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Novel planar glucose biosensors for continuous monitoring use

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Abstract

Novel planar glucose biosensors to be used for continuous monitoring have been developed. The electrodes are produced with the "screen printing" technique, and present a high degree of reproducibility together with a low cost and the possibility of mass production. Prior to enzyme immobilisation, electrodes are chemically modified with ferric hexacyanoferrate (Prussian Blue). This allows the detection of the hydrogen peroxide produced by the enzymatic reaction catalysed by GOD, at low applied potential (ca. 0.0 V versus Ag/AgCl), highly limiting any electrochemical interferences. The layer of Prussian Blue (PB) showed a high stability at the working conditions (pH 7.4) and also after 1 year of storage dry at RT, no loss of activity was observed. The assembled glucose biosensors, showed high sensitivity towards glucose together with a long-term operational and storage stability. In a continuous flow system, with all the analytical parameters optimised, the glucose biosensors detected glucose concentration as low as 0.025 mM with a linear range up to 1.0 mM.

These probes were also tested over 50–60 h in a continuous flow mode to evaluate their operational stability. A 0.5 mM concentration of glucose was continuously fluxed into a biosensor wall-jet cell and the current due to the hydrogen peroxide reduction was continuously monitored. After 50–60 h, the drift of the signal observed was around 30%.

Because of their high stability, these sensors suggest the possibility of using such biosensors, in conjunction with a microdialysis probe, for a continuous monitoring of glucose for clinical purposes.

Keywords: Glucose; Continuous monitoring; Screen-printed; Mediator; Microdialysis

1. Introduction

Approximately, 17 million people in the US have Diabetes, a life-long disorder that is, as yet, incurable. A number of long-term complications arise due to the damage caused by diabetes on small and large blood vessels. Blindness, kidney failure, amputation of the lower limbs and nerve damage are only some of the major consequences of diabetes. Long periods of hyperglycaemic account for these long-term complications but also hypoglycaemia (blood glucose level below 50 mg/dl) represents a major issue in diabetes therapy since it can cause sudden coma and brain damage (Goldstein et al., 1984; Zimmermann et al., 2003). Development of these complications can be prevented or slowed down by controlling blood glucose level. Periodic fingerthick tests have found in this perspective a large use but they often fail to detect all hypoglycaemic and hyperglycaemic events since glucose levels can change rapidly (2.25 mg/dl/min). Especially nocturnal hypoglycaemia often remains undetected. For this reason continuous, automated and non-invasive blood glucose monitoring have been for a long period a main goal to assist management of diabetes (Cameron and Ambler, 2004; Pickup and Alcock, 1991).

In the last years several attempts have been made to find the most suitable mean for a continuous monitoring of di-

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abetes in order to check and monitor the glucose level in diabetic patients during the whole day (Turner et al., 1999; Aussedat et al., 1997, 2000; Mastrototaro, 1999). In this perspective the use of a microdialysis probe coupled on-line with an electrochemical sensor modified with glucose oxidase has provided very good results (Moscone and Mascini, 1993a,b; Meyeroff et al., 1992; Moscone et al., 1992). Compared with implantable enzyme sensors (Koudelka et al., 1991; Pickup, 1993) for real-time in vivo analyses, the coupling of microdialysis sampling with external enzyme sensors was shown to be less invasive, more stable, and easier to sterilise.

A wearable system (Glucoday®) based on this principle was developed by A. Menarini Diagnostics in 2001 and is currently commercialised (Poscia et al., 2003; Varalli et al., 2003; Maran et al., 2002). The instrument is based on the use of a wall-jet cell in which a glucose biosensor is located. The glucose biosensor is obtained by the immobilisation of glucose oxidase on a platinum electrode using a nylon membrane. Different membranes are then used to obtain the required sensitivity and selectivity. The biosensor is connected to a microdialysis probe made of a hollow fiber inserted in the subcutaneous tissue. The whole system consists of a specially developed microperistaltic pump, a miniaturised wall-jet cell, which contains the glucose biosensor, and the electronic circuit. The device was demonstrated to be able to overcome the problem of sensitivity variation of glucose biosensors reported in the past and showed an optimal stability for more than 48 h of continuous work (Poscia et al., 2003; Varalli et al., 2003).

