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Ferrocenyl and permethylferrocenyl cyclic and polyhedral siloxane polymers as mediators in amperometric biosensors

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Abstract

Cyclosiloxane and silsesquioxane-based ferrocenyl and permethylferrocenyl polymers have been used as mediators in amperometric enzyme electrodes for the detection of glucose. Biosensors have been prepared by electrostatically immobilizing the enzyme glucose oxidase (GOx) on electrodes modified with the polymers. The steady-state amperometric response of the sensors is investigated as a function of the applied potential and substrate concentration. The dependence of the sensors response on the structure of the siloxane-framework and on the presence or not of methyl groups on the ferrocenyl units is discussed.

Keywords: Ferrocene; Cyclosiloxanes; Silsesquioxanes; Glucose-sensor; Modified electrode

1. Introduction

In recent years various amperometric electrodes with immobilized oxidases have been developed for the detection of their corresponding substrates. For continuous measurements the activity of the enzymes should be regenerated after the catalytic reaction. There are two conventional types of oxidase enzyme-modified electrodes: mediated and mediatorless. Mediated enzyme electrodes are based on monitoring the electro-oxidation of the mediator reduced by the oxidase enzymatic reaction. Mediatorless enzyme electrodes based on monitoring direct electro-reduction or electro-oxidation of H_2O_2 generated by the enzymatic reaction can be used when oxygen acts as the natural electron acceptor for restoring the active form of the enzymes. The oxidase-based reaction can be also electrochemically followed by measuring the decreased oxygen content in the solution with an oxygen probe (Clark type electrode) as was done in the very first enzyme sensors [1] or by the direct electrochemical reduction of oxygen at a single working electrode. The Clark oxygen electrode requires the use of a membrane to prevent interfering reactions to occur at the electrode surface largely decreasing its sensitivity and a substantial negative electrode potential (≅−0.6 V vs SCE) is needed leading to a noisy background current. Electrodes modified with metal complexes have been used as catalysts for monitoring the oxygen consumption by substrates in the presence of enzymes [2,3].

Under anaerobic conditions other electron acceptors, such as ferrocene, replace oxygen and so ferrocenyl-based polymers are useful materials as mediators for

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electrochemical biosensors. Various methods have been studied for the immobilization of ferrocenes on electrode surfaces to produce chemically modified electrodes. Covalent binding to platinum or carbon is frequently used. Other approaches include the incorporation of the ferrocene moiety to polymeric materials [4,5].

It has been shown that polycationic redox polymers adsorbed on graphite can strongly bind the enzyme glucose-oxidase and that the bound enzyme can then be electrochemically oxidized by the base electrode through mediation by the redox polymer [6]. Subsequent papers have described the catalytic oxidation of glucose in two- and three-dimensional structures in which GOx is "molecularly wired" by the redox polymer [7]. Heller and co-workers [8,9] demonstrated that the polymer/enzyme assembly immobilized onto the surface could eliminate the need for a membrane barrier to avoid interferences. Also there have been several reports of the immobilization of GOx into a three-dimensional redox polymer mediator, poly(vinylferrocene) (PVFc), via electrochemical oxidation [10,11].

In the last years we have prepared and studied new classes of ferrocenyl and permethylferrocenyl polymers containing cyclic and polyhedral siloxanes as frameworks [12–14]. Structurally well-defined silsesquioxanes are a versatile class of three-dimensional oligomeric organosilicon compounds with the silicon atoms as corners and oxygen atoms between them and with various degrees of symmetry. Due to their stereochemical properties both, cyclosiloxanes and polyhedral silsesquioxanes, are good candidates as sterically controlled multifunctional catalyst systems.

While polymers containing ferrocenyl moieties in the backbone or as pendant substituents are numerous, polymers constructed from permethylferrocene monomers have been relatively less explored. However, organometallic compounds containing permethylcyclopentadienyl ligands are interesting since they often exhibit significantly different properties than their non-methylated analogues. As a result of the enhanced electron donating ability of the permethylated cyclopentadienyl rings, polymethylferrocenyl derivatives exhibit lower redox potentials. In addition, it is well-known from studies of monomeric ferrocene mediators that modification of the cyclopentadienyl ring with methyl substituents can cause changes in the rate of the electron transfer between the reduced form of the enzymes and the oxidized ferrocene. It has been reported that 1,1′-dimethylferrocene reoxidises reduced glucose-oxidase at a rate that was nearly three times higher than the unsubstituted ferrocene. This improved mediating ability of dimethylferrocene is maintained after the species is covalently attached to a siloxane polymer [15,16]. These facts promise to be of importance when these polymers are used as mediators for biosensors in order to increase their efficiency and minimize interferences.

In this study we report features and applications of enzyme electrodes based on glucose oxidase immobilized on Pt electrodes modified with ferrocenyl and permethylferrocenyl polymers, containing a cyclotetrasiloxane (**1** and **2**) or an octasilsesquioxane (**3** and **4**) (Chart 1) in the backbone, which act as mediators. On the other hand, the ferrocenyl moieties show electrocatalytic activity towards the reduction of oxygen, which allows the determination of the dissolved oxygen content at less negative potentials and with enhanced sensitivity than with a bare electrode.

