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# ELECTROPOLYMERIZATION OF HYDROXYBENZENE AND AMINOBENZENE ISOMERS ON PLATINUM ELECTRODES TO ASSEMBLE INTERFERENCE-FREE ELECTROCHEMICAL BIOSENSORS

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**Abstract**—Hydroxybenzene and aminobenzene isomers have been electropolymerized on platinum electrodes together with oxidase enzymes for preparation and analytical evaluation of electrochemical biosensors. A conventional three electrode system was assembled for the electropolymerization of these compounds which led to the formation of poly(hydroxybenzene) and poly(aminobenzene). Different scan rates and scan ranges of potential were investigated and selected according to the monomer used. The time of electropolymerization was from 7 min to 4 h. All the electrodes with the polymer films formed were tested for $H_2O_2$ by cyclic voltammetry. Compounds as ascorbate, urate and acetaminophen, which are a common source of interferences in electrochemical biosensors analysis, based on $H_2O_2$ detection, were tested. Results showed an oxidation peak of $H_2O_2$ at the bare electrode and in all the electropolymerized probes, whereas for the interfering compounds the oxidation peak at electropolymerized probes was considerably reduced or not observed. When the electropolymerization was carried out in presence of the enzyme glucose oxidase, again the probe did not respond to the interferents, but gave a current signal to glucose in the range of 0.05 to 5 mmol/L. Copyright © 1996 Elsevier Science Ltd

*Key words:* electrochemical transducers, electropolymerization, glucose oxidase, electrochemical biosensors, interferences.

## INTRODUCTION

Electropolymerization of organic compounds on electrochemical transducers is at present one of the most studied strategies to assemble electrochemical biosensors with peculiar characteristics which mainly lead to avoid interferences when applied in real matrix[1].

Conventional electrochemical biosensors, based on amperometric $H_2O_2$ detection on Pt electrodes, assembled with three membrane on the transducer surface, are, at present, the most used biosensors for practical applications[2]. This assembling is required when the analysis is performed in biological, food and environmental samples, which often contain bacteria and interferents as ascorbic acid and uric acid which are electroactive at the same applied potential for $H_2O_2$ detection. However, this assembling poses some limitations as the difficulty to avoid the interferences as acetaminophen[3, 4]. Also the thickness of the membranes increases the probe response time, which usually ranges from one to five minutes.

To improve the performances of these biosensors, some efforts in the past few years have been devoted to the formation of thin (few microns) films[1, 5] electropolymerized on the electrode surface, which could perform as the conventional probes, but with improved characteristics of selectivity and response time. Electropolymerization of organic compounds in presence of oxidase enzymes on Pt electrodes, or on platinized, reticulated and vitreous carbon electrodes for assembling of electrochemical biosensors with characteristics of improved selectivity and response time has been reported[1, 5]. Also analytical applications of these probes have been proposed during the past few years[6].

In this paper we investigate the electropolymerization of hydroxybenzene and aminobenzene isomers on Pt electrodes by cyclic voltammetry in absence and in presence of the enzyme glucose oxidase.

The novelty of our work consists in the study of new compounds as phenol and aniline derivatives which lead to the construction of a new glucose bio-

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Table 1. Experimental conditions of electropolymerization of different monomers

| | Scanning rate mV/s | Scanning potential Volts | Electropolymerization time/hours |
|---|---|---|---|
| Diaminobenzene monomers | 50 | +0.6/+0.8 | 0.1/0.2 |
| Hydroxybenzene monomers | 2/20 | 0.0/+0.8 | 4/6 |
| Mixed monomers | 2 | 0.0/+0.8 | 4 |

sensor with improved characteristics in terms of selectivity and response time.

These parameters have been extensively studied in the literature[1(a), 1(d)] using different monomers electropolymerized together with the enzyme GOD or using similar monomers[1b] with the enzyme glucose oxidase covalently immobilized on the polymer surface. Electropolymerized films on platinized, reticulated, and vitreous carbon electrodes have been reported by Sasso *et al.* and Geise *et al.*[1, 7]. In this case the enzyme glucose oxidase was covalently immobilized on the electrode surface; then, the electropolymerization was performed using hydroxybenzene and aminobenzene isomers.

