Talanta, Vol. 39, No. 8, pp. 1039–1044, 1992
Printed in Great Britain.

0039-9140/92 $5.00 + 0.00

SUBCUTANEOUS MICRODIALYSIS PROBE COUPLED WITH GLUCOSE BIOSENSOR FOR *IN VIVO* CONTINUOUS MONITORING

D. MOSCONE and M. PASINI
Dipartimento di Scienze e Tecnologie Chimiche, Universitá di Roma Tor Vergata, 00173 Roma, Italy

M. MASCINI*
Dipartimento di Sanitá Pubblica, Epidemiologia e Chimica, Analitica Ambientale, Universitá di Firenze, Via Gino Capponi 9, 50121 Firenze, Italy

(*Received* 3 *June* 1991. *Revised* 2 *December* 1991. *Accepted* 4 *December* 1991)

Summary—Microdialysis probes have been tested to evaluate the influence of flow-rate, probe dimensions and temperature when used as sampling systems inserted subcutaneously for *in vivo* monitoring of glucose. The probe was coupled with a glucose biosensor obtained from a thin layer electrochemical cell generally used as detector for liquid chromatography. Glucose oxidase was immobilized on a nylon net membrane and it was placed over an acetate cellulose membrane into the cell in contact with the platinum anode.

Microdialysis is a new sampling technique for *in vivo* analysis and continuous monitoring.[1–3] The technique has been mainly developed for measurement of neurotransmitters in the brain and coupled to liquid chromatography to identify specific compounds.[4–6]

The *in vivo* continuous measurement of glucose is a challenge for developing a wearable artificial pancreas.

Needle shaped glucose biosensors have been developed for *in vivo* analysis and have been inserted subcutaneously or in the bloodstream of anaesthetized animals. The technique has been developed in a few laboratories[7–13] and several problems have arisen.

The production of useful biosensors is low (only 20–50% of sensors produced are useful); the biosensors have only been inserted in anaesthetized animals because any movement affects the output signal; the biosensors should be sterilized which impairs the enzyme activity; there is a marked variation in the sensitivity when biosensors are implanted *in vivo*;[8–10] this can only be controlled with an *in vivo* calibration of the device, which can only be done indirectly.[12]

There is also a potential health hazard. To leave a needle in the human body is considered a low risk if the site is subcutaneous, medium risk if intraperitoneal and high risk if it is placed directly into the blood stream.

However diabetics need a continuous glucose control and the dream of a closed loop artificial pancreas where the continuous output of a glucose biosensor drives the insulin infusion into a diabetic patient is a challenge for many research teams.

In this paper we show a complete apparatus suitable for *in vivo* glucose monitoring obtained by coupling a microdialysis probe and a glucose biosensor in a thin layer flow cell configuration. The system could be easily miniaturized for the development of a wearable glucose monitor.

The principle is outlined as follows: a physiological buffer is pumped at constant flow (10–30 μl/min) into a thin dialysis fiber with diameter of 200 μm (the microdialysis probe) placed subcutaneously (or in the bloodstream); the buffer equilibrates with the subcutaneous liquid (or with blood), then flows into a thin layer cell provided with a glucose biosensor which continuously monitors the glucose value. As microdialysis probe we compared *in vitro* a commercial probe and a single sterilized thin dialysis hollow fiber.

The system has several advantages over the insertion of a bioprobe-needle into the body; the sensitivity of the biosensor can be checked regularly, the hollow fiber used as microdialysis probe can be easily sterilized, it can be inserted

*Author for correspondence.

in animals and humans while awake; the overall process of the *in vivo* implantation can be controlled much more and processes such as inflammation and clotting (when inserted in bloodstream) can easily be followed and eliminated.

Preliminary experiments with a hollow fiber sterilized on animals and humans are discussed.

EXPERIMENTAL

Materials and apparatus

Glucose oxidase (GOD, E.C.1.1.3.4, from Aspergillus Niger, type VII, 132,000 U/g) was obtained from Sigma.

A GOD immobilized-nylon net membrane was prepared as previously reported.[14]

The glucose solution was prepared with β-$D(+)$glucose from Farmitalia Carlo Erba (Milano, Italy), allowed to equilibrate overnight and suitably diluted.

The buffer solution, Dulbecco's physiological buffer (pH = 7.4), was prepared in doubly distilled water.

All chemicals were analytical grade.