2. Experimental

2.1. Reagents

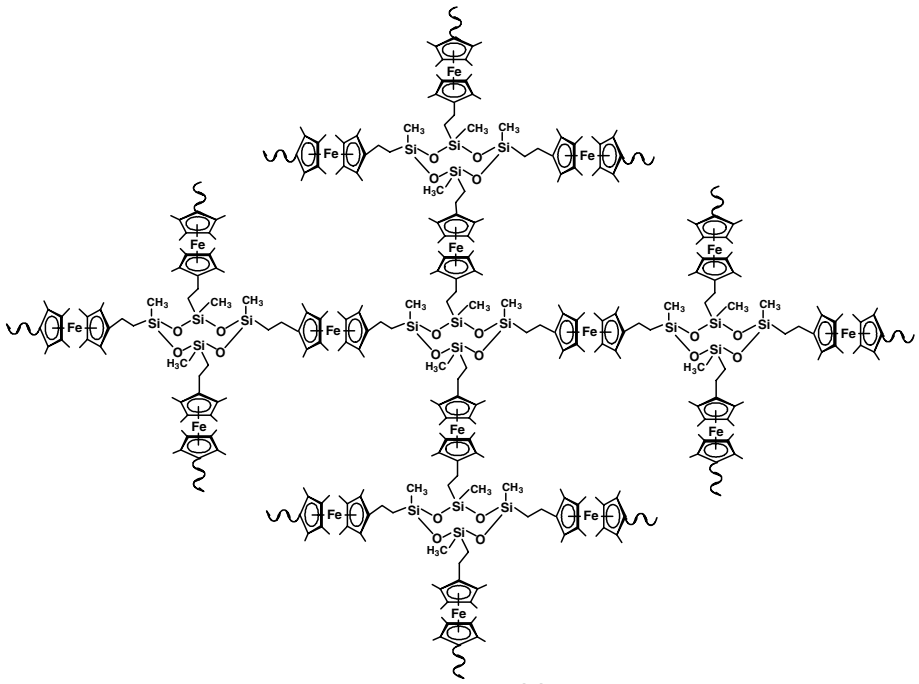
The polymers were synthesized via hydroxilylation reactions of 1,1′-divinylferrocene or 1,1′-divinyl(octamethyl)ferrocene with 1,3,5,7-tetramethylcyclotetrasiloxane or octa(hydrodimethylsiloxy)octasilsesquioxane according to the procedure described earlier [12,13]. Glucose oxidase from *Aspergillus niger* (type VII, 185,000 U/g) and glucose were supplied from Sigma. Glucose solutions were allowed to reach mutarotational equilibrium at room temperature for 24 h before use. All other chemicals were analytical grade and were used without further purification. All solutions were prepared with doubly distilled water.

2.2. Apparatus

Electrochemical measurements were performed using an Ecochemie BV Autolab PGSTAT 12. All experiments were carried out in a conventional three-electrode cell at 20–21 °C. A Pt disk of 3 mm diameter served as working electrode, a Pt wire as auxiliary electrode, and a saturated Calomel reference electrode (SCE) were employed. In steady-state measurements, a Metrohm 628-10 rotating electrode was used. All amperometric measurements were performed in 0.1 M phosphate buffer with 0.1 M $NaClO_4$ (pH 7.0). The solutions were air saturated in all enzyme sensors measurements. The background current was allowed to decay to a steady value before aliquots of substrate solution were added.

2.3. Electrode preparation

The Pt disk electrode was polished using 0.1 μm alumina powder and rinsed with water in an ultrasonic bath. The 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. **1** and **2** polymer films were deposited on Pt electrodes from an electrolyte bath containing



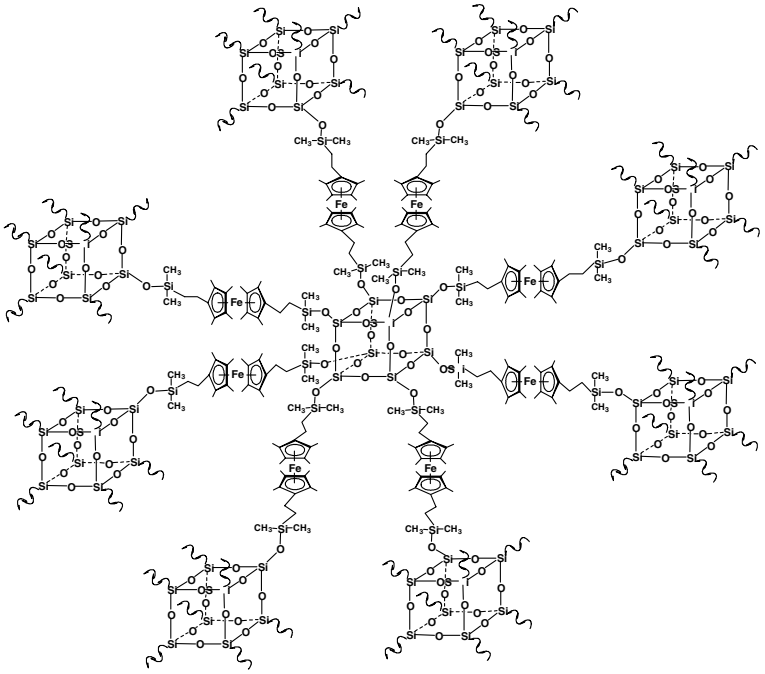


Chart 1.

