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Carbon-paste biosensors array for long-term glucose measurement

J. Kulys & H. E. Hansen

Novo Nordisk A/S, Roskildevej 56, 3400 Hillerød, Denmark

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Abstract: An amperometric glucose biosensor based on a new mediator and carbon paste acts at 0·08 V vs. an Ag/AgCl electrode *in situ* and exhibits a linear response up to 16 mM of glucose. The measurements are performed on freshly renovated electrode surfaces, and every sensor can be used for 100 measurements.

The long-term stable biosensors were obtained using stabilized glucose oxidase and an array of biosensors. The application of a chemometric method permits exploitation of the biosensor during a period of more than 166 days at 35°C and relative humidity 75%. The relative error of chemometric prediction was less than 5·4%, and the response changes less than 0·15% per week.

Keywords: biosensor, enzyme electrode, reference electrode, glucose, phenylenediamine derivative, chemometrics.

INTRODUCTION

A remarkable variety of schemes have been developed for biosensing (Cass, 1990). However, the long-term stability of biosensors remains a *bona fide* problem. This problem arises because the sensitivity of the biosensor changes with time. Factors such as temperature, humidity, etc. modify the response of the biosensor. In principle, there are two separate contributions to the change in sensitivity of a biosensor with time: one has to do with the physical surroundings and the other with the intrinsic properties of the biosensor. The physical surroundings of a biosensor may change when it is placed *in situ*. The enzyme activity, mediator amount and membrane permeability will change over time, and it is not possible to prevent these processes completely. This paper will not cover these aspects of the response performance of biosensors.

In this work, a conceptually different approach to the improvement of the long-term response stability of biosensors is analyzed. This approach is based on chemometrics. Chemometric data processing (the use of mathematics and statistics on chemical data) has become a routine procedure in analytical practice (Martens & Næs, 1989). Vapor and odor can be detected from a solid-state multisensor array using pattern-recognition networks (Gardner *et al.*, 1992). Electrochemical devices based on a sensor array for carbohydrates, amino acids and other analytes have been described (Chen *et al.*, 1993), and it is considered that multi-electrode arrays will open up a new dimension in electrochemical analysis (Svendsen, 1993). The chemometric method explored in this work is applied to biosensors. It will be shown that a set of biosensors can be made that significantly ameliorates the problem of the long-term response of biosensors; with sufficient

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quantitative knowledge, the calibration relationship between the measured quantity and the analyte concentration can be adjusted to correct for natural drift in the quantitative response of the biosensor.

The glucose biosensors used are based on an array of two carbon-paste electrodes. Details of a glucose biosensor in which all components are mixed and embedded in the pasting oil was published by G.J. Patriarche and co-workers in 1989, and inlcuded dimethylferrocene as mediator (Amine *et al.*, 1989). Later publications described a number of glucose sensors which used as electron transfer mediators dimethylferrocene and polyethylene glycol-modified enzyme (Mizutani *et al.*, 1991), ferrocene-poly(ethylene oxide) (Hale *et al.*, 1991), tetrathiafulvalene derivatives (Lee *et al.*, 1992), tetracyanoquinodimethane (Pandey *et al.*, 1992), graphite–Teflon ferrocene (Wang *et al.*, 1993) and Meldola blue (Kulys *et al.*, 1994a). These studies indicated that the biosensors had different stabilities. Amine *et al.* (1991) investigated the operational stability of their sensors for 4 weeks and came to the conclusion that mediator leaking and gradual surface erosion are the main factors limiting their analytical performance. Sakura and Buck (1992) asserted that enzyme electrodes based on graphite powder with poly(vinylchloride) as a binder and ferrocene monocarboxylic acid as the mediator remained active and stable for more than a year in a dried state in a refrigerator. It was shown that the modification of glucose oxidase with poly(ethylene glycol) did not improve the stability of sensors (Mizutani *et al.*, 1991).

