ELSEVIER

Biosensors & Bioelectronics 16 (2001) 391–397

BIOSENSORS & BIOELECTRONICS

www.elsevier.com/locate/bios

# A flow-through amperometric sensor based on dialysis tubing and free enzyme reactors

S. Böhm *, D. Pijanowska, W. Olthuis, P. Bergveld

*MESA+ Research Institute, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands*

Received 11 May 2000; received in revised form 1 March 2001; accepted 8 March 2001

**Abstract**

A generic flow-through amperometric microenzyme sensor is described, which is based on semi-permeable dialysis tubing carrying the sample to be analyzed. This tubing (300 μm OD) is led through a small cavity, containing the working and reference electrode. By filling this cavity with a few μl of an appropriate enzyme solution, an amperometric enzyme sensor results. As the dialysis tubing is impermeable for large molecular species such as enzymes, this approach does not require any immobilization chemistry, and as a consequence the enzyme is present in its natural free form. Based on this principle, amperometric sensors for lactate, glucose, and glutamate were formed by filling cavities, precision machined in Perspex®, with buffered solutions containing respectively, lactate-, glucose-, and glutamate-oxidase. All sensors showed a large linear range (0–35 mM for glucose, 0–3 mM for lactate, and 0–5 mM for glutamate) covering the complete physiological range. The lower detection limit was in the order of 15–50 μM. Applicability in flow injection analysis systems is demonstrated. 

*Keywords:* Flow-through; Amperometric; Enzyme sensor; Semi-permeable membrane

## 1. Introduction

The accurate and automated determination of enzyme substrates (e.g. glucose, lactate, etc.) is of key importance in many industrial and clinical situations. For this purpose, laboratories are often equipped with flow injection analysis (FIA) systems (Ruzicka and Hansen, 1988; Karlberg and Pacey, 1989; Schmid, 1991), which are able of automatically performing a large number of assays per unit of time. Much research has been devoted to the development of flow-through enzyme sensors for use in combination with FIA. The principle of measurement in the majority of cases is the amperometric detection of hydrogen peroxide released by the enzyme/substrates reaction. Therefore, the described sensors can be classified mainly according to the method of enzyme immobilization. In most of the cases, the enzyme is immobilized in the vicinity of the working electrode required for hydrogen peroxide detection, either by entrapping the enzyme in a polymer matrix or hydrogel (Ito et al., 1995; Zilkha et al., 1995; Mizutani et al., 1996; Schneider et al., 1996; Steinkuhl et al., 1996; Pfeiffer et al., 1997; Perdomo et al., 1999), which is often electrodeposited directly on to the electrode (Umana and Waller, 1986; Mastrototaro et al., 1991; Palmisano et al., 1994; van Os et al., 1995; Adeloju et al., 1996), or by covalent attachment to an appropriate support resulting in an enzyme reactor (de Boer et al., 1994a,b; Yao et al., 1994; Laurell and Drott, 1995; Kaptein et al., 1998). The described sensors are in principle suitable for integration in FIA systems. However, the described immobilization methods are in some cases rather elaborate and often result in only a monolayer of enzyme having a limited effective activity. It is also suggested that enzyme immobilization affects enzyme activity and stability (Almeida et al., 1993; Danilich et al., 1993; Camacho-Rubio et al., 1996; Seo et al., 1998). Moreover, immobilization techniques are not always completely understood and sometimes not reproducible with respect to resulting enzyme activity, enzyme leakage, etc. It can therefore be concluded that preferably the enzyme in the sensor should be present in its native free form. Examples of this class of enzyme

* Corresponding author. Tel.: +31-53-4892724; fax: +31-53-4892287.

*E-mail addresses:* s.bohm@el.utwente.nl (S. Böhm), w.olthuis@el.utwente.nl (W. Olthuis).