In our work the enzyme was copolymerized together with the monomer in solution. This resulted in a growing film containing the enzyme electrochemically immobilized.

The advance in using a copolymerization in the presence of the enzyme is the easier assembling of the probe, and faster response time. The immobilized enzyme is entrapped in the polymer structure, so it is available for the substrate (glucose) giving an immediate catalytic reaction with consequent production of $H_2O_2$ which diffuses through the polymer and produces a current signal more rapidly than a sensor assembled with the glucose oxidase immobilized in the inner side of a blocking membrane[1(b), 1(c)].

The response of the probes prepared in the absence and in the presence of the enzyme was investigated by cyclic voltammetry of $H_2O_2$, ascorbic acid, uric acid and acetaminophen.

Parameters as the monomer amount and/or the ratio of two different mixed monomers, the scan rate, the electropolymerization time and the current signal/noise ratio of the compound tested were investigated. Then the best electrochemical enzyme glucose probes were prepared and tested for amperometric measurements of glucose, ascorbic acid, uric acid and acetaminophen.

Probe stability, reproducibility and response time is also reported.

## EXPERIMENTAL

### *Apparatus*

An AMEL polarographic system model 433A (AMEL, Milan, Italy) was used for voltammetric studies. Amperometric measurements were carried out with a 559 HPLC Detector from AMEL, Milan, Italy. Current was recorded using a LKB 2210 strip chart recorder from Delft (Holland).

The Pt electrodes, surface nominal area 0.071 $cm^2$, were from AMEL (Milan, Italy).

### *Materials*

1,2-Diaminobenzene (1,2-DAB) 99%, 1,3-diaminobenzene (1,3-DAB) 99%, 1,4-diaminobenzene (1,4-DAB) 99%, resorcinol (99%) and pyrogallol (99%) were purchased from FLUKA and used as received.

Catechol (98%) (FLUKA) was crystallized from $CHCl_3$ and its m.p. was 101–103°C (lit.[8] m.p. 101–104°C).

$\beta$D + -glucose, acetaminophen, ascorbic acid and uric acid were from SIGMA. Glucose oxidase (GOD), type VII from *Aspergillus Niger*, 137 000 units/g, was purchased from SIGMA and used as received.

Glucose solutions were allowed to mutarotate overnight at room temperature before use. All the other chemicals were analytical grade.

Phosphate buffer (0.1 mol/L, pH 6.5; or 0.1 mol/L pH 7.2) was prepared with distilled-deionized water using sodium dihydrogen phosphate dihydrate (FLUKA Biochemica Micro Select for molecular biology). The pH was adjusted to 6.5 or 7.2 with pellets of sodium hydroxide.

Hydrogen peroxide solutions were prepared in phosphate buffer pH 7.2 by appropriate dilution of a 30% solution (Merck).

### *Procedure*

The bare electrode used for the preparation of the sensors consisted of a Pt disk (2 mm diameter) sealed in a tygon tube. The working electrode surfaces were polished with alumina powder ($Al_2O_3$, Buehler) of various particle sizes (1, 0.3 and 0.05 $\mu$m) before use. After a careful rinsing with distilled water, the electrodes were pre-treated by potential cycling in 0.5 mol/L $H_2SO_4$ from −0.2 V to +1.2 V (vs *sce*) at a scan rate of 20 mV/s, until no changes were observed in the cyclic voltammograms[9].

### *Electropolymerization in the absence of enzyme*

For the electropolymerization on platinum electrodes the following monomers have been used: 1,2-DAB, 1,3-DAB, 1,4-DAB, resorcinol, catechol, pyrogallol.

