

Development of Redox-Mediated Oxysilane Sol–Gel Biosensors on Carbon-Film Electrode Substrates

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ABSTRACT: Three types of carbon-film electrodes, made from electrical resistors with 1.5-, 15-, and 140- Ω nominal resistances, were used to develop redox-mediated sol–gel biosensors, and the results were compared with those from previously investigated 2- Ω carbon-film electrodes. Two different redox mediators, copper hexacyanoferrate and poly(neutral red), were deposited onto the carbon-film electrodes, with the latter showing good electrochemical properties for electroanalytical applications, which were best on electrodes made from 15- Ω carbon-film resistors. It was not possible to deposit mediator film on the carbon-film resistor electrodes of 140- Ω nominal resistance. Glucose oxidase was immobilized on poly(-

neutral red) modified electrodes with sol–gel encapsulation from a mixture of 3-glycidoxypropyltrimethoxysilane and methyltrimethoxysilane precursors at a volume ratio of 2 : 1. The best sensor electrochemical properties and response to glucose in model solution were found with electrodes constructed from 15- Ω resistors, although the stability under the same conditions was better in the biosensors constructed with 2- Ω nominal resistance electrodes. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 112: 505–512, 2009

Key words: biological applications of polymers; electrochemistry; polysilanes; redox polymers

INTRODUCTION

The sol–gel encapsulation of bioactive substances, particularly for the development of biosensors, has been an attractive field for investigation during the last 2 decades.^{1–5} Silica-based organic–inorganic network composites are attractive materials because they combine, in a single solid, both the properties of a rigid three-dimensional porous silica network and the particular chemical reactivity of the organic components.^{4,5} Moreover, the pH, gelation time, transparency, and hydrophobicity can be adapted to encapsulate protein molecules such as enzymes.^{1–3} Usually, sol–gel is formed from oxysilane in two steps, hydrolysis and then condensation, with the latter being responsible for the building of the SiO₂ cage for each enzyme molecule capsule.³

Sol–gel enzyme or protein encapsulation has been applied in different electrochemical^{3,6–15} and optical^{16,17} biosensors. The most commonly used sol–gel precursors are trioxysilanes or tetraoxysilanes with

simple organic components, usually methyltrimethoxysilane (MTMOS)^{18,19} or tetraethoxysilane.^{19–23} Oxysilanes with complex organic groups have been used much less; however, 3-aminopropyltriethoxysilane^{14,15,19,24} and 3-glycidoxypropyltrimethoxysilane (GPMOS) were recently introduced for the first time for use in biosensors in our previous articles.^{14,15} Mixtures of sol–gel precursors have also been used for biosensor preparation to change the hydrophobic and hydrophilic properties and the nanopore structure of the resultant sol–gel to be more compatible with protein encapsulation.^{3,25} Such precursor mixtures, especially those containing substituted long organic chains mixed with short-chain oxysilanes, provide unique properties that cannot be achieved with only one precursor.⁴

Different electrode substrates have been used in the development of electrochemical biosensors based on sol–gel.^{3,4} However, the most used electrodes in the last 2 years have been based on carbon, for example, graphite,^{10,26} carbon ceramic,^{7,17} and screen-printed graphite.⁹

Several years ago, a new carbon-film electrode was introduced for use in electroanalysis.^{27–29} These carbon films are obtained by the coating of a ceramic substrate with a thin deposit of pyrolytic carbon^{28,29} or by the sputtering of graphite.³⁰ Electrodes made from carbon-film electrical resistors have the anisotropic nonporous properties of glassy carbon and a large potential window after pretreatment, are

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reproducible, and do not need to be polished before use.²⁸ Such electrodes are inexpensive and offer an easy way to develop oxidase enzyme biosensors but require a high overpotential to detect hydrogen peroxide, which reduces the sensor selectivity.³¹ To improve selectivity, these electrochemical biosensors have been modified with redox mediators, particularly copper¹⁴ or cobalt³² hexacyanoferrates; with methyl viologen,³³ and with poly(neutral red) (PNR).^{15,34,35} It was demonstrated with cyclic voltammetry (CV) and electrochemical impedance spectroscopy in the study cited in ref. 29 that electrodes made from carbon-film electrical resistors of 2- Ω resistance have better properties as biosensor electrode substrates than those of 20- Ω resistance due to a larger potential window at the lower resistance electrodes, so that these electrodes were used for application as electrode substrate for biosensors.³²⁻³⁵

In this study, three new types of carbon-film electrical resistors, with nominal resistance values of 1.5, 15, and 140 Ω , were investigated. Redox mediator films were deposited first and characterized electrochemically. The modified electrodes with the best electrochemical properties were then applied to the development of glucose biosensors with sol-gel encapsulated enzyme and compared with those made from previously investigated 2- Ω electrodes.

