BUTTERWORTH HEINEMANN

Polymer Vol. 36 No. 14, pp. 2813–2818, 1995
Elsevier Science Ltd
Printed in Great Britain

The effect of structure on poly(quinone) systems for amperometric glucose sensors

Takahiko Kaku and Yoshiyuki Okamoto*
Department of Chemistry and Herman F. Mark Polymer Research Institute, Polytechnic University, 6 Metrotech Center, Brooklyn, NY 11201, USA

and Lyndon Charles, Wilfred Holness and Hiroko I. Karan
School of Science, Health and Technology, Medgar Evers College, City University of New York, Brooklyn, NY 11225, USA
(Received 12 September 1994)

2,5-Etheramino-*p*-benzoquinone **I**, poly(2,5-etheramino-*p*-benzoquinone)s **IIa–c** ($x = 3$, 6 and 33), poly(2,5-ω,ω'-hexamethylenediamino-*p*-benzoquinone) **IV** and poly(3,6-etheramino-*o*-benzoquinone) **V** ($x = 6$) (x is the number of propylene oxide repeating units) were synthesized and their efficiencies as electron relay systems for amperometric glucose sensors were investigated and compared. Cyclic voltammetry and steady state potential measurements showed that the quinone monomer and polymers efficiently mediated electron transfer from reduced glucose oxidase to a conventional carbon paste electrode. Sensors constructed with 2,5-etheramino-*p*-benzoquinone showed higher efficiency than those constructed with polymeric systems. Among the poly(2,5-etheramino-*p*-benzoquinone)s, the length of the propylene oxide chain between quinone moieties was found to be critical for electron relay efficiency. Sensors constructed from polymers with shorter propylene oxide chains between the quinone moieties demonstrated greater efficiency than those constructed from polymers with longer propylene oxide chains ($x = 3 > 6 \gg 33$). The sensor stability was investigated using sensors containing monomeric quinone compound **I**, non-crosslinked polymer **IIb** and crosslinked polymer **VI**. The sensors with crosslinked polymer were the most stable and maintained their efficiency over 50 days.

(Keywords: poly(quinone) systems; amperometric sensors; glucose)

INTRODUCTION

Amperometric glucose electrodes based on glucose oxidase (GO) undergo several chemical or electrochemical steps which produce a measurable current that is related to the glucose concentration. In the initial step, glucose converts the oxidized flavin adenine dinucleotide (FAD) centre of the enzyme into its reduced form ($FADH_2$). Because these redox centres are electrically insulated well within the enzyme molecule, direct electron transfer to the surface of a conventional electrode does not occur to any measurable degree. The most common method[1–4] of indirectly measuring the amount of glucose present relies on the natural enzymatic reaction

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \delta\text{-gluconolactone} + H_2O_2$$

where oxygen is the electron acceptor for glucose oxidase. The oxygen is reduced by the $FADH_2$ to hydrogen peroxide, which may then diffuse out of the enzyme and be detected electrochemically. The working potential of such a device is quite high (H_2O_2 is oxidized at approximately +0.7 V vs. the saturated calomel electrode) and the sensor is therefore very sensitive to many common electroactive species such as uric acid, ascorbic acid and acetaminophen. Hydrogen peroxide is also known to have a detrimental effect on glucose oxidase activity.

In recent years, systems have been developed which use a non-physiological redox couple to shuttle electrons between the $FADH_2$ and the electrode by the following mechanism

$$\beta\text{-D-glucose} + GO(FAD) \longrightarrow \delta\text{-gluconolactone} + GO(FADH_2)$$

