Glucose sensor utilizing polypyrrole incorporated in track-etch membranes as the mediator

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(Received 11 September 1991; revised version received 3 March 1992; accepted 27 March 1992)

Abstract: Template synthesis inside track-etch membranes is employed to create conducting microtubules of polypyrrole. Within these tubules, glucose oxidase can be adsorbed irreversibly, while retaining its catalytic activity. A glucose sensor is described utilizing the polypyrrole microtubules as mediator. With this sensor glucose can be measured amperometrically in the concentration range 1-30 mM. The sensor is highly selective with respect to other substrates and the sensor response is independent of oxygen concentration. Evidence is presented that in the biosensor direct electron transfer may occur between glucose oxidase and the polypyrrole.

Keywords: glucose sensor, direct electron transfer, conducting polymer, amperometry, microtubules.

INTRODUCTION

Biosensors based on oxido-reductases normally detect a substance indirectly, e.g. by measuring the decrease in oxygen concentration or the increase in hydrogen peroxide concentration (Galiatsatos et al., 1990; Mann-Buxbaum et al., 1990). The reason for this is that large redox proteins cannot communicate directly with ordinary electrodes (Degani & Heller, 1989). The active centres of these proteins are insulated by a thick protein shell which makes direct electron transfer to an electrode surface impossible (Heller, 1990).

A traditional glucose sensor is based on glucose oxidase (GOD), which contains the
flavin adenine dinucleotide (FAD) redox centre. It measures glucose by the following series of reactions:

\[
\text{GOD-FAD} + \text{glucose} \rightarrow \text{GOD-FADH_2} + \text{gluconolactone} \\
\text{GOD-FADH_2} + \text{O}_2 \rightarrow \text{GOD-FAD} + \text{H}_2\text{O}_2
\]

The hydrogen peroxide produced is detected at the anode. Hydrogen peroxide detection has the disadvantage that high voltages have to be applied to the sensor. Furthermore, it degrades the enzyme (Sasso et al., 1990). An alternative approach is to use small diffusion mediators like ferrocene and ferrocene derivatives as artificial electron acceptors for redox enzymes (Dicks et al., 1989). For glucose oxidase the regeneration of reduced flavin (2) is accompanied by the reduction of the mediator (3).

\[
\text{GOD-FADH_2} + 2\text{Med}^{\text{Ox}} \rightarrow \text{GOD-FAD} + 2\text{Med}^{\text{Red}} + 2\text{H}^+ 
\]

The reduced mediator is subsequently electrochemically oxidized. Sensors using this principle often suffer from leakage of the mediator out of the system (Schuhmann et al., 1990). Since most mediators are toxic, this restricts the use of such sensors.

Creating an environment where a redox enzyme, despite its insulating protein shell, can communicate directly with an electronically conducting material, would produce a reagentless biosensor. In this paper we describe such a biosensor. We have immobilized the redox enzyme glucose oxidase on a conducting polymer (e.g. polypyrrole) which is located within the pores of a track-etch membrane (Nuclepore® or Cyclopore®). Polyconjugated conducting materials like polypyrrole and polythiophene can form microtubules in the pores of such membranes. These microtubules display enhanced electronic conductivity, as was shown earlier by Cai & Martin (1989). Our rationale was that the mesoscopic space inside the pores of the track-etch membranes, together with the morphology of the conducting polymer, would allow immobilization of GOD and might favour direct electron transfer between the redox enzyme and the conducting polymer. It is most preferable to perform enzyme immobilization without additional reagents. Simple adsorption on the conducting polymer surface will allow the enzyme to remain catalytically active. We expected that the confined space in the pores would contribute to this desired property. Not only will the protein molecules inside the pores have a better chance to retain their native structure, but also these molecules will be protected from their environment. Sheer forces and sensor manipulation will have a less damaging effect. We will show that the immobilization of glucose oxidase on conducting polymers inside the pores of track-etch membranes indeed gives rise to a stable, reagentless biosensor. This sensor is able to detect glucose in an amperometric way. The reduced enzyme becomes reoxidized by donating its electrons directly to the conducting polymer (Scheme 1). Optimal interaction between glucose oxidase and the conducting polymer results in an oxygen-independent detection.

\[\text{solution} \rightarrow \text{membrane pore} \rightarrow \text{anode}\]

**Scheme 1.** Electron shuttle, showing how electrons are transferred from glucose to the anode, mediated by the conducting polymer.