Cellulose acetate (53% acetyl) and polyvinyl acetate of high molecular weight were obtained from Farmitalia Carlo Erba (Milano, Italy). For casting the cellulose membrane a precision gauge tool (from Precision Gage and Tool Co., Dayton, OH, U.S.A.) was used. This membrane, with about 100 Dalton MWCO, was prepared as previously reported.[15-17]

Peristaltic pump, Minipuls 3, for flow analysis was from Gilson (France). Thermostat model F3 was from Haake.

Rheodyne valve (U.S.A.) Model 7125 was used as injection valve.

A complete thin-layer transducer cell for LCEC (liquid chromatography/electrochemistry) was obtained from BAS (BioAnalytical Systems, IN, U.S.A.). A LC-4B Amperometric detector from BAS was connected to an Amel Model 868 recorder.

The CMA/10 Microdialysis probe (fitted with a polycarbonate–polyether co-polymeric membrane, i.d. 400 μm, wall thickness 60 μm and molecular cut-off approximately 20,000 Dalton) was obtained from Carnegie Medicine (Stockholm, Sweden).

The hollow fiber Filtral 12 AN 69 HF polyacrylonitril sodium metalil sulphonate (i.d. 200 μm and molecular cut-off approximately 35,000 Daltons) was obtained from Hospal Industie (Meyziev, France).

Silicone tubing (i.d. 0.300 mm, o.d. 0.630 mm, wall thickness 0.165 mm) from A-M Systems and Teflon tubing (i.d. 0.330 mm, o.d. 0.482 mm, wall thickness 0.152 mm) from Firie (Genova, Italy) were used to connect hollow fibers to the flow-system.

Assembling of the sensor

A complete thin-layer transducer cell includes three separate electrodes: the working electrode (platinum disk with diameter of 3 mm), the reference (Ag/AgCl) and the auxiliary electrode (stainless steel). Figure 1 shows the assembly of the sensor.

A thin (20 μm) membrane of cellulose acetate was stretched over the entire plastic block where the electrode area was located; it removes the electrochemical interferences (uric acid, ascorbate, *etc.*) with its nominal MWCO of 100;[15-17] this figure is obtained as a rough number and mainly means that ascorbic and uric acids do not reach the electrode surface while hydrogen peroxide passes through easily. A dialysis membrane with a nominal claimed MWCO of 100

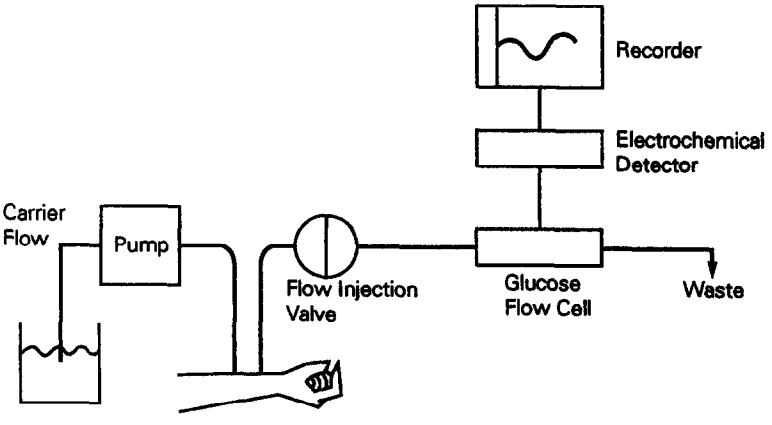

Fig. 1. Schematic diagram of the thin layer cell.

recently became commercially available from Spectrum Med. Ind. (CA, U.S.A.). Unfortunately this last membrane cannot be used, because it shows high interferences from uric and ascorbic acids, common substances in blood and subcutaneous liquid.

A nylon net (thickness 100 μm, with diameter of 6 mm) with the immobilized glucose oxidase enzyme was placed over the electrode area. Then a gasket is put on the half cell before assembling the cell. The upper half of the cell contains two ports to insert the inlet and outlet tubing.

The microdialysis probe is connected to the inlet tubing, the outlet is connected to the reference electrode and then to waste.

Procedures

The flow system for *in vitro* experiments is shown in Fig. 2. The peristaltic pump drives carrier solution through the microdialysis probe immersed in glucose standard solutions at constant rate. A steady-state current is obtained.

The standard solutions where the microdialysis probe was immersed were manually changed.

The CMA/10 probe was used as received. The hollow fiber probe (200 μm) was connected to the Teflon tubes of the flow system. *In vivo* experiments have been performed only with a sterilized hollow fiber.

