Kinetic study of the performance of third-generation biosensors

Cornelis G.J. Koopal

TNO Nutrition and Food Research, Institute of Biotechnology and Chemistry, PO Box 360, NL-3700 AJ Zeist (Netherlands)

Roeland J.M. Nolte

Nijmegen SON Research Center, University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen (Netherlands)

(Received 30 July 1993)

Abstract

A kinetic study of the performance of third-generation biosensors for glucose based on glucose oxidase immobilized on a microporous matrix of the conducting polymer poly(pyrrole) is presented. The mechanism of the enzymatically catalyzed oxidation of glucose will be different in this type of biosensor as the natural electron acceptor oxygen is replaced by the conducting polymer. Different kinetic parameters are found for the immobilized glucose oxidase than for the enzyme in solution. Mediation by the conducting polymer is found to be very effective and no significant electron transfer to oxygen is observed. In addition to substrate transport limitation in the microporous matrix, the enzymatic reaction in the biosensors is limited by the applied potential.

1. Introduction

Enzyme immobilization on conducting-polymer-modified electrodes has received much attention recently with reference to amperometric biosensor research [1–5]. In amperometric biosensors the reaction between the biological receptor, i.e. the enzyme molecule, and its substrate can be detected as an electrical current [6]. The interest in conducting polymers as the enzyme-immobilizing material is rationalized by the finding that these polymers can physically entrap enzyme molecules [7–9] and appear to stabilize the immobilized enzyme [10]. Enzyme-based amperometric biosensors can be subdivided into first-, second- and third-generation biosensors [11]. For example, a first-generation amperometric biosensor for glucose makes use of the electrochemical detection of the species produced (hydrogen peroxide) or consumed (oxygen) by the enzyme glucose oxidase which is immobilized on an electrode surface [12,13]. Conducting-polymer-modified electrodes have been developed in which the electrode surface (platinum) acts as both the substrate for the immobilization of the conducting polymer and the electrochemical detector for the hydrogen peroxide that is produced [8,9,14,15]. The substitution of oxygen by artificial mediators as a cosubstrate for glucose oxidase has led to the second generation of glucose sensors. The use of these mediators in conjunction with conducting polymers as the immobilization matrix stabilizes the enzyme to some extent, but leakage of mediator out of the polymer matrix rapidly decreases the biosensor response and introduces toxic species into the sample solutions [16–19]. Biosensor systems in which the redox enzyme operates without the use of natural cosubstrates or soluble mediators are called third-generation biosensors. Third-generation biosensors based on conducting-polymer-modified electrodes benefit from stabilization by the polymer and the absence of inactivation by hydrogen peroxide [20]. Glucose electrodes have been developed based on direct communication of glucose oxidase with conducting organic salt electrodes [21] or with polymers that are modified with redox active molecules [17,18,22–24].

In previous papers we have described two novel third-generation biosensor systems—a track-etch membrane sensor and a latex + poly(pyrrole) membrane sensor [25,26]. These membrane sensors contain a microporous matrix in which the conducting polymer poly(pyrrole) is deposited. It was shown that glucose oxidase can be immobilized in the modified matrix by physical adsorption while retaining its activity. The
resulting systems displayed excellent sensor properties, which were ascribed, amongst others, to the fact that the redox enzyme transfers its electrons directly to the conducting matrix. Figure 1 shows a schematic representation of our two biosensors. It also shows how the electrons involved in the enzymatic oxidation of glucose are transferred to the conducting polymer and eventually to the electrode where they are measured as a current.

In this paper we deal with a kinetic study of the performance of the two biosensor systems mentioned above. The kinetics of the oxidation of glucose by glucose oxidase in homogeneous aqueous solution has been studied in detail [27]. Molecular oxygen is the electron acceptor in this case. The reaction involves five steps:

\[ E_{ox} + S \xrightarrow{k_4} E_{ox}S \xrightarrow{k_1} E_{red}P \]  
\[ E_{red}P \xrightarrow{k_2} E_{red} + P \]  
\[ E_{red}P + S \xrightarrow{k_3} E_{red} + S + P \]  

Three steps are related to the oxidation of glucose and the dissociation of the product complex. Two other steps bear upon the binding of molecular oxygen, its conversion into hydrogen peroxide, and the dissociation of the latter molecule from the enzyme. This part of the sequence transfers the reduced enzyme back to the biologically active state.