**MATERIALS AND METHODS**

**Materials and apparatus**

Glucose oxidase (EC 1.1.3.4) type II (25 000 U g\(^{-1}\)) from Aspergillus niger and catalase (EC 1.11.1.6, 2800 U mg\(^{-1}\)) from bovine liver were obtained from Sigma. Benzoquinone was from Aldrich (Germany) and was sublimed prior to use. Pyrrole was from Merck and was distilled under nitrogen before use. 3-Methylthiophene was purchased from Sigma. 3-Hexylthiophene was synthesized according to a described procedure (Pham et al., 1986). Anhydrous iron(III) chloride (98%) was obtained from Fluka and was used as received. The Nuclepore membranes were purchased from Ankersmit (The Netherlands). The Cyclopore membranes were a gift from Cyclopore SA (Belgium). All other reagents were commercial products and of analytical grade.

All electrochemical measurements were performed with an Autolab potentiostat.
controlled by an Olivetti M24 personal computer and general-purpose electrochemical system (GPES) software (Eco Chemie, The Netherlands). Current output was recorded on a Yew 3056 pen recorder. The pore size distribution of the filter membranes was measured with a Coulter Porometer II (V3B) at the Cyclopore Co. Electron micrographs were made on a CAMSCAN scanning electron microscope (Cambridge Instruments) at the University of Utrecht.

**Preparation of polymeric modified membranes**

Pyrrole was chemically polymerized within the pores of Nuclepore or Cyclopore membranes in a specially made reaction vessel (Fig. 1). Polymerization was achieved with freshly made solutions of 0.6 M pyrrole and 2 M iron(III) chloride in distilled water. Membranes with different pore diameters were used. The polymerization time varied between 30 s and 10 min depending on the diameter of the pores. In the polymerization experiment the membrane separated the pyrrole solution from the oxidizing solution. The reagents meet in the pores and react to give polypyrrole. After the appropriate time, the polymerization reaction was quenched by rinsing the membrane with distilled water. Surface material was removed by carefully wiping the membrane with a tissue.

The same apparatus was used for the polymerization of 3-methylthiophene and 3-hexylthiophene. The polymerization agent in this case was 1 M Fe(C104)3 in CH3CN and the monomer concentration was 0.6 M in CH3CN.

The resistance of the composite membranes was measured according to a procedure described in the literature (Cai & Martin, 1989). A bed of silver powder served as the auxiliary electrode in the resistance measurement. A membrane was placed on this bed and a second electrode, consisting of a platinum wire sealed into a 7 mm diameter glass tube, was placed on top of the membrane. A pressure of 1.3 kg cm⁻² was applied to the latter electrode to ensure a uniform contact. The resistance was measured with a Fluke model 45 digital multimeter.

**Coating with metal layer**

Platinum or gold was applied to one side of the membrane with an Edwards sputtercoater S150B.

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**Glucose sensor utilizing polypyrrole**

![Diagram](image)

*Fig. 1. Layout of the apparatus used in the polypyrrole microtubule synthesis.*

The layer thickness was monitored with an Edwards FTM5 unit. The thickness of the metal layers varied between 50 and 350 nm.

**Immobilization of enzyme**

Enzyme immobilization was achieved by agitating (Gyrotory Shaker model G2, New Brunswick Scientific, USA) membranes in 3 ml of 5 mg ml⁻¹ of GOD at a temperature of 277 K for at least 0.5 h. The membranes were successively dried overnight on CaCl₂ in a desiccator.

**Measurement of enzyme activity**

Enzyme activity was assayed with a three-electrode cell containing 70 ml phosphate-buffered saline (PBS) pH 7.4, 5 mM benzoquinone and 0.5 mM glucose. Prior to use, the glucose solution was allowed to mutarotate for at least 24 h. The assay was performed with a platinum rotating disk electrode (RDE, 6 mm diameter) equipped with an Electrocraft corporation model E550 motor and E552 speed control unit. The platinum working electrode was set at a potential of 0.350 V (Ag/AgCl reference) and was rotated at a speed of 2000 rpm. A platinum wire was used as auxiliary electrode. The solution was flushed with argon before each experiment. During the assay argon was blanketed over the solution.

