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Third-generation amperometric biosensor for glucose. Polypyrrole deposited within a matrix of uniform latex particles as mediator

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Abstract

Uniform latex particles and agarose gels are utilized to create porous membranes in which polypyrrole is electrochemically synthesized. Within the pores of these modified membranes glucose oxidase can be adsorbed irreversibly while its catalytic activity is retained. A glucose sensor which has a considerable lifetime under continuous use is constructed from the enzyme membrane. With this sensor glucose can be measured amperometrically in the concentration range 1–60 mM. The sensor response is independent of oxygen concentration.

INTRODUCTION

Third-generation amperometric biosensors [1] are based on the principle of direct electron transfer from the biological receptor to a modified or unmodified
electrode [2–5]. Until now, only a few cases have been reported where large redox enzymes communicate directly with such electrode materials [6]. The response current in these cases was very low [7]. This poor electrochemistry is due to the existence of a thick insulating protein shell around the active center of the enzymes [5,8]. To realize direct electron transfer, it is necessary to create an electrode material that can interact efficiently with the active center of the redox enzyme [2].

Electrochemically synthesized polypyrrole has been used by many researchers as an environment for redox enzymes [7,9–13]. Recently, we reported on a glucose sensor which contains glucose oxidase immobilized within electron-conducting polypyrrole microtubules. These microtubules had been incorporated in track-etch membranes [14,15]. We have presented evidence that this glucose sensor works according to the principle of direct electron transfer from the redox enzyme to the conducting polymer. It was proposed that the strong adsorption of the glucose oxidase molecules in the confined space of the polypyrrole microtubules and the morphology of the internal conducting polymer surface made direct electron transfer possible. The track-etch glucose sensor can be used for prolonged periods of time in continuous glucose monitoring. However, from a technological point of view this principle is not suitable for disposable sensor applications. Such disposable sensors are generally prepared by a printing technique [16] which is not possible with our track-etch membranes. We had the idea that uniform latex particles (ULPs) might form an excellent porous matrix for polypyrrole coating and successive enzyme immobilization. This in turn could open a route to develop disposable biosensors as the ULP matrix can be applied as an ink.

Polypyrrole synthesized in aqueous media [17,18] has a surface structure which is very rough and corrugated [11,19]. Such an amorphous structure present inside the confined space of a ULP matrix could provide a very favorable environment for a redox enzyme. In this paper we shall show that our approach can indeed be used to construct a stable third-generation biosensor which is able to detect glucose in an amperometric way (Scheme 1 and Fig. 1).

MATERIALS AND METHODS

Glucose oxidase (EC 1.1.3.4) type II (25,000 U/g) from Aspergillus niger and catalase (EC 1.11.1.6, 2800 U/mg) 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 before use. Latex suspensions with particles of 112 and 220 nm were from Perstorp Analytical. Agarose type VIII was purchased from Sigma. All other reagents were of analytical grade.

The galvanostat was a home-made instrument. Its current output was monitored with a Fluke 45 digital multimeter. All electrochemical measurements were performed using an Autolab potentiostat controlled by an Olivetti M24 personal computer and General Purpose Electrochemical System (GPES) software (Eco Chemie, Utrecht, The Netherlands). Current output was recorded on a Yew 3056 pen recorder. Electron micrographs were obtained using a Camscan scanning electron microscope (Cambridge Instruments).

**Platinum coating**

Glassy carbon disks 8 mm in diameter (Antec, Leiden, The Netherlands) were used as the base electrode. The electrodes were polished with Alpha Micropolish alumina no. 1C (1.0 µm, Buehler Ltd., USA). Platinum was applied to the polished surface with an Edwards sputter-coater S150B. A platinum target of diameter 8 cm and thickness 0.5 mm was used as the platinum source. The layer thickness was monitored with an Edwards FTM5 unit. Sputtering was continued until the thickness of the platinum layers was 300 nm.
Preparation of latex membranes

Agarose type VIII was dissolved by boiling the appropriate amount (0.1 or 0.25 wt.%) for 2 min in distilled water. Freshly made solutions, which were still hot, were used to make the latex membranes. A volume of agarose solution was mixed thoroughly with an equal volume of latex suspension. A 75 µl droplet was applied to a freshly platinum sputtered glassy carbon disk. After application, the electrode was placed overnight in the refrigerator. The dried latex electrode was subsequently heated in an oven at 333 K for 1 h.

