TRACE GLUCOSE ELECTRODE FOR CLINICAL, FOOD AND ENVIRONMENTAL DETERMINATIONS

Robert Sternberg, Arnaud Apoteker, Daniel Thevenot

To cite this version:

Robert Sternberg, Arnaud Apoteker, Daniel Thevenot. TRACE GLUCOSE ELECTRODE FOR CLINICAL, FOOD AND ENVIRONMENTAL DETERMINATIONS. W. Franklin Smyth. ANALYTICAL CHEMISTRY SYMPOSIA SERIES - vol. 2. Electroanalysis in hygiene, environmental, clinical and pharmaceutical chemistry, Apr 1979, London, United Kingdom. ELSEVIER SCIENTIFIC PUBLISHING COMPANY, pp.461-473, 1980. <hal-01179332>

HAL Id: hal-01179332
https://hal.archives-ouvertes.fr/hal-01179332
Submitted on 23 Jul 2015

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.
electroanalysis in hygiene, environmental, clinical and pharmaceutical chemistry

Proceedings of a Conference, organised by the Electroanalytical Group of the Chemical Society, London, held at Chelsea College, University of London, April 17th–20th 1979

decided by

W. Franklin Smyth

Department of Chemistry, Chelsea College, University of London, Manresa Road, London SW3
TRACE GLUCOSE ELECTRODE FOR CLINICAL, FOOD AND ENVIRONMENTAL DETERMINATIONS

Robert STERNBERG, Arnaud APOTEKER and Daniel R. THEVENOT*
Equipe de Bioélectrochimie et d'Analyse du Milieu
Laboratoire d'Énergétique Biochimique
Université PARIS Val de Marne, Avenue du Général de Gaulle
94010 CRÉTEIL Cédex (France)

ABSTRACT

A very sensitive method has been devised for the determination of glucose using electrochemical sensors. The differential device includes a glucose electrode, consisting of a platinum disk covered by a β-D-glucose oxidase collagen membrane, and a compensating electrode mounted with a non-enzymatic collagen membrane. Current outputs of both electrodes are substracted and differentiated giving steady-state and dynamic responses proportional to glucose concentration in the 100nM - 2mM range.

The glucose sensor has been successfully tested in clinical (human whole blood, plasma or serum and human seminal plasma), food (fruit, wines, preserved food) and environmental analysis (polluted river, whey) illustrating the high selectivity and versatility of the device.

I - INTRODUCTION

The association of electrochemical sensors with enzymatic preparations, either in solution or immobilized on various supports, leading to the so-called enzyme electrodes, is, since the first report by Clark and Lyons (ref.1), a rapidly expanding analytical method (ref.2 - 4). In the presence of β-D-glucose oxidase, glucose is oxidized by dissolved oxygen leading to gluconic acid and hydrogen peroxide. An alternative method to the colorimetric detection of hydrogen peroxide is its electrochemical oxidation on a solid electrode (ref.1,5). Among the several supports available for enzyme immobilization, reconstituted collagen membranes appear very convenient and stable especially when a mild acyl-azide activation is performed ensuring a covalent binding of the enzyme (ref.6-8).
In a previous work we developed a trace-glucose sensor associating a β-D-glucose oxidase collagen membrane and an electrochemical detection of hydrogen peroxide (ref.9 - 11): the high sensitivity and selectivity of the differential device including a glucose electrode and a compensating non-enzymatic electrode lead us to test its performances in clinical, industrial and environmental laboratory conditions.

2 - EXPERIMENTAL
2.1 - Solution and reagents
Insoluble films of highly polymerized reconstituted collagen (20cm wide) were a gift of the Centre Technique du Cuir (Lyon, France). Their thickness is about 0.1mm in dry state and from 0.3 to 0.5mm when swollen. Lyophilized β-D-glucose oxidase (GOD, E.C. 1.1.3.4.) grade 1 (210 U/mg) and GOD-Perid clinical glucose kit were supplied by Boehringer, France. Unless otherwise mentioned all chemicals used were reagent grade. The stock solution of 0.1M glucose was allowed to mutarotate at room temperature at least 3 h before use, and was stored at 4°C. The stock solution of 0.1M H₂O₂ was frequently assayed by titration with 0.1N Ce(SO₄)₂ using ferrous O-phenanthroline as indicator.

