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A flow-through amperometric sensor for micro-analytical systems

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Abstract

In this paper, the performance of the flow-through amperometric sensor based on semi-permeable dialysis tubing implemented in silicon is presented. The sensor is designed in the form to be an integral component of a lab-on-a-chip systems and it has been successfully incorporated into an integrated microdialysis system. Results concerning glucose sensors based on a flow-through sensor implemented in silicon operating as part of the integrated microdialysis system are presented. Moreover, comparison of the performances of the glucose sensor implemented in Perspex in the earlier work and the sensor implemented in silicon are discussed.

Keywords: Biosensors; Amperometric detection; Flow-through systems; Glucose sensors; Lab-on-a-chip; μTAS

1. Introduction

Continuous monitoring, or near continuous intermittent monitoring of substances of clinical interest can improve medical diagnosis, therapy and post-treatment care. In general it can be stated that in order to provide a continuous monitoring system, a number of requirements can be defined—small overall size, short response time, high sensitivity and selectivity to one species in a sample matrix. To satisfy the above requirements chemical sensors and biosensors have been used for decades. In the last decade, adaptation of silicon micromachining technology gives the possibility for miniaturisation of components of (bio)chemical analytical systems and building up micro-analytical systems (μTAS) or lab-on-a-chip with integrated sensors [1]. In general, all these components are connected via a system of microchannels. One component that is required for instance for the on-line monitoring is a set of miniaturised flow-through sensors.

A number of micro glucose sensors have been described. The majority of the sensors is based on amperometric detection so that the classification of the sensors is based on the methods of enzyme immobilization, such as (1) entrapment within polymeric membrane or behind dialysis membrane [2–12], or (2) covalent attachment to the sensor or microreactors surface [13–18]. Some of the sensors are designed in the form of microreactors fabricated using micromachining or containment technology [2,4,11,13,15–19]. Although they can be operated in FIA or microsystems, the integration of these type of sensors impose some problems if they have to be closely integrated within microfluidic channels, mainly due to their geometry (actual planar final structure).

A generic flow-through amperometric microenzyme sensor has been previously described by Böhm et al. [20]. The flow-through sensor was designed for integration in a micro-analytical system and/or sensor array. In this concept, the microchannel itself is an integral part of the sensor geometry and is formed by a length tubular semi-permeable membrane. The initial construction of the sensor was made in a Perspex block in the form of a microreaction cell filled with an enzyme solution separated from the analyte by a tubular semi-permeable membrane. The sensor based on this concept has now been successfully implemented in silicon as well as incorporated into an integrated microdialysis system. As a proof of principle glucose measurement was chosen. This paper presents the results of the flow-through glucose sensor implemented in silicon, operating as a part of the integrated microdialysis system. Moreover, the performances of the previous glucose sensors implemented in Perspex and the new system in silicon are compared.

1.1. Principle of the flow-through glucose amperometric sensor operation

In general, the principle of operation of the above-mentioned glucose sensor is based on electrochemical detection of hydrogen peroxide being a product of an enzymatic

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reaction. In the case of a flow-through glucose amperometric sensor, the glucose particles transported by the carrier solution from the sample region diffuse through a semi-permeable membrane to the reaction cell of the sensor. The reaction cell is filled with a solution containing glucose oxidase (GOx). Here the glucose is oxidised in a reaction catalysed by the GOx in the presence of a natural mediator, i.e. oxygen, according to the following reaction:

$$\text{glucose} + O_2 + H_2O \xrightarrow{GOx} \text{gluconolactone} + H_2O_2 \quad (1)$$

The hydrogen peroxide generated in reaction (1) is electrochemically oxidised at an anode:

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

The current resulting from the reaction is a measurand as it is proportional to the glucose concentration in the sample.

2. Experimental

2.1. Materials

Dialysis tube from regenerated cellulose of MWCO 20 kDa was adapted from an artificial kidney Filtral® 6, AN69 HF, Hospal, France.

A conducting silver–silver chloride paste Electrodag® 6088 SS was obtained from Acheson Colloiden B.V., The Netherlands.

Enzyme. Glucose oxidase (GOx) type II of activity 15 500 U/g solid was purchased from Sigma. For hydrogen peroxide standards preparation, 35% H_2O_2 stock solution from Merck was used. Glucose standards were prepared with use of 45 mM phosphate buffer containing 10 mM KCl (pH 7.3) for at least 24 h before use. Other reagents were of analytical grade.