0956-5663/01/$ - see front matter 
PII: S0956-5663(01)00153-1

sensors are given in Clark and Lyons (1962), Nilsson et al. (1973), Rechnitz (1978), Nikolesis (1984). However, the construction of these sensors is rather complicated and fragile, whilst the resulting size does not allow the easy integration of a sensor array in a typical FIA system for multi-analyte detection. For this purpose, a microbioreactor for glucose detection has been proposed (Son et al., 1996). In this device, a microcavity ($500 \times 500 \times 200\ \mu m^3$) with integrated platinum working electrode is filled with a solution containing the free enzyme GOx. After filling, a semi-permeable nylon membrane closing the cavity is polymerized in situ. However, for the in situ polymerization of the membrane, it was necessary to add the monomer in a high concentration (0.3 M hexamethylene diamine). It was suggested by the authors that a part of the free enzyme is involved in the polymerization reaction resulting in difficulties regarding enzyme activity and stability as mentioned before. Besides this disadvantage, the manufacturing of the sensor is complicated.

In this work, a flow-through enzymatic sensor is proposed, which is based on a microenzyme reactor formed around a semi-permeable membrane tubing (Fig. 1). Through a small cavity, typically with a volume of a few μl, a semi-permeable passage is formed by a piece of dialysis tubing. This cavity is filled with an aqueous solution containing the free enzyme. The molecular weight cut-off (MWCO) of the tube membrane material is chosen small enough to prevent any leakage of the enzyme, but allows the free inward diffusion of small molecular substrate (analyte) from the sample flowing through the tube. As this substrate enters the enzyme reactor, it is converted and hydrogen peroxide is released giving rise to a current through the working electrode, which is depending on the analyte concentration. To enlarge the surface of the working electrode and at the same time cover the area, where most of the analyte is being converted, in this work a spiral electrode is applied. Major benefits of this approach is that no immobilization is required, enabling the incorporation of enzyme in any desired concentration. Thus, the required sensitivity and detection limit for a particular application can be obtained. Another advantage is the generic design, which allows the easy construction of an array of similar cavities around a single dialysis tube, which after filling each cavity with the appropriate enzyme solution results in a multi-analyte sensor array. Moreover, the use of dialysis tubing enables easy integration with a so-called microdialysis probe for in vivo sampling of the blood stream or subcutaneous tissue (Morrison et al., 1991; Bergveld et al., 1999; Torto et al., 1999).

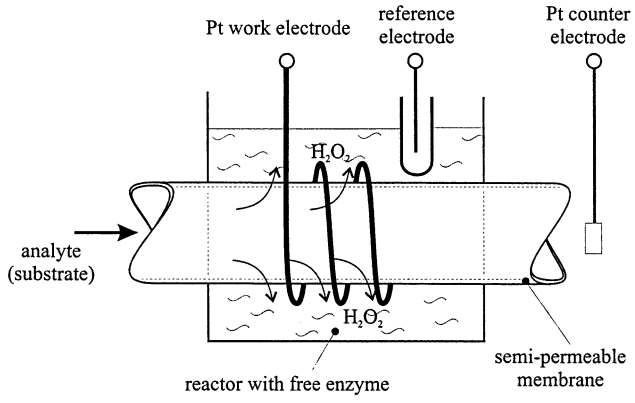


Fig. 1. Proposed amperometric enzyme sensor based on a semi-permeable dialysis tubing.

Based on this geometry, sensors for lactate, glucose, and glutamate were constructed by precision machining in Perspex®. Both sensors were evaluated in continuous flow mode as well as in a FIA system.

## 2. Experimental

### 2.1. Sensor construction

A number of electrode housings were machined from a piece of Perspex® by conventional precision engineering. A few millimeter length of a silver rod having a diameter of about 1.5 mm was inserted in a drilled vertical hole for receiving the enzyme solution. To yield a stable Ag/AgCl reference electrode, the silver surface of the rod was carefully cleaned prior to electrochemical chlorination in a 0.1 M HCl solution at a current density of 1 $mA/cm^2$ for 1 h. Platinum wire (127 μm diameter, Aldrich, The Netherlands) was wound around a 400 μm capillary to yield a spiral having a width of about 1.3 mm (8 turns). Prior to assembly this working electrode was electrochemically cleaned in 0.5 sulfuric acid. Next a length of semi-permeable tubing (300 μm outer diameter, 50 μm wall thickness) adapted from an artificial kidney (regenerated cellulose, MWCO 20 kD, Filtral® 6, AN69 HF, Hospal France) was inserted in the housing, through the platinum spiral and fixed with epoxy resin. The epoxy resin only contacts the dialysis tubing at the in- and out-let of the cell and does not influence the properties of the semi-permeable membrane in the enzyme-filled cavity. Finally, two glass fluidic connectors were attached at the sides forming the in- and out-let of the flow-through sensor. A photograph of the sensor is shown in Fig. 2. A platinum counter electrode was positioned downstream of the sensor.