Human whole blood, plasma or serum samples were a gift of the Diabetic Center of Hotel-Dieu Hospital, Paris. After an eventual deproteinization by uranyl acetate and glucose determination by the GOD-Perid clinical kit (ref. 12), they were kept at -20°C before their analysis with the glucose sensor.

Human seminal plasma samples were provided by the Histoembryological Laboratory of Kremlin-Bicêtre Hospital, (Paris) and were all deproteinized by 25 min. filtration on Amicon cones under 3000 r.p.m. centrifugation and were kept at -20°C before their electrochemical analysis. Concentrates of hydrolysed whey were a gift of Bel cheese factories, Vendôme, France.

2.2 - Preparation of β-D-glucose oxidase collagen membranes
The previously described acyl-azide procedure for activation of collagen followed by enzyme coupling (ref.6 - 8) was used with β-D-glucose oxidase: the activity of these membranes was generally 50 nmol.min⁻¹.cm⁻²(ref.11). Enzyme membranes exhibited high mechanical resistance without any tanning, and no bacterial degradation was detected when the membranes were stored in 50mM acetate buffer pH 4.5 for more than 2 years at 4°C or for more than 6 months at room temperature.
2.3 - Glucose sensor

The glucose sensor consisted of two electrodes; both contained a platinum disk and a collagen membrane maintained in close contact by a screwed cap (ref.10 - 11). Common auxiliary electrodes were platinum wires and references were Ag/AgCl. Electrodes $E_1$ and $E_2$ were filled with 0.2M acetate buffer, 0.1M KCl, pH 5.6, and mounted with both a β-D-glucose oxidase collagen membrane and a non-enzymatic one respectively. A potential difference of 650mV was generally maintained between the platinum working anodes and the references by a potentiostat. Previously described electronics (ref.11) allowed the recording of four different current vs time curves (Fig.1): $(I_1 - I_2)$, $I_2$, $d(I_1 - I_2)/dt$ and $d^2(I_1 - I_2)/dt^2$. Electronics were supplied by Solea-Tacussel (Villeurbanne, France) and consisted of a Deltapol differential current amplifier, a PRG5 potentiostat and a Deri vol derivating amplifier used with a time-base of 1sec. Recorders were Solea-Tacussel EPL2, with TV 11 GD plug-in unit, and three traces Linear 395.

2.4 - Procedure

Electrodes $E_1$ and $E_2$ were allowed to equilibrate in 20 ml 0.2 M acetate buffer pH 5.6 during 15 to 30 min. after stepping the potential of the working electrodes to + 650 mV vs Ag/AgCl references. Unless otherwise mentioned, the temperature of all solutions was carefully thermostated at 30.0 ± 0.1°C using a Huber ministat cryo-thermostat. The electrodes were either dipped into the buffer solution into which the sample was added, or mounted with flow-through caps. With the latter, the flow rate in the two-channel Gilson polystaltic pump was constant and equal to 0.85 ml/min and the circulating solution was either the buffer or a mixture of the buffer and of the sample (1:1). Blood samples were centrifuged on a Janetzki TH 12. Solid samples such as fruit were homogenized on a Virtis 45 at half maximum speed during ca 3 min.

3 - BASIC PROPERTIES OF THE GLUCOSE SENSOR

The association of a β-D-glucose oxidase collagen membrane and of an amperometric detection of hydrogen peroxide on a platinum disk, yields a very reliable glucose electrode $E_1$. In order to compensate electrochemical interferences, the differential device includes an electrode $E_2$ mounted with a non-enzymatic collagen membrane. When a glucose-containing sample is added to the buffer solution either into which both electrodes are dipped, or circulating through adequate caps, 4 different current vs time curves may be recorded (Fig. 1):
* $I_2$ is the current output of the non-enzymatic compensating electrode $E_2$; $I_2$ is usually very low except if the sample contains electrochemical reducing species;

* $(I_1 - I_2)$ corresponding to the detection of hydrogen peroxide enzymatically generated, reaches a steady-state value after 2-3 min; this is the steady-state response of the sensor:

* $d(I_1 - I_2)/dt$ is maximum after 30 to 50 sec; the height of this peak is the dynamic response of the sensor;

* $d^2(I_1 - I_2)/dt^2$ may control a switch when reaching a null point, and be used for printing the dynamic response (ref. 11).