$$GO(FADH_2) + 2M_{ox} \longrightarrow GO(FAD) + 2M_{red} + 2H^+$$

$$2M_{red} \longrightarrow 2M_{ox} + 2e^- \quad \text{(at the electrode)}$$

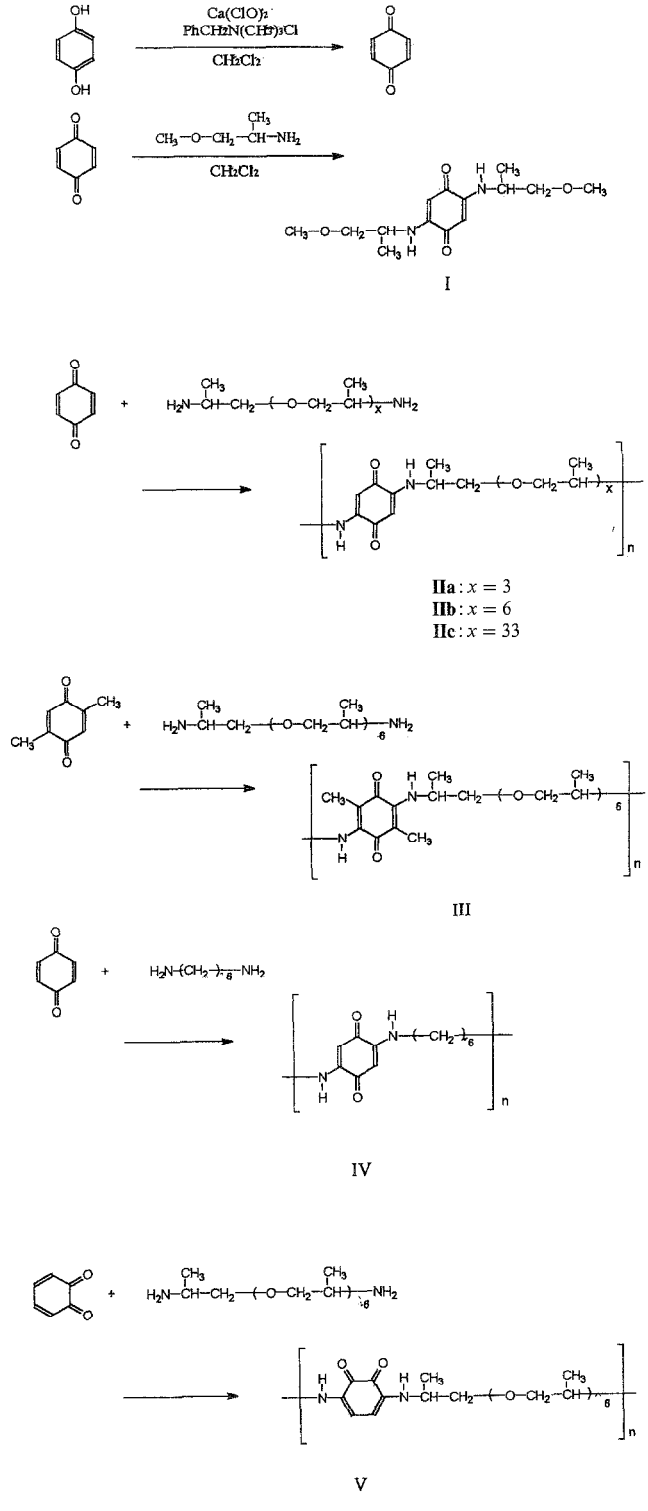
In this scheme, GO(FAD) represents the oxidized form of glucose oxidase and $GO(FADH_2)$ refers to the reduced form. The mediating species M_{ox}/M_{red} is assumed to be a one-electron couple. Sensors based on derivatives of the ferrocene/ferricinium redox couple[5–8] and on quinone derivatives[9–11] have been reported. In potential clinical applications, however, sensors based on electron-shuttling redox couples suffer an inherent drawback: the soluble, or partially soluble, mediating species can diffuse away from the electrode surface into

* To whom correspondence should be addressed

the bulk solution, which would preclude the use of these devices as implantable probes in clinical applications and restrict their use in long term *in situ* measurements (e.g. fermentation monitoring).