EXPERIMENTAL

Reagents

Graphite powder and paraffin oil were received from Fluka, poly(ethylene glycol) (PEG; MW 20 Kda), D(+) trehalose dihydrate, glucose oxidase (GO), *Aspergillus niger* (EC 1.1.3.4) with activity 211 U mg^{-1} and Triton X-100 were obtained from Sigma. Cellulose acetate was acquired from Aldrich, phenylmethyldimethylsilicone (OV-11; Cat. No. OV-11-100) was purchased from Ohio Valley Specialty Chemical Inc. (Marietta, USA). 1-(N,N-dimethylamine)-4-(4-morpholine)benzene (AMB) was synthesized by Niels Clauson-Kaas A/S (Denmark) according to a patent application (Buch-Rasmussen *et al.*, 1992). Perhydrol (30% of hydrogen peroxide) and all other compounds were received from Merck.

Dialysis of glucose oxidase was carried out using 1 g ml^{-1} of enzyme solution in water at 4°C against 300 ml of water (3 h), and after changing the water the dialysis was performed for an additional 9 h. The dialysis tubings used were MWCO 12,000–14,000; SPECTRA/POR. Samples of glucose oxidase with trehalose were prepared by adding to the dialyzed glucose oxidase an equal amount (by weight) of trehalose dihydrate. The dialyzed solution of glucose oxidase or a solution containing additional trehalose was lyophilized, and samples were kept in vacuum desiccators under phosphorus pentoxide for at least 2 days.

Mediator and enzyme activity determination

Mediator concentrations in buffer solutions were determined using cyclic voltammetry and a glassy carbon electrode. Cyclic voltammetry (CV) was performed using a computer-controlled electroanalytical system (model CS-1087, Cypress Systems, Inc., USA) in the three-electrode regime. The filtering time of the system was 50 μs. As a reference, a saturated calomel electrode (SCE, saturated with KCl, mod. K-401, Radiometer, Denmark) and an auxiliary electrode made from Pt-wire (diameter 0·2 mm, length 4 cm) mounted on the end of the reference electrode were used. Mediator amounts in carbon paste were determined after extraction from the carbon paste with petroleum or heptane by measuring the absorbance at 263 nm ($\epsilon = 1{\cdot}74 \times 10^4$ M^{-1} cm^{-1}) and at 320 nm ($\epsilon = 2{\cdot}4 \times 10^3$ M^{-1} cm^{-1}), or by measuring the fluorescence at 386 nm. The extinction coefficient of AMP was determined in heptane.

The mediator distribution between paraffin (silicon) oil and 0·1 M Na-phosphate buffer solution, pH 7·4, containing 0·1 M NaCl, was investigated at 24·4°C. The 1·0–1·2 g of mediator solution with concentrations 43·5–65·9 mM in paraffin oil and silicon oil (d was assumed to be 1 g cm^{-3}) was added to the surface of 15·0 ml buffer solution and incubated under intensive mixing with a rotating bar under anaerobic conditions. The concentration of mediator in the buffer solution was measured using a glassy carbon electrode inserted into the solution. The

equilibrium concentration between buffer solution and oil was established after 2 h of incubation. The concentration of the mediator was measured using a calibration curve. The established diffusion coefficient of the mediator was $6{\cdot}7 \times 10^{-6}$ cm^2 s^{-1}. The distribution coefficients were 6·0 and 10·7 for paraffin and silicon oil, respectively.

The amount of catalytically active glucose oxidase in lyophilized samples was determined by measuring the absorbance change of a deaerated glucose oxidase solution in 0·1 M phosphate buffer, pH 7·4, comprising 0·1 M NaCl and 0·11 M of glucose, using the absorbance coefficient $1{\cdot}31 \times 10^4$ M^{-1} cm^{-1} at 456 nm (Weibel & Bright, 1971). It was shown that lyophilized samples of glucose oxidase contain 74% of catalytically active FAD, whereas samples with trehalose contain 35·6% of catalytically active FAD. The relative enzyme activities in the pastes were calculated by multiplying the activity (211 U mg^{-1}) and percent of catalytically active FAD.

For the determination of glucose oxidase activity in paraffin oil, a thermostated glass cell with a peroxide electrode (Kulys, 1992) was used. The platinum anode of the peroxide electrode was covered with a semipermeable membrane prepared from a 2% solution of cellulose acetate in acetone. 50 ml of incubation mixture contained 0·1 M sodium acetate buffer solution, pH 5·6, with 0·1 M sodium chloride and 0·1% Triton X-100. The measurements were performed at 25°C after a weighed amount (19–31 mg) of enzyme/paraffin oil mixture was added to the solution and it had been incubated under extensive mixing using a magnetic bar for 5 min. The reaction started after 1 ml of 2·5 M glucose solution was added to the buffer solution. The peroxide electrode was calibrated using hydrogen peroxide in the same reaction mixture.