This paper reports the results obtained with the use of a planar electrode obtained by the screen-printed technology as electrochemical probe to be coupled with a microdialysis fiber for continuous glucose monitoring. In this study only the analytical performances of these sensors will be addressed without studying the microdialysis fiber which has already been thoroughly investigated in previous works (Poscia et al., 2003; Varalli et al., 2003). Immobilisation of glucose oxidase is accomplished in this case by the use of a cross-linking method avoiding the use of any membrane. However, the most significant advance is represented by the use of a mediator (Prussian Blue) as principal factor for hydrogen peroxide measurement. Prussian Blue is in fact able to catalyse the reduction of the hydrogen peroxide produced by the enzymatic reaction catalysed by glucose oxidase (Itaya et al., 1984; Karyakin and Karyakina, 1999; Karyakin et al., 2000). The electrochemical detection of hydrogen peroxide becomes possible at very low applied potentials (ca. 0.0 V versus Ag/AgCl), greatly reducing any possible electrochemical interference and avoiding the use of any cut off membrane at the electrode surface.

After 1984 (Cass et al., 1984), the use of mediator-based amperometric sensors for glucose measurements have found an incredible improvement with a wide application in the production and commercialisation of fingerthick probe (Roche Diagnostics, Bayer, Lifescan as well as Medisens biosensors are all based on the use of an electrochemical mediator). However, this is in our knowledge the first case in which a Prussian Blue (mediator) based sensor is continuously used for more than 48 h.

The use of this electrochemical mediators (PB) deposited on the surface of an electrode has in fact always been affected by low stability, especially at pH above 7 (Zhang et al., 1999; Garjonyte and Malinauskas, 1998; Karyakin et al., 1998). The optimised procedure for Prussian Blue preparation adopted in this study (Moscone et al., 2001; Ricci et al., 2003) allows for a continuous use of this mediator for a long period even at alkaline pH, thus making the use of such a mediator suitable for the on-line monitoring of glucose in diabetic patients. Moreover, the modification procedure is extremely easy and reproducible and also suitable for mass production of modified electrodes. Results will demonstrate the analytical characteristics of the sensors modified with Prussian Blue and glucose oxidase towards hydrogen peroxide and glucose. The stability of these sensors under the desired conditions (i.e. continuous flow at 10 μl/min) will be also presented together with the storage stability tests. Preliminary results based on the use of biological samples to test the stability of these sensors in real conditions are also presented.

2. Materials and methods

2.1. Materials and reagents

The perfusion solution (i.e. buffer solution) was prepared by adding 1 g/l of sodium benzoate to a Dulbecco's physiological buffer (136.9 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2PO_4, pH 7.4).

The glucose test solution (i.e. control solution) was prepared by using the same perfusion solution with a concentration of 5×10^{-4} M of glucose and 0.1% of Kathon added as microbial preservative. Hydrogen peroxide or glucose solutions were prepared by adding different amounts of hydrogen peroxide and glucose, respectively, in the perfusion solution.

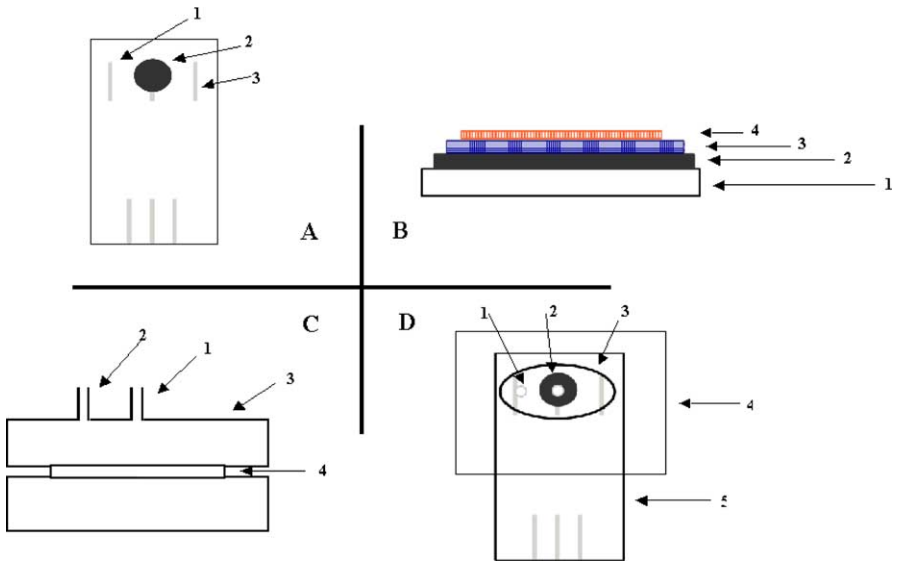
All the solutions were of analytical grade. Ferric chloride, potassium ferricyanide, glutaraldehyde, hydrogen peroxide and β-D-glucose were obtained from Sigma.

Glucose oxidase from Aspergillus Niger (181 U/mg) was obtained from Sigma.

Human serum was obtained from A. Menarini Diagnostics and is a lyophilised human-based control serum usually used for quality control in clinical chemistry. The serum was reconstituted prior to use with distilled water.