approx. 0.5 mM Fc and 0.1 M tetra-*n*-butylammonium hexafluorophosphate (TBAH) in dichloromethane, which was deaerated with nitrogen prior to electro-oxidation, by cycling the electrode potential at 100 $mV\,s^{-1}$ between 0 and 1.0 V (vs SCE). The coated electrodes were rinsed with dichloromethane. However polymers **3** and **4** cannot be electrodeposited and their films were prepared by dipping 5 μl of the corresponding polymer

solution onto the electrode surface and then allowing them to dry in air at room temperature.

The surface coverage of electroactive ferrocenyl sites in the film, Γ, was determined from the integrated charge of the cyclic voltammetric waves, and similar coverage values were used in anaerobic and aerobic measurements.

The immobilization of the enzyme was carried out by immersing each polymer coated electrode in an electrochemical cell containing an enzyme solution (0.1% of GOx in acetate buffer, pH 5.3, and $NaClO_4$ 0.1 M). A +0.7 V vs SCE potential was applied to the polymers coated electrodes during 30 min under stirring and nitrogen bubbling, subsequently rinsed in deionised water and air-dried before use. The pH of the enzyme solution was kept at a value of 5.3 where the enzyme exists in the anionic form, GOx^{m-} [17] facilitating its interaction with the oxidized polymer and then the electrodes were kept for 10 min in glutaraldehyde vapour at room temperature. The prepared enzyme electrode was allowed to dry in air, and rinsed thoroughly with the buffer. The polymer modified and enzyme electrodes were stored at room temperature and at 4 °C respectively, when not in use.

3. Results and discussion

3.1. Electrode characterization

The voltammetric response of the polymer films deposited onto platinum electrodes exhibits, in aqueous and non-aqueous solutions, a well-defined symmetrical oxidation–reduction-wave characteristic of the ferrocenyl moieties (Fig. 1).The formal potential values are presented in Table 1. The potentials observed for the methylated polymers (**2** and **4**) are more negative than those corresponding to the films of the respective non-methylated ferrocenyl polymers (**1** and **3**). This is the result of the strong electron-donating effect of the eight methyl groups on the ferrocene rings.

Glucose-oxidase was immobilized into conducting films of polymers by electrostatic interactions, employing the procedure described in Section 2. In all cases, the optimal operational conditions in the immobilization process were employed. Immersion periods longer than 30 min and GOx concentrations higher than 0.1% did not give rise to variations in the results.

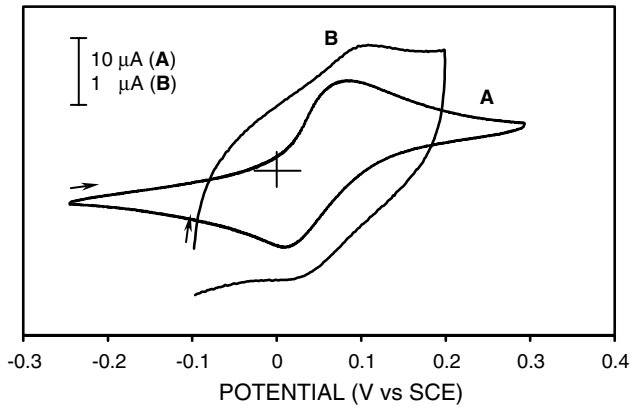


Fig. 1. Cyclic voltammograms of polymer **2** electrodeposited at a platinum electrode ($\Gamma = 2.14\times10^{-9}$ mol ferrocene cm^{-2} thickness film) in (A) deaerated CH_3CN/TBAH 0.1 M, and (B) deaerated phosphate buffer (pH 7.0)/$NaClO_4$ 0.1 M. Scan rate: 100 $mV\,s^{-1}$.

Fig. 2 shows typical cyclic voltammograms of a polymer/GOx-modified electrode taken in phosphate buffer (pH 7) before (curve A) and after (curve B) glucose was added to the solution. It should be appreciated that the redox peaks in Fig. 2(A) are less prominent and intense than those of the polymer-modified electrodes