2.2. A flow-through amperometric microenzyme sensor

As it was mentioned, the initial construction of a flow-through amperometric sensor with microreaction cell (7 μl volume) was made in Perspex [20]. Based on this principle, an amperometric microsensor has now been implemented in a silicon glass sandwich construction (Fig. 1). The tubing (200 μm i.d.) is led through a cavity etched in silicon. The cavity of 5 μl volume contains an electrochemical cell with a working, counter (CE) and reference (RE) electrodes, forming a standard three-electrode amperometric detection system. The platinum electrodes were patterned on the Pyrex wafer. Both wafers, silicon and Pyrex, were anodically bonded. Next, to form a reference electrode, the silver–silver chloride paste was deposited on top of the Pt electrode. The paste was annealed at 80 °C for 3 h. Then, the cavity around the dialysis tubing was closed with a cover glass fixed with UV curable glue (Loctite 350) and filled with an appropriate enzyme solution, which ends the fabrication procedure.

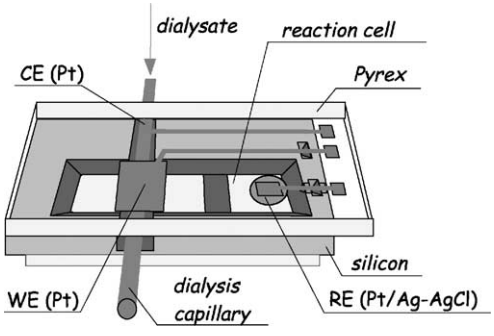

Fig. 1. Layout of a flow-through sensor.

In the sensor realised in Perspex, the working electrode (WE) was a Pt wire coiled around the dialysis tube resulting in a large coaxially located electrochemically active surface (2.6 mm^2), whereas in the silicon sensor, the working electrode is a planar Pt layer of 1.7 mm^2 surface area evaporated onto the Pyrex wafer.

2.3. An integrated microdialysis system

The integrated microdialysis system consisting of sensor array, microdialysis probe and calibration facilities, was fabricated with use of a monolithic technology [21]. The system was constructed of two wafers: (i) a Pyrex glass wafer with patterned platinum electrodes and (ii) a silicon wafer with the system of channels and sensors cells etched with a reactive plasma. The two wafers were anodically bonded and then diced into chips. To improve adhesion of the platinum electrodes to the glass, an intermediate titanium layer was applied.

A cannula type microdialysis probe was made in the form of coaxially fixed tubes. As the internal tube, providing perfusate to the microdialysis probe, a fused silica tube (o.d. = 150 μm, i.d. = 100 μm from Alltech Nederland B.V., Breda, The Netherlands) was used, whereas the external one was a semi-permeable (dialysis) capillary. The tubes were fixed with epoxy resin (Hysol®, Dexter, USA). The total length of the microdialysis probe was 22 mm, while the effective length of the dialysis membrane was 20 mm.

2.4. Measurements

2.4.1. Sensors characterisation

Two amperometric sensors, made in Perspex and in silicon, respectively, were both operated as a three-electrode system. The potential of the working electrode was held constant at +650 mV versus the Ag/AgCl reference electrode. The oxidation current at the working electrode was measured. The platinum counter electrode was placed downstream. The measurements were performed with the use of a potentiostat Model 263A, EG&G. The sensors were tested in a closed loop flow-through system, where the flow was driven by a peristaltic pump (Pharmacia, P-1, Sweden) at a flow rate of 10 ml/h. The concentration of hydrogen

peroxide and glucose in the sample solution was changed by a standard addition method.

Tests were performed for two different glucose oxidase concentrations in the reaction cell: 22 and 132 U/ml. The enzyme was dissolved in a phosphate buffer solution at pH 7.3. In the case of the sensor tests for sensitivity to hydrogen peroxide, the reaction cell was filled with the phosphate buffer solution without the enzyme.

2.4.2. Tests of the sensors in an integrated microdialysis system—sampling via microdialysis probe

The glucose amperometric sensor was tested as a part of an integrated microdialysis system. The sensor was operating in a three-electrode system as described above. The carrier solution (perfusate — 45 mM phosphate buffer at pH 7.3) was driven with a constant flow rate of 2 μl/min by a microdialysis pump (CMA 102, CMA Microdialysis AB, Sweden). The reaction cell was filled with glucose oxidase solution (31 U/ml of GOx in the phosphate buffer solution).

The sample solutions containing different concentrations of glucose were prepared in 45 mM phosphate buffer. Sampling was performed under flow of perfusate via a microdialysis probe which was inserted into sample solutions containing glucose of different concentrations: 1.25, 3, 6.25, 12.5 and 25 mM for time intervals of 25 s. Between the consecutive samplings, the microdialysis probe was immersed in a phosphate buffer and perfused with the carrier solution.