Before use, the cavity was filled with the appropriate enzyme solution ($\approx 4$ μl) and the sensor was perfused with buffer to remove any glycerol from the membrane pores. The composition of the applied enzyme solutions are listed in Table 1. The presence of a constant 10 mM KCl concentration guarantees a stable potential at the Ag/AgCl (pseudo)reference electrode.

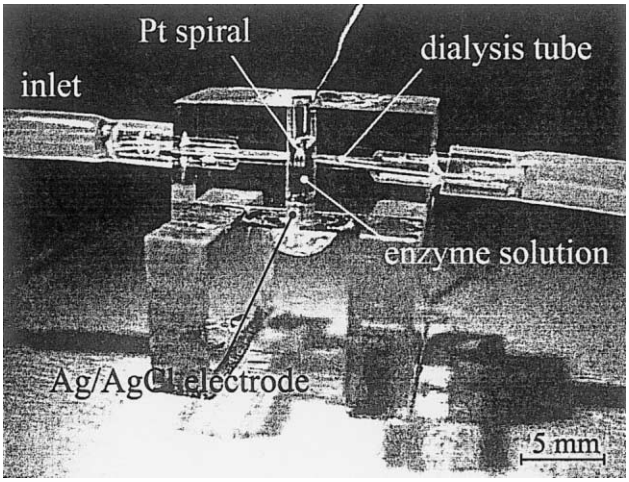


Fig. 2. Photograph of a realized flow-through sensor.

### 2.2. Instrumentation and measurement procedure

Sensors were connected to a precision peristaltic pump (P1, Pharmacia, Sweden, tubing: Teflon® tubing, 0.6 mm ID) for driving the sample solutions (substrate + 20 mM phosphate buffer, pH 7.3) from a stirred beaker through the sensor at a continuous flow rate. For the amperometric detection, a potentiostat (EG&G model 263A, Princeton Applied Research, UK, 650 mV vs. Ag/AgCl) was used, linked to a computer for data storage and analysis.

Continuous flow-through measurements were performed by pumping increasing concentrations of substrate through the sensor at a continuous flow rate (100 μl/min). To demonstrate the applicability as a sensor in FIA systems, various plugs of different concentration were manually injected in a continuous carrier stream (FIA set-up: 13 μl plug volume, tube: $L = 63$ cm, ID = 0.6 mm, carrier: 20 mM phosphate buffer, pH 7.3, flow rate 100 μl/min).

As for the enzymatic reaction oxygen is required, the amount of dissolved oxygen can influence the sensitivity. To investigate this influence, first the amount of dissolved oxygen in the laboratory sample solution was determined (oxygen probe, Orion, model 810). Then, by bubbling with pure oxygen gas, the dissolved oxygen level was increased and the sensitivity of the sensor was determined again.

To establish the stability of this type of enzyme sensors, a lactate sensor was continuously perfused for 24 h with a 0.5 mM lactate solution. Also, the sensitivity was determined after 1 month of intermittent use and wet storage.

As the design allows the easy implementation of an array of enzyme-filled cavities surrounding a single dialysis tube, the cross-talk between different sensors was determined by placing two reactors in series. Hydrogen peroxide produced in the upstream sensor can potentially leak out and can be carried over to the downstream sensor-giving rise to an erroneous sensor signal. This process of hydrogen peroxide carry over was investigated by placing a lactate sensor downstream of a glutamate sensor and pumping glutamate samples through both sensors. Any current through the lactate sensor indicates a carry over of hydrogen peroxide generated in the upstream glutamate sensor (assuming absolute selectivity of the LOD enzyme).