Glucose oxidase inactivation in paraffin oil was investigated at 40°C and relative humidities (RH) 0 and 75%. The reaction mixture (~1 g) contained 0·9–1·6% of lyophilized and equilibrated (at RH 0 or 75%) glucose oxidase and 0·5–0·64% of mediator or enzyme only. The RH 0% ($5{\cdot}7 \times 10^{-3}$%) was created in a vacuum desiccator using phosphorus pentoxide; for generation of RH 75%, a saturated sodium chloride solution was used (Hägerdal & Löfqvist, 1973).

Carbon paste formulation

The carbon pastes were prepared by mixing 2 g of heated (at 650°C for 5 min) and cooled (in a vacuum desiccator) graphite with 1 g of paraffin oil (or silicon oil) containing 0·05–10 mg g^{-1} of AMB and samples of enzyme. The graphite was heated in order to remove the adsorbed water and organic volatile compounds and to standardize the preparation of samples. The amount of enzyme added to the paste varied from 10 to 400 mg per g of graphite. The amounts of enzymes and mediator in the carbon pastes studied are shown in Table 1.

Electrode preparation

Carbon-paste electrodes were manufactured by filling two channels (inner diameter 2·3 mm) of injection-moulded high-density polyethylene rods (diameter 5·8 mm, length 40 mm). The filling was performed in vacuum (2–3 mbar) by an automatic machine with a piston pressure of 5·5 bar. Two smaller channels (inner diameter 1·3 mm) in the rods housed two silver electrodes prepared from silver wires of diameter 0·2 mm. Electrical contacts were made by gold-plated copper connector pins.

Measuring procedure

The electrode current was measured using a three-electrode system. The reference silver/silver chloride electrode was prepared *in situ* immediately before every measurement (Kulys *et al.*, 1994b). The sensor current was measured in an 'inverted' drop cell in such a manner that all the electrodes were facing upwards. Every sensor response was measured using a new electrode surface. The renewal of the surface was performed by cutting slices (thickness 0·3 mm) off the polyethylene rod, carbon paste, and auxiliary and reference electrodes by means of a stainless steel knife in a special mechanical device.

The procedure of sensor calibration includes placing a drop (about 0·04 ml) of 0–16 mM glucose solution onto the surface of a vertically orientated electrode followed by 10 s of reference electrode preparation *in situ*. Then the potential (80 mV) is applied to the working electrodes and after a 10 s delay the current is measured for 30 s. The potential of the reference *in situ* electrode was 45 mV vs. SCE (Kulys *et al.*,

TABLE 1 Glucose biosensors parameters in 0·1 M Na-phosphate buffer solution pH 7·4 containing 7 mM glucose, 0·1 M NaCl and 2% PEG

Enzyme form	*Amount of enzyme (U/mg graphite)*	*Pasting oil (amount of mediator, %)*	*Testing time (days at 35°C, RH 75%)*	*Predicted parameters**		
				a, μC	*b, %/week*	*RSE, %*
Native**	31/1·6	Paraffin (0·5)	91	348·1	0·15	3·2
Native/trehalose**	29/1·5	Paraffin (0·5)	91	348·9	0·09	2·4
Native/trehalose***	15/0·9	Paraffin (0·5)	124	278·5	−0·11	5·2
Native/trehalose***	15/0·9	Silicon oil (1·0)	166	299·6	−0·14	2·4

*a and b are the initial response and the slope of predicted response change; RSE is a relative standard error.
**Measurements were performed using independent sensors with different amounts of the enzyme.
***Measurements were performed using a single sensor; each channel of sensor contains different amounts of the enzyme.

1994b). In the series of experiments with biosensors containing the same amount of enzyme in both channels, the current of the sensors during this period was integrated: ten independent measurements from both channels were averaged and shown in graphs as 'RESPONSE, μC'. In the other series of experiments in which the sensor channels were filled with carbon paste containing different amounts of enzyme, the average values of ten simultaneous measurements from both channels are presented.