Aminobenzene and hydroxybenzene isomers were electropolymerized using a 5 mmol/L solution of monomers in sodium phosphate buffer (0.1 mol/L, pH 6.5) which was deareated for 30 min. with Argon

Table 2. Voltammetric data relative to polymer/Pt electrodes in the presence of $H_2O_2$ and interferents (scan rate 50 mV/s). Monomers used are diaminobenzene isomers, diaminobenzene mixed with dihydroxy benzene isomers and hydroxybenzene isomers

| | | $ip_a$ ($\mu$A) at covered electrode | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $ip_a$ ($\mu$A) at bare electrode | Poly (1,2-DAB) | Poly (1,3-DAB) | Poly (1,4-DAB) | Poly (1,2-DAB-1,3 DAB) | Poly (1,3-DAB/ CAT) | Poly (1,2-DAB/ RES) | Poly (1,2-DAB/ CAT) | Poly (1,3-DAB/ RES) | Poly (1,2-DAB-1,4 DAB) | Poly (RES) | Poly (CAT) | Poly (PYR) | Poly (RES/ CAT) |
| $H_2O_2$ | 8.00 | 7.65 | 8.01 | 7.99 | 7.97 | 7.95 | 8.01 | 8.03 | 8.04 | 8.00 | 8.02 | 8.01 | 7.97 | 7.63 |
| 5 mM | | (−4%) | (−) | (−) | (−0.3%) | (1%) | (−) | (−) | (−) | (−) | (−) | (−) | (−0.3%) | (−5%) |
| Interferents | | | | | | | | | | | | | | |
| Uric Acid 4 mM | 8.80 | 1.05 (−83%) | 1.42 (−84%) | 0.29 (−97%) | 0.85 (−90%) | 0.29 (−97%) | 0.78 (−89%) | 1.38 (−84%) | 1.43 (−80%) | 1.22 (−86%) | 0.86 (−90%) | 0.71 (−92%) | 0.85 (−90%) | 4.85 (−45%) |
| Ascorbic Acid 4 mM | 7.14 | 1.02 (−86%) | 0.87 (−88%) | 0.85 (−88%) | 1.21 (−83%) | 0.91 (−87%) | 1.25 (−82%) | 1.27 (−82%) | 1.15 (−84%) | 1.22 (−86%) | 0.78 (−89%) | 1.04 (−89%) | 1.43 (−80%) | 1.14 (−84%) |
| Acetaminophen 4 mM | 6.85 | 0.88 (−87%) | 1.28 (−81%) | 1.43 (−79%) | 0.85 (−87%) | 0.28 (−98%) | 1.28 (−82%) | 1.32 (−81%) | 1.27 (−81%) | 1.14 (−84%) | 1.25 (−82%) | 0.98 (−86%) | 6.82 (−0.4%) | 3.42 (−51%) |

(99% Air Liquide). For the preparation of films containing two monomers, the concentration was 2.5 mmol/L of each monomer.

The electrochemical cell consisted in a Pt working electrode, a *sce* reference electrode and a Pt counter electrode. The solution was a phosphate buffer 0.1 mol/L pH 6.5 ($I$ = 0.1 mol/L).

Mixed polymers films were obtained by combination of 1,2-DAB/1,3-DAB; 1,3-DAB/catechol; resorcinol/catechol. The monomer concentration was 5 mmol/L. The technique used was the cyclic voltammetry.

Table 1 illustrates the working conditions used for the electropolymerization of the above monomers. The potential was continuously cycled until a minimum value of current which remained constant after further cycling was observed. This indicates that the electrode surface was completely covered by the polymer. The time of the maximum current decrease is slightly different from the electropolymerization time reported in Table 1, varying from 30 s to 30 min to obtain a compact film, depending from the electropolymerization rate (see Table 1).

The electropolymerization time depends on the monomer nature. The response to the interferents was monitored weekly for about one month and normalized to the initial response.

*Electropolymerization in the presence of the enzyme*

The experimental conditions of the electropolymerization in the presence of the enzyme were the same of those carried out in absence of the enzyme (Table 1). The amount of enzyme used was 500 U/ml.

*Glucose analysis*

The electrodes posed at +0.60 V applied potential were equilibrated in phosphate buffer solution until a constant current baseline was reached. Then aliquots of glucose were injected and the current variations recorded.

## RESULTS AND DISCUSSION

*Electropolymerization studies*

Platinum electrodes were used for all of the studies performed because of their excellent properties in $H_2O_2$ detection[1(b)].