EXPERIMENTAL

Chemicals and solutions

GOPMOS, MTMOS, and neutral red (*N8,N8,3-trimethylphenazine-2,8-diamine*) were obtained from Aldrich (Taufkirchen, Germany); their structure is presented in Figure 1. Glucose oxidase (GOx) from *Aspergillus niger* (EC 1.1.3.4), anhydrous α -D-(+)-glucose crystals, and 5% Nafion solution in alcohols were obtained from Sigma (Germany). $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{K}_3\text{Fe}(\text{CN})_6$ were from Merck (Darmstadt, Germany).

The electrolyte solution, 0.1M phosphate buffered saline (PBS) with a pH of 7.0, was prepared from sodium dihydrogenphosphate and disodium hydrogenphosphate (Riedel-de-Haën, Seelze, Germany), to which 0.05M NaCl was added. Millipore (Billerica, MA) Milli-Q nanopure water (resistivity > 18 M Ω cm) was used for the preparation of all solutions. The experiments were performed at room temperature ($25 \pm 1^\circ\text{C}$).

Electrode preparation

The electrodes were made from carbon-film resistors with 1.5-, 15-, and 140- Ω nominal resistances, as described elsewhere.^{27,29} Electrodes were also prepared from 2- Ω nominal resistance resistors. Briefly,

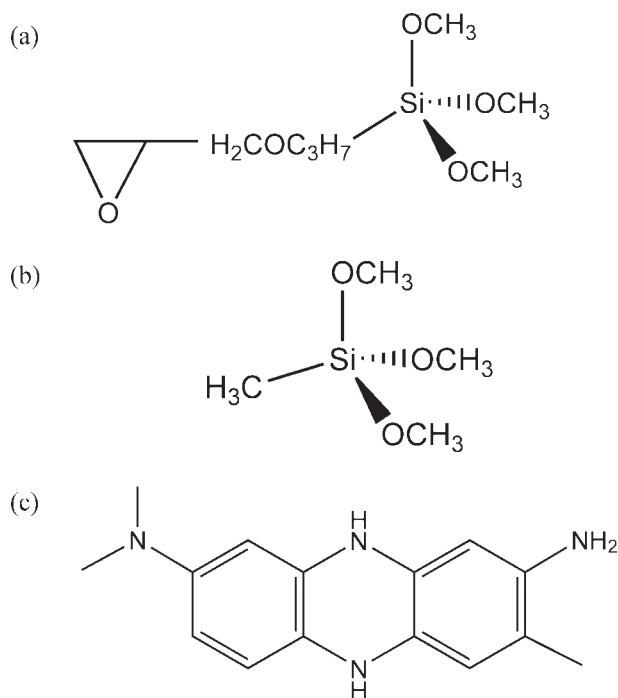


Figure 1 Structures of (a) oxysilane GOPMOS, (b) oxysilane MTMOS, and (c) neutral red monomer (*N8,N8,3-trimethylphenazine-2,8-diamine*).

the resistors were fabricated from ceramic cylinders with external diameters of 1.5 mm and lengths of 6.0 mm by the pyrolytic deposition of carbon in a rotating oven at 1100°C from methane in a flux of nitrogen. Tight-fitting metal caps with conducting wire were attached at each end. To make electrodes, one of the metal caps, plus conducting wire, was removed, and the other wire was sheathed in a plastic tube; the corresponding metal contact was covered with epoxy resin. In this way, a length of 4.0 mm was exposed, and the corresponding electrode geometric area was about 0.20 cm^2 .²⁸

Before use, the electrodes were electrochemically pretreated by potential cycling between 0.0 and 1.0 V versus a saturated calomel electrode (SCE) in a 0.1M KCl solution (before chemical deposition of a copper hexacyanoferrate (CuHCF) mediator) or between -1.0 and 1.0 V versus a SCE in a 0.1M KNO_3 solution (before electropolymerization of neutral red) for not less than five cycles, until stable cyclic voltammograms were obtained.

We chemically deposited CuHCF by electroless precipitation, immersing the electrodes for 50 min in a solution containing 10 mM CuCl_2 , 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$, and 100 mM KCl.³⁶ After this, the electrodes were dried in a hot air stream for 3 min and left for 24 h to stabilize.

PNR was prepared by electropolymerization from an aqueous solution of 1 mM neutral red in 0.05M phosphate buffer (pH 5.5) plus 0.1M KNO_3 , with the

applied potential cycled from -1.0 to 1.0 V 15 times at a potential sweep rate of 50 mV/s.¹⁵ They were also left to stabilize for 1 day in air at room temperature before use.

The sol-gel solution was prepared through the mixing of two oxysilanes with water at an optimized GOPMOS/MTMOS/H₂O ratio (μ L) of $130 : 70 : 600$.^{14,15} A volume of 2 μ L of $6M$ HCl solution was then added, and the mixtures obtained were stirred extensively for a few minutes and then sonicated for 15 min. Following this, the solutions were heated in a hot air stream ($\sim 70^\circ\text{C}$) to evaporate the alcohol formed during hydrolysis of the oxysilanes³⁷ until the solution lost 40% of its volume. It was left for 1 h at room temperature to cool and neutralized to pH 7.0, and 5% of glycerol was added to prevent the resulting xerogel from cracking. After this, 50 μ L of the solution was carefully mixed with 15 μ L of GOx (10%) solution in a $0.1M$ PBS solution at pH 7.0 and left for 1 h to equilibrate.