The actual assay was performed by monitoring the current output of the potentiostatted RDE while immersing a sample membrane into the solution.
Amperometric measurements

To perform amperometric measurements, the enzyme membrane was placed as working electrode in a three-electrode flow cell (Sparc Holland). The metal-coated rear of the membrane was pushed against a glassy carbon disk (diameter 8 mm). To insulate the active surface of the membrane from the auxiliary electrode, it was covered with a teflon spacer of 1 mm thickness. In the spacer a duct of ±0-15 cm² was left, allowing the membrane to make contact with the solution. An Ag/AgCl electrode was used as reference electrode. The base of the flow cell acted as auxiliary electrode (glassy carbon). Buffer solution was driven through at 1·75 ml min⁻¹ (Watson Marlowe 101U peristaltic pump). The potential of the membrane was set at 0.350 V. When the background current had been diminished (<200 nA), the buffer solution was replaced by the glucose solution and the current response was monitored.

RESULTS AND DISCUSSION

Modification of track-etch membrane

Track-etch membranes were modified with polypyrrole, poly(3-methyl)thiophene and poly(3-hexyl)thiophene, by means of an oxidative polymerization reaction. The polymerization equipment is shown in Fig. 1. Pyrrole polymerization was effected by allowing the membrane to separate an aqueous 0·6 M pyrrole solution from an aqueous 2·0 M FeCl₃ solution. In the pores of the membrane the pyrrole monomer and the oxidizing solution meet, whereupon polymerization takes place. In this way we were able to modify membranes with original pore sizes ranging from 200 nm to 8 μm. For the polymerization of (3-methyl)thiophene and (3-hexyl)thiophene solutions of 0·6 M of the monomer and 1·0 M of Fe(ClO₄)₂ in acetonitrile were used. We managed to immobilize GOD in conducting polymer-modified membranes with 600, 800 and 1000 nm pores (see below). Membranes with larger pores were not used for enzyme immobilization, because modification of these larger pores with conducting polymer does not lead to enhanced conductivities (Cai & Martin, 1989). Therefore, it was not expected that such membranes would lead to working biosensors.

The porosity of the modified membranes was varied by allowing the polymerization reaction to take place for increasing periods of time. Scanning electron microscopy showed how the pores of the track-etch membranes became filled with conducting polymer when the polymerization time increased (Fig. 2). In Table 1 the mean pore size as measured by differential flow porosimetry is given as a function of the polymerization time. The results in this table are for a membrane with originally 1 μm pores, treated with pyrrole. There is a correlation between the pore size of the membrane and the electrical resistance of the conducting polymer inside the pores (Table 1). When the pores become smaller, the amount of conducting polymer inside the pores increases and the resistance becomes lower. After 4 min, the porosity becomes insignificantly low and the

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Mean pore size (nm)</th>
<th>Resistance (Ω)</th>
<th>Enzyme immobilization</th>
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</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>985</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>0·5</td>
<td>889</td>
<td>52·0</td>
<td>++</td>
</tr>
<tr>
<td>1·0</td>
<td>603</td>
<td>13·1</td>
<td>++ +</td>
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<tr>
<td>2·0</td>
<td>315</td>
<td>3·9</td>
<td>++</td>
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<tr>
<td>3·0</td>
<td>264</td>
<td>2·1</td>
<td>+</td>
</tr>
<tr>
<td>4·0</td>
<td>212</td>
<td>1·9</td>
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<tr>
<td>5·0</td>
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<tr>
<td>10·0</td>
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</tr>
</tbody>
</table>

*Cyclopore membrane with originally 1000 nm pores. Treated with 0·6 M pyrrole and 2·0 M FeCl₃ in water.
Immobilization of glucose oxidase in polypyrrole-modified pores

Immobilization of glucose oxidase inside the conducting microtubules was achieved by soaking a modified membrane in an aqueous, buffered solution, containing the enzyme, for at least 0.5 h and successively drying overnight at 277 K.

The membranes, modified with conducting polymer and treated with GOD solution, were tested for enzymatic activity by means of the following assay (Aubrée-Lecat et al., 1989). The natural co-substrate (oxygen) was replaced by the artificial electron acceptor benzoquinone. Hydroquinone, which is formed in the catalytic cycle, was measured electrochemically at a rotating disk electrode (RDE). The regeneration of benzoquinone from hydroquinone takes place at a fixed potential (0.35 V versus Ag/AgCl). The resulting current is a measure of the enzymatic activity. Although there is a slight rise in current as a consequence of the uncatalysed oxidation of glucose by benzoquinone, the rise in current as a result of the catalytic action of the enzyme is large enough to give a significant difference in slope of the current-time plot.

