



HAL
open science

245 - A highly sensitive glucose electrode using glucose oxidase collagen film

Daniel R. Thevenot, Robert Sternberg, Pierre R Coulet, Danièle C Gautheron

► To cite this version:

Daniel R. Thevenot, Robert Sternberg, Pierre R Coulet, Danièle C Gautheron. 245 - A highly sensitive glucose electrode using glucose oxidase collagen film. *Bioelectrochemistry and Bioenergetics*, 1978, 5, pp.548 - 553. 10.1016/0302-4598(87)85047-X . hal-01083001

HAL Id: hal-01083001

<https://enpc.hal.science/hal-01083001>

Submitted on 14 Nov 2014

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

245 - A Highly Sensitive Glucose Electrode Using Glucose Oxidase Collagen Film *

by DANIEL R. THEVENOT ^o,* PIERRE R. COULET ^{oo},
ROBERT STERNBERG ^o and DANIELE C. GAUTHERON ^{oo}

^o Equipe de Bioélectrochimie et d'Analyse du Milieu, Laboratoire d'Energétique Biochimique, Université Paris Val de Marne, Avenue du Général de Gaulle, 94010 Créteil Cédex, (France)

^{oo} Laboratoire de Biologie et de Technologie des Membranes, CNRS - Université Claude Bernard, 43, Boulevard du 11 Novembre 1918, 69621 Villeurbanne Cédex, (France)

Manuscript received April 7th 1978

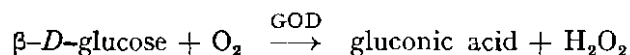
Summary

Industrially reconstituted collagen films have shown excellent properties for β -D-glucose oxidase coupling. Associated with a platinum anode for amperometric detection of hydrogen peroxide, these enzymatic films form a very simple and easy to handle glucose electrode; this device presents a very high sensitivity (*ca.* 10^{-8} M) giving responses proportional to glucose concentration over 5 orders of magnitude.

Introduction

The association of an enzyme in a soluble form with an electrochemical sensor was first reported by CLARK and LYONS, in 1962.¹ Since this date, the obtainment of carrier-bound enzymes has permitted the design of enzyme electrodes. In most cases, enzymes are trapped in gels surrounding the sensor² leading to systems which are difficult to handle.

In the present work, an enzymatic membrane was prepared from reconstituted calf skin collagen after acyl-azide activation and a coupling process giving a surface binding of enzymes of different classes.³⁻⁵ A glucose electrode using β -D-glucose oxidase (GOD) membranes was developed, using an amperometric method with a platinum electrode to detect hydrogen peroxide, which is a product of the enzymatic oxidation of glucose according to the reaction:



* Discussed at the 4th International Symposium on Bioelectrochemistry, Woods Hole (Mass.), 2-8 October 1977.

• To whom all correspondence should be addressed.

The potential was fixed at +650 mV *vs* Ag|AgCl, KCl 0.1 *M* and the anodic current was recorded. A stationary response was then obtained allowing the measurement of very low glucose concentrations. In a more sophisticated device, a second electrode involving a non-enzymatic membrane was used to compensate for the detection of other electroactive molecules and to enhance the selectivity of the glucose electrode.^{6,7}

Experimental

Instrumentation

The glucose electrode consisted of a modified gas electrode in which the pH detector was replaced by a platinum disk and the usual teflon film by a collagen membrane. In a differential device, electrode 1 was mounted with a β -D-glucose oxidase collagen membrane and electrode 2 with a non-enzymatic one.

Electronics were made by SOLEA TACUSSEL: current outputs of both working electrodes were first subtracted (Deltapol) and then twice differentiated (Derivol) with a time-base of one second (GSTP); thus different current *vs* time curves were available and usually recorded after a glucose pulse (SOLEA TACUSSEL EPL 2 with TV 11 GD plug-in unit, and three traces LINEAR 395 recorders).

Unless otherwise mentioned, the temperature of the solutions were carefully thermostated to 30.0 ± 0.1 °C (COLORA cryothermostat WK 5 DS).

Solutions and reagents

Insoluble films of highly polymerized reconstituted collagen (20 cm wide) were a gift of the Centre Technique du Cuir, Lyon (France); their thickness is about 0.1 mm in a dry state and 0.3–0.5 mm when swollen. They do not need to be tanned and can be stored several years without damage.³⁻⁵

Unless otherwise mentioned, all chemicals were reagent grade. The stock solutions of 0.1 *M* glucose were allowed to mutarotate at room temperature at least 3 hours before using and were stored at 4 °C. Both electrodes were filled with and dipped into 0.2 *M* acetate buffer, 0.1 *M* KCl solutions, pH 5.6.

Glucose oxidase binding on collagen membranes

The mild general acyl azide procedure for collagen membranes activation was used^{3,4} followed by the enzyme coupling.

Carboxyls were first esterified by immersion of crude membranes in a methanol/0.2 *M* hydrochloric acid solution for at least 72 hours, then treated overnight by 1 % hydrazine and soaked at 4 °C for 3 minutes in 0.5 *M* NaNO₂—0.3 *M* HCl mixture just before coupling. Thorough washings were performed between each step and at the end of the

activation process avoiding contact between reagents and enzyme solutions.