CuHCF- or PNR-coated carbon-film electrodes were then immersed in the sol-gel-enzyme solution for 5 min, removed, and left for sol-gel formation at 4°C for 3 days. Electrodes were stored at 4°C in buffer solution when not in use.

Methods and instruments

The three-electrode electrochemical cell contained a carbon-film or modified-carbon-film (with a redox mediator or redox mediator plus sol-gel enzyme layer) working electrode, a platinum foil as a counter electrode, and a SCE as a reference. Measurements were performed with a computer-controlled μ -Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Eco Chemie, Utrecht, Netherlands).

Examination of the electrode surfaces was done with a Wild M3 optical microscope (Zeiss, Wild Heerbrugg, Switzerland), with $25\times$ amplification.

RESULTS AND DISCUSSION

Characterization of the carbon-film resistor electrodes and redox mediator deposition

The resistor electrodes of 1.5 , 15 , and 140 Ω were previously characterized electrochemically with CV and electrochemical impedance spectroscopy and also by atomic force microscopy, confocal Raman spectroscopy, and X-ray diffraction.³⁸ The electrodes were pretreated in a buffer solution at a constant potential of 0.9 V versus a SCE, and the model redox system, $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$, was investigated before and after electrode pretreatment. Well-defined reversible peaks were obtained for the 1.5 - Ω carbon-film electrodes after pretreatment, which became

less reversible with increasing electrode resistance. Irreversible redox peaks with low currents were obtained for the carbon-film electrodes of 140 - Ω nominal resistance, which suggested that these resistors could not be used as supports for sensors or biosensors.

The different surface morphologies of the different types of resistor were evidenced by their reflectivities. Resistors with a low resistance of 1.5 Ω , which corresponded to thicker carbon films, had shiny surfaces observed visually and by optical microscope (not shown); they became progressively less light-reflecting as the resistance became higher. This was in agreement with the atomic force microscopy morphology images in ref. 38, which gave mean surface roughnesses of 88 nm for 1.5 - Ω and 270 nm for 140 - Ω resistors. Nevertheless, an increased roughness may provide a better surface for modification by a mediator and enzyme layer, so it was worth it to investigate all three new types of resistors and compare them to the previously used 2 - Ω resistors.

The redox mediator CuHCF was deposited chemically and PNR was deposited by the electropolymerization of neutral red in all three types of resistor electrodes; these were compared with the previously used carbon-film resistor electrodes of 2 Ω . The procedures used are described in the Experimental section.

CuHCF redox mediator

CuHCF films on carbon-film substrates prepared by chemical adsorption for 50 min were dried in a hot air stream for 3 min and left for 1 day in air at room temperature to stabilize. There was a clear visible difference between the resistor without mediator and that with deposited CuHCF: the CuHCF film was less shiny and had a darker color than the bare carbon-film electrode.

Cyclic voltammograms were recorded in $0.1M$ KCl [Fig. 2(a)] and $0.1M$ PBS at pH 7.0 [Fig. 2(b)] to investigate the electrochemical behavior of the redox mediator in the different types of resistor electrodes. Figure 2 shows that the voltammetric behavior depended on the electrolyte and the carbon-film resistor electrode.

In $0.1M$ KCl [Fig. 2(a)], the cyclic voltammogram showed a typical reversible redox couple of $\text{Fe}^{\text{II}}/\text{Fe}^{\text{III}}$ at 0.62 – 0.67 V versus the SCE, whereas peaks of $\text{Cu}^{\text{I}}/\text{Cu}^{\text{II}}$ (at ~ 0.5 V) were much smaller than those of $\text{Fe}^{\text{II}}/\text{Fe}^{\text{III}}$, which were suppressed and hardly visible. The redox behavior of CuHCF films was reported in detail elsewhere.³⁶ The highest peak current was obtained in carbon-film resistors with 15 - Ω nominal resistance. Carbon-film resistors of 1.5 Ω had slightly lower peak currents, whereas no CuHCF film was obtained at the resistors with the highest nominal resistance.