In Fig. 3 the effect on the measured current is shown when a GOD-treated polypyrrole membrane with 800 nm pores is introduced into the electrochemical cell. As can be seen, the current increases immediately after introduction of the membrane. Membranes with 600 and 1000 nm pores also showed this behaviour, whereas membranes with pores smaller than 600 nm did not display any enzyme activity. The fact that after withdrawal of the membrane the resistance reaches a constant value of 1.9 Ω. Longer polymerization times gave non-porous membranes. Enzyme treatment of these membranes did not lead to a working biosensor (see next section).

Fig. 2. Scanning electron micrographs of track-etch membranes at various polymerization times: (a) untreated membrane, original pore size 800 nm; (b) 2 min; (c) 5 min. — 1 μm.

Fig. 3. Measurement of enzyme activity by monitoring enzymatically produced hydroquinone at an RDE. (1) and (3): introduction of membrane; (2) and (4): withdrawal of membrane; (5) introduction of 0.125 U GOD.
activity returns to its initial value is an indication that the enzyme is properly immobilized. Not properly immobilized material would stay in solution and the slope of the line after point 2 in the figure would be higher. This is actually found for membranes treated with GOD without conducting polymer present in the pores. In this case any enzyme present in the pores is washed out.

Also drying of the membrane after adsorption of the enzyme is essential. Enzyme is washed out completely when membranes are used directly after enzyme treatment. We may conclude, therefore, that drying results in proper immobilization of the conducting polymer surface.

From Table 1 (last column) it is evident that the enzymatic activity of the polymer-modified membranes disappears when they are no longer porous. The various polymerization times gave different enzyme activities. For the membrane in Table 1 the highest activity was reached at a polymerization time of 1-2 min. Experiments with fluorescine-labelled GOD showed that the enzyme is only present in the pores of the membrane and not on the surface. Therefore, we may conclude that the enzyme immobilization is most effective inside the pores and not on the membrane surface. Figure 3 shows that repeated introduction and withdrawal of the polypyrrole membrane does not change the amount of active, immobilized enzyme (points 1-4). At the end of the measurement, a known quantity (5 µg, 0.125 U) of enzyme was added to calibrate the activity (point 5 in Fig. 3). The electrode reaction and the transport of hydroquinone to the electrode (forced convection was applied) are very fast. As a result, the oxidation of benzoquinone by the enzyme will be the rate-determining step. This means that the slopes resulting from immobilized and free enzyme can be correlated, at least in a semi-quantitative way. From the calibration it was concluded that about 0.125 U of active GOD is present in the membrane of Fig. 3.

The three types of conducting polymers that were used all showed good immobilization ability; in every polymer the enzyme was properly anchored. The absolute amount of enzyme that became immobilized varied, however. Polypyrrole membranes showed the highest activity. Poly(3-methylthiophene) showed an intermediate activity, and the activity of poly(3-hexylthiophene) was the lowest. The conductivities of the three polymers decreased in the same order. Polypyrrole membranes showed a high conductivity (1-2 S cm⁻¹), whereas the conductivities of poly(3-methylthiophene) and poly(3-hexylthiophene) membranes were relatively low (<1 × 10⁻³ S cm⁻¹). As the surface area of these microscopic hollow conducting cylinders cannot be determined exactly, it is not possible to give accurate conductivity values. The electrical resistances in Table 1, however, are accurate.

The higher electrical resistance of the membranes filled with poly(3-methylthiophene) and poly(3-hexylthiophene), together with the lower ability of these membranes to immobilize the enzyme, made us decide to use polypyrrole in the construction of an amperometric biosensor.

**Amperometric track-etch membrane biosensor**

In order to construct an electrode from the polypyrrole-modified membrane, we coated one of its sides with a conducting metal layer. A layer thickness of 50 nm proved to be sufficient. Subsequent to coating, the membranes were treated with GOD solution. The metals tested were platinum and gold. Sensors coated with platinum gave the highest response currents to glucose. Both platinum and gold electrodes did not respond to glucose when the membranes were not previously treated with enzyme. Therefore, non-specific oxidation of glucose did not take place.