Recently, these studies have been extended in our laboratories to systems in which the mediating redox moieties such as ferrocene[12–16], 4,4-bipyridyl[17], tetrathiafulvalene[18] and quinone[19] were covalently attached to polymer backbones. These polymeric systems were reported to catalyse efficiently the electrooxidation of glucose. We have recently reported the synthesis of non-crosslinked and crosslinked poly(etheraminoquinone)s and their application as electron relay systems to amperometric glucose sensors[20]. Sensors containing these relay systems were stable and responded rapidly to low glucose concentrations (<0.1 mM) and reached steady state current responses in less than one minute.

In this study, we synthesized a series of poly(etheraminoquinone)s (i.e. poly(2,5-etheramino-*p*-benzoquinone)s **IIa–c** ($x = 3$, 6 and 33), poly(2,5-ω,ω'-hexamethylenediamino-*p*-benzoquinone) **IV** and poly(3,6-etheramino-*o*-benzoquinone) **V** ($x = 6$)) (x is the number of propylene oxide repeating units) and investigated their efficiencies as electron relay systems in amperometric glucose sensors. The effect of using *p*-quinone and *o*-quinone in the polymeric relay systems and the influence of the length of the propylene oxide chain between quinone moieties in poly(etheraminoquinone)s on the efficiency of the sensors were also studied. In addition, monomeric etheraminoquinone, 2,5-etheramino-*p*-benzoquinone **I** and the crosslinked polymer **VI** were prepared and the efficiency and stability of sensors constructed with monomeric and polymeric etheraminoquinone systems and non-crosslinked **IIb** and crosslinked **VI** polymer systems were compared.



Scheme 1 Syntheses of diamine-substituted quinone compounds

EXPERIMENTAL

Chemicals

Glucose oxidase (EC 1.1.3.4, type VII, from *Aspergillus niger*) and glucose were obtained from Sigma. Graphite powder, paraffin oil and calcium hypochlorite were obtained from Fluka. Hydroquinone, catechol, 2-amino-1-methoxypropane, hexamethylenediamine and benzyltrimethylammonium chloride (phase transfer agent) were purchased from Aldrich. Jeffamine and poly(oxypropylene)diamines were obtained from Texaco. Glucose solutions were prepared by dissolving appropriate amounts in a 0.1 M phosphate buffer/0.1 M KCl mixture (pH 7.0); the glucose was allowed to reach mutarotational equilibrium before use (ca. 24 h). All other chemicals were reagent grade and were used as received.

Synthesis of polymers

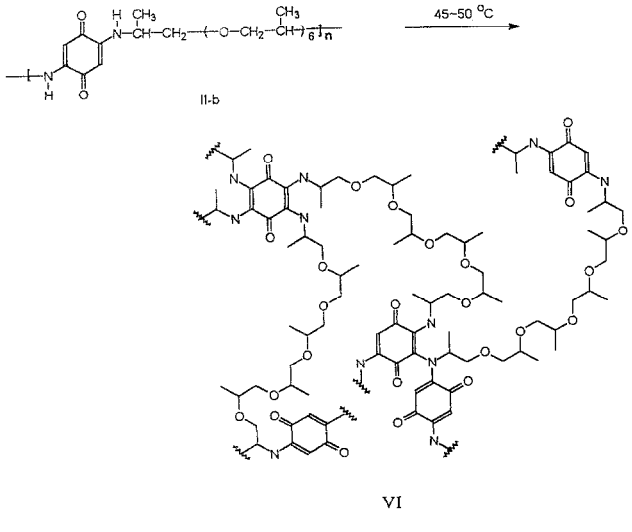
Diamine-substituted quinone compounds were prepared by the modified method reported by Nithianandam and Erhan[21]. 2,5-(1′-Methyl-2′-methoxyetheramino)quinone, poly(etheraminoquinone)s and poly(hexamethylenediaminoquinone)s were prepared according to *Scheme 1*. A typical synthetic process for the quinone polymers was as follows. Hydroquinone (4.4 g, 0.04 mol) was suspended in 50 ml of methylene chloride in a round-bottomed flask equipped with a reflux condenser and a mechanical stirrer. Hypochlorite (11.5 g, 0.08 mol; 65–70% of available chlorine) was added to the suspension with vigorous stirring, followed by addition of 0.18 g (2.5 mol% of the hydroquinone) of the phase transfer catalyst benzyltrimethylammonium chloride. The solution was refluxed for 2 h. When all of the hydroquinone was oxidized, the reaction mixture was cooled to ~20°C and 0.04 mol of Jeffamine (9.2 g of Jeffamine D-230, an ω,ω'-diamine with two to three units of propylene oxide) in 50 ml of methylene chloride was added slowly, to prevent an increase in reaction temperature. The reaction mixture was stirred vigorously for 4 h at 10–15°C. The reaction was followed