2.2. Glucose biosensor

Screen-printed electrodes (SPEs) were home produced with a 245 DEK (Weymouth, England) screen printing machine. Graphite-based ink was used to print the working electrode, while the silver ink was used for the reference and counter electrodes (see Scheme 1A). The substrate was a flexible polyester film obtained from Autotype Italia (Milan,



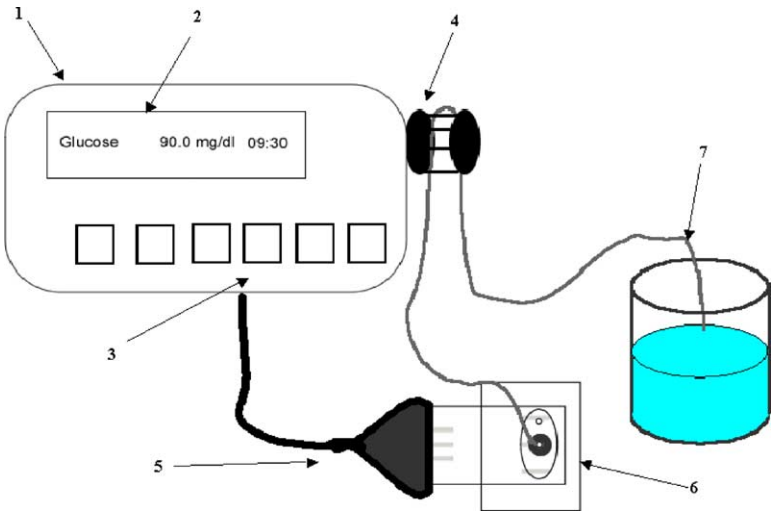
Scheme 1. Schematic diagram of biosensor and wall-jet cell. (A) Screen-printed electrode front-view: (1) silver ink acting as reference electrode, (2) graphite ink acting as working electrode successively modified with Prussian Blue and glucose oxidase, (3) silver ink acting as counter electrode. (B) Screen-printed glucose biosensor side-view: (1) Polyester film as support for printing step, (2) graphite ink, (3) Prussian Blue layer, (4) glucose oxidase layer. (C) Wall-jet flow cell side-view: (1) inlet of the flow, (2) outlet, (3) cell made of Teflon, (4) glucose biosensor. (D) Wall-jet flow cell front-view: (1) outlet, (2) inlet of the flow, (3) O-ring, (4) flow-cell, (5) glucose biosensor.

Italy). The working electrode (diameter 0.2 cm) was modified with Prussian Blue using a chemical procedure already optimised in a previous work (Ricci et al., 2003).

Glucose biosensors were obtained by immobilising glucose oxidase onto the Prussian Blue modified electrode surface (see Scheme 1B). The procedure used consisted in a cross-linking method employing glutaraldehyde and nafion.

The glucose biosensors obtained in this way were then stored dry in different conditions in order to study their storage stability.

When tested for glucose or hydrogen peroxide, the biosensors were inserted into the wall-jet cell (Scheme 1C and D) and connected with a peristaltic pump fixed at a rate of 10 μl/min. The signal due to glucose or hydrogen peroxide was continuously recorded using a potentiostat capable of recording and storing the amperometric signal (see Section 2.3 for details). In Scheme 2, a diagram of the system used for this study is shown. The cell is connected with an instrument capable of applying a constant potential (−50 mV) and controlling a peristaltic pump with a flow rate of 10 μl/min.



Scheme 2. Schematic diagram of the system: (1) Portable instrument capable of applying a constant potential, measuring the current produced in the cell and controlling a peristaltic pump. The data are stored in the instrument and then downloaded in a PC. (2) Display showing values of glucose concentration (mg/dl), current, battery and pressure status. (3) Keyboard. (4) Peristaltic pump controlled by the instrument (10 μl/min). (5) Connections for glucose biosensor poised at −50 mV. (6) Wall-jet flow cell. (7) Solution of buffer or glucose which reaches the electrode surface.

2.3. Instruments

To test the stability and response of the hydrogen peroxide sensors and glucose biosensor, a prototype portable instrument was obtained by A. Menarini Diagnostics. The portable system comprises of a potentiostat with a fixed applied potential of −50 mV which is connected to the screen-printed electrode (hydrogen peroxide or glucose sensor) inserted in the cell. The potentiostat is able to continuously record the current produced in the cell and to store current values. A programme allows the downloading of all the stored values in a PC. Moreover, the system controls a microperistaltic pump which continuously pumps a solution to the measuring cell at 10 μl/min (see Scheme 2).