Results of the electropolymerization studies and the response of the probes to hydrogen peroxide and ascorbic acid, uric acid and acetaminophen are reported in Table 2. These data are the mean of four determinations and the relative standard deviation ranged from 0.5 to 2%.

Figure 1 shows a cyclic voltammogram obtained by electropolymerization of 1,3-DAB at a scan rate of 50 mV/s. It is interesting to note the rapid decrease of the peak current after few cycles. Values are recorded as current peak ($i_{pa}$) in $\mu$A. The bare electrode gave a current peak of 8.00 $\mu$A for hydrogen peroxide 5 mmol/L and a current peak of 8.80, 7.14 and 6.85 $\mu$A respectively for uric acid, ascorbic acid and acetaminophen at a concentration of 4 mmol/L. When the probe was tested with the electropolymerized films the current peak of $H_2O_2$ remained almost unchanged while for the interferents a consistent decrease of current peak was observed. During the electropolymerization the potential was continuously cycled until a minimum constant value of current was observed.

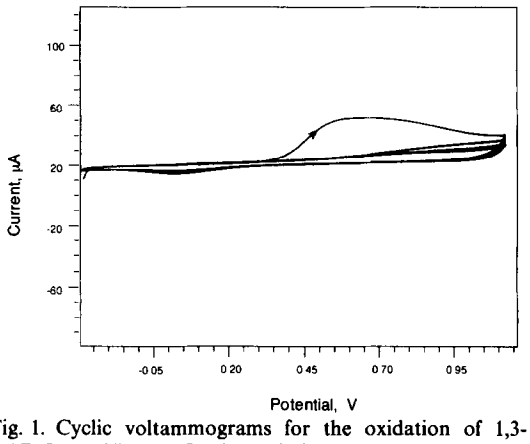


Fig. 1. Cyclic voltammograms for the oxidation of 1,3-DAB 5 mmol/L at a Pt electrode in a deoxygenated phosphate buffer (pH 7.2 $I$ = 0.1). Scan rate 50 mV/s.

The current decrease was between 80–90% of the oxidative peak observed for the monomer. This is an indirect demonstration of the formation of a compact film on the electrode surface.

The efficiency of the polymeric film was investigated by the evaluation of the decrease of the oxidation current of the interferents tested.

In this case the concentration of the interferents used is at least ten times higher than that present in physiological samples (physiological concentrations are 0.05 mmol/L for uric acid and 0.11 mmol/L for ascorbic acid).

In general, except for the poly(pyrogallol) and poly(resorcinol/catechol) a peak current decrease between 80–90% was observed. The most efficient polymer to prevent the interference of the uric acid was the poly(1,3-DAB/catechol) which gave a current decrease of 97%. For the ascorbic acid the poly(resorcinol) gave a current decrease of 89% and for the acetaminophen the highest current decrease was observed with poly(1,3-DAB/catechol).

The new polymer studied poly(pyrogallol) showed a good current decrease for uric and ascorbic acid (90% and 80% respectively) but was the less efficient towards the acetaminophen whose current peak signal remained almost unchanged.

Normally, when using cyclic voltammetry for electropolymerization, the current reaches a minimum after a time varying from 30 s to 30 min, depending on the electropolymerization rate. While this time is long enough to prevent interferences initially, it does not result in a film with long term stability. Generally, we have carried out the electropolymerization for a longer time, and electrodes prepared with times reported in Table 1 show a negligible response to the interferents at least for a month. This suggests[1(b), 1(c)] that after initial coverage of the electrode the