by u.v.–visible spectroscopy (Shimadzu UV 2100). The mixture was then filtered and the filtrate was evaporated under vacuum to remove the methylene chloride. The polymer obtained was purified by dissolving in a small amount of ethanol and then precipitating into cold water. This step was repeated a few times and the polymer was dried *in vacuo*. The molecular weight was estimated by gel permeation chromatography (g.p.c.) (Shimadzu LC-10AD with refractive index detector RID-6A, u.v. detector SPD-10A, chromatopack C-R4A and polystyrene columns 80M and 801). The structure of the polymer was characterized using i.r. (Shimadzu IR-60 and Nippon Bunko IR-810) and n.m.r. (JEOL GSX-400) spectra. The crosslinked polymer was prepared using poly(etheraminoquinone) **IIb**, which is partially soluble in water. The aqueous solution of polymer **IIb** was mixed with glucose oxidase and heated (45–50°C) in air for 24 h, yielding a crosslinked polymer **VI** insoluble in water and organic solvents (*Scheme 2*).

Electrode construction

The modified carbon paste electrode for construction of the glucose sensors was prepared by thoroughly mixing 100 mg of graphite powder and a measured amount of the quinone compound dissolved in either THF or methylene chloride; the molar amount of the quinone moiety was the same for all electrodes (10.8 μmol of quinone per 100 mg of graphite powder) unless indicated otherwise. The concentration of quinone was determined by steady state current response experiments with electrodes containing various concentrations of poly(etheraminoquinone) **IIb** (*Figure 1*). Sensor efficiency reached a maximum at ~10 μmol of quinone per 100 mg of graphite powder.

An aqueous solution of glucose oxidase (10 mg of GO per 1 ml of water) was added to the mixture of quinone polymer and graphite. Water was removed from the mixture and 20 μl of paraffin oil was blended in to form a paste. The paste was packed into the cavity of a commercial plastic electrode (BAS, W. Lafayette, IN) previously partially filled with unmodified graphite paste, leaving an approximately 2 mm deep well at the base of the electrode (3.0 mm outer diameter, 1.6 mm inner diameter). The resulting surface area of the carbon



Scheme 2 Synthesis and proposed structure of crosslinked poly(etheraminoquinone) **VI**

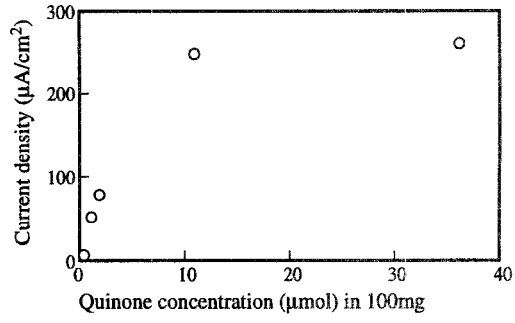


Figure 1 Steady state potential experiment for electrodes with different concentrations of poly(etheraminoquinone) **IIb** at an applied potential of +300 mV vs. Ag/AgCl at 25°C

paste electrode was 0.02 cm^2. The electrodes were generally stored under dry conditions at 5°C. Electrodes with crosslinked polymer were prepared by mixing the crosslinked polymer (10.8 μmol of quinone moiety) containing glucose oxidase (10 mg) with 100 mg of graphite powder, and the resulting mixture was blended with 20 μl of paraffin oil into a paste.