After the first ten measurements, the sensors were placed in the thermostat and kept at 35°C and RH 75% for a specified period of time. After this incubation time, 90 measurements were performed and the average value of the responses was used for calculation. All calibrations were done at room temperature, *i.e.* in the interval from 22·4 to 27·3°C. The sensor temperature was monitored using a thermoresistor mounted in the electrode frame, and all sensor responses were re-calculated to 25°C using a temperature coefficient of 3·4% per degree centigrade.

For the biosensors response measurements, glucose solution equilibrated with air in 0·1 M Na-phosphate buffer, pH 7·4, comprising 0·1 M NaCl and 2% PEG, was used. All bionsensor calibrations made in the long-term stability test were performed using the same solution containing 7 mM of glucose.

Apparatus and calculations

The biosensor responses were determined using a dedicated computerized self-made potentiostat. The program is written in 'C' for IBM compatible computers. A/D converter resolution is 16 bits; integration sampling time is 20 ms.

The acquired data were transferred to Mathcad[3·1] Windows version and were used for the calculations and plots below. The approximation of data was performed by minimizing the mean squared error.

RESULTS AND DISCUSSION

The principle of a carbon-paste biosensor containing a bioelectrode, and reference and auxiliary electrodes in one piece, with renewal of the electrode surface, was described in 1990 (Colin, 1990). Amongst other features, the biosensors investigated here differ from those previously described by using a new mediator which is soluble in oil. The use of a low potential redox mediator (0·15 V vs. SCE at pH 7·0) (Kulys *et al.*, 1994c) permits a glucose sensor response at 125 mV (vs. SCE). The sensor action can be explained by the following scheme. The glucose oxidase (GO) adsorbed on graphite is reduced by glucose (S). The reduced mediator dissolved in paraffin ($M_{(par)}$) diffuses into the buffer solution at the interface, is oxidized electrochemically at the 125 mV potential and interacts with the reduced glucose oxidase.

$$GO_{(ad)(ox)} + S \Rightarrow GO_{(ad)(red)} + P$$

$$M_{(par)} \Rightarrow M_{(sol)} - e \Rightarrow M^{+}_{(sol)}$$

$$GO_{(ad)(red)} + M^{+}_{(sol)} \Rightarrow GO_{(ad)(ox)} + M_{(sol)}$$

The sensors exhibit linear calibration

(correlation coefficient 0·998) from 1 to 16 mM of glucose (Fig. 1). Only at glucose concentrations less than 1 mM a small deviation from the linearity of the calibration graph can be observed. By employing a renewed electrode surface for every measurement, surface erosion and mediator leaking are hindered and the sensors show a good statistical performance: the coefficient of variation of 10 × 2 measurements varied in the range 0·8–2·7% at 7 mM glucose.

Figure 2 shows the long-term responses of biosensors containing different samples of enzyme. The data presented indicate that the decrease in response depends on the amount of enzyme as well as on the type of enzyme utilized in the carbon paste. When sensors contain lyophilized glucose oxidase and a low enzyme activity (1·6 U mg^{-1}), the response decreases from 230 to 52 μC over a period of 13 weeks. During the same period of time, the response of sensors containing a high amount of enzyme (31 U mg^{-1}) only changes from 364 to 261 μC. The kinetics of the decrease in the response of the sensors with low enzyme activity fit a bi-exponential decay curve with half-times of inactivation of 0·8 and 9 weeks and partial magnitudes of 60 and 40%, respectively.

Introducing trehalose into the carbon paste significantly decreases the sensor inactivation rate (Fig. 2). At low enzyme activity the response decreases by 34%, whereas at high activity it changes only 5% during both 13 and 12·1 weeks. The kinetics of the changes of the sensor with