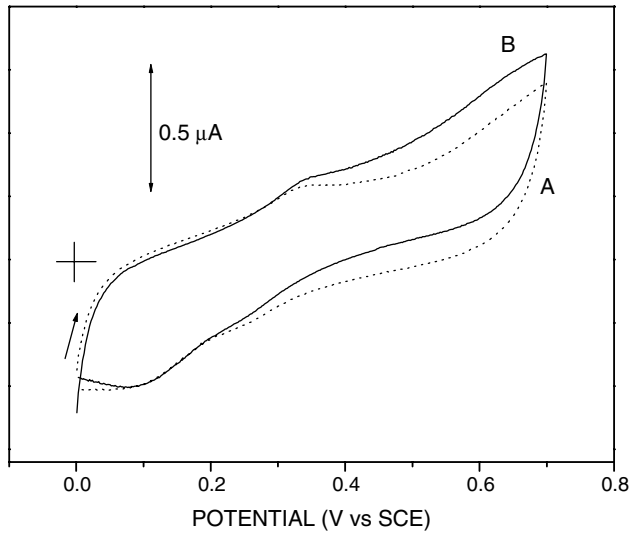


Fig. 2. Cyclic voltammograms of a platinum disk electrode modified with a film of polymer **1** GOx ($\Gamma = 2.5\times10^{-9}$ mol ferrocene cm^{-2} thickness film) in deaerated phosphate buffer (pH 7.0) in absence (A) and presence (B) of glucose 10 mM. Scan rate 5 $mV\,s^{-1}$.

Table 1
Cyclic voltammetric data for electrodes modified with **1**–**4** ferrocenyl polymers

	1		**2**		**3**		**4**	
	CH_3CN	H_2O	CH_3CN	H_2O	CH_3CN	H_2O	CH_3CN	H_2O
$E_{1/2}$ (V vs SCE)	0.35	0.31	0.05	0.04	0.49	0.29	−0.01	−0.02

without GOx immobilized. This change in the shape of the cyclic voltammogram can been attributed to the effects of the GOx molecules (GOx^{m-}), incorporated in the film structure by electrostatic interactions, on the charge transfer process. A similar electrochemical behaviour due to the counteranion effects has been previously reported for electrodes modified with films of polymers containing ferrocene units [11,18–20].

The voltammogram in presence of glucose (Fig. 2(B)) indicates a catalytic behaviour. It can be observed that the anodic current for the polymer-GOx electrodes is increased on addition of glucose to the solution, whereas the reduction current decreases, which is consistent with a catalytic process.

The glucose response of platinum electrodes modified with GOx and different polymers was studied as a function of the working potential (Fig. 3). As expected, the polymeric permethylated relays can mediate electron transfer from reduced glucose oxidase more efficiently than the corresponding non-methylated ferrocenyl polymer.

Fig. 3 also indicates that the sensors based on cyclotetrasiloxane polymers (**1** and **2**) display a larger response to glucose than the sensors based on the polymeric silsesquioxanes (**3** and **4**), however the later show electrocatalytical signal at lower potentials than the corresponding cyclotetrasiloxanes. This fact can be related to the lower redox potentials of the ferrocenyl moieties in the silsesquioxane polymers due to the somewhat stronger electron-donating effect of the substituents attached to the cyclopentadienyl rings, which allows that the reoxidation of the reduced enzyme molecules takes place at more negative potential values.

A significant effect of the polymer film coverage on the bioelectrocatalytic signals of the enzyme electrodes was detected. The maximum response was exhibited with a polymer coverage of about $\Gamma = 1\text{–}3\times10^{-9}$ mol ferrocene cm^{-2}. With respect to pH effects, maximal current values were obtained at pH 7.0, though the current response is essentially independent of the pH over the physiologically relevant range; i.e. pH 6.0–9.0.

In order to evaluate the analytical performance of the enzyme electrodes, calibration curves were obtained for electrodes containing similar ferrocenyl surface coverage values. Fig. 4 shows the steady state currents for electrodes modified with the four different ferrocenyl polymers (**1**–**4**) as a function of the glucose concentration. As can be seen, the sensors based on polymers **1** and **2**, in which the ferrocenyl units are attached to cyclotetrasiloxane frameworks, display at high potentials (see, for example Fig. 4(a)) a marked increase in the bioelectrocatalytic signals and in the sensitivity in comparison with those of the sensors containing ferrocenylsilsesquioxanes as relay systems (**3** and **4**). In the other hand, it should be noted (Fig. 4(b)) that the permethylated polymers (**2** and **4**) allow to use lower working potentials in glucose measurements.

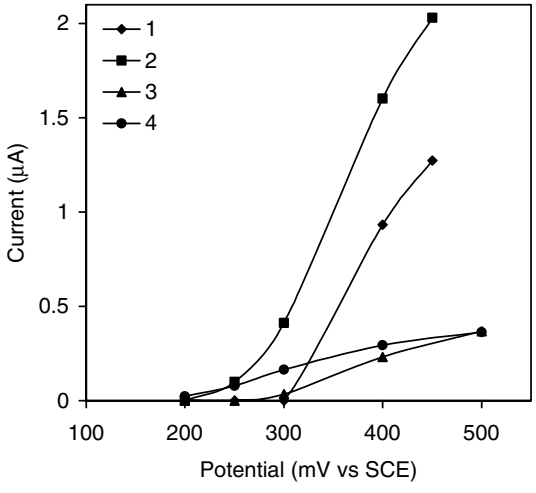


Fig. 3. Effect of the working potential on the response of the four sensors ($\Gamma \approx 2\times10^{-9}$ mol ferrocene cm^{-2} thickness). Steady-state currents measured in 0.1 M deaerated phosphate buffer (pH 7.0). Each value is the mean result of five electrodes.