Electrochemical measurements

Cyclic voltammetry and stationary potential measurements were performed using a Princeton Applied Research polarographic analyser (model 174A), a Princeton Applied Research universal programmer (model 175) and a Princeton Applied Research potentiostat/galvanostat (model 363/173). All experiments were carried out in a conventional cell containing 0.1 M phosphate buffer with 0.1 M KCl (pH 7.0) at 23 ± 2°C. All experimental solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 10 min. In the stationary potential experiments, a gentle flow of nitrogen was also used to facilitate stirring. In addition to the modified carbon paste working electrode, an Ag/AgCl reference electrode and a platinum wire auxiliary electrode were employed. In the stationary state potential experiments, the background current was allowed to decay to a constant value before samples of a stock glucose solution were added to the buffer solution.

RESULTS AND DISCUSSION

In the syntheses of poly(etheraminoquinone)s, the condensation between quinone and diamine results in a colour change. The colour of the final products was dark brownish purple and the resulting polymers were generally soluble in organic solvents such as THF and methylene chloride. After condensation began, a new absorption at λ_{max} of 432 nm (with Jeffamines), possibly from the conjugation between nitrogen and quinone, was observed. I.r. spectra of the polymers showed absorption peaks for each substituent: 3225 cm^{-1} (N–H); 2960, 2920 and 2860 cm^{-1} (C–H, CH_3); 1645 cm^{-1} (C=O); 1580 cm^{-1} (C=C); 1460 cm^{-1} (C–H, CH_3); and 1248, 1215 and 1020 cm^{-1} (C–N). The infra-red spectrum of the crosslinked polymer **VI** became broad with a decrease in the N–H stretching band (around 3200 cm^{-1}) and the aromatic hydrogen band (around 3000 cm^{-1}). The average yield of polymers was ~ 45%. The polymers contained three, four or five repeating units. The proton n.m.r. spectra of the quinone **I** and the quinone polymers

were observed in $CDCl_3$ solution. The n.m.r. spectrum of the quinone **I** showed peaks at 0.94 (d, 6H, CH_3), 1.33 (d, 6H, OCH_3), 3.10 (d, 4H, CH_2), 3.31 (t, 2H, CH) and 6.53 ppm (s, 2H, Ar), and the polymer **IIc** showed peaks at 0.91 (CH_3), 2.90–3.15 (m, CH_2), 3.00–3.30 (m, CH) and 6.45 ppm (Ar).

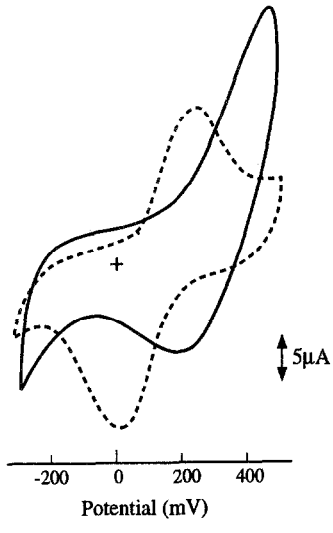


Figure 2 Cyclic voltammograms of a poly(etheraminoquinone) **IIb**-containing enzyme-modified carbon paste electrode with no glucose present (dashed line) and in the presence of 0.1 M glucose (solid line) (in 0.1 M phosphate buffer solution, vs. Ag/AgCl; sweep rate 5 mV s^{-1})

Table 1 Redox potentials of various quinone compounds in the solution state[a]

Compound	E_{pa} (mV)	E_{pc} (mV)
1,4-Benzoquinone	−60	[b]
2,5-Dimethyl-1,4-benzoquinone	−280	−190
I	−260	−430
IIb	−240	−480
III	−480	−550

[a] Cyclic voltammetry was carried out in CH_2Cl_2 with tetrabutylammonium perchlorate (0.1 M) as the electrolyte, using an Ag/AgCl reference electrode at 25°C. The working electrode was Pt. The sweep rate was 50 mV s^{-1}. E_{pa} and E_{pc} are the anodic and cathodic peak potential, respectively

[b] The reduction potential was not clear

Table 2 Redox potentials of quinone compounds and quinone polymers in the modified carbon paste electrode[a]