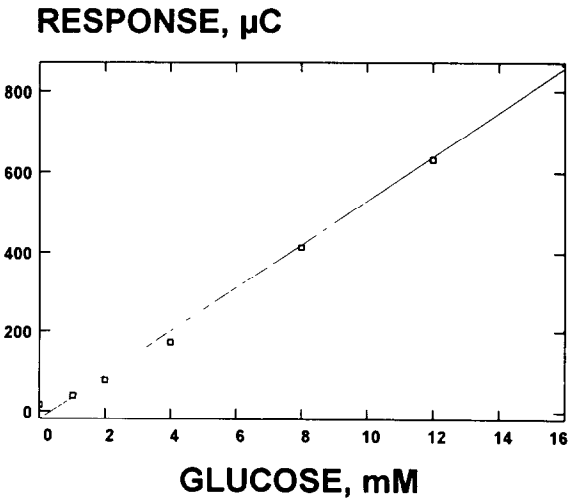

Fig. 1. Calibration graph of a glucose biosensor at pH 7·4 and electrode potential 80 mV vs. Ag/AgCl electrode in situ.

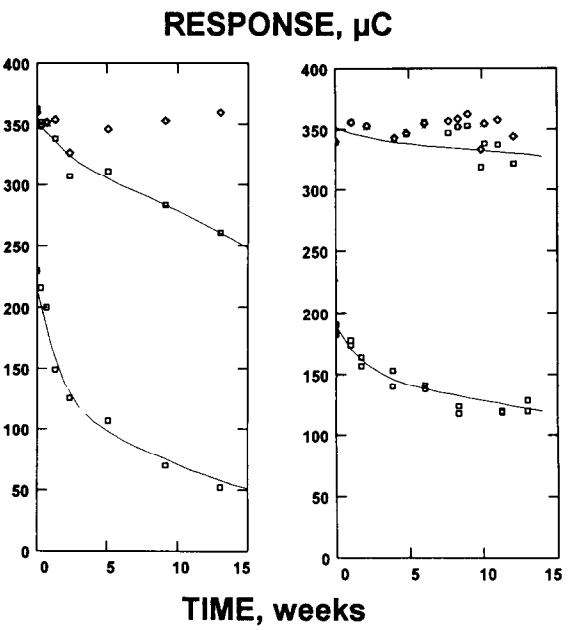

Fig. 2. Long-term glucose biosensor response at 35°C, RH 75%. Left-hand side, biosensors containing glucose oxidase carbon paste; right-hand side, biosensor containing enzyme/trehalose carbon paste. Square points, experimental data; solid lines, calculated values; diamonds, predicted values; dotted lines, linear approximation of predicted values.

low activity was approximated by a bi-exponential decay curve with half-times of 1 and 25 weeks and partial magnitudes of 35 and 65%, respectively.

The biosensors contain two components the loss of which might account for a decrease in response: the mediator and the enzyme. Thus an immediate and necessary question is which factor contributes to the long-term response loss of the biosensor. Figure 3 shows the dependence of the sensor response on the amount of mediator in the carbon paste. The estimated apparent Michaelis constant for this mediator in paraffin oil was 0·54 mM or, using the distribution coefficient, in buffer solution it was 0·09 mM. In paraffin oil this value is about 36 times higher than the concentration of mediator (19·4 mM) that was used for long-term sensor testing. In buffer solution, the apparent K_m of the mediator calculated at 7 mM of glucose was 0·044 mM, with catalytic and reductive constants of 898 s^{-1} and 1·21 × 10^4 M^{-1} s^{-1}, respectively (Weibel & Bright, 1971), and a value of 1·14 × 10^6 M^{-1} s^{-1} for the oxidative constant of the mediator (Kulys *et al.*, 1994c). The rather small difference (~2 times) between the constant measured in buffer

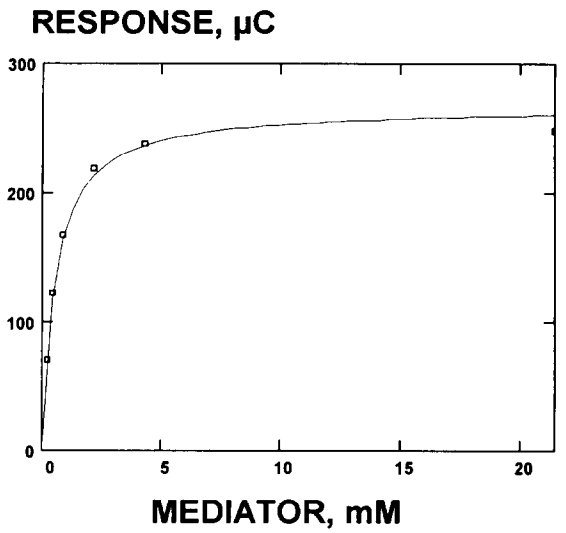

Fig. 3. Dependence of biosensor response on the amount of mediator in carbon paste. Glucose concentration 7 mM; carbon paste contains 4 U mg^{-1} of glucose oxidase; electrode potential as in Fig. 1.

