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Glucose electrochemical probe with extended linearity for whole blood*

M. MASCINI,† S. SELLERI and M. RIVIELLO

Institute of Analytical Chemistry, University of Firenze, Via G. Capponi, 9-50121 Firenze, Italy

Abstract: A glucose probe based on a suitable modification of the silanization process of commercially available polycarbonate membrane was assembled and evaluated. The silanization procedure was carried out in gaseous phase in an anhydrous atmosphere, with dimethyldichlorosilane in a closed vessel. This new procedure seems to be highly reproducible and has the distinct advantage of fast response time and extended linearity up to 0.1 mol l^{-1} of glucose. Application to serum samples shows the possibility of application in clinical chemistry.

Keywords: *Glucose biosensor; enzymatic determination; polycarbonate silanized membrane; coupling with amperometric and enzymatic methods; pharmaceutical and clinical analysis.*

Introduction

The measurement of glucose by the determination of hydrogen peroxide obtained through enzymatic reaction with glucose oxidase forms the basis of several research papers and commercially available analysers for clinical analysis. However, dilution of samples in these systems is always necessary because of the limiting concentration of the cofactor oxygen and of the magnitude of the K_m (Michaelis–Menten constant).

The interest to develop a biosensor for glucose with an extended range up to 30 mmol l^{-1} resides in the possibility of direct measurements of several biological, pharmaceutical and industrial samples [1–5]. Generally, this problem can be solved by placing an extra membrane over the immobilized layer of glucose oxidase to minimize the glucose diffusion without affecting the oxygen transport. Several procedures to obtain a suitable membrane have been reported [6].

This paper presents a new procedure based on silanization of a commercially available polycarbonate membrane carried out in gaseous phase in an anhydrous atmosphere which is highly reproducible; the assembled biosensor has a fast response time and extended linearity up to 0.1 mol l^{-1} of glucose.

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†To whom correspondence should be addressed.

Experimental

Material and procedures

The hydrogen peroxide sensor was a platinum anode of 2.0 mm dia. surrounded with a ring of silver–silver chloride acting as reference and counter electrode. The probe was provided by the Instrumentation Laboratory (Milano, Italy). The polarization unit was a Polarecord (Metrohom model E 506) with recorder. For casting the cellulose membrane, a precision gauge tool was used (Precision Gage and Tool Co., Dayton, OH, USA). The polycarbonate membranes (porosity 0.03 μm) were supplied by Nucleopore (Pleasanton CA, USA). Glutaraldehyde (for electronic microscopy) was obtained from Merck. Glucose oxidase was obtained from Sigma (Type VII). Dimethyldichlorosilane, and all other products were of the highest purity available.

Assembly of the sensor

On the platinum surface we fixed a set of membranes obtained by spreading glucose oxidase with glutaraldehyde on a cellulose acetate membrane and by covering it with a silanized polycarbonate membrane. The cellulose acetate membrane is used to eliminate interferences from electroactive chemicals (ascorbic acid, etc.). The glutaraldehyde forms with the glucose oxidase an intermediate layer of immobilized enzyme. The overall set, 50 μm thick, is fixed after 1 h on the platinum sensor by means of an O-ring. Then the excess of glutaraldehyde is washed out with 0.1 mol l^{-1} of glycine.

Casting the cellulose acetate membrane

Cellulose acetate (1.98 g) and polyvinylacetate (20 mg) were dissolved in 20 ml of cyclohexanone and 30 ml of acetone. The solution was cast on a glass plate with a thickness of 200 μm with the aid of a casting tool. After complete evaporation (10 h) the membrane was immersed in water and peeled off the glass surface. The dried membrane has a thickness of about 20 μm.

Silanization of polycarbonate membrane

Membranes were suspended as received in a closed vessel in the presence of a desiccant (silica gel) and the vapour of a solution of dimethyldichlorosilane in 1,1.1 trichloroethane (10%) for 50–60 s. Then membranes were washed with water and with $NaHCO_3$ solution to eliminate all traces of hydrochloric acid formed.

Procedure for evaluating membranes

Calibration curves were obtained by addition of suitable glucose concentrated solution to 20 ml of buffer in a beaker, or by flowing, by a peristaltic pump, the standard solutions in a suitable flow cell obtained by Instrumentation Laboratory S.p.A. (Milano, Italy). A potential value of +0.7 V versus silver–silver chloride was applied through the polarization unit.

Results and Discussion

The research for finding a procedure that gives reproducible polycarbonate silanization was prompted by the poor reproducibility of the procedure described previously [3–6].