The top surface area of the cylindrical enzyme-filled cavity was open to air. The relatively large volume to surface ratio of this provisional set-up largely prevented any substantial evaporation during the experiments. In the next generation of this device, which is currently being processed, using bulk and surface micromachining of silicon and glass, the cavities will be closed after filling.

Table 1
Composition of the applied enzyme solutions

| Analyte | Enzyme solution |
|---|---|
| Lactate | LOD (Sigma-Aldrich, EC 1.4.3.11, from Streptomyces sp.) 80 U/ml[a], Phosphate buffer 20 mM, pH 7.3, 10 mM KCl. |
| Glucose | GOD (Sigma-Aldrich, EC 1.1.3.4, type II from *Aspergillus niger*) 100–644 U/ml[a], Phosphate buffer 20 mM, pH 7.3, 10 mM KCl. |
| Glutamate | GlOD, 5 U/ml[a], Phosphate buffer 20 mM, pH 7.3, 10 mM KCl. |

[a] Mean activity indicated by supplier. All enzymes were from a single batch.

## 3. Results and discussion

### 3.1. Lactate sensor

#### 3.1.1. Continuous flow

Fig. 3 shows a typical response of a lactate sensor for a continuous flow rate (sensor G1). From this graph, it can be seen that the sensor currents are in the μA regime, indicating a high sensitivity. The response time is in the order of 45 s, whilst the background current is in the range of 0–50 nA for all tested sensors. As can be seen from the calibration curve plotted in Fig. 4, the linear range extends up to 3 mM lactate, which is well above the physiological range indicating applicability in in vivo monitoring (whole blood: 0.44–1.8 mM, Harper, 1975).

The amount of dissolved oxygen in the plain samples used during experiments was determined to be 1.4 mg/l. After bubbling with oxygen gas, this amount was increased to about 15 mg/l. However, the sensitivity remained unchanged indicating that no oxygen depletion occurs under normal laboratory conditions and as a consequence no sample pretreatment (i.e., bubbling with oxygen) is required.

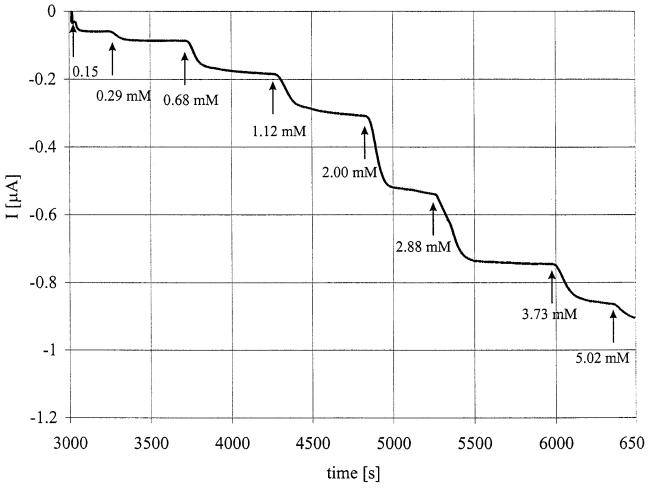


Fig. 3. Typical response of the enzyme sensor (no. L1) for a continuous flow rate (100 µl/min). Changes in lactate concentration are indicated by the arrows.

### 3.1.2. Flow injection analysis set-up

Fig. 5 shows a recording of the sensor response for consecutive injection of separate lactate plugs in the FIA set-up (sensor no. L1). After the injection of sample solution, the current increased to reach a peak within 50 s ( = travel time, $t_A$) and returned to baseline within 120 s ( = peak width $t_B$). This current-time profile provided a maximum sampling rate of $\approx 30$ samples/h. The experimental values of $t_A$ and $t_B$ are somewhat higher than those obtained from a semi-empirical model of Vanderslice et al. (1981) (theoretical values: $t_A = 47–94$ s, $t_B = 51–103$ s) in which the detector response has been assumed to be very fast. This is a result of the increased response time of the sensor caused by the fact that the substrate has to diffuse through the semi-permeable membrane towards the working electrode. The total path $\ell$ (m) for diffusion is