Both responses are proportional to glucose concentration in a very large range: ca 100nM to 2 mM. Lowest detection limit reaches 10nM (ref. 10 - 11). Precision equals 2 to 5 % in the 300nM - 1mM concentration range. Reproductibility, expressed as the relative variation from initial value, is better than 10 to 20 % after a storage of 3 months at room temperature or 10 months at 4°C. Selectivity depends both of the enzyme and of the electrochemical differential device: the selectivity coefficient is smaller than $5 \times 10^{-4}$ for glucose over fructose, lactose and sucrose and smaller than $5 \times 10^{-3}$ for glucose over non-enzymatically-generated hydrogen peroxide. If the presence of such electrochemical interferences are suspected a good balance of the steady-state responses of electrodes to addition of hydrogen peroxide must be realized by adjustment of the differential current amplifier. Steady-state responses of the sensor are proportional to $\beta$-D-glucose oxidase activity of the collagen membrane: in our case the ratio $(I/C)/activity$ is equal to ca 0.05 (mA/M$^{-1}$)/(mU.cm$^{-2}$). Thus sensor responses are as dependent on temperature as the $\beta$-D-glucose oxidase activity of collagen films. Because this dependence reaches 6 to 11 %/°C at 20°C and 4 to 5 %/°C at 30°C, it is necessary to carefully thermostate the solution in contact with both electrodes. Nevertheless, the sensor may be used over a large temperature range: 15 to 40°C.

4. - CLINICAL GLUCOSE DETERMINATIONS

4.1 - Human blood

We have studied the behaviour of the glucose sensor with different types of blood: deproteinized or non-deproteinized samples of whole blood, serum or plasma were successively analysed. When 50 to 100 μl of these samples are added to the 20 ml of buffer solution, in which both electrodes are immersed, a non-enzymatic current $I_2$ appears on electrode2(Fig. 1). This demonstrates the importance of compensating
electrochemical interferences. Comparison of steady-state or dynamic responses i.e. respectively the plateau of $I_1 - I_2$ and the peak of $d(I_1 - I_2)/dt$, to the corresponding responses with glucose standards, allows a simple, reagentless and rapid glucose determination. This method was successfully tested on 200 assays, most blood samples corresponding to induced glycemias tests realized in the Diabetic Department of the Hotel-Dieu Hospital in Paris (ref. 10).

The addition of 50 to 500 µl of blood samples into the 20 ml buffer of the reaction vessel decreases the slope of the calibration curve of 5 - 20 %. This difficulty may be easily avoided by two different ways: either a new calibration with a glucose standard is performed every 3-5 blood additions into the same solution, or the solution is frequently changed. The slight inhibition of the sensor by blood components is indeed reversible and initial calibration is recovered when washing the electrodes and the cell. Under these operating conditions, precision was ca 7 % as estimated by standard deviation from mean of 11 successive determinations on the same sample.

Table 1 presents data obtained with 11 different blood samples which were differently treated and separated into two fractions, one of which being centrifuged for analysis of the supernatant. When whole blood was centrifuged, yielding serum or plasma in absence or presence of heparin respectively, glucose content appeared significantly higher. This centrifugation seemed also to increase the importance of the non-enzymatic response $I_2$, showing a higher content in electrochemical reducers.

As shown on figure 2, both for different deproteinized whole blood and for plasma samples, non-enzymatic response $I_2$ seems independent of the enzymatic one i.e. $(I_1 - I_2)$: this means that the content in electrochemical oxidable species is not related to glucose concentration. Furthermore, comparison of data obtained at 20 and 30°C shows that unlike calibrated $(I_1 - I_2)$ values which within 10°C increases of about 180 %, $I_2$ values are either poorly or not temperature dependent. This result is probably related in the difference of rate-limiting factors in both cases; whereas $(I_1 - I_2)$ is proportional to the β-D-glucose oxidase activity of the collagen membrane and thus highly temperature dependent, $I_2$ is purely electrochemical and probably only related to diffusion reactions.

In order to check the accuracy of the glucose sensor responses, we correlated on 32 different deproteinized whole blood and 25 plasma samples the values obtained from steady-state and dynamic responses
Figure 1 - Response curves for a 50 \( \mu l \) blood plasma sample.