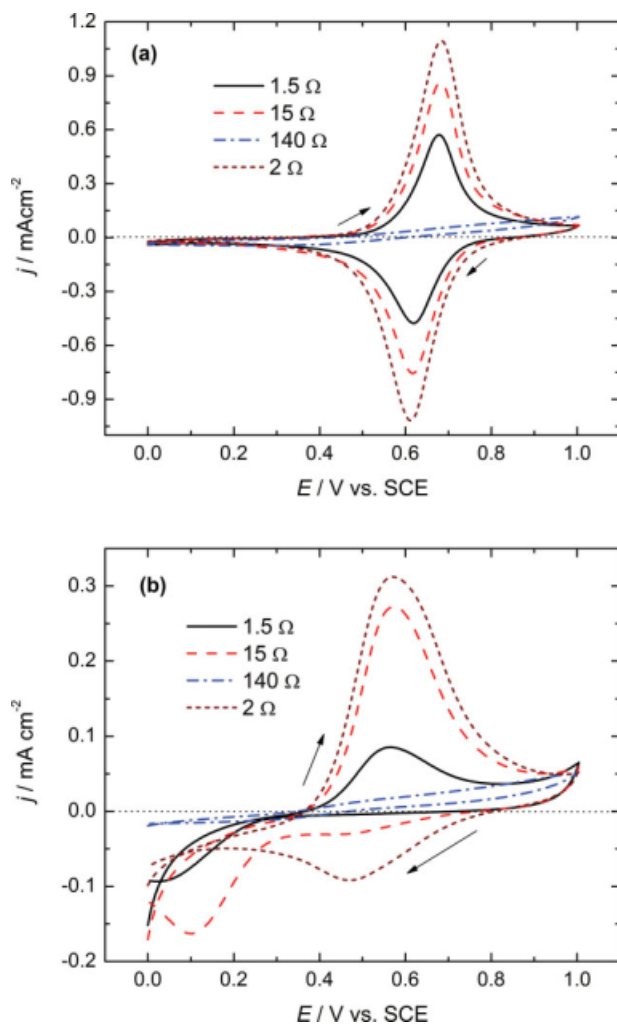


Figure 2 Cyclic voltammograms for (a) 0.1M KCl and (b) 0.1M PBS (pH 7.0) at CuHCF deposited chemically onto carbon-film electrodes (j = current density; E = potential). The potential sweep rate was 50 mV/s. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The cyclic voltammograms obtained with the same electrodes in PBS electrolyte solution at pH 7.0 [Fig. 2(b)] were different from those in KCl solution. In this case, the reduction peaks were smaller than those of oxidation, and the peak separation varied significantly between different carbon-film resistors, especially compared to the 2- Ω resistor electrodes. It was found in the study in ref. 35 that the electrochemical behavior of CuHCF in PBS solution depended on the potential window, and when the positive potential limit was 0.8 V versus the SCE, the peaks were well defined but much broader than in KCl. However, in carbon-film resistors of 1.5 and 15 Ω , no changes in the electrochemical behavior were seen when the positive potential limit was reduced to 0.8 V.

No peaks attributable to CuHCF were observed in the modified carbon-film electrodes made from

140- Ω resistors in either PBS or KCl electrolyte. These differences were perhaps due to the different morphologies of the carbon films and a different capability to adsorb CuHCF. It shows that CuHCF could not be easily used as a mediator in 140- Ω resistor electrodes.

These differences in the CVs in the different electrolytes depended on both the solution pH and the electrolyte, as described elsewhere.³⁶ The similarity of the electrochemical behavior at the CuHCF deposited on resistors of 1.5 and 15 Ω could be explained by the carbon-film structure. Confocal Raman spectroscopy and X-ray diffraction data showed that all types of carbon-film resistor (1.5, 2, 15, and 140 Ω) had a graphitic structure with sp^2 bonds. However, the most similar X-ray diffraction profile was found to be for resistors of 1.5 and 15 Ω , except the diffraction line at about 31° for 2θ , which was more intense for the resistor with the 15- Ω nominal resistance.³⁸

These differences could also have been due, but to a lesser extent, to different surface morphologies, as described previously.

PNR redox mediator

Neutral red was polymerized from a 1 mM aqueous solution of monomer in pH 5.5 0.05M phosphate buffer + 0.1M KNO_3 , the KNO_3 acting as a catalyst as well as an electrolyte.^{35,39} The PNR film also had a darker color than that the uncoated resistor and was less shiny, more uniform, and smoother than CuHCF.

Figure 3 shows the cyclic voltammograms of the polymerization of neutral red in the different carbon-film resistor electrodes. The tendency was very similar to that seen with CuHCF; that is, each resistor led to a slightly different electrochemical behavior. In all cases, the cyclic voltammograms had two redox couples and one irreversible oxidation peak with the same peak position. The first quasi-reversible oxidation peak at -0.45 V versus the SCE was attributed to overlapping monomer and polymer oxidation, the second irreversible oxidation wave at 0.2 V belonged to the PNR doping process, and the last oxidation wave at 0.8 V was due to the irreversible oxidation of the monomer, the result of which was a singly charged cation radical able to initiate polymerization.^{34,35,40,41}

The oxidation peak always increased in size with the number of potential scans, whereas the reduction peak increased until the third to fifth cycle and then began to shift to a more negative potential with a decrease in peak current because of some surface changes after polymer formation.^{35,41} However, the peak area became bigger with each cycle, which indicated film growth. The increase in the reduction peak current varied for different types of carbon-