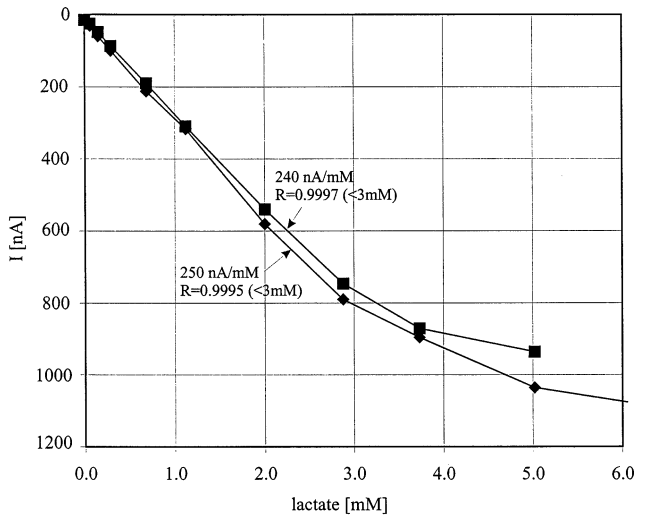


Fig. 4. Calibration curve of lactate sensor no. L1 and L2.

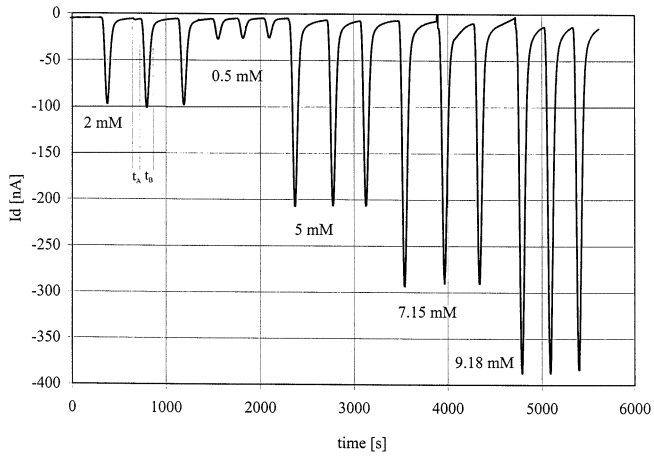


Fig. 5. Recorded calibration peaks for lactate (FIA set-up, sensor L1).

about 100 µm, which corresponds to a diffusion time $\tau$ of about 10 s ($\tau = \ell^2/D$, $D$: diffusion coefficient for lactate = $1.04 \times 10^{-9}$ m$^2$/s). If this delay is taken into account, the experimental values for $t_A$ and $t_B$ correspond with theory. For this FIA configuration, the peak height vs. concentration and correlation coefficient $R$ are 42.9 nA/mM and 0.9992, respectively (for the summarized FIA performance, see Table 2). The relative mean standard deviation for 50 consecutive injections of 1 mM lactate was found to be 0.7%.

### 3.1.3. Stability

After continuous perfusion for 24 h with lactate, which is comparable to about 2000 FIA injections of about 7 mM (mean current 130 nA), the sensor did show only a negligible change of sensitivity, which was within the accuracy of the measurements (about 3%). Also, after intermittent use of the sensor for one month, the sensitivity remains constant within this accuracy.

## 3.2. Glucose sensor

### 3.2.1. Continuous flow

A number of glucose sensors with increasing enzyme concentration were manufactured and evaluated. From Table 2, it can be seen that a higher enzyme activity in the reactor yields a higher sensitivity, although saturation occurs above an activity of 300 U/ml. This indicates that if the sensor is filled with a high (excess) enzyme concentration, any small decrease in enzyme sensitivity will result in a negligible decrease in sensitivity.

The sensitivity, response time and background current are comparable to the lactate sensor. The linear range for glucose extends up to about 35 mM, which is well beyond the physiological range (whole blood: 0.45–6 mM, for diabetes up to 40 mM in severe hyperglycemia, Harper, 1975).