The dimethyldichlorosilane reacts continuously and rapidly with water, and air humidity forming hydrochloric acid. This reaction varies the percentage of reagent and

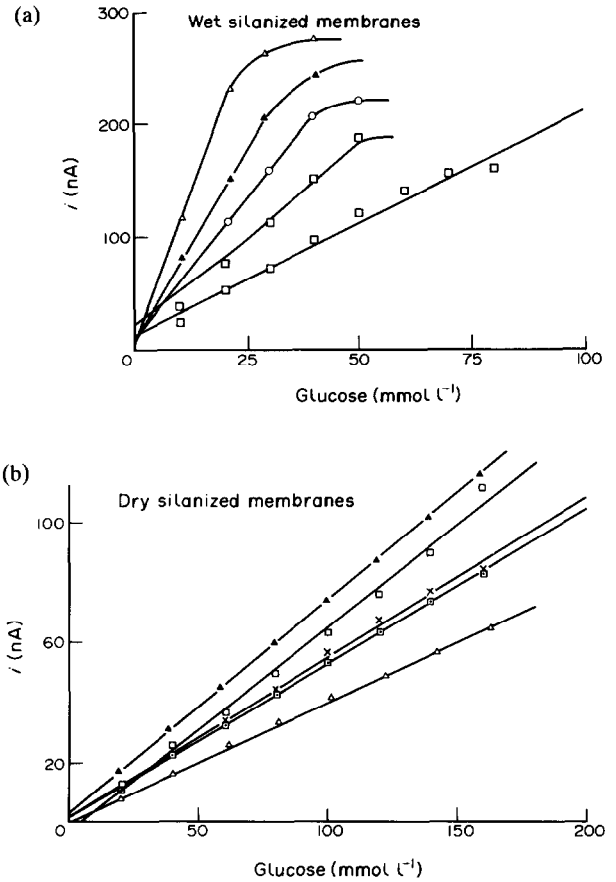

Figure 1
Calibration curves of five biosensors obtained with procedure described in ref. 6 (a) and in this paper (b).

its features when it comes in contact with polycarbonate. Figure 1 reports the comparison between some membranes prepared with the reported procedure [3, 6] and the new procedure. In Fig. 1(a) the calibration curve (current versus glucose concentration) obtained using the silanization procedure described previously is reported. Only seldom was a suitable membrane with extended linearity obtained. In Fig. 1(a) the five electrodes were assembled on the same day; all of them show an extended linearity (a normal glucose probe is linear only up to 1–2 mmol l^{-1}, different sensitivity (slope of the linear part), but only one is linear over 50 mmol l^{-1} (20% is really the general reproducibility of this procedure in our laboratory experience). In Fig. 1(b), the calibration curves of five different biosensors obtained on the same day, with the silanizing procedure described in this paper are reported. All of them gave distinct linear behaviour over 150 mmol l^{-1} with different sensitivity (slope). The high reproducibility of the gas phase treatment was surprising. The difference in the sensitivity (slope) depends on the difference in the assembled stage; once assembled if for any reason the membrane was disassembled from the electrochemical probe and then reassembled again, this could show a difference in the sensitivity similar to that shown by the five biosensors in Fig. 1(b).

It is believed that the reason is the higher purity of the vapour phase in comparison with the solution where hydrochloric acid concentration is variable and its effect is deleterious to polycarbonate structure. The anhydrous ambient also eliminates the formation of the acidic component in the vapour phase and consents such reproducible procedure.

In Fig. 2 the dynamic response to several additions of a glucose standard solution in phosphate buffer is reported. From the plot it is possible to evaluate the fast response obtained with this procedure up to high concentration value. With the silanized membrane the glucose biosensor has a response time smaller of the untreated membrane [6].

The sensor was also employed in a flowing apparatus to obtain fast analysis throughput and optimize the reproducibility.

When a flow cell is employed, with 1 ml min^{-1} as the flow rate, results reported in Fig. 3 were obtained, and a rapid response (<30 s) can be calculated to obtain a steady state. Also in this case, linear response to high concentration (over 0.1 mol l^{-1}) was reached.