In eqns. (1)–(5) \( E_{ox} \) and \( E_{red} \) are the oxidized and reduced forms of glucose oxidase, \( S \) is \( \beta \)-D-glucose and \( P \) is D-gluconolactone. Oxygen mediation does not occur in the track-etch and latex + poly(pyrrole) membrane sensors (eqn. (4)). Re-oxidation of reduced enzyme takes place by electron transfer to the conducting polymer. Equations (4) and (5) are no longer valid in our case. They are replaced by the single step

\[ E_{red} + O_2 \xrightarrow{k_4} E_{ox}H_2O_2 \]  
\[ E_{ox}H_2O_2 \xrightarrow{k_5} E_{ox} + H_2O_2 \]  

where \( p_{ox} \) and \( p_{red} \) represent the oxidized and reduced states of poly(pyrrole) respectively. The constant \( k_6 \) is the heterogeneous electron transfer rate constant. Because of the presence of an oxidizing potential, electroactive sites on the conducting polymer that are reduced by the enzyme are immediately reoxidized.

2. Materials and methods

The construction of the biosensors is described in detail elsewhere [25,26]. When not in use, the sensor membranes were stored in Oxysept 2 (Allergan Benelux, The Netherlands) to preserve them and to avoid bacterial or fungus contamination.

Glucose oxidase (E.C. 1.1.3.4) type II (25 000 U g\(^{-1}\)) from Aspergillus niger and catalase (E.C. 1.11.1.6, 2800 U mg\(^{-1}\)) from bovine liver were obtained from Sigma. Phosphate-buffered saline (PBS) (pH 7.4, 10 mM phosphate) was prepared using distilled water which was filtered over a microfiltration membrane (Millipore). The PBS was sterilized after preparation. Before use, four Omnicare tablets (Allergan Benelux, Netherlands) were added to every litre of buffer solution. In this way, at least 20 U ml\(^{-1}\) catalase were present beforehand in all preparations made with PBS. All other reagents were of analytical grade.

Electrochemical measurements were performed using a CU-04-AZ electrochemical controller (Antec Leyden, The Netherlands). This apparatus allowed for current offsets up to 1000 nA. After filtering (RC time 2 s), the current output was recorded on a Yew 3056 pen recorder. For the activity measurements, the enzyme membrane was used as the working electrode in a three-electrode flow cell (AMOR flow cell, Antec Ley-
An Ag/AgCl electrode was used as the reference electrode. The base of the flow cell (glassy carbon) acted as the auxiliary electrode. In the case of the track–etch membrane sensor the platinum-coated rear of the membrane was pushed against a glassy carbon disc (diameter, 6 mm). The latex + poly(pyrrole) membrane was deposited on a glassy carbon disc which had been coated with a thin layer of platinum (300 nm). To insulate the active surface of the sensor membrane from the auxiliary electrode, it was covered with a Teflon spacer 1 mm thick. A duct of approximately 0.15 cm$^2$ was left in the spacer, allowing the membrane to make contact with the solution. PBS was driven through the cell at a rate of 1.75 ml min$^{-1}$ using a Watson Marlow 503S 4 channel peristaltic pump. The membrane potential was set at the desired value by means of the electrochemical controller. During the measurements, the buffer solution was replaced by a glucose solution and the current response was monitored.

The current response to glucose (concentration 1–2 mM) as a function of pH was determined using the appropriate buffer salts and adjusting them to the desired pH value with 2 M HCl on 1 M NaOH. Buffer solutions of 10 mM sodium phosphate + citric acid, 10 mM sodium tetraborate + HCl and 10 mM sodium tetraborate + NaOH were used to cover the pH range 2.6–9.6. All buffers contained 0.15 M NaCl. The pH values of the buffers and of the resulting glucose solutions were measured using a Metrohm 691 pH meter.

The temperature measurements were performed in a thermostated water bath (Julabo U3, Julabo Labortechnik GMBH, Germany). The flow cell was isolated from the water by the application of a layer of grease (Glisseal, Borer Chemie AG, Switzerland). Crushed ice was added for the measurements at temperatures below room temperature. The cell was incubated at the various temperatures for at least 15 minutes before the steady-state current response to a 2 mM glucose solution was measured.