2.4. Procedures

2.4.1. Operational stability study

The operational stability of the glucose biosensors was studied as follows: the biosensors were inserted in the wall-jet cell (Scheme 1C and D) and a perfusion solution was driven into the cell by use of a peristaltic pump. After a stable baseline was obtained (ca. 3 min), the perfusion solution was substituted by a control solution (0.5 mM glucose) and the signal due to glucose was continuously monitored.

The operational stability of the hydrogen peroxide sensors (Prussian Blue modified sensors without glucose oxidase) was also studied. In this case the control solutions were different concentrations of hydrogen peroxide solutions. The hydrogen peroxide solutions, due to its low stability, were daily prepared.

2.4.2. Operational stability with serum sample

To evaluate the stability of the glucose biosensor with biological samples, the continuous flow system was connected with the microdialysis probe. The probe was perfused with the physiological solution (perfusion solution). At the beginning of the experiment the probe was immersed in a perfusion solution to evaluate the background current. After this, the probe was inserted in a glucose control solution (5.0 mM) to test the response of the biosensor to glucose. The microdialysis probe was then placed in human serum samples under stirring conditions and occasionally alternated with the glucose control solution to test the stability of the biosensor. Also Kathon (0.1%) was added as preservative to avoid the decrease of glucose concentration.

2.4.3. Storage stability

Sensors were stored at room temperature (RT) (ca. 25 °C) or at 45 °C in an oven. The sensors were left dry in dark. The storage stability of the glucose biosensors was evaluated by measuring the signal due to the glucose control solution after a fixed period. The signal from the control solution was obtained in a continuous flow mode (10 μl/min) as explained above for the operational stability.

3. Results and discussion

3.1. Hydrogen peroxide probe

A series of experiments were devised to evaluate factors such as the efficacy of the Prussian Blue modification of the screen-printed electrodes, the hydrogen peroxide signal obtained, electrochemical interferences as well as operational and storage stability. The results of these studies were utilised to determine whether these sensors could be used in flow mode for glucose continuous monitoring after the coupling with glucose oxidase.

The flow rate of the perfusion solution in a microdialysis system is usually between 1 and 10 μl/min. In this case we have adopted a flow rate of 10 μl/min in order to evaluate the stability of the electrodes in the worst possible condition.

The analytical performance of the modified electrodes was then tested in a continuous flow mode using the same flow rate (10 μl/min) and the wall-jet cell that will be adopted for glucose monitoring. The detection limit for H_2O_2 (s/n = 3) obtained was 10^{-6} M while the linearity range was between 10^{-6} and 10^{-3} M, the noise current 15–20 nA and the sensitivity towards hydrogen peroxide was 213 mA/M cm^2. The time needed to reach a stable background was around 2 min, while 1 min was sufficient to reach 90% of the final signal after introduction of the hydrogen peroxide solution (i.e. 10^{-4} M). Reproducibility of these sensors was 5% ($n = 5$).

The effect of the substances that usually interfere in the electrochemical determination of glucose was also examined. The signal due to ascorbic acid, uric acid, 4-acetamidophenol was evaluated using a fixed concentration of 0.5 mM. No response was observed for these electrochemical interferents except for ascorbic acid where a signal equal to 3% of the hydrogen peroxide signal was detected. However, it should be pointed out that the normal concentration of ascorbic acid in blood is around 25–100 μM with a maximum value of 200 μM. This means that after the dilution due to the dialysis step (i.e. around 10 times), a maximum concentration of about 10–20 μM would give an interference signal of ca. 0.2%.

3.2. Operational stability of the Prussian Blue modified electrodes

The effect of the flow rate on the stability of the Prussian Blue layer was first evaluated. In this case, the current for a fixed concentration of hydrogen peroxide was measured prior to the experiment in a batch mode. Then the electrode was inserted into the cell and the flow was continued for a total period of 100 h at open circuit (no current passing). Both the perfusion solution and a hydrogen peroxide control solution (0.2 mM) were used in this study. After 100 h the electrode was removed from the cell and the current due to hydrogen peroxide (0.2 mM) was measured again in batch mode. In both cases the results showed a very good stability (100% of initial signal) of the Prussian Blue layer after 100 h of continuous flow. This demonstrates that the effect of the flow on

the surface of the electrode does not affect the activity of the Prussian Blue deposited and also shows that the flow does not bring the mediator leaking from the electrode surface. This high mechanical stability of the Prussian Blue layer is probably to be ascribed to the strong absorption of Prussian Blue particles onto the surface of the screen-printed electrodes and to the low solubility of Prussian Blue.