Incorporation of polypyrrole

Latex electrodes were sealed with Teflon tape in such a way that only the latex surface made contact with the polymerization medium. An aqueous solution containing 0.9% sodium chloride and 10 mM phosphate buffer (phosphate buffered saline (PBS)), together with 0.3 M pyrrole was used in the polymerization reaction. To allow the solution to penetrate the membrane sufficiently the latex membrane was placed in the solution at least 1 min before polymerization was going to take place. Subsequently, a constant current (20 mA/cm²) was supplied to the cell for the appropriate amount of time. A platinum plate acted as the counter-electrode. After polymerization the electrodes were rinsed with PBS.

Immobilization of enzyme

Enzyme immobilization was achieved by agitating (Gyrotory Shaker model G2, New Brunswick Scientific, USA) the composite membranes in 3 ml of 5 mg/ml glucose oxidase at a temperature of 277 K for 4 h. The membranes were subsequently dried overnight on CaCl₂ in a desiccator.

Enzyme activity assay

Enzyme activity was assayed with a three-electrode cell containing 5 mM benzoquinone and 0.5 M glucose in 20 ml PBS (pH 7.5). 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 in 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) and was rotated at a speed of 2000 rev/min. A platinum wire was used as the 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 RDE while immersing a sample membrane into the solution.
Amperometric biosensor measurements

To perform amperometric measurements, the enzyme membrane was placed as working electrode in a three-electrode flow cell (Sparc Holland). To insulate the active surface of the membrane from the auxiliary electrode, it was covered with a Teflon spacer 1 mm thick. In the spacer a duct of approximately 0.15 cm² was left, allowing the membrane to make contact with the solution. An Ag/AgCl electrode was used as the reference electrode. The base of the flow cell acted as auxiliary electrode (glassy carbon). Buffer solution was driven through the cell at a rate of 1.75 ml/min (Watson Marlow 101U peristaltic pump). The potential of the membrane was set at 0.350 V. When the background current had decreased sufficiently, the buffer solution was replaced by the glucose solution and the current response was monitored.

Determination of glucose concentrations in fermentation samples

Biosensor measurements

For measurements of glucose concentrations in fermentation samples the sensor was first calibrated by measuring known amounts of glucose in the medium which was also used in the fermentation process. The fermentation process was a batch cultivation of *Aspergillus niger* in a 3 l fermentor at room temperature. The fermentation medium consisted of 3 g/l (16.7 mM) glucose, 0.66 g/l diammonium-tartrate, 0.15 g/l MgSO₄ · 7H₂O, 30 mg/l CaCl₂ · 2H₂O and 5.55 mg/l FeSO₄ · 7H₂O. The pH of the medium was adjusted to pH 4.5 with phosphoric acid. The glucose concentrations of the samples from the fermentor were measured in the same way as described above.

Spectrophotometric determinations

Glucose concentrations in fermentation samples were measured by means of a commercial glucose kit (β-glucose UV method; Boehringer) using a Perkin-Elmer Lambda 5 spectrophotometer.

RESULTS AND DISCUSSION

Latex membranes were cast on a freshly sputtered platinum surface from an aqueous solution containing the suspended latex particles and agarose. The agarose was added to achieve a better fixation between the latex spheres and between these spheres and the electrode [20]. After casting, the latex was dried at low temperature (277 K) to obtain a uniform layer without cracks on the surface. After drying, the modified electrode was heat treated which resulted in a very strong layer.
Agarose content of the latex membranes

The amount of agarose present in the latex suspension was varied to determine the minimum agarose content required to yield good membranes. Latex membranes were cast from solutions containing 0.125, 0.100, 0.075 and 0.050 wt.% agarose. An agarose content of 0.125 wt.% led to latex membranes which were less accessible for polypyrrole coating. Biosensors constructed from such membranes displayed lower activity and very long response times (see below). Amounts of agarose below 0.125 wt.% resulted in strong latex membranes which could successfully be treated with polypyrrole. The lowest agarose content tested (0.050 wt.%) still yielded membranes with strong adhesive properties. Therefore, in subsequent experiments latex suspensions with 0.05 wt.% agarose were used.