### 3. Results

We determined the apparent kinetic parameters of glucose oxidase in our sensors under steady-state conditions. This means that the current response due to varying concentrations of glucose is measured under conditions where the bulk substrate concentration does not change in time. A Michaelis–Menten equation of the following form was used [10]:

$$I_{ss} = \frac{I_{\text{max}} [S]}{K_M + [S]}$$  \hfill (7)

where $I_{ss}$ is the measured steady-state current and $I_{\text{max}}$ is the maximum catalytic current for the sensor. The biosensors operate under conditions where internal diffusion limitation of substrate may be significant. Therefore only apparent $K_M$ values (denoted by $K_M'$) are determined. The maximum current, $I_{\text{max}}$ may vary from sensor to sensor. Its value depends on the enzyme loading, the available surface and the amount of biochemically active enzyme. Therefore $I_{\text{max}}$ is not an intrinsic parameter of the immobilized enzyme.

#### 3.1. Effect of glucose

The biosensor was used as the indicator electrode in a three-electrode flow cell. The potential applied was 0.35 V vs. Ag/AgCl. This is a very low potential considering the fact that no additional redox mediators are present in our biosensors. The measurements were conducted under an argon atmosphere using argon-flushed solutions. All solutions contained at least 20 U ml$^{-1}$ catalase to destroy any hydrogen peroxide produced accidentally. Therefore any response current observed upon the addition of glucose is presumed to result from direct electron transfer between the enzyme and the conducting polymer [7,25,28]. The glucose concentration was varied between zero and 100 mM. We observed that the biosensors did not respond reliably to concentrations higher than 60 mM. Figure 2 shows the steady-state current as a function of glucose concentration for a representative track–etch membrane electrode and a latex + poly(pyrrole) membrane electrode. Lineweaver–Burk plots of the data are given in Fig. 3. The kinetic parameters were calculated using three different procedures: the Lineweaver–Burk [29], Hanes–Woelf [30] and Wilkinson [31] procedure. All three gave the same results. The parameters are summarized in Table 1. For comparison, literature values for free and immobilized glucose oxidase are also given in the table. These literature values show a great discrepancy. Despite this, we can conclude that our values for $K_M'$ are significantly lower than those reported in the literature *.

#### 3.2. Effect of applied potential

The biosensor response in the glucose concentration range 0–20 mM was determined at various potentials versus Ag/AgCl. The measurement conditions were the same as those described above. A representative result for the track–etch membrane sensor is presented

* We encountered one publication in which a $K_M'$ for glucose of 11.5 mM is mentioned [36]. The authors claim that their $K_M'$ value is an order of magnitude greater than the $K_M$ for the solubilized enzyme ($K_M = 33$ mM [34]). As this claim is not consistent with the $K_M'$ value given, we believe that the reported value of 11.5 mM is incorrect.
in Fig. 4. The latex + poly(pyrrole) membrane sensor gave similar response curves. In order to establish whether the biosensor response is predominantly limited by substrate transport or by interfacial dynamics at the electrode, Tafel plots [37,38] of the data points were made. The results for the track-etch membrane sensor at glucose concentrations of 2.5 and 20 mM are shown in Fig. 5. As can be seen in this figure, Tafel-like behaviour is evident at low potentials (straight lines drawn through the data points) but not at high potentials (see also Section 4).

3.3. Effect of pH and temperature

The pH dependence of our biosensors was determined at a glucose concentration of 2 mM. This relatively low concentration was chosen to avoid local variations in acidity due to the enzymatic production of gluconic acid. The result for the track-etch membrane sensor (220 nm beads, 5 µm thick, poly(pyrrole) deposition using a total charge dose of 400 mC cm⁻²) is given in Fig. 6. The other latex + poly(pyrrole) membrane or track-etch membrane sensors gave similar profiles. For comparison, a pH curve from the literature is also given in Fig. 6. It represents glucose oxidase entrapped in a poly(pyrrole) matrix during electrochemical immobilization [2,10].