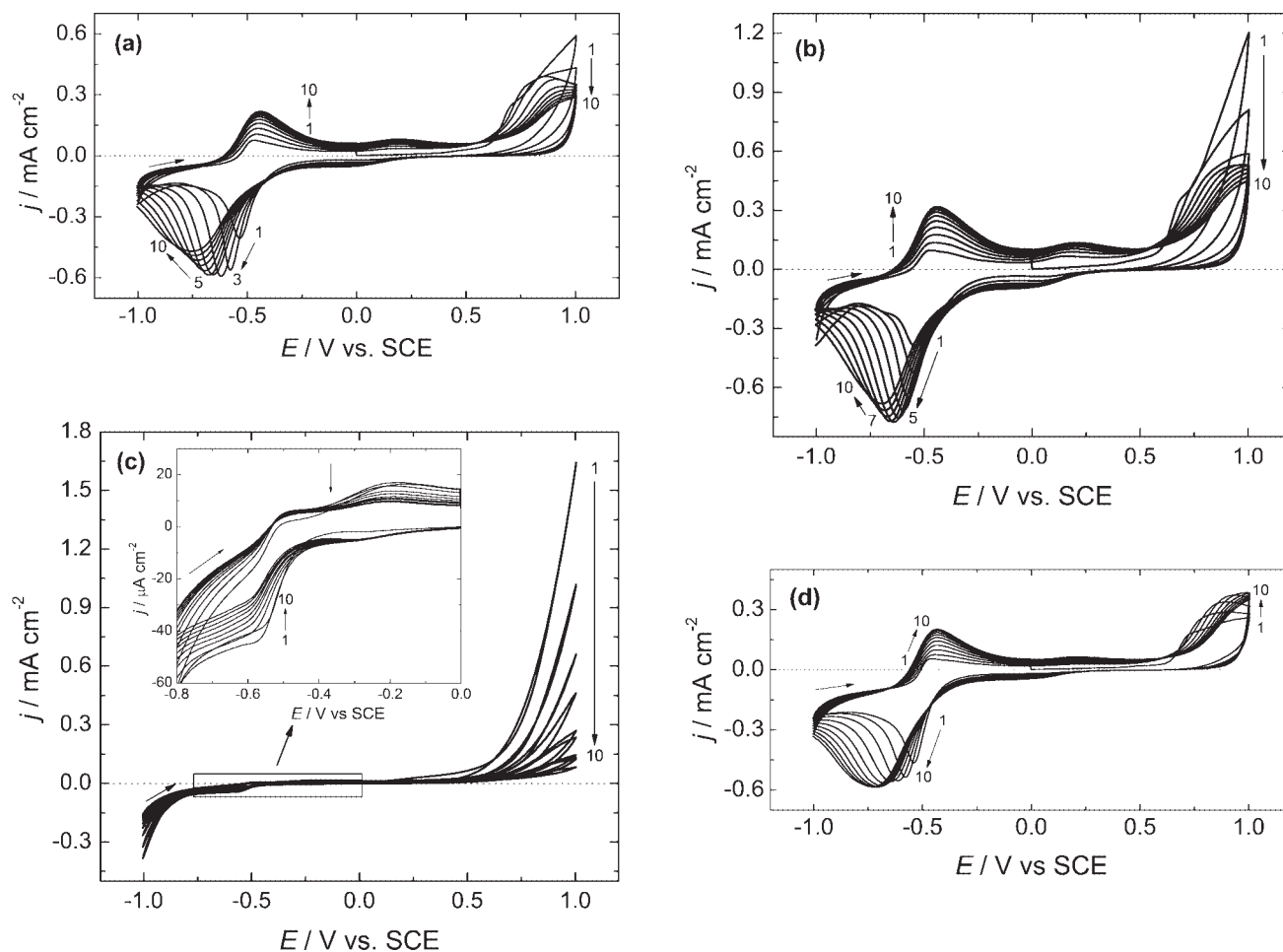


Figure 3 Polymerization of neutral red at different carbon-film resistor electrodes: (a) 1.5, (b) 15, (c) 140, and (d) 2 Ω (j = current density; E = potential). The solution composition and experimental conditions are listed in the Experimental section.

film electrodes. The 1.5- and 15- Ω electrodes showed a decrease in the reduction peak current after the third and fifth potential cycles, respectively [Fig. 3(a,b)]. Figure 3(d) shows that for 2- Ω electrodes, this peak increased significantly in height until the fourth cycle, after which, the increase was negligible, and the peak just became broader.

The monomer oxidation wave shifted to more positive potentials, and the current decreased with the number of cycles in the 1.5- and 15- Ω nominal resistance electrodes, as occurs in glassy carbon electrodes;^{35,40} in glassy carbon, the monomer oxidation potential was also found to vary with pH.⁴¹ The same tendency regarding the potential shift was found in carbon resistors of 2.0- Ω nominal resistance, but the current increased with the number of cycles.

In the case of the 140- Ω carbon-film resistor electrodes, as with CuHCF, the electrochemical behavior was completely different. As shown in Figure 3(c), redox peaks were much lower compared to other resistors and did not increase during potential cy-

cling: the oxidation peak remained constant, and the reduction peak decreased in size. Also, no monomer oxidation occurred: the faradaic current decreased with the number of scans as found for PNR polymerization from room-temperature ionic liquids.⁴² These factors indicated that the PNR film was not forming (as happened with CuHCF), and so these 140- Ω carbon-film resistors could not be used as electrode substrates for sensors or biosensors as predicted from the electrochemical characterization before modification.