Next the operational stability in a closed circuit system was evaluated for the Prussian Blue modified electrodes. The electrodes were inserted in the wall-jet cell, the potential fixed at −50 mV, and the current generated at the electrode surface was continuously recorded. Firstly, a perfusion solution was flowed onto the electrode for 100 h. To check the stability of the electrode a hydrogen peroxide (0.2 mM) solution was pumped to the cell before and after the whole period of experiment (i.e. 100 h). At the end of the experiment the signal observed for hydrogen peroxide (0.2 mM) was the same obtained at the beginning demonstrating that the applied potential, in absence of hydrogen peroxide, does not negatively affect the Prussian Blue stability.

After this, the response was monitored for two different solutions of 0.1 and 0.2 mM (Fig. 1a) of hydrogen peroxide. The solutions were continuously passed in the wall-jet cell and the signal due to the reduction of hydrogen peroxide was recorded for a total of 48 h. A decrease of around 10 and 15% was detected at the end of 48 h monitoring for 0.1 and 0.2 mM, respectively. In the case of the use of these concentrations of hydrogen peroxide, however, it should be considered that the reduced Prussian Blue in the presence of hydrogen peroxide coupled with the applied potential is forced to continuously change its oxidative state according to the following equation (1) (Itaya et al., 1984):

$$(PB_{red})K_4Fe_4{}^{2+}[Fe^{2+}(CN)_6]_3 \rightleftarrows (PB_{ox})Fe_4{}^{3+}[Fe^{2+}(CN)_6]_3 + 4K^+ + 4e^- \quad (1)$$

From the results obtained in this study, it seems that the crucial point concerning the stability of the Prussian Blue is represented by this reaction. When condition are such that the Prussian Blue is not forced to go in the oxidised form (less stable) the stability of the Prussian Blue layer is very high. This is probably the reason why, in absence of an applied potential and of hydrogen peroxide, the Prussian Blue retained 100% of its activity even after 100 h of continuous flow, while in the case of the applied potential and the presence of hydrogen peroxide a decrease was observed. Moreover, it should be pointed out that even taking into consideration the slight decrease, the stability of the Prussian Blue layer is extremely good and quite new for Prussian Blue based sensors which have been always affected by a low stability. As already pointed out in our previous papers (Moscone et al., 2001; Ricci et al., 2003), the high stability is probably the result of the newly developed chemical modification procedure which leads to a stronger adsorption of the Prussian Blue particles on the electrode surface. In contrast to the Prussian Blue layer obtained with the more commonly used electrochemical procedures, these modified electrodes are in fact more stable at basic pH and their continuous use is possible with a minimal loss of activity after several hours. Moreover, with respect to the electrochemical procedure, our chemical deposition is much more suitable for mass production since no electrochemical steps are required and a highly automated process could be adopted.

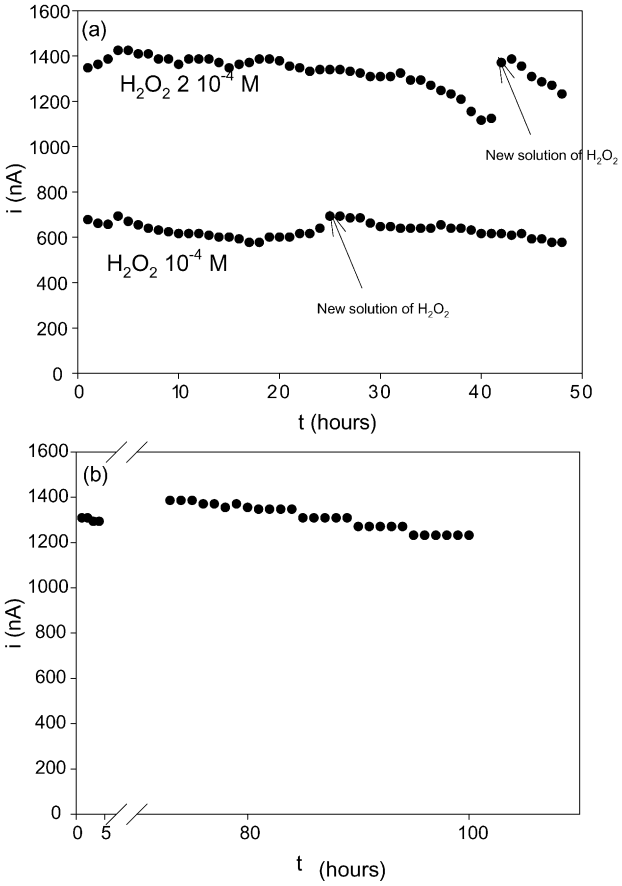


Fig. 1. Operational stability of hydrogen peroxide sensors (PB modified sensors). Applied potential −50 mV vs. int. ref. Continuous monitoring of current in continuous flow mode (10 μl/min). Solution of hydrogen peroxide were renewed where indicated by arrows. (a) Two sensors tested with 10^{-4} and 2×10^{-4} M of hydrogen peroxide. (b) Sensor 6-month-old with a solution of 2×10^{-4} M of hydrogen peroxide.