The temperature dependence of the biosensors was determined by measuring the steady-state current due to the addition of a fixed amount of glucose (1 mM) in the temperature range 4–45°C. Fig. 7(a) shows the resulting temperature profile for a track-etch membrane sensor. As can be seen, the response of the sensor increases monotonically from 4 to 37°C and then decreases. This behaviour was found to be reversible. An Arrhenius-like plot of the data from Fig. 7(a) is shown in Fig. 7(b). The temperature behaviour of the latex + poly(pyrrole) membrane sensor was very similar. The following relation was used to calculate the activation energy $E_a$ from the data:

$$\ln(I) = -\frac{E_a}{RT} + \text{constant}$$

(8)
TABLE 1. Kinetic parameters of the membrane sensors and some literature systems

<table>
<thead>
<tr>
<th>System</th>
<th>$K_M$ or $K_M / \text{mM glucose}$</th>
<th>$I_{max} / \mu\text{A}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidase in track-etch membrane</td>
<td>14.7 ± 0.8 a</td>
<td>3.05 ± 0.07 a</td>
<td>32</td>
</tr>
<tr>
<td>Glucose oxidase in latex membrane</td>
<td>15.7 ± 2.2 a</td>
<td>4.1 ± 0.3 a</td>
<td></td>
</tr>
<tr>
<td>Glucose oxidase entrapped in poly(pyrrole)</td>
<td>11.2 ± 0.4 a</td>
<td>1.13 ± 0.02 a</td>
<td>32</td>
</tr>
<tr>
<td>Glucose oxidase entrapped in poly(pyrrole)</td>
<td>10.9 ± 0.2 a</td>
<td>1.12 ± 0.3 a</td>
<td></td>
</tr>
<tr>
<td>Glucose oxidase covalently bound to glassy carbon</td>
<td>33.4 ± 0.7 a</td>
<td>7.2 ± 0.06 a</td>
<td>2</td>
</tr>
<tr>
<td>Free glucose oxidase</td>
<td>31</td>
<td>4.3</td>
<td>10</td>
</tr>
<tr>
<td>Free glucose oxidase</td>
<td>60</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Free glucose oxidase</td>
<td>33</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Free glucose oxidase</td>
<td>120</td>
<td>$V = 235 \text{ s}^{-1}$</td>
<td>35</td>
</tr>
</tbody>
</table>

a Values for independently prepared electrodes; standard errors calculated by the Wilkinson method [31].
b Measured at 0°C.
c Maximum velocity.

For the track-etch membrane sensor $E_a = 41 \text{ kJ mol}^{-1}$; for the latex + poly(pyrrole) membrane sensor a value of $E_a = 44 \text{ kJ mol}^{-1}$ was obtained. These activation energies are similar to those reported by others for glucose oxidase entrapped in poly(pyrrole) (see Section 4) [2].

3.4. Lifetime

The lifetime of the two biosensors was evaluated under conditions of continuous operation at room tem-
The immobilized glucose oxidase in our biosensors obeys Michaelis–Menten kinetics. Table 1 shows that the apparent Michaelis–Menten constant $K'_M$ (rows 1 and 2) is lower than $K_{M(\text{glucose})}$ for glucose oxidase in a homogeneous air-saturated solution (rows 6–8) [27]. As a first tentative conclusion we can say that this low $K'_M$ value suggests that the matrix of our sensor electrodes is not covered with a multilayer of enzyme. Such a multilayer of immobilized enzyme would have imposed major diffusional restrictions on substrate transport and this would have been manifested as a significantly increased apparent Michaelis–Menten constant value compared with the free enzyme [33]. The decrease in the Michaelis–Menten constant suggests that the reaction rate is partly limited by interfacial dynamics; electron transfer from the enzyme to the conducting polymer is probably rate determining in the total reaction sequence [39–41]. This conclusion is supported by the results from the experiments carried out at different electrode potentials (Figs. 4 and 5). At low potentials (100–200 mV vs. Ag/AgCl) a Tafel-like behaviour is observed at both low and high glucose concentrations. This is characteristic for a reaction sequence in which a heterogeneous electron transfer step at the electrode.

**Fig. 7.** Steady-state current response of a track-etch membrane biosensor as a function of temperature. (a) Track-etch membrane with 800 nm pores treated with pyrrole+FeCl$_3$ for 1 min and was incubated with 5 mg ml$^{-1}$ glucose oxidase for 30 min: $+$ first temperature experiment; $\bullet$ second experiment after cooling the system to 15°C. (b) Arrhenius plot of the data points.

A concentration of 2 mM glucose was continuously measured during the experiments. At fixed times a glucose concentration of 5 mM (latex sensor) or 10 mM (track–etch sensor) was introduced into the carrier stream and the increase in current was measured. In this way we were able to account for the baseline drift that could occur during the experiments. A representative lifetime curve for both sensors is given in Fig. 8.