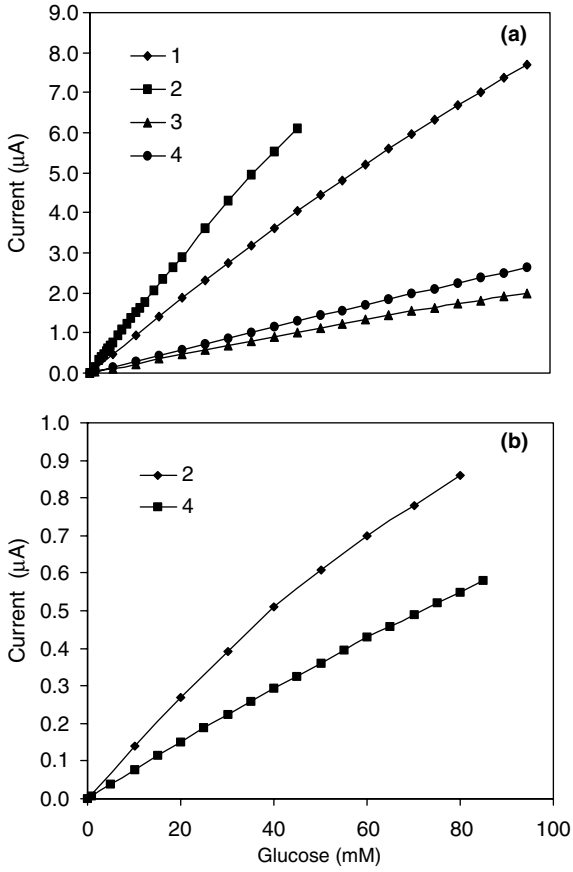


Fig. 4. Glucose calibration plots of polymer–enzyme sensors ($\Gamma \approx 2\times10^{-9}$ mol ferrocene cm^{-2} thickness films). Steady-state currents measured at (a) +0.40 V and (b) +0.25 V (vs SCE), in 0.1 M deaerated phosphate buffer (pH 7.0). Each value is the mean result of five electrodes.

In all cases the bioelectrocatalytic signals reached the steady-state value within 5–10 s after addition of the glucose samples and the steady-state currents were maintained with no fluctuation. The sensitivities calculated from the linear region of calibration curves are shown in Table 2.

The Lineweaver–Burke (L–B) plots for the steady-state response of the various sensors built with the four ferrocenyl-siloxane polymers under the studied conditions are non-linear and concave down in the high glucose concentration range. In agreement with the model developed by Savinell and co-workers [11] a non-linear L–B plot is qualitatively indicative of a mass transport limitation, and taking into account that the operating potentials are sufficient to eliminate the electrolysis rate from consideration, this behaviour corresponds to a sensor in which the substrate diffusion becomes slower than other reaction steps [21]. This behaviour is consistent with the high values of the apparent Michaelis–Menten constants calculated from the slope of the L–B plots and the wide linear ranges observed in the calibration graphs (Table 2). Due to the diffusion limited process the values of $K_{M,app}$ obtained are larger than the intrinsic value and the linearly measurable range is greatly enhanced.

This behaviour is likely a consequence of the morphological characteristics of the films formed in the electrodeposition process. Fig. 5 displays the microstructure of films of polymers **1** and **3** electrochemically deposited on a platinum electrode shown by scanning electron microscopy (SEM). The SEM micrographs reveal that the film of polymer 3 (Fig. 5(b)) presents a slightly compact structure and some porosity can be detected. In contrast to this, the polymer **1** film is (Fig. 5(a)) constituted by cross-linked fibres. In the less permeable films of the polymers based on polyhedral silsesquioxanes the diffusional mobility of the enzyme is probably reduced and the increased diffusion resistance may complicate the ability of the immobilized GOx to interact with the analyte. Films of the cyclosiloxane-based polymers exhibit a more permeable structure that leads to facilitate the diffusion of glucose minimizing the transport barrier, which also contributes to the signal enhancement. Another reason for a better response may be a higher flexibility of the cyclotetrasiloxane backbone. When cyclosiloxane-based polymers **1** and **2** are compared there are not significant differences in the polymer structure and similar current responses were obtained at high potentials with the two redox mediators. The comparison of **3** and **4** leads to the same results. In addition to the fact that a variable fraction of the enzyme molecules in the films may not be properly wired by the redox polymers due to conformational factors, the flexibility might be an important factor in the ability to facilitate electron transfer from the reduced enzyme, because it provides a more efficient interaction between the polymeric relay systems and the enzyme molecules [16,22]. Moreover, the decrease of flexibility of the polymer backbone and a longer separation between ferrocenyl neighbours resulting in a decreased electron transfer efficiency between ferrocene moieties may also cause a lower biocatalytic response of the sensors based on silsesquioxane backbones compared to cyclotetrasiloxane polymers.