Activated films of $2 \times 1.5 \text{ cm}^2$ were dipped (2 hours at 4°C) into 1.5 cm^3 of borate buffer at pH 9 containing 2.5 mg β -D-glucose oxydase (GOD, E.C. 1.1.3.4, BOEHRINGER lyophilizate, grade I). Excess of soluble glucose oxydase was washed away for about 100 minutes in 1 M KCl and surface activity was in the range $30\text{--}60 \text{ nmol. min}^{-1} \text{ cm}^{-2}$. Enzyme collagen membranes were stored in 0.2 M acetate buffer, 0.1 M KCl (pH 5.6).

Procedure

Both electrodes were allowed to equilibrate in the buffer solution for 15 to 30 minutes after stepping the potential of the platinum disks to $+650 \text{ mV vs Ag|AgCl, } 0.1 \text{ M KCl}$. This potential corresponds to a diffusion-limited current for H_2O_2 oxidation. Calibrations were performed by successive microadditions (10 to 50 mm^3) of stock solutions of 10^{-6} to 10^{-1} M glucose to 10 to 20 cm^3 of buffer. The stationary response was the variation of the steady state values of $I_1\text{--}I_2$ when a sample of a glucose containing solution was added and the dynamic response was the height of the peak of the first derivative $d(I_1\text{--}I_2)/dt$. When successive additions were performed in the same solution, a current offset was used.

Results and discussion

When the enzyme electrode is immersed in a medium in which a pulse of glucose is added a steady state takes place after 2–3 minutes, as shown on Fig. 1, and the value of the anodic current reaches a plateau. The variation of intensity is directly dependent on the glucose concentration in the assay: this is the *stationary response*. On the other hand, the *dynamic response* is measured by the height of the peak obtained after 30–50 s by recording the first derivative of this current.

The lowest glucose concentration detected under these conditions is less than 10^{-8} M (Fig. 2). For values higher than 10^{-3} M , the responses become independent of the glucose concentration. The concentrations, which can be determined, range between *ca.* 10^{-8} and 10^{-2} M *i.e.* over 6 orders of magnitude and the linearity of the calibration curve is obtained between $(3\text{--}5) \times 10^{-8}$ and $(3\text{--}5) \times 10^{-3} \text{ M}$ *i.e.* over 5 orders of magnitude (Fig. 2).

In a typical experiment, the calibration curves remained linear even after 40 hours operation at 30°C and 250 days storage at 4°C , allowing accurate repeated determinations for 160 micro-assays tested. However, a daily calibration was necessary, because of the slight decreasing of the calibration curve slope: *ca.* 20% after 20 hours operation (cumulated time of measurements) and 20–50% after 40 hours. The accuracy of glucose determinations during a set of experiments was tested by successive additions of the same aliquot of glucose (Fig. 1);

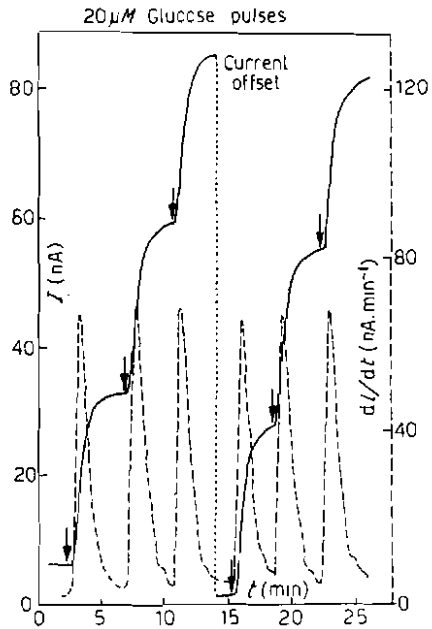


Fig. 1. Electrode responses when successive $20 \mu M$ glucose pulses are added to the buffer solution. Addition of $40 \text{ mm}^3 (\mu l)$ aliquots of 10 mM glucose solution into 20 cm^3 of 0.2 M acetate buffer, 0.1 M KCl (pH 5.6) solution. (—) direct current giving stationary responses, (---) first derivative of this current giving dynamic responses.

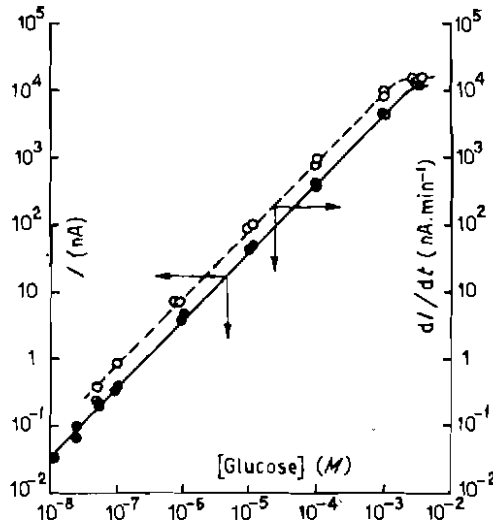


Fig. 2. Calibration curves of the glucose electrode. (—) stationary response; (---) dynamic response.

for 13 successive additions of 10^{-5} M glucose, the standard deviation from the mean is usually lower than 2%.