### 3.2.2. Flow injection analysis set-up

Sensor G4 was applied in the FIA set-up and was found to result in comparable performance with respect to the lactate sensor L1.

### 3.3. Glutamate sensor

The results for the glutamate sensor are comparable to the previous sensors for both continuous flow and FIA experiments (Table 2). Also, the linear range of both the continuous flow and the FIA experiments were well within the physiological range of 0.1–0.5 mM (Harper, 1975).

### 3.4. Sensor cross-talk

It was found that even injections of high concentrations of glutamate (up to 5 mM), did not give rise of a sensor current in the lactate sensor placed downstream. This indicates a large electrochemical conversion factor of hydrogen peroxide in the glutamate cell and that only negligible amounts leak out of the reactor. This indicates that arrays of enzymatic sensors can be implemented for monitoring a variety of analytes in series.

### 3.5. Redox interference

The next generation of an array of enzyme-filled cavities, currently being processed, may contain a cavity that is only filled with background electrolyte and the Pt spiral working electrode. This cavity, the first in the array, can catch possibly interfering redox species, before they can reach the enzyme-filled cavities, thus preventing an erroneous read-out in the enzyme reactors due to interfering species.

## 4. Conclusions

Enzymatic microenzyme sensors were constructed for the amperometric detection of lactate, glucose, and glutamate. In the generic design, a semi-permeable dialysis tube was applied to physically entrap the enzyme, thereby taking away the requirement of immobilization. All implemented sensors exhibit a large linear range covering the complete physiological range. It was found that the sensitivity of this type of sensor did not change after one month of intermittent use. Another benefit from this sensor geometry is that an excess amount of enzyme can be incorporated in order to establish a

Table 2
Summarized sensor characteristics

Continuous flow

| Sensor no. | Enzyme activity (U/ml) | Slope (nA/mM) | $R$ | Linear range (mM) | Detection limit (μM) |
|---|---|---|---|---|---|
| *Lactate*[a] | | | | | |
| L1 | 80 | 250 | 0.9995 | 0–3 | 25 |
| L2 | 80 | 240 | 0.9969 | 0–3 | |
| *Glucose*[b] | | | | | |
| G1 | 100 | 188 | 0.9990 | 0–26 | 50 |
| G2 | 320 | 297 | 0.9988 | 0–26 | |
| G4 | 644 | 312 | 0.9985 | 0–35 | |
| G3 | 520 | 308 | 0.9979 | 0–35 | |
| *Glutamate*[c] | | | | | |
| GL1 | 5 | 76 | 0.9984 | 0–5 | 15 |

*FIA performance*[d]

| Sensor no. | Related standard deviation (%) | Peak height (nA/mM) | $R$ | Range (mM) | $t_A$, $t_B$ experiment (s) | $t_A$, $t_B$ theory (s) |
|---|---|---|---|---|---|---|
| Lactate L1 | <2 | 42.9 | 0.9992 | 0–9.8 | 50, 120[e] | 47–94, 51–103 |
| Glucose G4 | <5 | 39.2 | 0.9998 | 0–10 | 52, 130[e] | 22–44, 55–116 |
| Glutamate GL1 | <1.5 | 8.35 | 0.9992 | 0–5 | 62, 135[e] | 28–56, 70–140 |

[a] Sample solution: 0–5 mM lactate in 20 mM phosphate buffer pH 7.3.
[b] Sample solution: 0–45 mM glucose in 20 mM phosphate buffer pH 7.3.
[c] Sample solution: 0–5 mM glutamate in 20 mM phosphate buffer pH 7.3.
[d] FIA configuration: 15 μl plug volume, tube: $L = 63$ cm, ID = 0.6 mm, carrier: 20 mM phosphate buffer, pH 7.3, flow rate 100 μl/min.
[e] See the text.

stable sensor as a small decrease in enzyme activity will result in a negligible decrease in sensitivity. The current design allows the implementation of enzyme sensor arrays for a variety of substrates and is applicable in miniaturized chemical analysis systems as the inner diameter of the flow-through sensor is only 0.2 mm. It followed that the hydrogen peroxide conversion is large enough to prevent cross-talk between sensors placed in series, enabling the integration of enzymatic sensor arrays. Moreover, the use of dialysis tubing enables easy integration with a so-called microdialysis probe for in vivo sampling of the blood stream or subcutaneous tissue (Bergveld et al., 1999). The integrated version of this probe with the sensors as proposed in this paper is currently being processed.