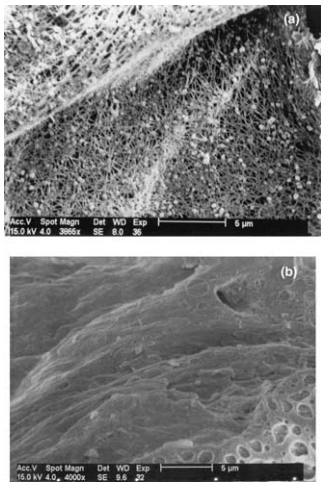


Fig. 5. SEM micrographs of a platinum disk electrode modified with films of (a) polymer **1** and (b) polymer **3**.

The sensor response was affected by the presence of ascorbic acid (AA) in the available potential range, producing a substantial increase in the steady-state response due to the direct or catalysed electro-oxidation of ascorbate ions. This interference is usually avoided by covering the electrode surface with Nafion. In fact, the signal due to the addition of 0.1 mM AA to a 1 mM glucose solution was practically cancelled. The additional nafion layers cause a decrease in the amperometric signal of ca. 40% related to the uncovered enzyme electrodes. However, the sensor response was essentially unaffected by the presence of oxygen indicating the great efficacy of the mediator.

Table 2

	1	**2**		**3**	**4**	
E (V vs SCE)	450	450	250	450	450	250
Linear range (up to)	50 mM	35 mM	35 mM	40 mM	70 mM	75 mM
Sensitivity (nA/mM)	130	230	17.4	40.6	35.8	7.5
Detection limit ($S/N=3$)	20 μM	40 μM	190 μM	150 μM	50 μM	1.01 mM
$K_{M,app}$	322 mM	242 mM		236 mM	216 mM	

3.2. Determination of glucose under aerobic conditions

The electrochemical reduction of oxygen and hydrogen peroxide by the siloxane-polymers-modified electrodes, was studied by rotated disk voltammetry. Electrodes modified with films of polymer **2** were found to catalyse the electrochemical reduction of oxygen and hydrogen peroxide. Fig. 6 shows the typical current potential curves for the reduction of oxygen and hydrogen peroxide at bare and polymer **2**-modified electrodes. As it can be seen, the reduction at a bare platinum electrode occurs at much more negative potentials than at a polymer-modified electrode which is clearly indicative of an appreciable electrocatalytic effect.

Furthermore, it is important to note that, at the polymer **2**-modified electrode, the voltammogram associated with the reduction of H_2O_2 is still shifted ca. 0.12 V to more positive potentials with respect to the voltammogram corresponding to the reduction of dissolved O_2 (in oxygen-saturated pH 7.0 aqueous phosphate buffer). A similar behaviour has been previously reported for the electrochemical reduction of hydrogen peroxide at TiO_2 and Prussian blue glassy carbon-modified electrodes [23,24]. In this case the electrocatalytic effect has been related to the geometrical positions and availability of high-spin Fe^{2+} ions.

Therefore, although polymer **2** appears to be an electrocatalyst for the reduction of both hydrogen peroxide and oxygen the voltammogram also shows that the dissolved oxygen does not influence the electrode response in the potential range from 200 to 100 mV (vs SCE). At these operating potentials the electrochemical reduction of the H_2O_2 produced by the oxidase-based reaction can be successfully used for direct measurement of glucose.

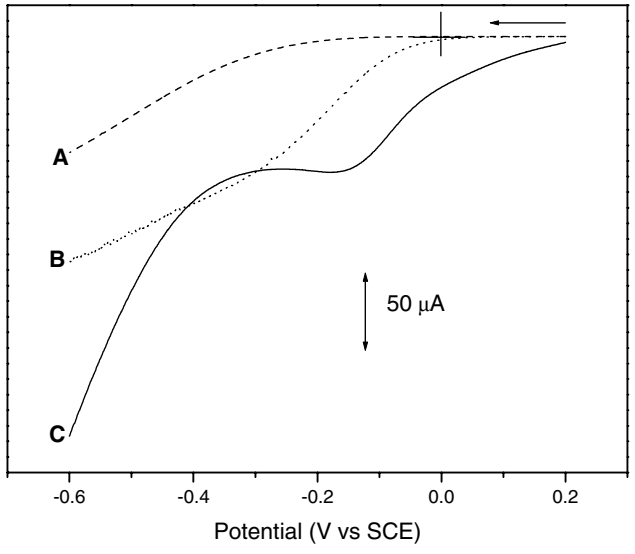


Fig. 6. Linear voltammograms with a rotated electrode (500 rpm) of (A) platinum bare, (B) GOx–polymer **2** ($\Gamma = 2.5\times10^{-9}$ mol ferrocene cm^{-2} thickness film) coated platinum electrode in O_2 saturated phosphate buffer (pH 7.0) solution, and (C) GOx–polymer **2** ($\Gamma = 2.5\times10^{-9}$ mol ferrocene cm^{-2} thickness film) coated platinum electrode in deaerated phosphate buffer (pH 7.0) solution with 10 mM H_2O_2. Scan rate: 5 mV s^{-1}.