To place the microdialysis hollow fiber subcutaneously a sterilized needle was inserted transcutaneously through the skin for about one centimeter and the needle tip was pulled out. Then the sterilized fiber was inserted from the needle tip and the needle taken out leaving the hollow fiber under the skin. The fiber was connected to Teflon tubes and fixed with epoxy glue.

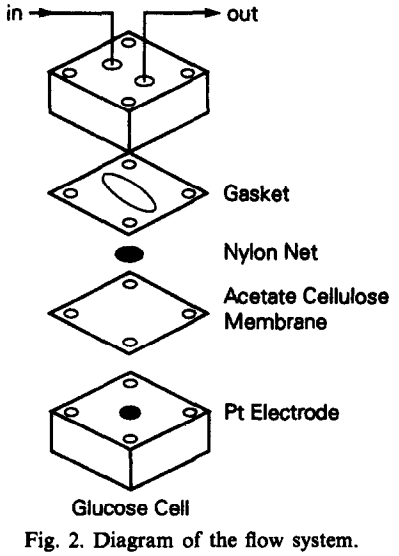

Fig. 2. Diagram of the flow system.

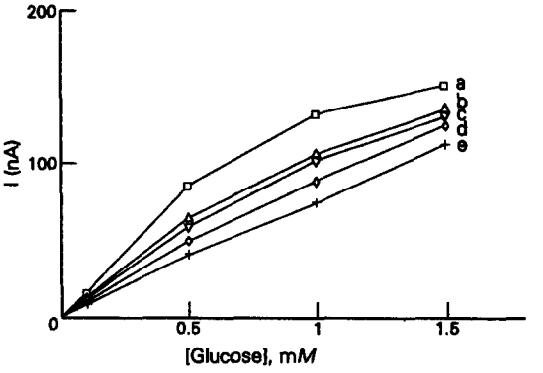

Fig. 3. Calibration curves of glucose biosensor at different flow-rates (without the microdialysis probe). (a) = 10 μl/min, (b) = 20 μl/min, (c) = 30 μl/min, (d) = 40 μl/min, (e) = 50 μl/min. Room temperature.

For checking variations in sensitivity during the experiments, a flow injection system was used. A Rheodyne injection valve with a 20-μl sample loop was introduced into the flow system just after the microdialysis probe. A glucose standard buffered solution filled the loop of the injection valve and flowed through the glucose biosensor.

Then we obtained a current profile similar to a peak. The dispersion coefficient of the apparatus was 1.1.

These peaks demonstrate the high reproducibility in "*in vitro*" experiments.

RESULTS AND DISCUSSION

In vitro *experiments*

Typical calibration curves for the glucose flow cell, without the microdialysis probe, at different flow-rates are shown in Fig. 3.

When the flow-rate is increased the linear range of the calibration curve increases and the current values decrease. This is a common experience with a glucose biosensor with hydrogen peroxide detection in a flow cell. The logical explanation is that hydrogen peroxide reaching the electrode surface decreases by increasing the flow-rate. This is due partly to a lower conversion of glucose and partly to a lower fraction of hydrogen peroxide reaching the electrode surface. Calibration curves obtained with hydrogen peroxide standards show a parallel variation with the flow-rate.

The non-linearity of the calibration curve at a concentration higher than about 1mM is