## Acknowledgements

The authors wish to thank Mr J. Bomer for the construction of the sensor bodies. This project is financially supported by the Dutch foundation for Fundamental Research on Matter (FOM), and the Royal Dutch Academy of Science (KNAW).

## References

Adeloju, S.B., Shaw, S.J., Wallace, G.G., 1996. Polypyrrole-based amperometric flow injection biosensor for urea. Anal. Chim. Acta 323, 107–113.

Almeida, N.F., Beckman, E.J., Ataai, M.M., 1993. Immobilization of glucose oxidase in thin polypyrrole films: influence of polymerization conditions and film thickness on the activity and stability of the immobilized enzyme. Biotech. Bioeng. 42, 1037–1045.

Bergveld, P., Böhm, S., Olthuis, W., 1999. Microdialysis-probe integrated with Si-chip. International Patent Application, PCT/NL99/00057.

Camacho-Rubio, F., Jurado-Almeda, E., González-Tello, P., Luzón González, G., 1996. A comparative study of the activity of free and immobilized enzymes and its application to glucose isomerase. Chem. Eng. Sci. 51 (17), 4159–4165.

Clark, L.C. Jr., Lyons, C., 1962. Electrode systems for continuous monitoring in cardiovascular surgery. Ann. NY Acad. Sci. 102, 29–46.

Danilich, M.J., Gervasio, D., Marchant, R.E., 1993. Activity of free and immobilized glucose oxidase: an electrochemical study. Ann. Biom. Eng. 21, 655–668.

de Boer, J., Korf, J., Plijter-Groendijk, H., 1994a. In vivo monitoring of lactate and glucose with microdialysis and enzyme reactors in intensive care medicine. J. Art. Org. 17 (3), 163–170.

de Boer, J., Baarsma, R., Okken, A., Plijter-Groendijk, H., Korf, J., 1994b. Application of transcutaneous microdialysis and continuous flow analysis for on-line glucose monitoring in newborn infants. J. Lab. Clin. Med. 124 (2), 210–217.

Harper, H.A., 1975. Review of Physiological Chemistry. Lange Medical Publications, Los Altos, CA.

Ito, N., Matsumoto, T., Fujiwara, H., Matsumoto, Y., Kayashima, S., Arai, T., Kikuchi, M., Karube, I., 1995. Transcutaneous lactate monitoring based on a micro-planar amperometric biosensor. Anal. Chim. Acta 312, 323–328.

Kaptein, W.A., Zwaagstra, J.J., Venema, K., Korf, J., 1998. Continuous ultraslow microdialysis and ultrafiltration for subcutaneous sampling as demonstrated by glucose and lactate measurements in rats. Anal. Chem. 70 (22), 4696–4700.

Karlberg, B., Pacey, G.E., 1989. Flow injection analysis: a practical guide. In: Techniques and Instrumentation in Analytical Chemistry, vol. 10. Elsevier, Amsterdam.

Laurell, T., Drott, J., 1995. Silicon wafer integrated enzyme reactors. Bios. Bioel. 10, 289–299.

Mastrototaro, J.J., Johnson, K.W., Morff, R.J., Lipson, D., Andrew, C.C., Allen, D.J., 1991. An electroenzymatic glucose sensor fabricated on a flexible substrate. Sens. Act. B 5, 139–144.

Mizutani, F., Yabuki, S., Hirata, Y., 1996. Flow injection analysis of lactic acid using an enzyme-polyion complex-coated electrode as the detector. Talanta 43 (10), 1815–1820.

Morrison, P.F., Bungay, P.M., Hsiao, J.K., Dykstra, I.N., Dedrick, R.L., 1991. Quantitative microdialysis. In: Robinson, T.E., Justice, J.B. Jr. (Eds.), Techniques in the Behavioral and Neural Sciences in Microdialysis in the Neurosciences, vol. 7. Elsevier, Amsterdam, pp. 47–80.