PNR films deposited on different carbon-film electrode resistors were characterized by CV in 0.1M phosphate buffer at pH 7.0 (Fig. 4). The observed peak current of the neutral red/leuco (neutral red) redox peak in modified electrodes prepared from the different resistors can be described by the sequence: 15 > 1.5 \geq 2 \gg 140 Ω . Again, the sequence reflects the surface morphology and the surface structure obtained by X-ray diffraction.³⁸ Electrodes from 15- Ω resistors seemed to be the best for neutral red polymerization and good electrochemical properties of PNR.

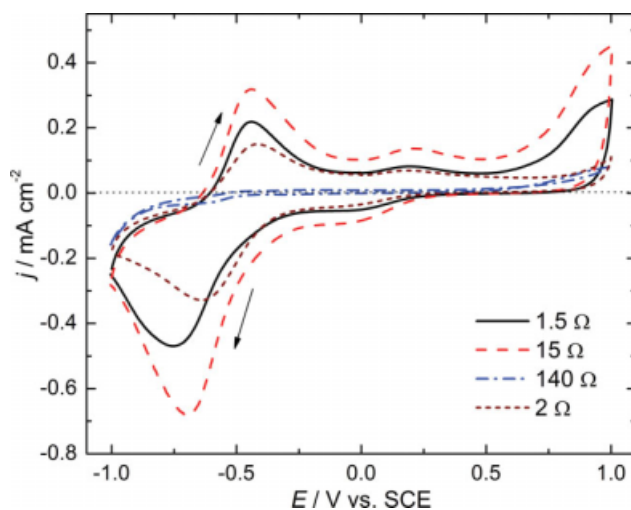


Figure 4 Cyclic voltammograms of PNR films deposited on different carbon-film resistors (j = current density; E = potential). The supporting electrolyte was a 0.1M phosphate buffer (pH 7.0); the potential sweep rate was 50 mV/s. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Sol-gel biosensors

Preparation

Sol-gel biosensors from CuHCF- or PNR-modified carbon-film electrodes were prepared by sol-gel enzyme encapsulation by a similar protocol to that reported in ref. 15 with a mixture of two sol-gel precursors, GOPMOS and MTMOS, without the addition of any alcohol. This precursor mixture was used to control pore size in the resulting sol-gel. As was shown before in ref. 15, the GOPMOS-based sol-gel had a lot of nanopores of diameters up to 50 nm^{15,43} whereas the sol-gel based on MTMOS had few, much larger, pores with enzyme leaching out through them. It was expected that the mixture of these precursors would form sol-gels with smaller pores. After following the procedure described in the Experimental section, we kept the electrodes in buffer at 4°C when not in use because, when it was kept under dry conditions, the xerogel cracked, and in buffer, it remained intact. Optical microscope examination showed that the sol-gel did not have a uniform thickness on the electrode surface because dip coating was used to apply sol-gel to the electrode.

Only carbon-film resistors of 1.5- and 15-Ω nominal resistance were used to prepare biosensors because that of 140 Ω was found not to be useful as a biosensor substrate, as shown in the previous section. Figure 5 presents an example of the cyclic voltammograms of PNR without and with sol-gel at the carbon-film resistor of 1.5-Ω nominal resistance. Sol-gel redox-mediated biosensors based on CuHCF and PNR as a mediator were examined in detail in

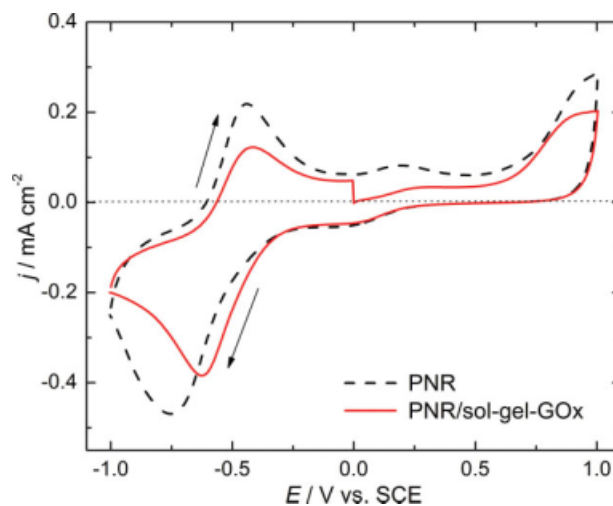


Figure 5 Cyclic voltammograms of a 1.5-Ω carbon-film resistor modified with PNR and PNR/sol-gel-GOx (for all other conditions, see Fig. 4; j = current density; E = potential). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

previous studies in 2-Ω electrodes,^{14,15,35,44} and the same tendency was obtained here in different carbon-film resistors, that is, some decrease in current corresponding to the PNR redox couple due to the sol-gel membrane layer.