**Fig. 8.** Stability plots of the biosensors in continuous operation (2 mM glucose) under ambient atmosphere (the activity of the sensors was measured by introducing an additional amount of glucose to the carrier stream (see text)): $+$ 800 nm track–etch membrane sensor treated for 1 min with poly(pyrrole)+FeCl$_3$ and subsequently incubated with 5 mg ml$^{-1}$ glucose oxidase for 30 min; $\bullet$ 220 nm beads latex+poly(pyrrole) membrane of approximately 5 μm thickness electropolymerized with pyrrole using a total charge dose of 400 mC/cm$^{-2}$ (the membrane was treated with 5 mg ml$^{-1}$ glucose oxidase for 4 h).
(see eq. (6)) is rate controlling. At higher potentials (250–350 mV vs. Ag/AgCl) a deviation from this Tafel behaviour occurs (Fig. 5), indicating that electron transfer is facilitated due to the higher overpotential [36]. When the potential is sufficiently high (e.g. 0.35 V vs. Ag/AgCl), the reaction is controlled by a combination of substrate transport limitation (see below) and Michaelis–Menten type enzyme kinetics [11].

The Lineweaver–Burk plot [29] (Fig. 3) and the Hanes–Woolf plot [30] (not shown) of the data revealed that a deviation from linearity occurs at higher glucose concentrations. This indicates that internal mass transport of glucose is limited at these high concentrations [42]. The result is that the concentration of glucose near the surface of the biosensor is lower than in solution, leading to a smaller current response.

Thus two rate-limiting processes take place at the same time in our biosensors. First, substrate transport limitation is imposed by the membrane structure, leading to a moderate increase in $K_M'$ [33]. Under normal operating conditions ($E = 0.35$ V) this limitation becomes detectable at high substrate concentrations. However, this effect is dominated by a second process, i.e. the interfacial dynamics governing the transport of electrons to the electrode. This causes a substantial decrease in $K_M'$ [33]. Such a reduction of $K_M'$ has also been observed in first-generation biosensors, i.e. sensors in which the electron acceptor is molecular oxygen. Glucose oxidase has a very high affinity for this second substrate; $k_3$ in eqn. (3) is $1.26 \times 10^8$ M$^{-1}$ min$^{-1}$ at 15°C [27]. Because of this, oxygen is easily depleted at the surface of the matrix in which glucose oxidase is immobilized, leading to a decrease in apparent $K_M'(g_{lucose})$ [33,43].

The question as to how the interfacial electron transport can still be so effective that it competes successfully with electron transport to molecular oxygen now arises. We believe that the active centers of the immobilized glucose oxidase molecules can be considered to be electrically wired to the oxidizing electrode by the poly(pyrrole) matrix [44]. The mediating moieties, i.e. the redox active sites on the conducting polymer, transport the electrons over a very short distance (< 5 nm) [45]. In addition, the conducting polymer can cycle the redox state of its electroactive sites with high electrochemical efficiency [46]. This creates a fairly constant surface concentration of sites able to communicate with the active centers of glucose oxidase. Molecular oxygen, in contrast, has to diffuse from the bulk solution into the biosensor membrane over a fairly long distance (normally of the order of 5–10 μm) before it can bind to the enzyme and accept electrons. Therefore oxygen cannot compete effectively with the poly(pyrrole) molecules for regenerating the reduced enzyme molecules [43].

The optimum pH value for glucose oxidase in the two biosensors is higher than the value for the free enzyme in solution (Table 2) [34,49]. We tentatively attribute this shift in optimum pH to the different process of reduced enzyme regeneration in our systems, which is expected to lead to the production of excess protons. The effect of the immobilization matrix itself could also be contributing to the shift in optimum pH. This has actually been found for glucose oxidase immobilized on various materials, e.g. carbon fibre [48], graphite [47], activated carbon [46] and poly(pyrrole) [2] (see Table 2). The most interesting result is that the optimum activity of our sensor systems ranges over approximately 1 pH unit [50], in contrast with what is found for other sensors (Fig. 6) [36,46,48]. This means that the enzyme has become relatively insensitive to (small) changes in bulk pH. Apparently, the poly(pyrrole) matrix creates a microscopic environment which stabilizes the response with respect to variations of pH in the bulk solution [51].