Nikolesis, D.P., 1984. Construction of an immobilised asparaginase sensor and determination of asparagine in blood. Anal. Chim. Acta 161, 343–348.

Nilsson, H., Akerlund, A., Mosbach, K., 1973. Determination of glucose, urea and penicillin using enzyme pH electrodes. Biochim. Biophys. Acta 320, 529–534.

Palmisano, F., Centonze, D., Zambonin, P.G., 1994. An in-situ electrosynthesized amperometric biosensor based on lactate oxidase immobilized in a poly-o-phenylenediamine film: determination of lactate in serum by flow injection analysis. Bios. Bioel. 9, 471–479.

Perdomo, J., Sundermeier, C., Hinkers, H., Martinez Morell, O., Seifert, W., Knoll, M., 1999. Containment sensors for the determination of L-lactate and glucose. Bios. Bioel. 14, 27–32.

Pfeiffer, D., Yang, L., Scheller, F.W., Kissinger, P.T., 1997. Continuous measurement of lactate in microdialysate. Anal. Chim. Acta 351 (1–3), 127–132.

Rechnitz, G.A., 1978. Biochemical electrodes using tissue slice. Chem. Eng. News 56, 19.

Ruzicka, J., Hansen, E.H., 1988. Flow injection analysis. In: Elving, P.J., Winefordner, J.D. (Eds.), Chemical Analysis, vol. 62, second ed. Wiley, New York.

Schmid, R.D., 1991. Flow injection analysis (FIA) based on enzymes or antibodies. In: Schmid, R.D. (Ed.), GBF Monographs, vol. 14. VCH, NewYork.

Schneider, J., Gründig, B., Renneberg, R., Cammann, K., Madaras, M.B., Buck, R.P., Vorlop, K., 1996. Hydrogel matrix for three enzyme entrapment in creatine/creatinine amperometric biosensing. Anal. Chim. Acta 325 (3), 161–167.

Seo, H., Itoyama, K., Morimoto, K., Takagishi, T., Oka, M., Hayashi, T., 1998. Spacer effects on enzymatic activity of bromelain immobilized onto porous chitosan beads. Eur. Polym. J. 34 (7), 917–922.

Son, M., Peddie, F., Mulcahy, D., Davey, D., Haskard, M.R., 1996. A microbioreactor based on interfacial polymerisation and application to flow injection analysis of glucose. Sens. Act. B 34, 422–428.

Steinkuhl, R., Sundermeier, C., Hinker, H., Dumschat, C., Cammann, K., Knoll, M., 1996. Microdialysis system for continuous glucose monitoring. Sens. Act. B 33, 19–24.

Torto, N., Laurell, T., Gorton, L., Marko-Varga, G., 1999. Recent trends in the application of microdialysis in bioprocesses. Anal. Chim. Acta 179, 281–305.

Umana, M., Waller, J., 1986. Protein modified electrodes: the glucose oxidase/polypyrrole system. Anal. Chem. 58, 2979–2983.

Vanderslice, J., Stewart, K.K., Rosenfeld, A.G., Higgs, D.J., 1981. Laminar dispersion in flow injection analysis. Talanta 28, 11–18.

van Os, P.J.H.J., Bult, A., van Bennekom, B.W., 1995. A glucose sensor, interference free for ascorbic acid. Anal. Chim. Acta 305 (1–3), 18–25.

Yao, T., Satomura, M., Nakahara, T., 1994. Amperometric flow-injection determination of glucose, urate and cholesterol in blood serum by using some immobilized enzyme reactors and poly(1,2-diaminobenzene)-coated platinum electrode. Anal. Chim. Acta 296, 271–276.

Zilkha, E., Obrenovitch, T.P., Koshy, A., Kusakabe, H., Bennetto, H.P., 1995. Extracellular glutamate: on-line monitoring using microdialysis coupled to enzyme-amperometric analysis. J. Neurosci. Meth. 60, 1–9.