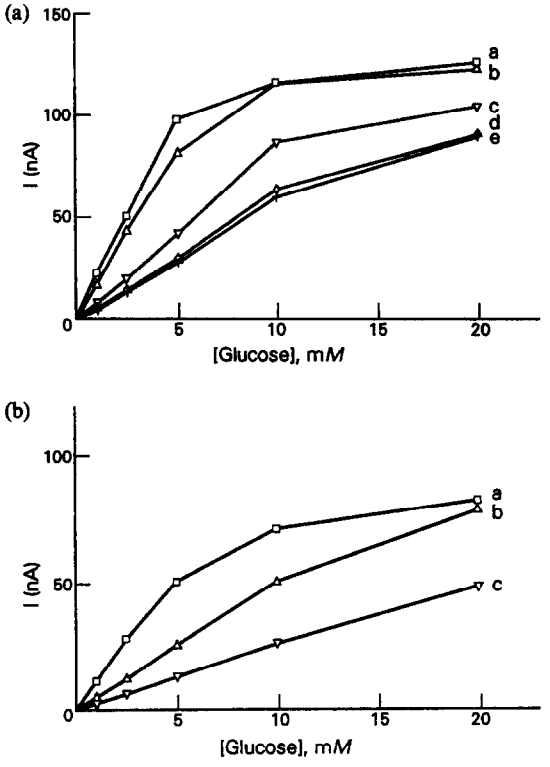

Fig. 4. Calibration curves of glucose with the microdialysis probe at different flow-rates. (a) CMA/10, length = 10 mm, $T = 37°$; (a) = 10 μl/min, (b) = 20 μl/min, (c) = 30 μl/min, (d) = 40 μl/min, (e) = 50 μl/min; (b) Hollow fiber, length = 20 mm, $T = 37°$; (a) = 10 μl/min, (b) = 30 μl/min, (c) = 50 μl/min.

mainly due to depletion of molecular oxygen, a cofactor in the glucose oxidase reaction.[18]

In Fig. 4(a) and 4(b) calibration curves are shown where the glucose biosensor is coupled with a microdialysis probe (CMA/10 and hollow fiber).

The upper limit of concentration attained in this case (20m*M*) is much higher and due to the limited diffusion of glucose through the microdialysis probe and reaching the glucose biosensor.

Besides this effect we observe a flow-rate influence as in Fig. 3, but in this case the diffusion rate through the microdialysis probe also affects the results.

It is interesting to notice how the two microdialysis probes show similar results in spite of the different materials and the different geometries (Carnegie length 10 mm and diameter of 400 μm, hollow fiber length 20 mm and diameter of 200 μm).

In Fig. 5 the length of the microdialysis probe (hollow fiber) was varied and the linearity range and the current values are greatly affected by this parameter. This is shown for the hollow fiber microdialysis probe, but similar results were obtained with the commercial probe.

To obtain a linear calibration curve up to 20m*M* (the high value for glucose in blood for diabetes) we chose a hollow fiber one centimeter long and a flow-rate of 30 μl/min. This flow is feasible for a wearable instrument, since it corresponds to less than 50 ml/day which can be stored easily.

In Fig. 6 dynamic curves are reported for the hollow fiber. The system, microdialysis and biosensor, shows such a fast response and recovery that it was difficult to forecast; only a few seconds are necessary to reach a stable current value corresponding to a defined concentration. The reproducibility of the current is very high, it was evaluated in several experiments as less than 5% over 10 consecutive assays.

Delay time was greatly reduced by using narrow bore tubing (Teflon tube 0.3 mm) between the microdialysis probe and the glucose biosensor (under 50 μl of volume).

The influence of temperature on the dialysis probe was evaluated. At 37° the current is about 30% higher and the linearity range is slightly reduced; this reflects the variation of the diffusion coefficient of the glucose through the microdialysis probe.

The stability of the signal with a hollow fiber during a ten-hour period was followed *in vitro*. Fluctuations smaller than 15% were generally obtained due to random variations in the experimental parameters.

Glucose biosensor is known to be stable during such interval of time, so it is not the primary source of fluctuation.

In vivo *experiments*

Fast response and recovery, simple apparatus and procedures have allowed the proposed

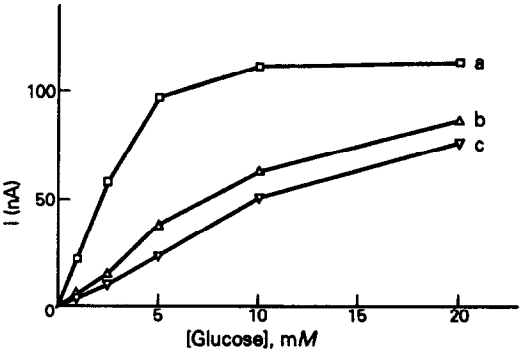

Fig. 5. Calibration curves of glucose with different membrane length microdialysis probes. Flow-rate = 30 μl/min, $T = 37°$, (a) = 40 mm, (b) = 20 mm, (c) = 10 mm.