The biosensor thus constructed is shown in Fig. 4. The membrane was placed in a three-
Fig. 5. Schematic representation of the flow cell.

electrode cell and acted as the working electrode (Fig. 5). The cell was taken up in a flow system, with the possibility to switch between a buffer solution and a solution that contained a known amount of glucose.

All experiments were carried out under an argon atmosphere and with at least 25 units ml$^{-1}$ of catalase present in the test solutions, including the buffer solution. The latter enzyme was added to destroy any H$_2$O$_2$ produced. In Fig. 6 the increase in current upon the addition of 10 mM glucose is shown. The electrochemical response to glucose in this figure is representative for membranes containing 600, 800 and 1000 nm pores. A calibration curve was made by successively measuring various amounts of glucose with the same sensor in a flow cell (Fig. 7). The current response to glucose was linear up to 10 mM. Measuring under argon with catalase present in solution gave good results up to 30 mM of glucose. The applied potential was 0.350 V versus Ag/AgCl reference, which is a very low potential, considering the absence of additional redox mediators (see below) (Scheller et al., 1991). Even lower potentials were possible. We measured the response of the biosensor to glucose at a potential of 100 mV versus Ag/AgCl. We also measured the response of the same sensor to H$_2$O$_2$ separately. At a potential of 100 mV versus Ag/AgCl the current due to the addition of glucose was still positive, whereas even small amounts of purposely added H$_2$O$_2$ (10$^{-4}$ wt% in phosphate buffer, pH 7.4) gave a strongly negative response (Delahay, 1965; Hoare, 1985). The positive response to glucose at such a low potential is possible because the redox potential of the flavin unit is much more negative; FAD, free in solution, has a redox potential of $-480$ mV versus Ag/AgCl (Narasimhan & Wingard, 1986b). The actual redox potential of FAD inside our biosensor will be different, but is probably of the same order of magnitude as in solution (Stankovich et al., 1989). Electron transfer from FAD of glucose oxidase to an (aminophenyl)boronic acid-modified glassy carbon electrode has been reported to take place at a potential of $-505$ mV versus Ag/AgCl (Narasimhan & Wingard, 1986a). In our opinion, electron mediation by FAD, which is released from the enzyme, can be excluded. The flavin cofactor is in essence irreversibly bound to the protein (Swoboda & Massey, 1965). As to this, our measurements were
performed in a flow system. Any released FAD is immediately washed out and cannot accidentally mediate the electron transfer. Therefore, we may be reasonably sure that the enzyme transfers its electrons directly to the conducting polymer. The confined space in the pores, together with the amorphous structure of the polypyrrole interior (Tamiya et al., 1989), apparently brings the active centres of the enzyme molecules in close contact with the conducting polymer. In its oxidized state polypyrrole is a polycation (Fortier & Belanger, 1991). Glucose oxidase, on the other hand, has at least ten net negative charges on its surface (Szucs et al., 1989). The interaction between polymer and enzyme, therefore, is expected to be electrostatic in nature and may be very strong. Drying of the membrane after enzyme treatment appeared to be essential for good enzyme immobilization and for the communication between the enzyme and conducting polymer. We believe that water and enzyme are competing species with regard to binding to the polymer. When water is removed by evaporation, enzyme adsorption is favoured (Armstrong et al., 1987) and the active centres of the enzyme can approach the conducting polymer sufficiently to make direct electron transfer possible. The possibility of realizing direct electron transfer from biocatalysts to electrodes has been mentioned in the literature (Delaney et al., 1986) and direct electron transfer between glucose oxidase and polypyrrole has been described before (Yabuki et al., 1989; Aizawa et al., 1990). However, no glucose-dependent current values were reported and it was said that the direct interaction of glucose oxidase with polypyrrole is weak (Kajiya et al., 1991). Electron transfer between glucose oxidase and an (aminophenyl)boronic acid-modified glassy carbon electrode has been described but measurements of glucose concentration were not reported (Narasimhan & Wingard, 1986a). The direct interaction of glucose oxidase with conducting organic salt electrodes has been claimed by Albery and coworkers (Albery et al., 1985), but their results are not unambiguous (Cenas & Kulys, 1981). We observed a distinct current response and the possibility of mediated electron transfer can probably be excluded, as discussed above.