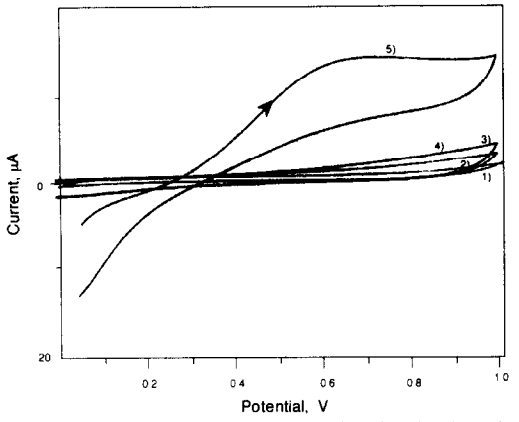


Fig. 2. Cyclic voltammograms at poly(1,3-DAB)/Pt electrode in the presence of: (1) buffer solution; (2 ascorbic acid 4 mmol/L; (3) acetaminophen 4 mmol/L; (4) uric acid 4 mmol/L; (5) $H_2O_2$ 5 mmol/L (in a deoxygenated phosphate buffer (pH 7.2 $I = 0.1$). Scan rate 50 mV/s.

films appears to undergo a rearrangement in structure that increases their porosity. When electropolymerizing for a longer time, new polymer is deposited in pores opened by this rearrangement.

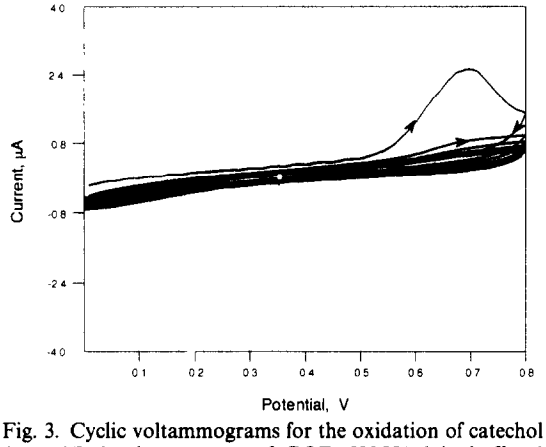


Fig. 3. Cyclic voltammograms for the oxidation of catechol 5 mmol/L in the presence of GOD 500 U/ml in buffered deareated solution (pH 7.2 $I = 0.1$). Scan rate 2 mV/s.

*Probe stability*

As reported previously the choice of the $H_2O_2$ as a testing compound for these probes lies on the fact that many oxidase enzymes catalyze the oxidation of their substrates in accordance with the following reaction:

$$\text{Substrate} + O_2 + H_2O \xrightarrow[\text{enzyme}]{\text{Oxidase}} \text{Product} + H_2O_2.$$

So the detection of $H_2O_2$ can be regarded as a signal proportional to the concentration of the substrate investigated.

Figure 2 shows the voltammograms of $H_2O_2$ 10 mmol/L, on 1,3-DAB electropolymerized probe, compared with the ascorbic acid, uric acid and acetaminophen. It is evident how the polymer slows down the diffusion of interferents while the $H_2O_2$ goes through the polymer structure giving a current of 14.29 μA. Since the $H_2O_2$ produced by the enzymatic reaction crosses the polymer structure to reach the electrode, we investigated the effect of a selected concentration of $H_2O_2$ on the polymer films. We stored the electropolymerized film electrodes in buffer and in buffer plus $H_2O_2$ 5 mmol/L. After one month the electrodes stored in buffer showed no change in the current response for $H_2O_2$ and for the interferents. During the same period the electrodes stored in $H_2O_2$ showed a current increase for all the interferents tested.

*Electropolymerization in the presence of enzyme*

The enzyme selected was the glucose oxidase because of its well known behaviour in amperometric enzyme electrode studies since 1973[10]. Among the monomer tested in the previous phase we selected five monomers: 1,2- and 1,3- and 1,4-DAB, resorcinol and catechol. The experimental conditions were the same used for the electropolymerization without the enzyme. Figure 3 reports one example of cyclic voltammograms for electropolymerization of catechol in presence of 500 U/ml of glucose oxidase.