The temperature dependence of the biosensor response, as shown in Fig. 7(a), shows a maximum around 37–40°C and then decreases. This behaviour has been reported before in the literature for glucose oxidase immobilized in poly(pyrrole) [2]. However, in our case this effect is reversible, suggesting that it is not caused by enzyme denaturation. The decrease in response can probably be attributed to a change in the protein structure, making the enzyme less catalytically active with respect to its substrate glucose [52]. The activation energies that have been calculated from the temperature dependent data (41 kJ mol$^{-1}$ 44 kJ mol$^{-1}$ for the track–etch membrane sensor and the latex + poly(pyrrole) membrane sensor respectively (see Section 3) agree well with the value reported by Fortier et al. [2] for glucose oxidase entrapped in a poly(pyrrole) film (41 kJ mol$^{-1}$). At this stage it is not justifiable to

### Table 2. Optimum pH values for free glucose oxidase and for glucose oxidase immobilized in various matrices

<table>
<thead>
<tr>
<th>Matrix</th>
<th>pH</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Track–etch sensor</td>
<td>6.2–7.1</td>
<td>32</td>
</tr>
<tr>
<td>Latex sensor</td>
<td>5.9–6.9</td>
<td>32</td>
</tr>
<tr>
<td>Poly(pyrrole)</td>
<td>6.0</td>
<td>2</td>
</tr>
<tr>
<td>Activated carbon</td>
<td>6.3</td>
<td>47</td>
</tr>
<tr>
<td>Graphite</td>
<td>7.3</td>
<td>36</td>
</tr>
<tr>
<td>TTF + TCNQ</td>
<td>7.4</td>
<td>21</td>
</tr>
<tr>
<td>Carbon fibre</td>
<td>7.8</td>
<td>48</td>
</tr>
<tr>
<td>Free enzyme</td>
<td>5.5–6.0</td>
<td>10,34,49</td>
</tr>
</tbody>
</table>

*Glucose oxidase from *A. niger*.

*Electrochemical entrapment.*

*TTF, tetra(thio)fluvale; TCNQ, tetracyanoquinodimethane.*
draw conclusions from the temperature experiments with regard to the reaction mechanism. However, it can be said that the catalytic action of glucose oxidase in our systems has not altered significantly compared with other systems in which poly(pyrrrole) is used as the immobilization matrix. In addition to this, we find that our matrices stabilize the enzyme molecules at higher temperatures, as is revealed by the reversible character of the temperature-dependent response (see Section 3).

5. Conclusions

Our kinetic analysis of the track-etch membrane and latex + poly(pyrrrole) membrane biosensors has shown that the measurement potential is an important parameter. For a properly functioning third-generation biosensor it is required that mass transport, electron transport and enzymatic catalysis are in balance [6,11,39,40]. The electrical potential of the sensor electrode strongly affects this balance. When this potential is low (less than ca. 0.2 V vs. Ag/AgCl) the rate of the enzymatic reaction becomes too low compared with the rate of substrate transport. It is reasonable to conclude that re-oxidation of the reduced enzyme is the rate-limiting process at these low potentials (see Fig. 1(c) [37]. The curves in Figs. 4 and 5 support this conclusion. When the potential is sufficiently high, the steps involved in enzymatic conversion of substrate become rate determining. Substrate transport limitation becomes important when high glucose concentrations are offered to the sensor membrane. As a consequence, biosensors operating at an appropriate potential will display approximately linear behaviour over a considerable range of substrate concentration [11,38,53]. When the sensor is operating at the correct potential small variations in the amount of enzyme will not affect the current response. As long as the amount of catalytically active enzyme is high enough to create a condition where substrate transport is rate controlling, a constant sensor response will be obtained. When enzyme denaturation occurs, this will not immediately be reflected in the current response. When it has proceeded to such an extent that the amount of active enzyme inside the sensor becomes rate determining, the response suddenly drops [9,15,36]. This is what is actually found for our biosensor systems (Fig. 8).

The lifetime of the latex + poly(pyrrrole) membrane sensor is significantly lower than the lifetime of the track-etch membrane sensor. This is a result of the poorer mechanical stability of the latex membrane. The continuous flow of solution which is passed over the sensor during the lifetime measurements produces slow degeneration of this membrane.

Acknowledgements

The authors wish to thank Martin Feiters for his helpful contributions to the discussion. This research was supported by the Technology Foundation (STW).

References