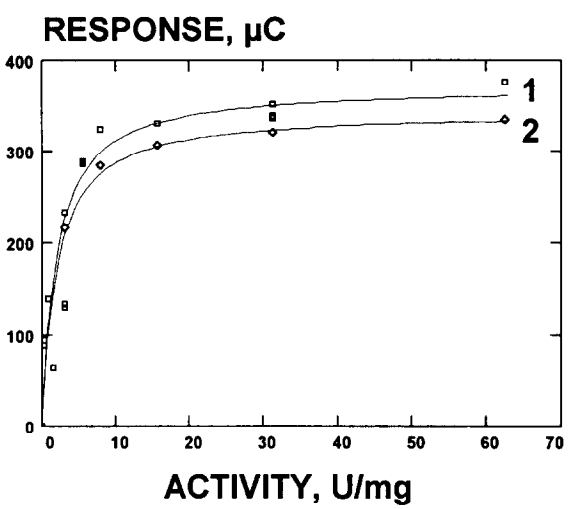

Fig. 4. Dependence of biosensor response on the amount of enzyme in carbon paste containing (1) paraffin oil and (2) silicon oil. Glucose concentration 7 mM; mediator amount (1) 0·5% and (2) 1%.

solution and calculated using glucose oxidase kinetic data can be explained by some diffusion limitation, and indicates that on the electrode surface an equilibrium distribution of mediator exists between buffer solution and paraffin.

AMP determinations performed over 103 days at 40°C and RH 75% show that the mediator concentration in the paraffin oil and glucose oxidase mixture decreases by only 17·5%. These data, together with the large excess amount (in comparison to K_m) of mediator in paraffin oil, indicate that the mediator concentration change is not a dominant factor in the long-term decrease in sensor response.

The sensor response has a saturating dependence on the amount of enzyme (Fig. 4). In the case of a paste containing paraffin oil, the limiting value of the response is about 5% higher than that of a silicon oil paste. This can be attributed to a difference of effective surface areas of the two carbon pastes (Wang *et al.*, 1993). For biosensor action, the saturating nature of these curves is very important. It is suggested here that the saturation of response is caused by external diffusion limitation of the substrate (glucose). This interpretation is confirmed by measurements of ferrocyanide oxidation on mediator-free carbon pastes in PEG buffer solution. When diffusion coefficients of $7{\cdot}3 \times 10^{-6}$ cm^2 s^{-1} and $6{\cdot}7 \times 10^{-6}$ cm^2 s^{-1} for ferrocyanide and glucose, respectively (Lide, 1993–1994), are used, the diffusion-limiting current of ferrocyanide oxidation and biocatalytical glucose oxidation on carbon-paste electrodes agree within 3·6%. Very recently, external diffusion control was also indicated for a glucose bioelectrode based on carbon paste containing a high proportion of dimethylferrocene (Amine *et al.*, 1993).

An expression for the dependence of the biosensor current on enzyme activity was established for different substrate concentrations (Kulys & Vidziunaite, 1990). At a glucose concentration (S_b) of 7 mM, the values of S_b/K_m of 0·072 and 0·07 for paraffin and silicon oil, respectively, can be taken immediately from the kinetic studies of glucose oxidase (Weibel & Bright, 1971; Kulys *et al.*, 1994c). Using these parameters, the dependence of the biosensor current on enzyme activity in carbon paste was calculated, and is shown by the solid line in Fig. 4.