A test of the operational stability has also been carried out using two electrodes after their storage at room temperature for 6 months. The electrodes showed the same behaviour during 100 h of monitoring. As shown in Fig. 1b the stability was very good with a loss of ca. 15% observed after 100 h. This demonstrates that the stability of the Prussian Blue layer is not decreased even after a long period of storage.

Finally, the Prussian Blue electrodes have been tested in terms of their storage stability. To do this, the electrodes were left dry at room temperature in dark after the surface modification. One year after their production the Prussian Blue electrodes tested for H_2O_2, retained 90 ± 10% ($n = 10$) of their initial signal response to hydrogen peroxide.

These results as to both the sensitivity and storage and operational stability of the sensors modified with a Prussian Blue layer are extremely promising to assemble glucose biosensors.

3.3. Glucose biosensors

In the next phase of the study, Prussian Blue modified electrodes were used as a support for glucose oxidase immobilisation in order to obtain biosensors that could be used for the continuous monitoring of glucose. First the performance of the glucose biosensors was tested in terms of glucose signal and analytical parameters. Calibration curve for glucose is shown in Fig. 2. A detection limit of around 2×10^{-5} M was obtained together with a linear range up to 1.0 mM. The reproducibility of these biosensors was 7% ($n = 5$). The sensitivity of the biosensors was 54 mA/M cm^2 and the current noise signal was almost the same of that obtained with the Prussian Blue modified electrodes. The sensitivity of the biosensors towards glucose was almost 25% with respect to that obtained for hydrogen peroxide. This result is probably related to the composition of the enzymatic membrane. The presence of Nafion, a polyanionic membrane, could in fact have a shielding effect which results in a lower signal of glucose relative to that of hydrogen peroxide.

A very important parameter for a system to be used for continuous monitoring of glucose is the response time which should be as low as possible in order to detect any changes of the analyte concentration in real time. The response time is dependent on the geometry and internal volume of the cell and on the flow rate (10 μl/min). To reach a 90% of the final signal for glucose (0.5 mM) starting from the background signal (perfusion solution), 2 min are needed. Another important characteristic to be taken in account is the time needed to reach the baseline. In this case, a time of 3–5 min is necessary to reach a stable current with the perfusion solution.

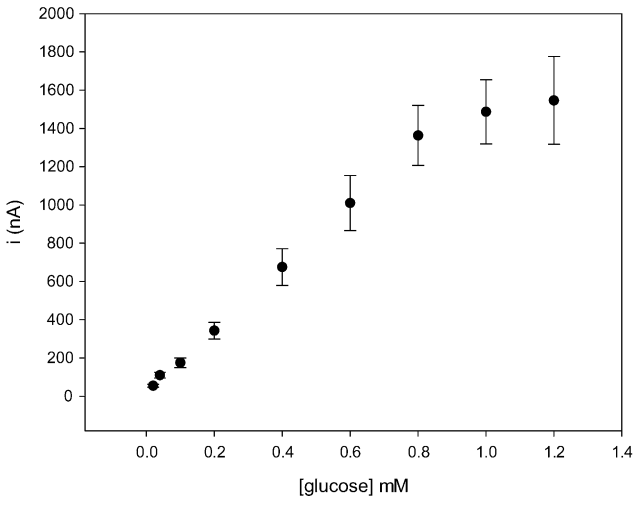


Fig. 2. Calibration curve for glucose (PB modified sensor + glucose oxidase). Applied potential −50 mV vs. int. ref. Continuous flow mode (10 μl/min). $n = 5$ biosensors tested.

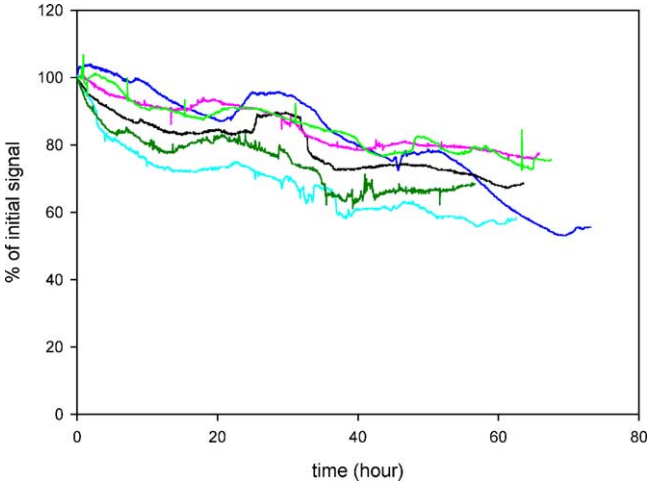


Fig. 3. Operative stability of six different glucose biosensors from a minimum of 50 to a maximum of 72 h. Continuous flow mode (10 μl/min). Glucose concentration: 0.5 mM. Applied potential −50 mV vs. int. ref. The current was continuously recorded for the whole experiment.