Substance	E_{pa} (mV)	E_{pc} (mV)	$E_{pa} - E_{pc}$ (mV)
1,4-Benzoquinone	110	−30	140
2,5-Dimethyl-1,4-benzoquinone	−20	−190	170
I	230	−40	270
IIa	225	10	215
IIb	220	10	210
IIc	320	[b]	
III	200	[b]	
IV	240	60	180
V	260	50	210
VI	285	10	275

[a] Cyclic voltammetry was carried out in 0.1 M phosphate buffer solution (0.1 M KCl, pH 7.0) vs. Ag/AgCl at 25°C. The sweep rate was 5 mV s^{-1}

[b] The reduction potential was not clear

Figure 2 shows typical cyclic voltammograms for a carbon paste electrode containing glucose oxidase and the poly(etheraminoquinone) **IIb**. With no glucose present, the voltammogram displays the typical anodic peak corresponding to the oxidation of the polymer-bound quinone moieties as well as the cathodic peak corresponding to the hydroquinone. Upon addition of glucose, the sensor undergoes a typical electrocatalytic reaction similar to those observed in sensors containing quinone-modified poly(siloxane)s[11,19].

Cyclic voltammetric data of the methylene chloride solutions and the carbon paste electrodes containing quinone monomers and various poly(etheramino-quinone)s are summarized in *Tables 1* and *2*, respectively. The oxidation potentials of the polymers were observed to be higher than that of 1,4-benzoquinone in the solid phase, but in the solution phase they were lower than that of 1,4-benzoquinone. The oxidation potentials of carbon paste electrodes containing quinone compounds were higher than those observed in the solution phase. This may be due to the rigidity of the solid system which retards the electron transfer process from enzyme to quinone as well as to the electrode surface. In solution, however, lower potentials are required because electron transfer is faster when quinones can move freely. In the solution phase, the oxidation of quinones with methyl or amino substituents occurred at lower oxidation potentials than for 1,4-benzoquinone, which was expected because of the electron-releasing properties of these substituents. However, this trend was not observed for carbon paste electrodes containing etheraminoquinone and poly(etheraminoquinone)s. It appears that in the solid matrix, various factors such as the flexibility of the polymer chains and polymer chain length influence the oxidation properties of the quinone.

The steady state response to glucose was measured at several applied potential values. The response to 21.6 mM glucose of the electrodes containing various poly(etheraminoquinone)s as electron transfer systems is shown in *Figure 3*. The anodic current increased with increasing positive applied potential and reached limiting values at approximately +400 mV vs. Ag/AgCl electrodes. The poly(etheraminoquinone) have shorter propylene oxide chains between quinone moieties was found to be a more efficient electron transfer system ($x = 3 > 6 \gg 33$). The electron transfer relay process between the FAD and $FADH_2$ centres of glucose oxidase at the carbon paste electrodes occurs through electron hopping among the redox groups. The chain length between quinone groups in poly(etheraminoquinone)s becomes longer when the number of repeating units (x) of propylene oxide increases. The number of repeating units of propylene oxide in polymer **IIa** ($x = 3$) is half that of **IIb** ($x = 6$) and approximately one-tenth that of **IIc** ($x = 33$) (*Figure 3*). Obviously, the quinone groups in the shorter propylene oxide chain are more favourably overlapping each other, resulting in an increased efficiency of electron transfer via these redox moieties. Similar measurements were carried out with carbon paste electrodes containing poly(etheramino-*p*-quinone) **IIb**, poly(hexamethylene-diamino-*p*-quinone) **IV** and poly(etheramino-*o*-quinone) **V** (*Figure 4*). Among these sensors, electrodes containing polymer **IIa** were found to be the most efficient.