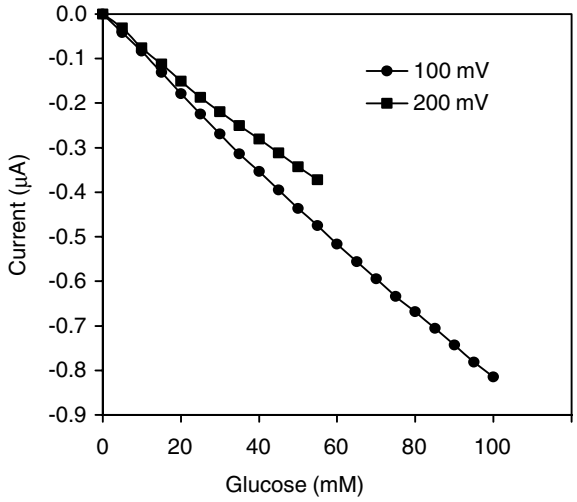


Fig. 7. Glucose calibration plots of the polymer **2**–enzyme sensors ($\Gamma \approx 2\times10^{-9}$ mol ferrocene cm^{-2} thickness films). Steady-state cathodic current measured as a function of the applied potential in O_2 saturated phosphate buffer (pH 7.0). Each value is the mean result of five electrodes.

The glucose response of the enzyme electrode was determined at several applied potentials (Fig. 7). As it is seen, the sensitivity of the biosensor is dependent on the applied potential and the cathodic current increases with decreasing applied potential, but 100 mV should be considered as the lowest potential suitable value since the cathodic reduction of molecular oxygen occurs at lower potentials interfering the glucose measurements. In all cases, the time elapsed to reach the steady state value of the current was less than 6 s. The linear ranges resulting from the calibration plots are up to 25 and 55 mM for a working potential of 200 and 100 mV with sensitivities (slopes of the linear portions) of 3.8 and 8.3 nA/mM, respectively.

At electrodes modified with electrodeposited films of polymers **3** and **4** the cathodic current is enhanced and the potential is shifted anodically by ca. 300 mV for the electrochemical reduction of oxygen, with respect to the bare electrode, indicating a lowering of the activation energy for this reaction. However, in this case, in contrast to electrodes modified with cyclotetrasiloxane polymers, the electrocatalytic reduction of oxygen occurs at less negative potentials than the reduction of hydrogen peroxide. Thus at applied potentials equal or less than 0.0 V the amperometric current detected corresponds to the reduction reaction of dissolved oxygen and it would mean less interference in the application of these electrodes as enzymatic sensors. By shifting the applied potential from 0.0 V to more negative potentials the reduction current of oxygen increases.

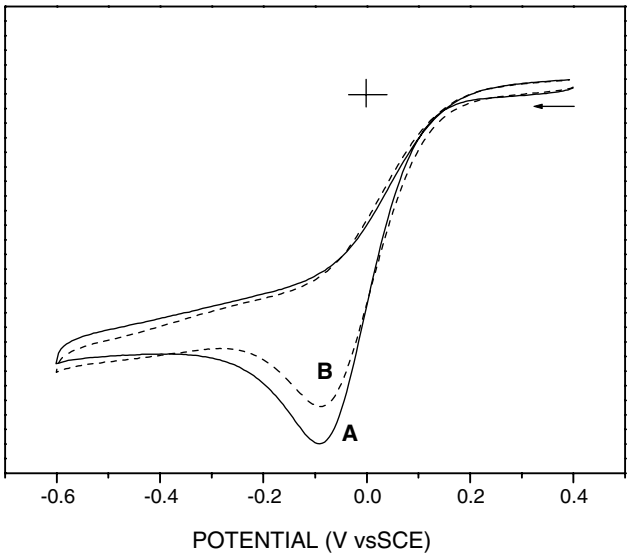


Fig. 8. Cyclic voltammograms of a platinum disk electrode modified with a film of polymer **4**–GOx ($\Gamma = 1.1\times10^{-9}$ mol ferrocene cm^{-2} thickness film) in O_2 saturated phosphate buffer (pH 7.0) in absence (A) and presence (B) of glucose 10 mM. Scan rate: 5 mV s^{-1}.

Due to their catalytic activity the silsesquioxane polymers/GOx-modified electrodes allows to carry out the determination of oxygen at a less negative potential and with higher sensitivity than the bare electrodes. Fig. 8 shows the cyclic voltammogram for a polymer **4**-GOx-modified electrode. As it can be seen the current due to oxygen reduction decreases substantially when glucose is added. Really, a typical response of **3** and **4** polymers-GOx-platinum electrodes to successive additions of glucose can be seen in Fig. 9(b) (inset), where the steady-state current due to oxygen reduction decreases as the concentration of glucose increases. The calibration curves (Fig. 9) plot the difference between the steady-state current measured at several operating potentials before and after each addition of glucose versus the glucose concentration for sensors prepared with polymers **3** and **4**. The analytical characteristics of these sensors applied to the cathodic determination of glucose via oxygen measure are listed in Table 3. These results reflect the variation of the electrocatalytic activity of the respective modified electrodes, discussed above and attributed to permethylferrocenyl groups.