Thickness of the latex membranes

Thick membranes (ca. 5 µm) [20] as well as thin membranes (ca. 1 µm) were prepared by using two different latex concentrations in the droplet that was cast on the electrode. The droplet size was kept constant. In both cases strong smooth layers were obtained. Latex particles of two different dimensions (112 nm and 220 nm diameter respectively) were tested to prepare the membranes. Both particle sizes yielded membranes with interspherical pores of the order of 50–200 nm, as determined by scanning electron microscopy (Fig. 2).

Synthesis and characterization of polypyrrole-coated latex membranes

The latex membranes were modified with polypyrrole by means of electrochemical polymerization. The polymerization medium was PBS, containing 0.3 M pyrrole. This medium was chosen because enzyme treatment of the polypyrrole-modified latex should preferably be performed in PBS. By using the same medium during polymerization and enzyme treatment the possibility of the exchange of dopant ions in the polymer with the solution was avoided. If this were to happen, major changes could occur in the conducting polymer properties [21]. The electrochemical polymerization was galvanostatically controlled. In this way we were able to vary the amount of polypyrrole in the latex membranes by changing the polymerization time. We found that galvanostatic polymerization gave much more reproducible results than polymerization under potentiostatic control. Potentiostatic control resulted in non-uniform coating of the latex membrane; large areas on the electrode surface were still white (clean latex), while other areas showed spots of very high polypyrrole content. Galvanostatic polymerization at moderate current densities (20 mA/cm²) resulted in an evenly spread polypyrrole coating of the latex.

The amount of charge (current multiplied by time) in the polymerization reaction was varied from 100 to 1000 mC/cm². The blackening of the originally white latex layers was proportional to the amount of charge passed. Although only
Fig. 2. Scanning electron micrographs of 5 µm latex membranes containing 220 nm particles: (a) untreated membrane; (b) membrane treated with pyrrole for 15 s to a charge dose of 300 mC/cm²; (c) membrane treated with pyrrole for 50 s to a charge dose of 1000 mC/cm².

qualitative, this was a good indication of the proper polymerization of pyrrole in the matrix of latex particles.

Scanning electron microscopy was used to image the coated latex membranes and to see how the membrane structure changed with polymerization time (Fig. 2). In Fig. 2(a) a very open structure is visible between the spheres that make up the bare latex layer. In Fig. 2(b), the latex particles are coated with a thin layer of polypyrrole (amount of charge passed is 300 mC/cm²). The internal surface now consists largely of polypyrrole but the porous structure is still present. Two uncoated latex spheres are visible at the surface. They can be used as a reference. When the latex membrane becomes coated with large amounts of polypyrrole, the porosity of the composite layer is lost. This is shown in Fig. 2(c) for a thick latex layer, which was treated with polypyrrole for 50 s. As will be shown below, no enzyme electrodes can be made from these nonporous latex membranes.
Preparation of enzyme electrodes and activity assay

An enzyme electrode was constructed from the polypyrrole-modified latex membranes by treating them with glucose oxidase. This was achieved by agitating the membrane in an aqueous PBS solution containing the enzyme for 4 h and subsequently drying overnight. The immobilization procedure was conducted at a temperature of 277 K.

The enzyme electrodes were tested separately (i.e. independent of the biosensor activity) for enzymatic activity by means of an assay described previously [22,23]. In this assay, the natural cosubstrate of glucose oxidase (oxygen) is replaced by the artificial electron acceptor benzoquinone. Hydroquinone, which is formed in the catalytic cycle, is measured electrochemically at an RDE. The regeneration of benzoquinone from hydroquinone takes place at a fixed potential (0.35 V/(Ag/AgCl)). The resulting current is a measure of the enzymatic activity. Although there is a slight rise in current caused by the spontaneous oxidation of glucose by benzoquinone, the current increase resulting from the catalytic action of the enzyme is large enough to give a significant difference in the slope of the current–time plot.