Response to glucose analyte

As was suspected from previous work,³⁶ the response to glucose in CuHCF-sol-gel glucose biosensors had a rather low sensitivity, so only PNR-modified sol-gel biosensors were used for the analysis of glucose in

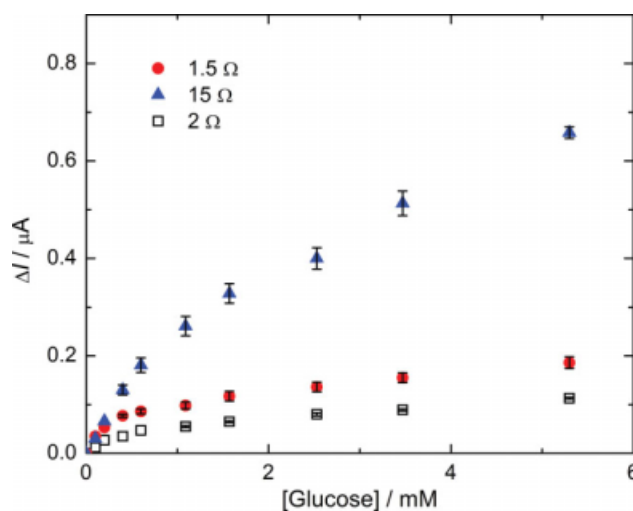


Figure 6 Calibration curves for different carbon-film resistors modified with PNR/sol-gel-GOx in 0.1M PBS (pH 7.0), at 0.25 V vs. SCE. The bars indicate the response error for three measurements. ΔI = current difference. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Parameters Calculated from the Calibration Curves ($n = 3$) in Different Carbon-Film Resistor Electrodes Modified with PNR as a Mediator and Sol-Gel Encapsulated GOx

Resistor nominal value (Ω)	Linear range (mM)	Slope (nA/mM)	Limit of detection (μ M)	K_M (mM)	R^2
1.5	0.05–0.40	181.5 ± 6.4	14.3	2.2	0.995
15	0.05–1.60	208.2 ± 7.3	15.2	4.3	0.993
2	0.05–0.60	74.7 ± 2.8	21.1	1.4	0.992

K_M = Michaelis Menten constant.

model solutions. The calibration curves are plotted in Figure 6. The response in carbon-film resistors of 1.5 and 2 Ω was rather similar under the same conditions, whereas in the electrodes from 15- Ω resistors, it was much higher. The standard deviation was similar in all resistance electrodes, and it varied from 3.5% (1.5- Ω electrode) to 3.8% (2- Ω electrode).

Data calculated from the calibration curves are presented in Table I. The widest linear range and highest sensitivity were obtained in carbon-film electrodes of 15- Ω nominal resistance, although the best limit of detection was found to be at the 1.5- Ω electrode. The sensitivity in the previously used 2- Ω resistance electrodes was lower. Nevertheless, the 2- Ω biosensors were stable for at least 1 month, with measurements once per day, and had 4–5 months of storage stability if they were kept in buffer at 4°C when not in use. The biosensors made from the new resistor electrodes (1.5 and 15 Ω) had a lower stability in use and slower kinetics, and the signal decreased constantly every day. This led us to the conclusion that, because of their different surface morphologies, the mediator and the sol-gel encapsulated enzyme were less well attached to the electrode than in the 2- Ω resistor electrodes.

CONCLUSIONS

Three different carbon-film resistor electrodes were investigated as possible biosensor electrode substrates and were prepared from resistors of 1.5, 15, and 140- Ω nominal resistances. The results were compared with those of previously investigated 2- Ω carbon-film electrodes. Two different redox mediators, CuHCF and PNR, were deposited onto the carbon-film electrodes, and of these, the PNR mediator showed good electrochemical properties after deposition, with the best properties in the 15- Ω carbon-film resistors. It was not possible to deposit the redox mediator on the carbon-film resistors of 140- Ω nominal resistance, so they could not be used for the redox-mediated biosensors described.

GOx enzyme was immobilized by sol-gel encapsulation. Sol-gel was prepared from the mixture of two precursors, GOPMOS and MTMOS, at a volume

ratio of 2 : 1. The best electrochemical properties and response to glucose in model solution were found in the enzyme-modified electrodes prepared from 15- Ω carbon-film resistors. However, the stability under the same conditions was not as good as that of the biosensors prepared in the 2- Ω nominal resistance electrodes. Glucose biosensors based on 1.5- and 2- Ω carbon-film electrodes had a similar performance, and the 15- Ω one showed an excellent amperometric response to the analyte.