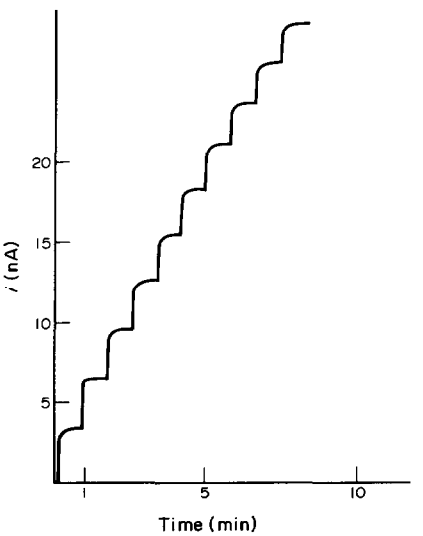

Figure 2
Dynamic response of glucose biosensor obtained by adding a glucose concentrated standard solution to a phosphate buffer; each addition corresponds to 10 mmol l^{-1}.

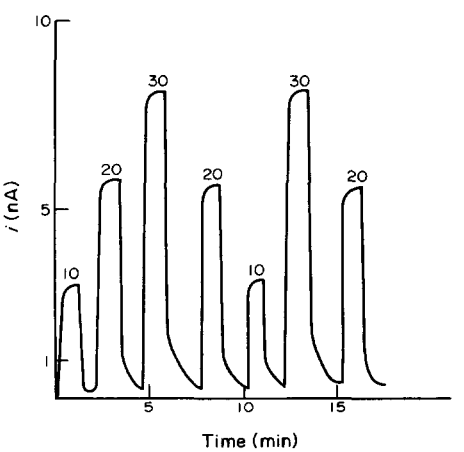

Figure 3
Flow-through response of glucose biosensor to standard solutions (conc. as marked in mmol l^{-1}).

The problem of biocompatibility with biological samples (serum and whole blood) was also investigated.

Some reconstituted serum samples gave an erroneous response as reported in Fig. 4. This is a common result when undiluted sera are processed, and it is generally ascribed to surfactants often present in such reconstituted samples. Therefore, fresh serum samples obtained in our laboratory were prepared and soon analysed and distinct peaks, as reported in Fig. 4, were obtained where the glucose value could be interpolated by calibration curves obtained with standard solutions.

Whole blood was also analysed by the flow technique. Therefore, cow whole blood was obtained by the slaughter service and heparinized with lithium heparin up to 50 IU ml^{-1}.

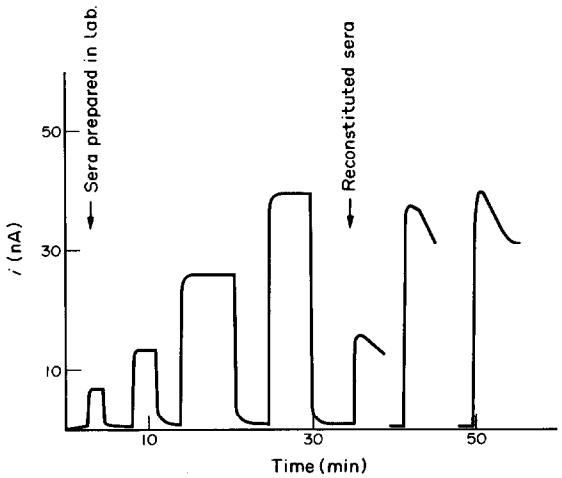

Figure 4
Compatibility of biosensor with fresh prepared serum samples diluted and with reconstituted serum samples.

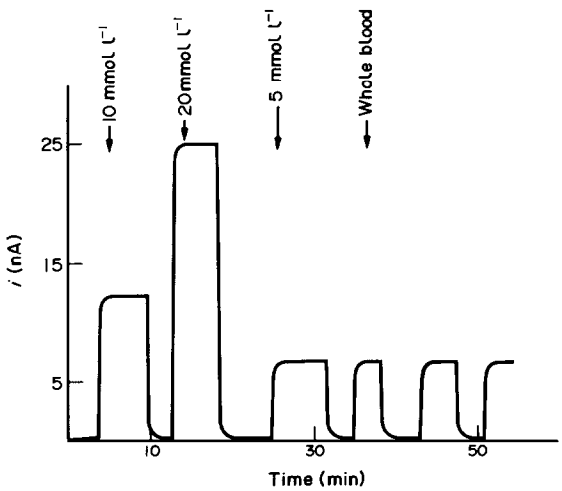

Figure 5
Biocompatibility of the glucose biosensor with whole blood and standard solutions.

Figure 5 reports a typical experiment where whole blood is compared with standard solution. Response times <1 min were obtained. Future work will be on the evaluation of biocompatibility with several kind of serum and human whole blood.

Conclusions

The reported procedure of silanization of polycarbonate membranes gives a distinct advantage of reproducibility, a large extended linearity calibration plot, rapid response time and an optimal biocompatibility with biological samples like serum and whole blood. Extension of such results will be confirmed in the near future for application with several biological compounds.

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