The determination of glucose was not possible by measuring of cathodic current produced by the hydrogen peroxide or the decrease of the oxygen wave with polymer **1**-modified electrodes, since both processes appear overlapped at any potential below 250 mV (vs SCE) giving rise to a very poor sensitivity.

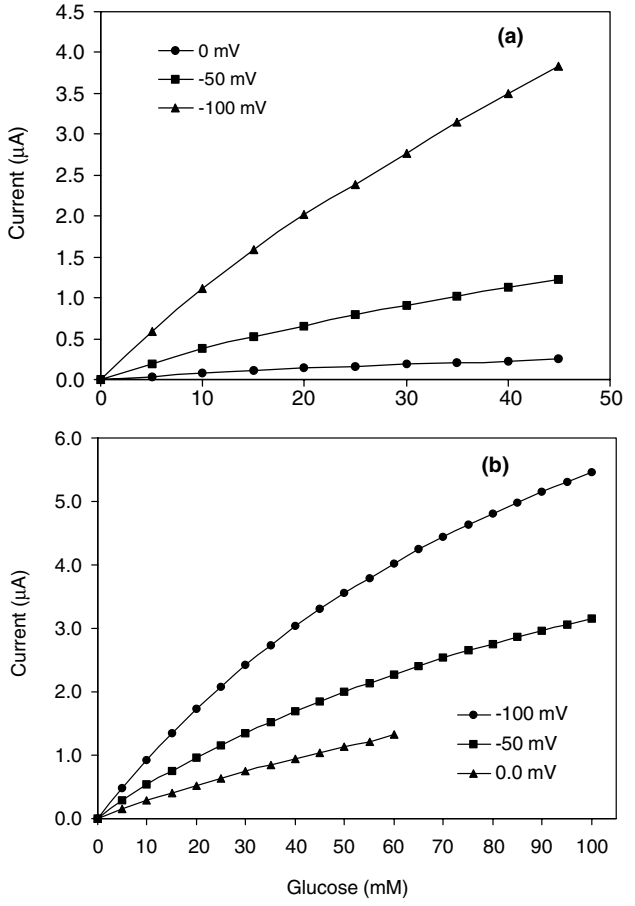


Fig. 9. Glucose calibration plots of a polymer **3**–enzyme sensor ($\Gamma = 2.3\times10^{-9}$ mol ferrocene cm^{-2} thickness film) (a) and a polymer **4**–enzyme sensor ($\Gamma = 2.1\times10^{-9}$ mol ferrocene cm^{-2} thickness film) (b). Steady-state cathodic current measured as a function of the applied potential in O_2 saturated phosphate buffer (pH 7.0). Each value is the mean result of five electrodes. Inset: typical reduction current responses of the electrodes to consecutive addition of 5 mM glucose aliquots to the electrolyte for polymer **4** sensor.

3.3. Stability and storage

The stability of the glucose sensor was evaluated by repetitive measurements of its response to 10 mM glucose

Table 3

	3			**4**		
E (mV vs SCE)	−100	−50	0.00	−100	−50	0.00
Linear range (up to)	15 mM	25 mM	20 mM	25 mM	25 mM	30 mM
Sensitivity (nA/mM)	111	38	7.7	92	54	29
Detection limit ($S/N = 3$)	160 μM	590 μM	850 μM	270 μM	400 μM	250 μM

within a period of 12 h. The response remained unchanged during this period and the relative standard deviation of 50 measurements was about 1%. Only a 25% decrease of their initial glucose response was observed for an electrode stored at 4 °C in air for 7 weeks. Interestingly, after cycling between 0.0 and 0.8 V, the conductivity is improved and the initial response is recovered.

4. Conclusions

A simple, versatile and easy procedure has been developed to prepare enzyme electrodes for sensor applications. An enzyme, GOx, is electrostatically immobilized into ferrocenyl and permethylferrocenyl polymers containing cyclic and polyhedral siloxanes as frameworks. The sensors behaviour is affected by structural characteristics of the polymers and the presence of methyl substituents.

The results obtained indicate that both anodic and cathodic operation modes may be used for glucose determination with these polymer-modified electrodes, so they can act as electrocatalyst in the oxidation of enzyme, the reduction of hydrogen peroxide generated and in the oxygen spent in the enzyme-catalysed reaction. In anaerobic conditions, the sensors based on polymers with cyclotetrasiloxane frameworks (**1** and **2**) display higher bioelectrocatalytic signals and sensitivities than silsesquioxanes (**3** and **4**).

In general, it is found that the permethylated polymers are more effective mediators and allow to use lower working potentials in the glucose measurements.

The biosensors developed in this work respond quickly to the substrate with a good linear response region and offered sensitivity and detection limits comparable or even better than other ferrocene-modified polymers mediated electrodes reported [11,25].

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