Electrodes with the monomeric quinone compound **I**

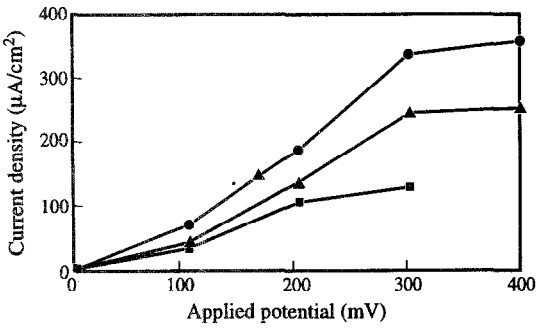


Figure 3 Steady state current response to 21.6 mM glucose of the poly(etheraminoquinone)s with various chain lengths of propylene oxide between quinones in quinone/glucose oxidase/carbon paste electrodes for several applied potentials. (●) **IIa**; (▲) **IV**; (■) **V**

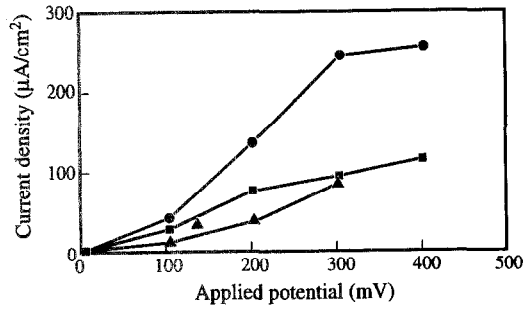


Figure 4 Steady state current response to 21.6 mM glucose of quinone polymer/glucose oxidase/carbon paste electrodes for several applied potentials. (●) **IIb**; (▲) **IV**; (■) **V**

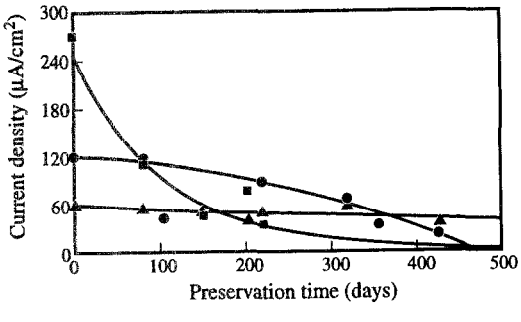


Figure 5 Stability test of enzyme-modified electrodes containing monomeric etheraminoquinone **I**, non-crosslinked polymer **IIb** and crosslinked polymer **VI**. Electrodes were tested twice a day, 2 h per test, in 0.1 M phosphate buffer solution (0.1 M KCl, pH 7.0) over several weeks. When the electrodes were not in use, they were stored in a dry chamber at 5°C. (●) Non-crosslinked polymer **IIb**; (▲) crosslinked polymer **VI**; (■) monomeric etheraminoquinone **I**

and the non-crosslinked **IIb** and crosslinked **VI** polymers were tested for their response to glucose twice a day, 2 h per test, in a buffer solution over several weeks, and were stored at 5°C in a dry chamber when they were not in use. The results are shown in *Figure 5*. The efficiency of electrodes containing monomeric compound **I** decreased rapidly with time and they showed almost no activity after 30 days. The efficiency of sensors with the non-crosslinked polymer **IIb** also decreased with time but not as rapidly as for the monomeric compound **I**. Electrodes with **IIb** appear to be more stable than those with the monomeric system **I**, but also lost most of their activity after 50 days. The efficiency of sensors with the crosslinked polymer **VI** was initially found to be about half that of non-crosslinked polymer electrodes; however, they maintained their efficiency over 50 days. These results may indicate that the enzyme (GO) was trapped in the crosslinked polymer matrix which prevented diffusion of GO from the electrode surface, contributing to the stability of the sensor even though the electron transfer efficiency decreased when the mobility of the quinone moieties was restricted by crosslinking.

CONCLUSIONS

2,5-Etheramino-*p*-benzoquinone and poly(etheramino-quinone)s were synthesized and their efficiencies as electron relay systems for amperometric glucose sensors investigated. The effect of crosslinking the polymers on the stability of the sensors was also studied. These studies show that poly(etheraminoquinone)s efficiently transfer electrons from reduced glucose oxidase to a conventional carbon paste electrode. The efficiency of sensors containing polymers with shorter propylene oxide chains between quinone moieties was higher than for sensors with polymers containing longer chains. By entrapping glucose oxidase in the crosslinked polymer matrix, the stability of the sensors was increased. The efficiency of the sensors decreased as quinone mobility was restricted by increasing the rigidity of the polymers. The oxidation potentials of poly(etheraminoquinone)s were higher in the solid state than in the solution phase owing to decreased mobility of the quinone moieties.