3.4. Operational stability of glucose sensors

The biosensors were then tested in terms of operational stability as explained in the experimental section. In this case the biosensors were inserted into the wall-jet cell and when the baseline due to the perfusion solution was reached, the control solution (glucose 0.5 mM) was passed into the cell. The signal due to glucose was recorded continuously for 50 and out to 72 h. Results are reported graphically in Fig. 3. From these data it could be concluded that the enzyme immobilisation procedure adopted provides a high operational stability at these operative conditions. The decrease of the signal is more pronounced in the first 12 h where an average loss of the signal of 20% is observed. After this initial period, the signal from the control solution of glucose is highly stabilised and a further decrease of 10–15% is observed during the next 40–50 h. In Fig. 3, the continuous monitoring (one point every 3 min) of some of the tested electrodes is shown. As already pointed out, the stability is very high and all the electrodes showed a similar trend. By comparing these results with those obtained with Prussian Blue modified electrodes it is possible to say that the decrease of the signal can be completely ascribed to enzyme inactivation. The initial drift of the signal in the first 12 h is also probably due to loss of part of enzyme which is not strongly bound to the membrane.

Results of the operational stability are extremely interesting for the future application of these probes for the continuous monitoring of glucose using a microdialysis probe.

A point that should at present be improved is the limited linear range of the biosensors which could cause some problem in the cases of high concentrations of glucose. Studies are in progress to solve this problem by finding a suitable micro-

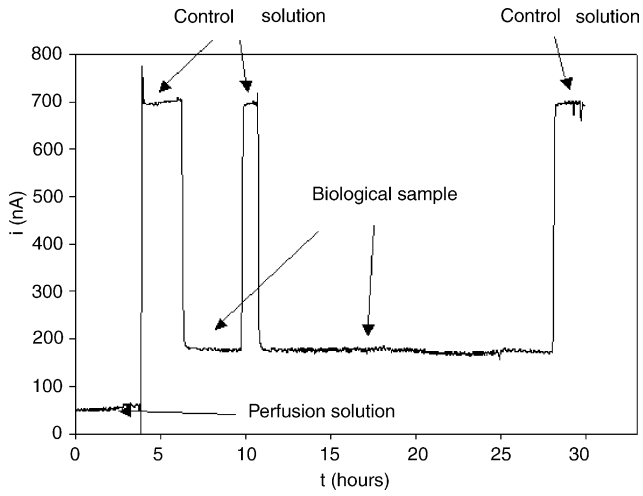


Fig. 4. Study of the biosensor stability with biological sample. Where indicated (i.e. biological sample) a solution obtained by dialysing a human serum with the microdialysis probe was flowed in the biosensor cell. At the beginning a perfusion solution and control solution (glucose 5 mM) were used instead of the serum to test the biosensor response. Control solution of glucose were also used during and at the end of the experiment to evaluate the stability of the biosensor. Continuous flow mode (10 μl/min). Applied potential −50 mV vs. int. ref.

dialysis probe which could be able to recover glucose from the subcutaneous cute in the desired range of concentrations.

It should also be pointed out that in the case of an in vivo measurement, the microdialysis probe will be able to recover not only glucose but also many other biological compounds with low molecular weight from the subcutaneous cute. The electrochemical interferences are highly reduced by the use of PB as well as a low applied potential. It could still be possible that other biological compounds could negatively affect the stability of the enzymatic membrane. It is possible to have a sort of passivation or fouling of the electrode surface due to the absorption of biological compounds on the enzymatic membrane. In this perspective, the slow flow rate could represent a disadvantage since it will not be able to rapidly remove these compounds from the surroundings of the electrode. An inhibition of the enzyme by certain compounds present in the subcutaneous tissue could also possibly lead to an underestimation of the glucose present in the blood.

For this reason experiments to test the operational stability of glucose sensors have been performed with dialysed biological samples. Microdialysis probes were inserted into a biological solution (i.e. human serum with glucose and a preservative added) and the signal due to glucose was continuously monitored for ca. 24 h. A control solution of glucose (5.0 mM) was also used to estimate the stability of the sensors. From the results shown in Fig. 4 it seems that the presence of biological compounds in the solution reaching the electrode surface does not contribute to a lower stability since the signal due to the control solution of glucose is almost unchanged after ca. 20 h. This is an important result because it means that even in the absence of any cut-off membrane on the electrode surface, a high stability could be achieved in the presence of complex fluids.