Figure 3 shows the effect on the measured current when a glucose oxidase treated polypyrrole latex membrane with spheres originally 112 nm in diameter, is introduced into the electrochemical cell. This latex membrane was treated with an amount of polypyrrole corresponding to passing a charge of 100 mC/cm². As can be seen, the current increases immediately after introduction of the membrane into the cell. Membranes with 220 nm spheres also showed this behavior. The fact that the activity returns to its initial value after withdrawal of the membrane is an
TABLE 1
Enzymatic activity of the various composite membranes as determined by the RDE assay

<table>
<thead>
<tr>
<th>Charge mC cm⁻²</th>
<th>1 µm b</th>
<th>5 µm b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>112 nm c</td>
<td>220 nm c</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>150</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>200</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>300</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>400</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>500</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1000</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+ Enzymatically active membranes; – nonactive membranes; × not tested.

a Thickness of composite membrane.

b Size of latex particles used in composite membrane.

Indication that the enzyme is properly immobilized in the pores. Material which was not properly immobilized would have stayed in solution and the slope of the line after point 2 in the figure would have been higher. This assay also showed that drying the membrane after adsorption of the enzyme is essential. Enzyme was washed out completely when membranes were tested for activity directly after enzyme treatment.

A number of enzyme electrodes based on the polypyrrole latex composite membranes were tested for enzymatic activity with this assay. The results for membranes composed of either 112 nm or 220 nm spheres, containing increasing amounts of polypyrrole, are listed in Table 1. The polypyrrole content is represented by the amount of charge passed in the electrochemical polymerization. It can be seen in Table 1 that up to a certain amount of charge enzymatically active membranes are obtained after enzyme treatment. Furthermore, the activity depends on the layer thickness of the membrane and not on the size of the particles. The thick layers were able to accommodate more polypyrrole before they became unfit for enzyme immobilization. This is to be expected because thick layers contain more interspherical space than thin layers. The enzyme is immobilized by physical adsorption and probably forms a monolayer on the polypyrrole surface. Thick latex membranes have a larger polypyrrole surface. Consequently, a higher enzyme loading is expected. The observed independence of the enzyme activity on the particle size probably stems from the fact that the membranes with 112 nm and 220 nm spheres have interspherical pores of approximately the same dimensions (Fig. 2).

Amperometric biosensor

In order to measure their biosensor activity the enzyme-treated composite membranes were used as the working electrode in an amperometric three-elec-
TABLE 2
Response times of the various composite membranes

<table>
<thead>
<tr>
<th>Charge mC cm⁻²</th>
<th>Response time/s</th>
<th>1 µm ²</th>
<th>5 µm ²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>112 nm b</td>
<td>220 nm h</td>
<td>112 nm b</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>12</td>
<td>x</td>
</tr>
<tr>
<td>150</td>
<td>20</td>
<td>20</td>
<td>x</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>300</td>
<td>30</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>500</td>
<td>-</td>
<td>-</td>
<td>95</td>
</tr>
</tbody>
</table>

- no enzyme activity; × not tested.

a Thickness of composite membrane.
b Size of latex particle used in composite membrane.

trode cell. The cell was part of a continuous flow system, which made it possible to switch between a buffer solution and a solution containing the substrate glucose. All experiments were conducted under an argon atmosphere and 25 U/ml catalase was present in all solutions. The latter enzyme was added to eliminate any enzymatically produced H₂O₂.

In general, only the electrodes which showed clear enzymatic activity in the RDE assay (Table 1) were tested as a biosensor. The amperometric measurements were performed at a potential of 0.35 V/(Ag/AgCl). In principle, nonspecific electrochemical glucose oxidation at the platinum surface could occur. Therefore the electrodes were also tested for glucose sensitivity before they had been treated with glucose oxidase. No current response was detected in these cases.

Enzyme electrodes based on latex membranes of thickness 1 µm showed a relatively low activity (approximately 10 ± 2 nA/mM glucose). However, the dynamic range was very good. The response time increased with the amount of charge passed during the pyrrole polymerization (Table 2). The response time is defined as the time needed to reach 95% of the steady-state current. The time to reach this steady-state current was only 12 s for the lowest polypyrrole content (polymerization charge 100 mC/cm²). When more than 400 mC/cm² of charge was passed during the polymerization, no biosensor activity was found for the resulting enzyme-treated electrodes. As already mentioned this is probably due to the fact that in these electrodes no interspherical surface is available for enzyme adsorption. For composite membranes prepared at 100-300 mC/cm², the biosensor activity was almost equal. The activity profile for one of these membranes (charge passage of 300 mC/cm²) is shown in Fig. 4. As can be seen, the current response to glucose is virtually linear in the range 0-20 mM. Also shown in Fig. 4 is the activity profile for a biosensor containing an amount of polypyrrole corresponding to 400 mC/cm². The dynamic range of this sensor is much lower. The use of 500 mC/cm² or higher amounts of charge yielded membranes which were
enzymatically inactive. The enzyme activity assay showed a similar trend (see above). No significant differences in activity were found when the thin latex membranes contained 112 or 220 nm spheres. The accessibility of the membrane to glucose oxidase seems to be the critical factor. Once the pores become too small, the enzyme cannot penetrate the membrane structure anymore and immobilization does not occur.