The characteristics of enzyme inactivation in carbon paste were obtained from studies of glucose oxidase inactivation in paraffin oil (Fig. 5). The data presented show that glucose oxidase activity decreases faster at RH 75% and in the presence of a mediator. Owing to the rather low reproducibility of the enzyme activity measurement caused by the heterogeneous nature of enzyme/paraffin oil suspension, the kinetic data

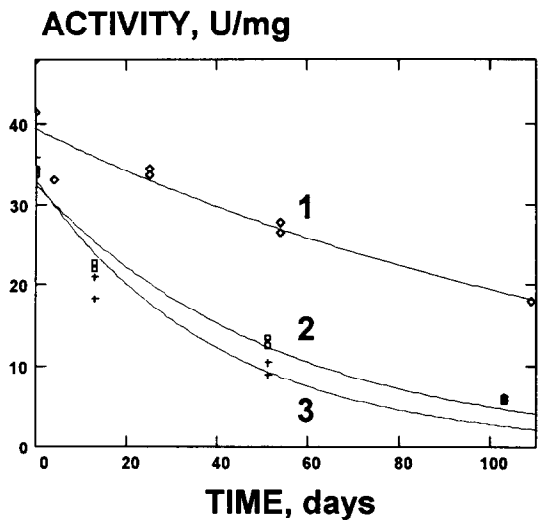

Fig. 5. Glucose oxidase inactivation in paraffin oil at 40°C and (1) RH 0% and (2,3) RH 75%. Concentration of mediator (1,2) 0% and (3) 0·64%.

were approximated using only a single exponential decay curve. The calculated life-times of glucose oxidase were 13·9, 5·2 and 3·9 weeks in 'dry' paraffin oil, in paraffin oil equilibrated with RH 75% air, and in the presence of 0·64% of mediator, respectively. The accelerated inactivation of enzymes in heptane in the presence of water was illustrated by Bo Mattiasson and coworkers (Kaul & Mattiasson, 1992). The action of water for long-term enzyme stability in nonpoliaric (non-protic) solvents is not clear. In a very recent publication (Hartsough & Merz, 1993), the dramatically increased hydrogen bond network of proteins in non-aqueous environments was established. In such media, water as well as mediator molecules can interact with hydrogen bonds and salt bridges. This may increase the flexibility of the peptide chain that will accelerate the refolding of proteins in hydrophobic surroundings.

The data of glucose oxidase inactivation in paraffin oil and in carbon paste can be compared with the restrictions that result from a difference in temperature. Moreover, the preferential adsorption of the protein globule on graphite is a commonly observed phenomenon that can increase or decrease the denaturation rate. However, these data demonstrate glucose oxidase stability in paraffin oil for the first time, and do not contradict the sensor stability results.

The additional information that the dominant factor influencing long-term sensor response is enzyme inactivation explains the increase in the stability of the sensor response when trehalose was used. Trehalose, like many other glass-forming carbohydrates, has been shown to be effective in protecting many enzymes from inactivation under dehydration (Roser, 1991; Levine & Slade, 1992). The increase of the life-time and the partial amount of stable enzyme (Fig. 2) also demonstrates the stabilization action of this carbohydrate to glucose oxidase in paraffin oil.

The model of the limitation of the external diffusion of biosensor action can be used to explain the change in biosensor response and to predict the response not perturbed by enzyme inactivation. At low enzyme activity the sensor acts in the kinetic regime and the decrease in response follows the change in enzyme activity (Fig. 2). Therefore, the half-time of the decrease in response corresponds to the enzyme life-time. At high enzyme activity and at the beginning of inactivation, the sensors act in the conditions which limit external diffusion and the enzyme inactivation has little influence on the decrease in response. The calculated currents rather convincingly approximate the experimental data of both types of sensors (Fig. 2). To compensate for the decrease in the sensor response, a correction factor (CF) should be calculated that corresponds to the relation between the responses of non-inactivated and inactivated sensors. CF is different for the two sensors, and is a function of the ratio (R) between the two sensor responses. Even if a precise model of the limitation of external diffusion is established, the functional dependence $CF = f(R)$ cannot be solved analytically, and therefore the response prediction was made numerically.

The diamonds in Fig. 2 represent such a prediction. The linear approximation of these data is shown by a dotted line. It can be seen that the predicted response hardly changes during the complete period of sensor testing in spite of large enzyme inactivation (Table 1). The relative standard errors, respresenting the predicted data scattering, were 3·2 and 2·4% for sensors with dialyzed and stabilized glucose oxidase, respectively (Table 1). The even smaller error of sensors containing stabilized enzyme is mainly caused by little inactivation of the sensors.