References

- Gill, I.; Ballesteros, A. *J Am Chem Soc* 1998, 120, 8587.
- Gill, I. *Chem Mater* 2001, 13, 3404.
- Pierre, A. C. *Biocatal Biotransform* 2004, 22, 145.
- Walcarius, A.; Mandler, D.; Cox, J. A.; Collinson, M.; Lev, O. *J Mater Chem* 2005, 15, 3663.
- Pagliari, M.; Ciriminna, R.; Palmisano, G. *Chem Soc Rev* 2007, 36, 932.
- Anitha, S.; Mohan, S. V.; Reddy, S. J. *Biosens Bioelectron* 2004, 20, 848.
- Lei, C. X.; Hu, S. Q.; Gao, N.; Shen, G. L.; Yu, R. Q. *Bioelectrochemistry* 2004, 65, 33.
- Rodríguez Gutiérrez, J. A.; Petit Domínguez, M. D.; Pinilla Macías, J. M. *Anal Chim Acta* 2004, 524, 339.
- Roman, G.; Pappas, A. C.; Kovala-Demertzi, D.; Prodromidis, M. I. *Anal Chim Acta* 2004, 523, 201.
- Salimi, A.; Compton, R. G.; Hallaj, R. *Anal Biochem* 2004, 333, 49.
- Teh, H. F.; Yang, X.; Gong, H.; Tan, S. N. *Electroanalysis* 2004, 16, 7690.
- Xu, J. Z.; Zhang, Y.; Li, G. X.; Zhu, J. J. *Mater Sci Eng C* 2004, 24, 833.
- Tu, Y. F.; Di, J. W.; Chen, X. J. *J Sol-Gel Sci Technol* 2005, 33, 187.
- Pauliukaite, R.; Brett, C. M. A. *Electrochim Acta* 2005, 50, 4973.
- Pauliukaite, R.; Brett, C. M. A.; Chiorcea Paquim, A. M.; Oliveira Brett, A. M. *Electrochim Acta* 2006, 52, 1.
- Pastor, I.; Esquembre, R.; Micol, V.; Mallavia, R.; Reyes Mateo, C. *Anal Biochem* 2004, 334, 335.
- Wu, H. J.; Choi, M. M. F. *Anal Chim Acta* 2004, 514, 219.
- Lev, O.; Wu, Z.; Bharathi, S.; Glezer, V.; Modestov, A.; Gun, J.; Rabinovich, L.; Sampath, S. *Chem Mater* 1997, 9, 2354.
- Wang, B.; Zhang, J.; Cheng, G.; Dong, S. *Chem Commun* 2000, 21, 2123.
- Künzelmann, U.; Böttcher, H. *Sens Actuators B* 1997, 38, 222.
- Wang, B.; Dong, S. *J Electroanal Chem* 2000, 487, 45.
- Gupta, B. D.; Sharma, D. K. *Opt Commun* 1997, 140, 32.

23. Martinez-Pérez, D.; Ferrer, M. L.; Mateo, C. R. *Anal Biochem* 2003, 322, 238.
24. Couto, C. M.; Araújo, A. N.; Montenegro, M. C. B. S. M.; Rohwedder, J.; Raimundo, I.; Pasquini, C. *Talanta* 2002, 56, 997.
25. Jerónimo, P. C. A.; Araújo, A. N.; Montenegro, M. C. B. S. M. *Talanta* 2007, 72, 13.
26. Andreescu, S.; Fournier, D.; Marty, J. L. *Anal Lett* 2003, 36, 1865.
27. Brett, C. M. A.; Angnes, L.; Liess, H. D. *Electroanalysis* 2001, 13, 765.
28. Filipe, O. M. S.; Brett, C. M. A. *Talanta* 2003, 61, 643.
29. Filipe, O. M. S.; Brett, C. M. A. *Electroanalysis* 2004, 16, 994.
30. Compton, R. G.; Foord, J. S.; Marken, F. *Electroanalysis* 2003, 15, 1349.
31. Florescu, M.; Brett, C. M. A. *Talanta* 2005, 65, 306.
32. Florescu, M.; Brett, C. M. A. *Anal Lett* 2004, 37, 871.
33. Ghica, M. E.; Brett, C. M. A. *Anal Chim Acta* 2005, 532, 145.
34. Ghica, M. E.; Brett, C. M. A. *Electroanalysis* 2006, 18, 748.
35. Pauliukaite, R.; Ghica, M. E.; Barsan, M.; Brett, C. M. A. *J Solid State Electrochem* 2007, 11, 899.
36. Pauliukaite, R.; Florescu, M.; Brett, C. M. A. *J Solid State Electrochem* 2005, 9, 354.
37. Ferrer, M. L.; del Monte, F.; Levy, D. *Chem Mater* 2002, 14, 3619.
38. Gouveia-Caridade, C.; Soares, D. M.; Liess, H. D.; Brett, C. M. A. *Appl Surf Sci* 2008, 254, 6380.
39. Karyakin, A. A.; Bobrova, O. A.; Karyakina, E. E. *J Electroanal Chem* 1995, 399, 179.
40. Chen, S. M.; Lin, K. C. *J Electroanal Chem* 2001, 511, 101.
41. Pauliukaite, R.; Brett, C. M. A. *Electroanalysis* 2008, 20, 1275.
42. Pauliukaite, R.; Doherty, A. P.; Murnaghan, K. D.; Brett, C. M. A. *J Electroanal Chem* 2008, 616, 14.
43. Chiorcea Paquim, A. M.; Pauliukaite, R.; Brett, C. M. A.; Oliveira Brett, A. M. *Biosens Bioelectron* 2008, 24, 297.
44. Pauliukaite, R.; Schoenleber, M.; Vadgama, P.; Brett, C. M. A. *Anal Bioanal Chem* 2008, 390, 1121.