The shape of the voltammogram was similar to that run without the enzyme. Also in this case a

Table 3. Voltammetric data at GOD/polymer/Pt electrodes in the presence of $H_2O_2$ and interferents (Scan rate 50 mV/s)

| | $ip_a$ (μA) at bare electrode | $ip_a$ (μA) at covered electrode | | | | |
|---|---|---|---|---|---|---|
| | | Poly (RES)/GOD | Poly (CAT)/GOD | Poly 1,2-DAB/GOD | Poly 1,3-DAB/GOD | Poly 1,4-DAB/GOD |
| $H_2O_2$ | 8.00 | 7.99 | 8.00 | 7.62 | 7.99 | 7.98 |
| 5 mM | | (−) | (−) | (−4%) | (−) | (−) |
| | | Interferents: | | | | |
| Uric Acid | 8.80 | 0.85 | 0.86 | 1.04 | 1.40 | 0.30 |
| 4 mM | | (−90%) | (−90%) | (−83%) | (−84%) | (−97%) |
| Ascorbic Acid | 7.14 | 0.77 | 1.03 | 1.02 | 0.88 | 0.87 |
| 4 mM | | (−89%) | (−86%) | (−86%) | (−87%) | (−88%) |
| Acetaminophen | 6.85 | 1.20 | 0.99 | 0.88 | 1.27 | 1.35 |
| 4 mM | | (−82%) | (−85%) | (−87%) | (−81%) | (80%) |

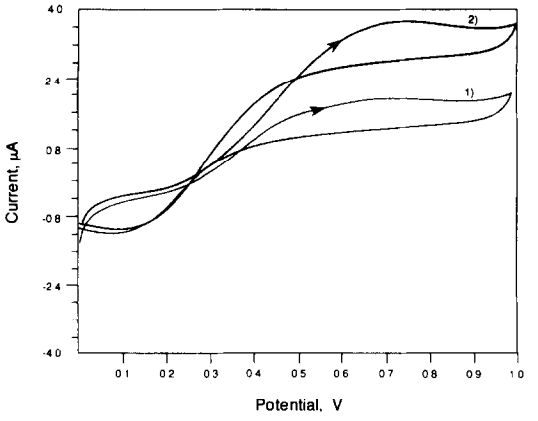


Fig. 4. Cyclic voltammograms at GOD/poly/catechol/Pt electrode of glucose: (1) 1 mmol/L; (2) 2 mmol/L in buffered solution (pH 7.2 $I = 0.1$). Scan rate 50 mV/s.

complete electropolymerization occurred after a few cycles. Results obtained with catechol were also obtained with all the others polymer prepared with GOD.

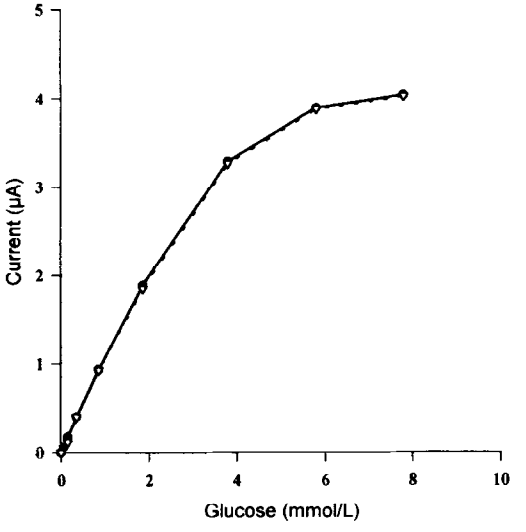


Fig. 5. Calibration curve for glucose at poly(catechol)/GOD/Pt biosensor and at poly(1,4-DAB)/GOD/Pt biosensor, in buffer solution pH 7.2. Applied potential +0.65 V vs. *sce*. Solid line is related to poly(catechol)/GOD/Pt biosensor and the dashed one to poly(1,4-DAB)/GOD/Pt biosensor.

These electrodes were then tested for $H_2O_2$ and interferents. Again all the probes responded to $H_2O_2$, but gave very low current signals to the interferents tested. Results are reported in Table 3.

The ascorbic acid, which is the same size as the glucose, does not pass the polymer film in the absence and in the presence of the enzyme, but a catalytic oxidation of glucose, which makes $H_2O_2$ detectable at the electrode surface, was observed. So we can suppose that the enzyme is available for glucose because it is also presently entrapped in the external side of the polymer structure[1(b), 11].