(—) \( I_1 - I_2 \) vs time: steady-state response; (— —) \( I_2 \) vs time: non-enzymatic response; (— . — . ·) \( d(I_1 - I_2)/dt \) vs time: dynamic response; (— ⋅ ⋅ ⋅ ⋅ ⋅) \( d^2 (I_1 - I_2)/dt^2 \) vs time: signal used in automated devices.

Figure 2 - Relation between non-enzymatic (\( I_2 \)) and enzymatic (\( I_1 - I_2 \)) responses to pulses of (A) deproteinized whole blood and (B) plasma samples.

Addition into 20 ml buffer of (A) 200 \( \mu l \) whole blood previously deproteinized and diluted (100 \( \mu l \) blood into 1 ml uranyl acetate) or (B) 50 \( \mu l \) non-deproteinized plasma. Determinations at (•) 20-21 and (•) 30°C.

with those of a standard clinical method i.e. the GOD-Perid Boehringer one. Figure 3 presents results of these correlation assays on deproteinized whole blood samples: linear regression slopes equal 1.053 and 0.998 for steady-state and dynamic responses respectively, \( r^2 \) coefficient being in both cases equal to 0.95. Corresponding results obtained with non-deproteinized plasma samples yield less accurate correlation; the linear regression slopes equal 1.012 and 0.905 for steady-state and dynamic responses, respectively, \( r^2 \) coefficient reaching only 0.7 - 0.8. This difference of behaviour is probably related to glucose evolution in the non-deproteinized samples prior.
Table 1 - Influence of the treatment of 11 different human blood samples on their apparent glucose concentration and on their content in electrochemical reducers, as demonstrated by the glucose sensor.

Centrifugation of whole blood samples yield plasma or serum in presence or absence of heparin respectively. Pulses of 50 μl samples into 20 ml of buffer.

<table>
<thead>
<tr>
<th>Glucose concentration (g/l) in whole blood</th>
<th>Non-enzymatic response $I_2$ (nA) in plasma</th>
<th>Non-enzymatic response $I_2$ (nA) in whole blood</th>
<th>Non-enzymatic response $I_2$ (nA) in plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.71</td>
<td>0.71 a</td>
<td>3.6</td>
<td>6.9</td>
</tr>
<tr>
<td>0.63</td>
<td>0.74 a</td>
<td>3.2</td>
<td>6.3</td>
</tr>
<tr>
<td>0.18</td>
<td>0.50</td>
<td>2.5</td>
<td>5.6</td>
</tr>
<tr>
<td>0.41</td>
<td>0.77</td>
<td>2.1</td>
<td>4.8</td>
</tr>
<tr>
<td>0.76 b</td>
<td>0.86 b</td>
<td>4.5 b</td>
<td>1.3 b</td>
</tr>
<tr>
<td>0.91 b</td>
<td>1.16</td>
<td>4.7</td>
<td>12.0</td>
</tr>
<tr>
<td>0.95</td>
<td>1.14</td>
<td>4.8</td>
<td>6.0</td>
</tr>
<tr>
<td>1.30</td>
<td>1.61</td>
<td>4.8</td>
<td>6.1</td>
</tr>
<tr>
<td>1.40 b</td>
<td>1.87 b</td>
<td>3.6 b</td>
<td>6.2 b</td>
</tr>
<tr>
<td>1.72</td>
<td>1.95</td>
<td>4.4 b</td>
<td>12.0</td>
</tr>
<tr>
<td>1.72</td>
<td>1.55</td>
<td>4.4</td>
<td>10.9</td>
</tr>
</tbody>
</table>

a: serum
b: values at 21°C

and after their storage at 20°C. The higher viscosity of non-deproteinized blood leading to a poorer precision in pipeting 50 μl aliquots has also to be taken into account.

4.2 - Human semen

Human seminal plasma contains large amounts of fructose; the simultaneous occurrence of much smaller amounts of glucose has been demonstrated by using soluble β-D-glucose oxidase (ref 13 - 15). Even after deproteinization by filtration on Amicon cones, seminal plasma contains high concentrations of electrochemically oxidable species: 50 μl pulses into 10 ml buffer gives non-enzymatic responses $I_2$ ranging between 6.5 and 25 nA, whereas corresponding enzymatic responses $I_1$ to $I_{2}$ reach only 0.25 to 8.5 nA. Thus, before glucose calibration, it is necessary to check the balance between both electrodes by an
Glucose determination in deproteinized whole blood: correlation between determinations by sensor and by GOD-Perid method.