The response time to glucose is 40 ± 2 s, which is fast considering the geometry of the sensor. External and internal resistances to substrate diffusion contribute to this response time. The individual effects of mass transport and diffusion resistance have to be evaluated separately before anything can be concluded about the intrinsic response time of the sensor. Kinetic measurements are in progress and will be presented elsewhere.

Effect of oxygen

When oxygen is present, it may become a competing species for the polypyrrole in accepting electrons from the flavin units of glucose oxidase. In that case not only does the current due to the direct electron transfer to the polymer become smaller, but also hydrogen peroxide will be formed. We observed no difference in response to glucose between sensors operating under an argon and under an oxygen atmosphere. To assure that absolutely no oxygen was present during the experiments the solutions were purged continuously with argon gas for several days and the response to glucose was measured at fixed intervals during this period. No decrease in response was observed in this case, revealing that the response current was indeed independent of oxygen. Taking this result into account, we might conclude that oxygen mediation does not take place.

Response under increased salt concentration

The response of the sensor to 10 mM glucose was measured under various salt (NaCl) concentrations. When the ionic strength of the solution was increased, the electron transfer from enzyme to electrode became diminished as was shown by the decreased current response (Fig. 8). However, the process is reversible as the response fully recovered upon switching back to the initial

![Fig. 8. Response of the glucose sensor to 10 mM glucose at different NaCl concentrations. *Addition of glucose.](image-url)
salt concentration (0.15 M). We believe that at high ionic strength the charges involved in the electrostatic interaction between enzyme and conducting polymer are screened, thereby suppressing the transfer of electrons.

**Selectivity and lifetime**

The sensitivity of the sensor to fructose, citrate, lactate, urea, uric acid, gluconate and pyruvate was tested separately. No significant response was observed to any of these components when they were present at millimolar concentrations. Ascorbate (vitamin C), a common interferent in amperometric biosensors, however, interfered strongly when present at 1 mM concentration. At concentrations larger than about 0.1 mM, an anion-excluding membrane is necessary.

The response of a freshly made biosensor to 10 mM glucose was tested over a period of three weeks in the presence of 25 units ml⁻¹ of catalase. The response was not stable during the first four days of operation (Fig. 9). In this period, the current response to 10 mM glucose decreased to 50% of the value measured on the first day. We presume that in the beginning an amount of enzyme, which is less firmly bound, is slowly washed out. After a few days, only firmly bound enzyme is left and a stable current response is obtained over an extended period of time. After the four-day period, the response of the sensor remained nearly the same for two weeks (Fig. 9).

In these two weeks, measurements of glucose were performed for prolonged periods of time and various experiments were conducted with the sensor membrane. For example, at day 7 the sensor was exposed to buffer solutions with various pH values. At high pH values (pH 10) some enzyme denaturation occurred, leading to a reduction in sensitivity. Between day 20 and 22, 10 mM glucose was measured continuously for 18 h. The current did not decrease significantly during this measurement.

We also measured glucose for extended periods of time under ambient atmosphere without catalase present. No significant loss of membrane sensitivity was observed in this case. If the reoxidation of GOD had been mediated by oxygen, this would have resulted in denaturation of the enzyme by hydrogen peroxide.

**CONCLUSIONS**

The biosensor described here can easily be constructed and is a simple device for measuring glucose. It can be used for extended periods of time. The selectivity for glucose is high. Our amperometric measurements were carried out at low potentials, which is very advantageous since interference by non-enzymatically oxidizable species will become less important, thereby improving the selectivity of the biosensor.

Evidence is presented that the response to glucose may be the result of direct electron transfer between enzyme and conducting polymer. The positive current response of the sensor on the addition of glucose even at low measuring potential (100 mV versus Ag/AgCl), the observed negative response to \( \text{H}_2\text{O}_2 \) at this potential, the reversible quenching of the response current under increased salt concentration, and the oxygen-independent signal are indications for this.

The idea of immobilizing redox enzymes inside conducting microcylinders can be translated to a wide range of redox enzymes and conducting polymers. We are currently investigating this.

**ACKNOWLEDGMENTS**

The authors wish to thank Mr W. van Bennekem and Mr J. Hoogvliet for their support in this
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