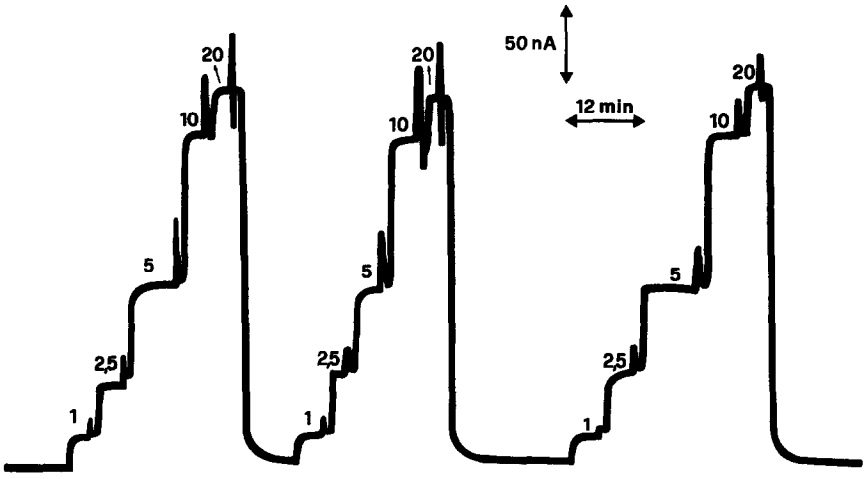

Fig. 6. Response time of the hollow fiber as microdialysis probe. Flow-rate = 30 μl/min, room temperature, membrane length = 20 mm.

method to be directly applied in *in vivo* experiments.

The fiber can be sterilized, it is rugged and easily handled and the material is reported to be highly biocompatible.[19]

Figure 7(a) shows preliminary results obtained monitoring glucose by sampling with a hollow fiber inserted subcutaneously in a rabbit [Fig. 7(a)] and in a human volunteer [Fig. 7(b)] during a glucose load experiment.

The stability of the signal before the glucose load shows how the removal of glucose by the probe does not disturb the physiological process. After a glucose load the current increases and then decreases following normal behavior. The variation of sensitivity was checked

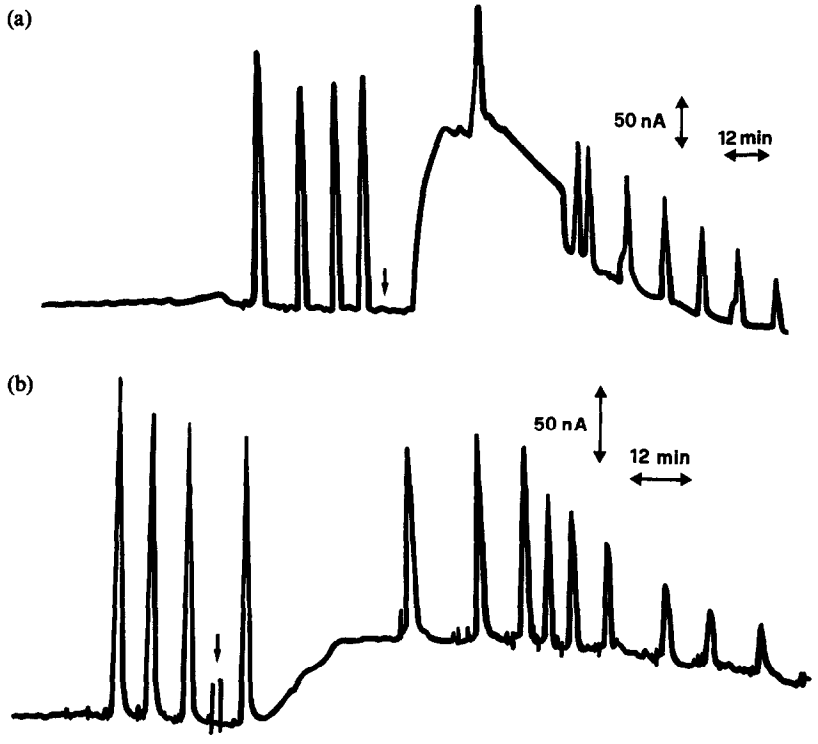

Fig. 7. *In vivo* experiments during glucose loading. Hollow fiber as microdialysis probe, flow-rate = 30 μl/min, membrane length = 10 mm; (a) rabbit weight 2.5 kg, fasting, unanesthetized (intravenous glucose loading = 3.5 g glucose). (b) On a human volunteer (oral loading = 70 g). The arrow shows the glucose administration. The peaks show the monitoring of sensitivity variation. The glucose standard solution was 1m*M*.

regularly by the flow injection apparatus described. A sharp decrease of the sensitivity is evident after about two hours in both experiments [Fig. 7(a) and (b)]. This effect was not previously detected.[20] If the microdialysis probe was disconnected and buffer as carrier was pumped through the cell, the glucose biosensor recovered the initial sensitivity in 15–30 min. We think an unknown substance is produced in the physiological liquid, probably in response to the fiber introduction. We believe it is released as a consequence of an inflammatory reaction and is able to diffuse through the hollow fiber and interfere with the enzyme or the electrode reaction.