Glucose determination in human semen. (—) Enzymatic and (— —) non-enzymatic responses to successive additions into 10 ml of buffer of (1) 25 µl of 0.64 mM H₂O₂, (2) 200 µl of 100 µM glucose and (3) 100 µl of deproteinized seminal plasma.

Hydrogen peroxide pulse, the balance of the differential current amplifier being settled to give no steady-state response (Fig. 4). Glucose determination is then realized by comparing the enzymatic steady-state response to sample with a precalibration assay.

This method gave, for 9 different samples, glucose concentration ranging between 1 and 10 mg/100 ml, which are in the previously reported range of 0 to 25 mg/100 ml (ref.15). As a significant glucose evolution was observed after several hours storage at room temperature and several days at -20°C, the accuracy of the sensor could only be established by simultaneous determination with a reference clinical method, immediately after deproteinization of the seminal plasma samples.
5. - FOOD GLUCOSE DETERMINATION

Glucose sensor was tested on a large number of food samples including fruit, drinks and preserved food. Liquid samples were simply analysed by addition of an aliquot of 10 to 100 μl into the reaction vessel. In the case of solid samples, such as apples or strawberries, a few minutes homogenization was necessary to obtain a liquid which was added to the vessel using Pedersen constriction micropipet.

Table 2 presents glucose concentration values obtained with the different samples; they range between 0.2 and 62 g/l. Corresponding values of the signal/electrochemical interference ratio \( \frac{I_1 - I_2}{I_2} \) can be as low as 0.7 in Muscadet white wine, demonstrating the importance of compensating electrode \( E_2 \) and of a good balance of both electrode responses to electrochemical interference such as external hydrogen peroxide (Fig. 5). In other cases, such as apple, grapefruit, stewed fruit and orange soda, \( I_2 \) variation is negligible and electrode \( E_2 \) may be disconnected. For all these samples, glucose determination was identical when using steady-state or dynamic responses of the sensor and precision was ca 7%. At the end of series of additions of samples into the same solution, a pulse of glucose standard was added and the corresponding responses were compared to those obtained during the precalibration: by this method we checked that the analyzed samples contained no inhibitor of the sensor.

6. - WASTES GLUCOSE DETERMINATIONS

6.1 - Polluted river

In order to study the influence of temperature on the mechanism of biodegradation of sugars at different temperatures, we have adapted the previously described sensor to the determination of mg/l amounts of glucose in Seine water samples (Ref. 16). The sample was diluted in a 1/1 ratio with buffer by a polystaltic pump and circulated in alternance with buffer into the flow-through caps of the electrodes (Fig. 6). Thus each response was the average of 2 signals: a/ the usual response to an increase of glucose concentration in the solution in contact with both electrodes and b/ the response to the symmetrical decrease of concentration during the washing up. There is indeed a good reversibility of both responses during these two phases: \( I_1 - I_2 \) decreases and returns to its original value, the first derivative \( \frac{d(I_1 - I_2)}{dt} \) is exactly symmetrical and yields a negative peak of equal height. Furthermore recording of \( I_2 \) showed
<table>
<thead>
<tr>
<th>Sample Origin</th>
<th>Volume (µl)</th>
<th>Glucose (g/l)</th>
<th>( \frac{I_1}{I_2} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Blood</td>
<td>50</td>
<td>0.6 - 2.3</td>
<td>2 - 20</td>
</tr>
<tr>
<td>(Whole blood, plasma or serum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Semen</td>
<td>50 - 100</td>
<td>0.01 - 0.10</td>
<td>0.01 - 0.7</td>
</tr>
<tr>
<td>(Plasma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - Food</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon</td>
<td>50</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Apple</td>
<td>50</td>
<td>15</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Orange</td>
<td>10</td>
<td>26</td>
<td>15 - 30</td>
</tr>
<tr>
<td>Strawberry</td>
<td>50</td>
<td>18</td>
<td>10 - 15</td>
</tr>
<tr>
<td>Drink</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange Soda</td>
<td>100</td>
<td>26</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>Cider</td>
<td>20</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>White Wine</td>
<td>100</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Preserved Food</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apricot-Apple</td>
<td>50</td>
<td>62</td>
<td>40</td>
</tr>
<tr>
<td>Raspberry-Apple</td>
<td>50</td>
<td>15</td>
<td>&gt; 220</td>
</tr>
<tr>
<td>3 - Environment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polluted River</td>
<td>6 - 8 ml a</td>
<td>0.004 - 0.05</td>
<td>&gt; 25</td>
</tr>
<tr>
<td>Hydrolysed Whey (concentrated)</td>
<td>50</td>
<td>200-300 b</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Ultrafiltrated Hydrolys ed Whey (concentrated)</td>
<td>50</td>
<td>300-400 b</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