The access of the redox couple to the metal–polymer interface, where the electrode process occurs, seems to be mainly governed by properties much more selective[12] than molecular size and charge, such as partition and mass transfer within the membrane controlled by specific chemical interactions between the individual solute and the polymer.

Moreover, the active site of the immobilized enzyme, when in the presence of glucose and ascorbate, will be engaged only with the glucose molecule, which is converted in gluconolactone and $H_2O_2$. We do not have any experimental evidence that the enzyme is covered by the polymer. However, from our experience of previous works[3], we know that any membrane which lowers the diffusion of ascorbic acid, slows down the diffusion of glucose, with an increase of the apparent $K_m$ of the enzyme. In our experiments the calculated apparent $K_m$ has found the same order of magnitude (see below) of that found with the immobilized enzyme not covered by any membrane[13]. So we think that the enzyme is available for the glucose as soon it reaches the outer part of the polymer structure, but the morphology of the polymer will remain the same (at least in the inner part) because ascorbic acid does not go through the membrane in the presence and in the absence of the enzyme.

It should be pointed out that the exact surface morphological relationship between the immobilized enzyme and the polymer film is not known and we can only hypothesize a possible representation of the biosensors surface, which also has been reported in previous works[1(c), 7, 11].

There is evidence that the films formed under similar conditions have the thickness of the order of 5–10 nm[5], so that the GOD molecules (diameter *ca.* 9 nm[14]) would be located at the polymer/electrolyte interface[7], otherwise $H_2O_2$ would not be produced and detected.

The probes were then tested as glucose enzyme electrodes. Results are reported in Fig. 4. Voltam-

Table 4. Voltammetric data at polymer/GOD/Pt electrodes in the presence of glucose at different concentrations in buffered solutions pH 7.2 (scan rate 50 mV/s)

| | $ip_a$ (μA) at covered electrode | | | | |
|---|---|---|---|---|---|
| [Glucose] | Poly (CAT)/GOD | Poly (RES)/GOD | Poly (1,2-DAB)/GOD | Poly (1,3-DAB)/GOD | Poly (1,4-DAB)/GOD |
| 0.5 mM | 1.08 | 1.02 | 0.99 | 0.98 | 1.04 |
| 1 mM | 2.00 | 1.95 | 1.97 | 1.96 | 2.04 |
| 2 mM | 3.98 | 3.90 | 3.71 | 3.94 | 3.88 |
| 10 mM | 7.98 | 7.80 | 7.42 | 7.90 | 7.64 |

mograms of catechol electropolymerized with GOD were obtained in the presence of different concentrations of glucose. The increase of the current peak by consecutive injections of glucose showed the catalytic effect of GOD immobilized in the electropolymerized film, which yields $H_2O_2$ as product. In fact the current increase was observed at the same potential obtained when cycling with $H_2O_2$.

Similar results have been observed for all the monomers selected and electropolymerized with GOD.

Table 4 reports the results obtained by glucose voltammograms in the presence of the electropolymerized glucose probes.

*Amperometric measurements*

Among all probes tested we selected those prepared with catechol and 1,4-DAB monomers plus GOD, which resulted more stable when tested for hydrogen peroxide.

Figure 5 shows respectively the calibration curves of glucose for catechol and 1,4-DAB plus GOD electropolymerized electrodes. Glucose was detected in the range of 0.05–5 mmol/L, with a linear range of 0.05–5 mmol/L and a detection limit of 1 $\mu$mol/L. From the data obtained in Fig. 5 a calculation of the $K_m$ apparent values was performed using the Lineweaver–Burk and Eadie–Hofstee plots. Values of $K_m$ apparent of the electropolymerized enzyme were respectively $2.0 \times 10^{-3}$ and $2.3 \times 10^{-3}$.

These values were comparable but lower than those obtained by GOD immobilized on collagen ($3.0 \times 10^{-3}$)[15], carbon ($3.1 \times 10^{-3}$)[16] and gelatine ($7.5 \times 10^{-3}$)[17].

This parameter depends on the thickness of the membrane and on the immobilization procedure. Also, it is controlled by the diffusion of the glucose which has to reach the immobilized enzyme.