ACKNOWLEDGEMENT

L.C. and W.H. acknowledge an NSF-RCMS grant (HRD 9255203) for partial support of this research.

REFERENCES

1 Clark, L. C. in 'Biosensors: Fundamentals and Applications' (Eds A. P. F. Turner, I. Karube and C. S. Wilson), Oxford University Press, New York, 1987, Ch. 1
2 Clark, L. C. and Lyons, C. *Ann. N. Y. Acad. Sci.* 1962, **102**, 29
3 Jönsson, G. and Gorton, L. *Anal. Lett.* 1987, **20**, 839
4 Heider, G. H., Sass, S. V., Huang, K.-M., Yacynych, A. M. and Wieck, H. J. *Anal. Chem.* 1990, **62**, 1106
5 Cass, A. E. G., Davis, G., Francis, G. D., Hill, H. A. O., Aston, W. J., Higgins, I. J., Plotkin, E. V., Scott, L. D. L. and Turner, A. P. F. *Anal. Chem.* 1984, **56**, 667
6 Lange, M. A. and Chambers, J. Q. *Anal. Chim. Acta* 1985, **175**, 89
7 Iwakura, C., Kajiya, Y. and Yoneyama, H. *J. Chem. Soc., Chem. Commun.* 1988, 1019
8 Jönsson, G., Gorton, L. and Petterson, L. *Electroanalysis* 1989, **1**, 49
9 Ikeda, T., Shibata, T. and Senda, S. *J. Electroanal. Chem.* 1989, **261**, 351
10 Kulys, J. J. and Cenas, N. K. *Biochim. Biophys. Acta* 1983, **744**, 57
11 Inagaki, T., Lee, H. S., Hale, P. D., Skotheim, T. A. and Okamoto, Y. *Macromolecules* 1989, **22**, 4641
12 Hale, P. D., Inagaki, T., Karan, H. I., Okamoto, Y. and Skotheim, T. *J. Am. Chem. Soc.* 1989, **111**, 3482
13 Inagaki, T., Lee, H. S., Skotheim, T. A. and Okamoto, Y. *J. Chem. Soc., Chem. Commun.* 1989, 1181
14 Hale, P. D., Inagaki, T., Lee, H. S., Karan, H. I., Okamoto, Y. and Skotheim, T. A. *Anal. Chim. Acta* 1990, **228**, 31
15 Gorton, L., Karan, H. I., Hale, P. D., Inagaki, T., Okamoto, Y. and Skotheim, T. A. *Anal. Chim. Acta* 1990, **228**, 23

16 Hale, P. D., Inagaki, T., Lee, H. S., Skotheim, T. A., Karan, H. I. and Okamoto, Y. in 'Biosensor Technology: Fundamentals and Applications' (Eds R. P. Buck, W. E. Hatfield, M. Umana and E. F. Bowden), Marcel Dekker, New York, 1990, Ch. 14
17 Eddowes, M. J. and Hill, H. A. O. *J. Chem. Soc., Chem. Commun.* 1977, 71
18 Lee, H. S., Liu, L.-F., Hale, P. D. and Okamoto, Y. *Heteroatom Chem.* 1992, **3,** 303
19 Karan, H. I., Hale, P. D., Lan, H. L., Lee, H. S., Liu, L.-F., Skotheim, T. A. and Okamoto, Y. *Polym. Adv. Technol.* 1992, **2,** 229
20 Kaku, T., Karan, H. I. and Okamoto, Y. *Anal. Chem.* 1994, **66,** 1231.
21 Nithianandam, V. S. and Erhan, S. *Polymer* 1191, **32,** 1146