- **a**: Flow-through device
- **b**: g/kg

A small or negligible content of the Seine river samples in electrochemically oxidizable species (table 2).

Under these conditions, 4 to 50 mg/l glucose concentrations were determined in samples incubated 0 to 120 hours at 20°C, the volume consumed by these analysis being usually 6-8 ml, and precision 5 to 7%.
Figure 5 - Glucose determination in food.

(— — ) Normal and (— - - ) derivative enzymatic and (— — ) normal non-enzymatic responses to successive additions, into 20 ml of buffer, of (1), (2) and (6) 100 µl of 1 mM glucose, (3) 50 µl of 640 µM hydrogen peroxide, (4) 100 µl of orange soda, already diluted 100 times in water, and (5) 100 µl of Muscadet white wine.

Figure 6 - Determination of traces of glucose in Seine river samples

(— — ) Normal and (— - - ) derivative enzymatic, and (— — ) normal non-enzymatic responses to the alternative circulation of buffer and of a 1:1 mixture of sample and buffer. (1) 15 mg/l glucose standard and 24 h incubated, at 20°C, Seine river samples containing initially (2) 15 (3) 25 and (4) 50 mg/l glucose. (1') to (4') buffer washing. (4) and (4') divide Y scales by 2.
6.2 - Cheese industry

Whey is the usual by-product or waste of cheese industry. After hydrolysis of lactose into glucose, the latter may be rapidly determined with glucose sensor. We performed these determinations on hydrolyzed whey samples, which has been eventually demineralized by ion-exchangers and/or deproteinized by ultrafiltration, before being concentrated by evaporation. As these samples were very viscous and contained 200 - 400 g glucose per kg, it was necessary to realize a predilution by a factor of ca 100. They exhibited negligible electrochemical interferences, $I_2$ being less than 100 times smaller than $I_1$. (Table 2)

7 - CONCLUSIONS

In a previous work, a trace glucose sensor was developed associating α-D-glucose oxidase collagen membrane and platinum disk for amperometric detection of hydrogen peroxide (ref. 9 - 11). The excellent performances of the sensor are more precisely its high sensitivity and selectivity lead us to test its behaviour in actual laboratory conditions. About 500 different assays were realized on clinical, food or waste samples. In most cases, two responses could be alternatively used. Dynamic response appearing 30 to 50 s after the sample injection was preferred when a rapid result was necessary; it enabled us to perform ca 13 determinations within 30 min. Steady-state response was chosen in tests simulating a glucose monitor in industrial process and using flowing-through device. Precision and accuracy were generally 5 to 7 %. The electrochemical compensating electrode appeared to be often necessary especially in biological fluids such as blood and semen. On the contrary to most commercial glucose analysers, a pre-deproteinization of the samples seemed to have no influence on precision or reproducibility of the sensor. The laboratory and industrial process potential of this simple, cheap and easy-to-realize sensor is very promising.

ACKNOWLEDGMENTS

We are indebted to Dr Jean Claude Soufir (Lab. d'hystoembryologie Hopital Kremlin Bicêtre, Paris) for his kindness in letting us use blood and semen samples of his laboratories and for collaboration on medical glucose determinations. We thank Dr. Pierre R. Coulet and Dr. Danièle C. Gautheron (Lab. de Biochimie et Technologie des Membranes, Université Claude Bernard, Lyon) for their collaboration on
enzyme immobilization and on the development of glucose sensors.

REFERENCES

8 P.R. Coulet, Thèse de Docteur ès Sciences, Université Cl. Bernard, Lyon, n° 78.03 (1978).
16 D.R. Thévenot and A. Apoteker, this vol., p. 445.