Charge doses smaller than 100 mC/cm² were not investigated because the polymerization time became too short to obtain reproducible conditions. Even at low current densities (20 mA/cm²), the polymerization time was less than 5 s which is too short to reach a constant current under galvanostatic control.

Sensors constructed from thick composite membranes (5 μm) containing 112 or 220 nm latex particles showed good activities (Table 2), as expected in view of the results in Table 1. However, significant differences in the absolute current values were measured when the two types of latex membranes (i.e. with 112 nm and 220 nm spheres respectively) contained the same amounts of polypyrrole. The calibration curves for enzyme electrodes composed of 112 nm diameter latex particles and amounts of polypyrrole corresponding to 200 and 400 mC/cm² are shown in Fig. 5(a). Lower amounts of polypyrrole were not tested (see also Table 1). A polymerization time corresponding to 500 mC/cm² yielded a sensor without activity. However, the RDE assay showed that enzyme is present in this case (Table 1). Probably this enzyme is not in direct contact with the conducting polymer.

Latex membranes with 220 nm particles and a polypyrrole deposition corresponding to 500 mC/cm² still displayed biosensor activity, in contrast with the
membranes with 112 nm particles. Higher polypyrrole loadings yielded inactive membranes (Fig. 5(b)). The optimum in Fig. 5(b) is for membranes with an amount of polypyrrole corresponding to a charge dose of 400 mC/cm². Lower amounts of polypyrrole yielded less active and poorly performing membranes.

The maximum activity of the sensors derived from the 5 µm membranes is $60 \pm 10 \text{nA/mM glucose}$ (calculated from the linear parts of the curves in Fig. 5(b)), which is higher than the activity of the 1 µm membranes ($10 \text{nA/mM glucose}$). This difference may be explained by the fact that the thick membranes can load more enzyme and have a larger surface of conducting polymer.

The response time of the biosensors depended on the amount of polypyrrole present, the thickness of the latex membrane and the particle size of the latex beads. The various response times were evaluated by measuring 5 mM glucose and are summarized in Table 2. It should be noted that this time is slightly dependent on the glucose concentration. However, the difference in response time for the measurement of, for example, 2 mM and 20 mM glucose was less than ca. 5 s.

**Selectivity and lifetime**

The sensitivity of the sensor to fructose, citrate, lactate, urea, uric acid and pyruvate was tested separately. No significant response was observed to any of these components when they were present at concentrations of 5 mM. Ascorbate (vitamin C) interfered strongly when present at 5 mM concentration. However, in real samples the ascorbate concentration is usually much lower [24] (e.g. 0.1 mM in milk).
The current response of freshly prepared biosensors was tested for prolonged periods of time. To this end, the sensors were taken up in a flow system which was kept at room temperature. The carrier stream contained 2 mM glucose, which was continuously measured during the lifetime experiment. At fixed intervals (every 24 h) an additional amount of glucose leading to a total concentration of 5 mM was introduced in the carrier stream and the increase in current was measured. In this way we were able to eliminate deviations due to baseline drift. In Fig. 6 the activity of a biosensor (5 μm membrane, 220 nm particles, charge dose 400 mC/cm²) to 5 mM glucose is plotted as a function of time. The current response is not stable during the first days of the measurement. Probably, in the beginning an amount of less firmly bound enzyme is slowly washed out. After 3 days, the sensor response remained the same for 10 days. This stability is sufficient for disposable applications.