This sensitivity variation can explain why the attempts to measure glucose *in vivo* by directly inserting needle glucose biosensors in blood or inserting subcutaneously fail more or less rapidly and it may explain the variations in sensitivity (slope of response *i vs.* concentration of glucose) reported in literature.[8–10] It is the first time that this phenomenon was followed during an *in vivo* experiment.

The problem of tissue reaction was reported recently in a few cases[10] to explain the high failure of subcutaneous glucose monitoring.

We are trying now to identify this compound and to use a suitable microdialysis fiber to overcome this problem.

However we did not notice this effect when the probe was inserted into the bloodstream; in this case normal behaviour was obtained and the output was related to the glucose concentration. Other experiments are now planned with clinicians for inserting the probes *in vivo* to obtain more data on this interesting effect.

CONCLUSIONS

The system shown is suitable for measuring glucose *in vivo* by coupling microdialysis with a glucose biosensor in the form of a thin layer cell. The system follows rapidly the variations of glucose concentration at the site where the "probe" is inserted. Preliminary results obtained placing the probe subcutaneously revealed the formation of an interferent species for the glucose oxidase enzyme or for the electrode. Such species are considered the result of an inflammatory reaction and it can explain the failure of experiments reported so far with the subcutaneous insertion of needle glucose biosensors.[8–10]

However, the same effect was not detected when the probe was placed in the bloodstream. We can explain it with the vascularized nature of the bloodstream but more *in vivo* experiments will clarify the nature of this effect.

Acknowledgement—This work has been supported by the C.N.R. Target Project Biotechnology and Bioinstrumentation, which is gratefully acknowledged.

REFERENCES

1. International Symposium on Microdialysis, Indianapolis, U.S.A., May 17–19, 1989.
2. C. F. Mandenius, *Anal. Letters*, 1988, **21**, 1817.
3. Bibliography on Microdialysis, by A. Eliasson, Carnegie Medicine, Stockholm, 1989.
4. P. K. Kissinger, *J. Chromatog.*, 1989, **488**, 31.
5. Y. L. Hurd, J. Kehr and U. Ungerstedt, *J. Neurochemistry*, 1988, **51**, 1314.
6. C. Speciale, U. Ungerstedt and R. Schwarcz, *Life Sci.*, 1988, **43**, 777.
7. M. Shichiri, N. Asakawa, Y. Yamasaki, R. Kawamori and H. Abe, *Diabetes Care*, 1986, **9**, 298.
8. R. Sternberg, M. B. Barrau, L. Gangiotti, D. Thevenot, D. Bindra, G. Wilson, G. Velho, P. Froguel and G. Reach, *Biosensors*, 1988, **4**, 27.
9. J. Pickup, G. Shaw, D. Claremont, *Diabetologia*, 1989, **32**, 213.
10. K. Rebrin, U. Fisher, T. Woedtke, P. Abel and E. Brunstein, *ibid.*, 1989, **32**, 573.
11. D. Matthews, E. Bown, T. Beck, E. Plotkin, L. Lock, E. Gosden and M. Wickham, *Diabet. Med.*, 1988, **5**, 248.
12. M. Koudelka, F. Rohner-Jeanrenaud, J. Terrettaz, E. Bobbioni-Harsch, N. de Rooij and B. Jeanrenaud, *Biosens. Bioelect.*, 1991, **6**, 31.
13. S. Churchouse, C. Battersby, W. Mullen and P. Vadgama, *Biosens.*, 1986, **2**, 325.
14. M. Mascini, G. Palleschi and M. Iannello, *Anal. Chim. Acta*, 1983, **156**, 135.
15. M. Mascini and F. Mazzei, *ibid.*, 1987, **192**, 9.
16. P. J. Taylor, E. Kmetec and J. M. Johnson, *Anal. Chem.*, 1977, **49**, 789.
17. G. Palleschi, M. A. Nabi Rahni, G. J. Lubrano, J. N. Ngwainbi and G. G. Guilbault, *Anal. Biochem.*, 1986, **159**, 114.
18. A. D. Gough, J. Y. Lucisano, P. H. S. Tse, *Anal. Chem.*, 1985, **57**, 2351.
19. Informations obtained by Hospal Industie.
20. T. Huang and P. Kissinger, *Current Separations*, 1989, **9**, (No. 1/2), 9.