Usually, membranes with controlled porosity placed on the immobilized enzyme slow down the diffusion of glucose, so that the enzyme "sees" a lower concentration of glucose than that really present in the solution and this results in an increase of the apparent $K_m$. As stated previously, our values are comparable with those obtained with immobilized enzyme not covered by a blocking membrane, we can suppose that our enzyme is entrapped, but not covered by the polymer structure.

The response time of those probes was 5–10 s which is considerably lower than that obtained with conventional glucose probes (1–2 min) and in agreement with those assembled with polymers[1, 5, 6].

The interferents were also tested with both the poly(catechol) and 1,4-DAB glucose probes. Poly(catechol) glucose probe showed no response for interferents injected at concentrations 0.1–1 mmol/L. This probe during the time was tested for glucose. The probe was stored in a buffer at 4°C, when not in use. After one month we observed a residual enzyme activity of about 30%, as shown in Fig. 6, while for poly(1,4-DAB)-glucose probe the residual activity was of about 20%, as shown in Fig. 7. During the same time the interferents injected at concentrations 0.1–1 mmol/L did not give any current signal.

The stability of the glucose sensors was also tested during the operation time. Consecutive calibration curves of glucose run during the same day showed a signal decrease of only 5% of the original values.

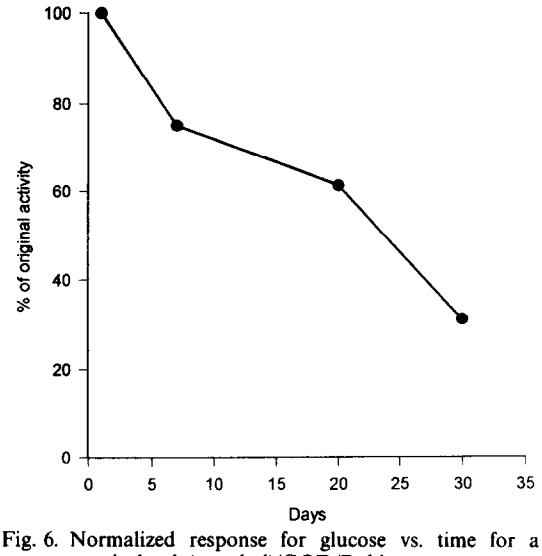


Fig. 6. Normalized response for glucose vs. time for a typical poly(catechol)/GOD/Pt biosensor.

When poly(1,4-DAB)-glucose probe was tested for ascorbic acid it showed that after the second day, a slight current signal increased during the time, after a week it was the same as that observed at bare electrode.

It is interesting to note that in this case acetaminophen and uric acid did not give any current signal and that the glucose probe did not lose its characteristics in terms of response time and linearity range. This seems to demonstrate that the polymer film is still functioning as a support for the enzyme, but it changes its diffusional characteristics during time. However, this point has to be further investigated.

## CONCLUSIONS

The investigation of the electropolymerization of different monomers on Pt electrodes has led to the preparation of glucose electrochemical biosensors with improved characteristics of stability and

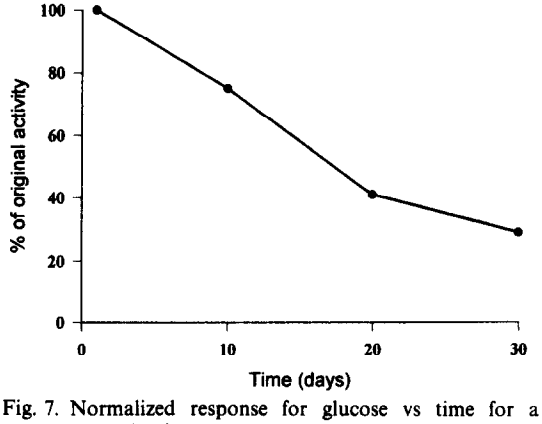


Fig. 7. Normalized response for glucose vs time for a typical poly(1,4-DAB1)/GOD/Pt biosensor.

response time. This system resulted a good alternative to the conventional three membrane electrodes and showed the possibility to apply these sensors in real matrix for glucose measurements.

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