In the case of the sensor arrays, where the two channels were filled with carbon pastes containing different amounts of enzyme, a more general chemometrical approach was used. The

ratio, R, was derived directly from the experimental data and the dependence of the correction factor on R was evaluated statistically using a third-order polynomial approximation. The experimental results and predicted values are presented in Fig. 6. It can be seen that the predicted response of the biosensor containing paraffin oil as well as silicon oil decreases by only 0·11 and 0·14% per week (Table 1). The relative standard errors of the predicted response are 5·4 and 2·4% during the periods of 124 and 166 days, respectively (Table 1).

For an evaluation of the chemometrical approach, it is important to stress two points. One point concerns the substrate concentration. It is easy to show that in external (as well as in internal) diffusion-limiting conditions, CF is not very sensitive to the substrate concentration when $S_b/K_m < 1$ and the diffusion module is larger than 1. The other point concerns the effect of temperature. Temperature influences the response as well as the rate of enzyme inactivation. The most important effect is response compensation. From analysis of the models of sensor action at external or internal diffusion-limiting conditions, it follows that the temperature coefficient can vary from 2% as in the case of mass-transport limitation (Bard & Faulkner, 1980) to about 10% as in the case of a kinetic regime (Kulys *et al.*, 1993). The ratio, R, as was discovered here, contains information about the temperature factor, but this functional dependence should be evaluated in independent measurements. A general scheme for chemometric prediction of biosensor response is suggested:

STATISTICALLY EVALUATED
INDEPENDENT MEASUREMENTS

$$\left.\begin{matrix}\text{Response 1}\\ \text{Response 2}\end{matrix}\right\} \Rightarrow \text{Ratio} \Rightarrow \underset{\downarrow}{\textit{Temperature Factor}}$$

$$\Rightarrow \text{Ratio} \Rightarrow \underset{\downarrow}{\textit{Correction Factor}} \Rightarrow \underset{\downarrow}{\textit{Predicted Response}}$$

In summary, we have reported (1) a previously undescribed mediator used for the preparation of glucose biosensors based on carbon paste, (2) a method for the stabilization of glucose oxidase in paraffin oil and a long-term stable glucose biosensor formulation, and (3) a novel, and perhaps generalized, chemometrical scheme for achieving enhanced long-term stability of biosensors. We expect that the principles discussed here will have the greatest practical relevance for a generation of biosensors based on other enzymes and transducers. This approach may not only provide new tools for the development of long-term stable biosensors, but also promote new opportunities to construct biosensors for the detection of inhibitors and for biosensors with other novel properties.

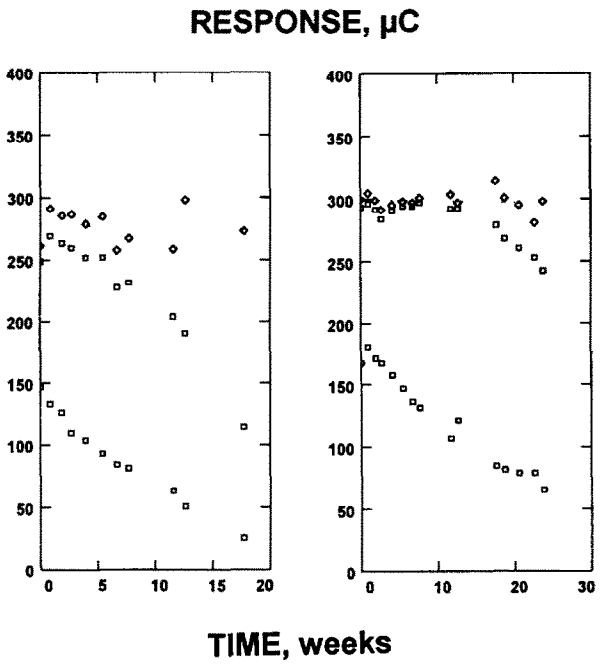

Fig. 6. Long-term response of a glucose biosensor array at 35°C, RH 75%. Left-hand side, biosensors containing enzyme/trehalose/paraffin carbon paste; right-hand side, biosensor containing enzyme/trehalose/silicon oil carbon paste. Square points, experimental data; diamonds, predicted values; dotted lines, linear approximation of predicted values.

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