of possibilities of experimental conditions, they seem very promising for varied applications in the field of biotechnology.

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