3.5. Storage stability of glucose biosensors

To evaluate the shelf life of the glucose biosensors, a series of sensors were produced and left dry at RT (i.e. 25 °C) and at 45 °C in an oven. Three biosensors for each period were then tested for glucose (0.5 mM) to determine their residual activity. Taking in account the good reproducibility of these biosensors (ca. 7%) it is possible to evaluate the residual activity by looking only at the signal of the electrodes after each storage interval studied and reporting as reference signal for the initial activity, an average value obtained with six new biosensors. The results are shown in Fig. 5. As it can be seen, the storage stability of these biosensors is extremely good and can be ascribed to the well-known stability of the glucose oxidase enzyme. These results also confirm the high stability of the Prussian Blue layer during storage. Moreover,

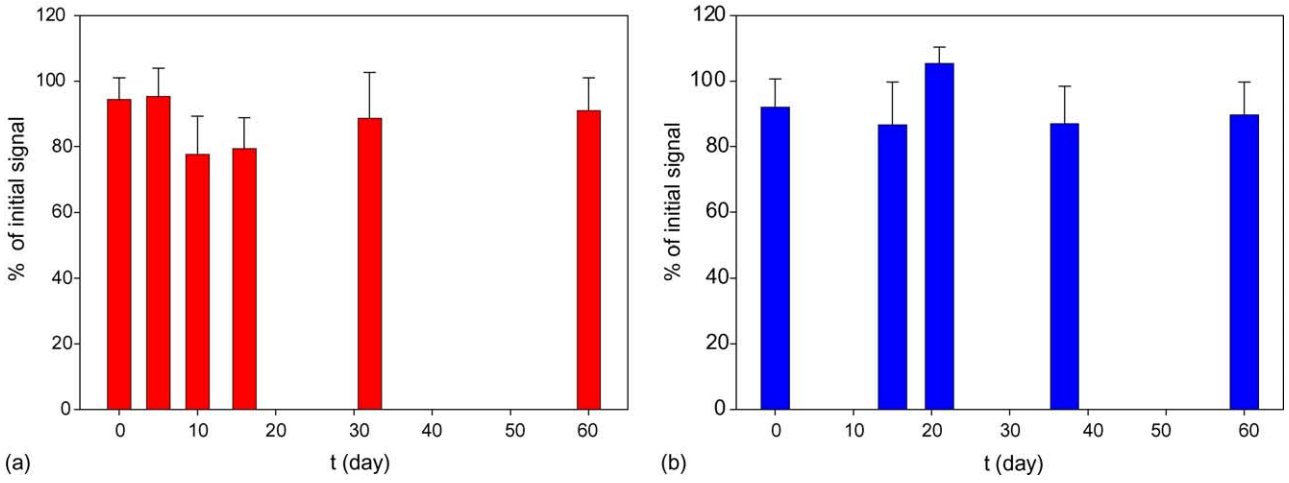


Fig. 5. Storage stability studies. Percentage of initial activity of different glucose biosensors after a storage time. (a) Biosensors stored at RT (i.e. 25 °C) dry. (b) Biosensors stored at 45 °C dry. For each period three biosensors were tested. See text for details.

looking at the results obtained at 45 °C (an average loss of ca. 10% after 60 days) it could be concluded that the shelf life of these biosensors is another encouraging point for their use in continuous glucose monitoring.

4. Conclusions

This paper presents a comprehensive evaluation of the first planar mediator (Prussian Blue) based biosensor applied in continuous glucose monitoring. The analytical performances of both the hydrogen peroxide sensors (screen-printed electrodes modified with Prussian Blue) and the glucose biosensors (Prussian Blue modified screen-printed electrodes with glucose oxidase immobilised) were very satisfactory even if the linear range has to be improved in order to measure glucose in all the pathologic states of diabetes. The operational stability was found to be suitable for the application proposed (i.e. ca. 30% loss of signal after 50–60 h of continuous flow). Moreover, studies with biological samples demonstrated that the operational stability was not affected by the presence of other biological compounds. Also storage stability was found to be very high with a loss of activity of ca. 10% after 2 months stored at 45 °C under dry conditions. This study has demonstrated that easy-to-produce planar sensor based on the use of Prussian Blue and glucose oxidase has been brought to an optimal level of performance. These biosensors are amenable for mass production, have long-term shelf life and a very high operational stability, a characteristic which is difficult to show for other similar mediator based biosensors.

These results showed promising applications of these biosensors in a continuous glucose monitoring system coupled with a microdialysis probe. Clinical tests application is now in progress to evaluate the possibility of using such biosensors for continuous monitoring in patients.

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