Glucose oxidase itself is very selective for $\beta\text{-D}$ -glucose; thus enzymatic electrode 1 presents a high selectivity for glucose compared

with usual sugars : selectivity ratios are higher than 2 000 / 1 for fructose, lactose and sucrose. As other species may diffuse through collagen membranes and may be oxidized on platinum at +0.94 V (N.H.E.), the use of a compensating non enzymatic electrode 2 eliminates possible interferences of species such as ascorbate, urate, tyrosine or hydrogen peroxide; the selectivity ratio for hydrogen peroxide ranges between 80/1 and 250/1 depending upon experimental parameters (accuracy of the balance of both electrodes and GOD activity of the film).

The use of non-enzymatic electrode 2 is specifically of greatest interest for blood glycemia determinations, I_2 usually reaching 10–50 % of I_1-I_2 . Fig. 3 presents the typical analysis of blood plasma samples after induced glycemia : in this case, a glucose calibration is necessary for each set of 3–4 blood additions. The dotted line, representing the second derivative of the current I_1-I_2 , has been successfully used for monitoring a printing device of the peak of the first derivative, *i.e.* the dynamic response.⁷

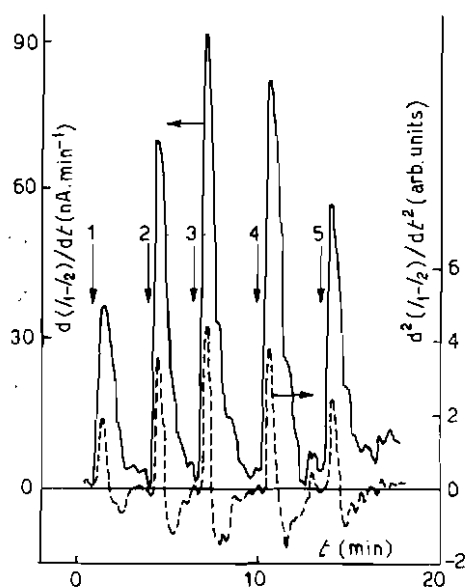


Fig. 3.

Induced glycemia. (—) first and (---) second derivative of the current after injection of 50 mm³ (μl) non deproteinized plasma samples. to 20 cm³ buffer (1) calibration of the electrode with a 10 μM glucose pulse, (2–5) glucose determination in plasma samples taken after 0 (2), 30 (3), 60 (4) and 120 minutes (5) after induced glycemia. Corresponding glucose content were respectively 1.1, 1.7, 1.4 and 1.0 g/l.

The glucose electrode may be used in a large temperature range, from 15 to 40 °C. As both responses are very sensitive to temperature (about 4–5 % / °C at 30 °C) it is necessary to carefully thermostate the solutions in contact with both electrodes.

Conclusions

Industrially reconstituted collagen films were found suitable for glucose oxidase immobilization; the enzymatic activity was maintained and its stability enhanced. A membrane loading of 50 to 100 mU per membrane (1 cm diameter) was sufficient to obtain a very sensitive glucose electrode when associated with amperometric hydrogen peroxide detection. Stationary and dynamic responses of this glucose electrode was proportionnal to glucose concentration from $3 - 5 \times 10^{-8}$ to $3 - 5 \times 10^{-3}$ M. Furthermore, this sensor was used for whole blood samples analysis in induced glycemia.

Acknowledgements

This work was partially supported by the Délégation Générale à la Recherche Scientifique et Technique, grant n° 76.7.0920.

References

- ¹ L.C. CLARK and C. LYONS, *Ann. N.Y. Acad. Sci.* **102**, 29 (1962)
- ² G.G. GUILBAULT, in *Immobilized Enzymes, Antigens, Antibodies and Peptides*, H.H. WEETALL (Editor), Marcel Dekker Inc., New York (1975) p. 293
- ³ P.R. COULET, J.H. JULLIARD, D.C. GAUTHERON, Brevet d'Invention Français (ANVAR) n° 2.235.133 (1973)
- ⁴ P.R. COULET, J. H. JULLIARD and D.C. GAUTHERON, *Biotechnol. Bioeng.* **16**, 1055 (1974)
- ⁵ P.R. COULET and D.C. GAUTHERON, in *Analysis and Control of Immobilized Enzyme Systems*, D. THOMAS and J.P. KERNEVEZ (Editors), North Holland, Amsterdam (1976) p. 165
- ⁶ D.R. THEVENOT, P.R. COULET and D.C. GAUTHERON, in *Enzyme Engineering* Plenum Press, New York (1978) Vol. **4**, p. 221
- ⁷ D.R. THEVENOT, P.R. COULET, R. STERNBERG, J. LAURENT and D.G. GAUTHERON to be published