Effect of oxygen

The effect of oxygen on the biosensor activity was studied at low potentials (0.10–0.35 V) in a continuous flow system. No additional electron mediators were present. The accidental mediation of electron transfer by free flavin molecules can be excluded, since any flavin cofactor, dissociated from the enzyme, is washed out immediately. The only low molecular weight mediator that can still be operative in the system is oxygen. However, we found no significant difference in activity when measurements under ambient atmosphere were compared with measurements under argon. Figure 7 shows a typical plot of the response (at 0.35 V/(Ag/AgCl)) under an argon atmosphere using argon-flushed solutions (full curve) and under
ambient atmosphere using air-saturated solutions (broken curve). The difference is less than 6%, which is within the range of experimental error.

Oxygen mediation would lead to hydrogen peroxide formation. At sufficiently low anodic potentials this would cause a strongly negative response because hydrogen peroxide is catalytically reduced [25,26]. In Fig. 8 the response of our sensor membrane to 10 mM glucose is compared with the response of the same membrane to 0.0025% hydrogen peroxide at a potential of 0.10 V/(Ag/AgCl). As can be seen the addition of hydrogen peroxide causes a large negative current. The addition of glucose leads to a positive current. The formation of even the smallest amount of hydrogen peroxide due to enzymatic glucose oxidation would have eliminated or probably inverted the current response to glucose. Therefore we can conclude that no significant oxygen mediation takes place in our system and that the measured current is due to direct electron transport from glucose oxidase to the polypyrrole.

Taking into account this direct electron transport, the working potential (maximum 0.35 V/(Ag/AgCl)) of our composite membrane electrode is very low. Polypyrrole is electroactive in its oxidized state and the charge carried by the conducting polymer can be cycled repetitively [27]. The confined space in the interspherical pores of the latex membrane apparently brings the active centers of the enzyme molecules into close contact with the conducting polymer. We found that drying after enzyme treatment of the membrane is essential for both the immobilization of the enzyme and the process of direct electron transfer. Water is likely to be a competing species for the enzyme with regard to adsorption on the polymer surface. When water is removed by evaporation, enzyme adsorption is
favorable [28]. Electrostatic interactions may play an important role in the immobilization process. Polypyrrole in its conducting state is a polycation [29]. Glucose oxidase at neutral pH has at least 10 negative charges on its surface [30]. Because

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**Fig. 8.** Current response of a membrane containing 220 nm latex particles and polypyrrole deposited at a charge dose of 200 mC/cm² (measuring potential 0.10 V/(Ag/AgCl)): (a) 10 mM glucose, sensor open to the air; (b) 0.0025% hydrogen peroxide.

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**Fig. 9.** Measurement of glucose concentrations during a fermentation process: broken curve, concentrations measured using the latex + polypyrrole biosensor described in Fig. 8; full curve, concentrations measured using a commercially available glucose kit.
of these features the electroactive sites on the conducting polymer can be expected
to interact strongly with the enzyme, thereby making direct electron transfer a
favorable process.

Measurements in fermentation samples

The performance of the latex + polypyrrole biosensor in real samples was
evaluated by measuring the biosensor response in samples directly drawn from a
fermentation process. Calibration of the sensor was achieved by adding fixed
amounts of glucose to the fermentation medium and recording the resulting
biosensor response. The pH of the medium was 4.5. At this pH value the biosensor
was sufficiently stable and sensitive to allow the decrease in glucose concentration
during the fermentation process to be measured successfully (Fig. 9). It can be
seen in Fig. 9 that the biosensor response correlates very well with glucose
determination using a commercial glucose kit. It can be concluded that the off-line
monitoring of glucose in fermentation samples using our latex + polypyrrole
biosensor is feasible.

CONCLUSIONS

We have shown that amperometric glucose sensors can be constructed from
polypyrrole-modified latex membranes which are cast on a platinum electrode.
Adsorption of glucose oxidase on the polypyrrole surface within the pores of the
membranes leads to immobilization without loss of enzymatic activity. Evidence is
presented which suggests that in the biosensor direct electron transfer occurs
between glucose oxidase and the conducting polymer. The absence of hydrogen
peroxide production and the stabilization of the enzyme in the pores of the
composite membrane results in a device which has a considerable lifetime under
continuous use. This device can be used successfully to measure glucose concen­
trations in, for example, fermentation processes.

The principle of immobilizing enzymes by adsorption on a conducting polymer
surface inside the pores of latex membranes can be applied to many combinations
of redox enzymes and conducting polymers. The construction is very solid and can
be utilized to develop disposable sensors. We are currently investigating these
possibilities.

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