To estimate the influence of sampling time on the sensor response, the microdialysis probe was immersed in a sample solution at constant concentration of glucose (12.5 mM) for different time intervals of 20, 25, 30, 40, 50, 80 and 200 s. After sampling the probe was inserted into the phosphate buffer solution.

The device was fastened in a holder system with vertically movable sample vial holder. By moving the sample vial holder, the microdialysis probe was easily positioned in the vial not to touch the sides of the vial. All measurements were performed at room temperature.

3. Results and discussion

The sensors were tested to estimate the sensitivity to hydrogen peroxide and to glucose for different glucose oxidase activity in the reaction cell (22 and 132 U/ml). An example of the sensor response to glucose is shown in Fig. 2. It can be seen that the response time is in the order of 60 s, whereas the background current is in the range 10–16 nA.

The calibration curve (Fig. 2b) indicates that the linear range expands up to 20 mM, which covers the whole tested range of glucose concentration. The sensitivity of the glucose sensor depends on the enzyme activity in the reaction cell. The higher sensitivity (16 nA/mM) was obtained for the

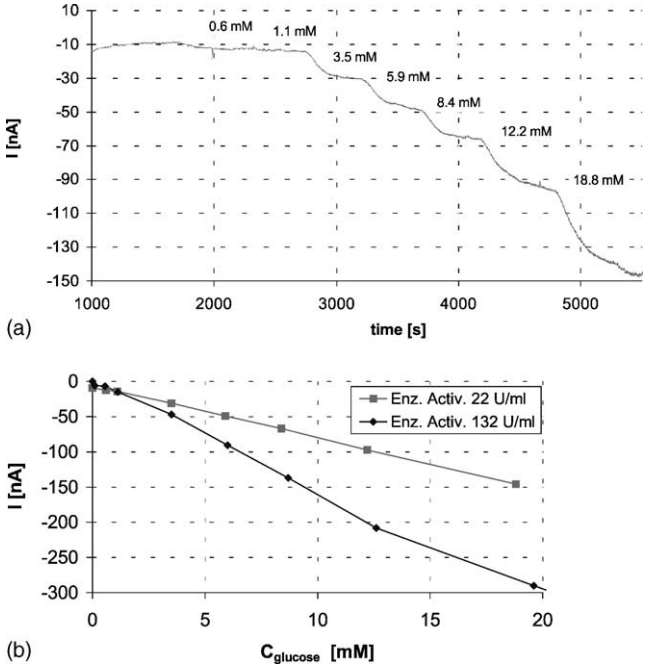

Fig. 2. Time response of a silicon sensor in continuous flow mode of operation (flow rate of 10 ml/h) (a), and calibration curves for the different enzyme activity: 22 and 132 U/ml (b). The upper calibration curve in figure (b) corresponds to the dynamic response in figure (a).

higher glucose oxidase concentration (132 U/ml) in the reaction cell (see Fig. 2b and Table 1).

As mentioned before, the construction of the silicon sensor was based on the previous construction of a flow-through amperometric sensor in Perspex. To compare parameters of both sensors (made in silicon and in Perspex), a sensitivity rate (SR) was defined as the ratio between the sensitivity to hydrogen peroxide and the sensitivity to glucose referred to the surface area of a working electrode — A, ($SR = S_{H_2O_2}/S_{glucose}/A$). The sensitivity rate expresses the efficiency of hydrogen peroxide conversion on a working electrode.

A comparison of the parameters for both glucose sensors during continuous flow is shown in Table 1. The sensitivity rate for the amperometric sensor made in a Perspex block ($SR = 2.4$) is lower than the sensitivity rate for the sensor made in silicon ($SR = 4.9$). This means that the electrochemical conversion of hydrogen peroxide (a product of glucose oxidation) was less effective for the silicon sensor than for the sensor made in Perspex. Another difference is much higher sensitivity for glucose of the sensor made in Perspex (179 nA/mM) than the silicon sensor (16 nA/mM) for both operating with the same enzyme concentration in the reaction cell (132 U/ml). This difference results from the design of the working electrodes for both sensors. The sensor realised in Perspex was equipped with the working electrode coiled around the dialysis tube resulting in a large coaxial sensing surface (2.6 mm^2), whereas the working electrode of the silicon sensor was planar and smaller (1.7 mm^2). In the later case, the sensor structure

Table 1
Parameters of glucose amperometric sensors for continuous flow with a flow rate of 10 ml/h

Species	Inner solution	Testing range (mM)	Sensitivity, S (nA/mM)	Linear range (mM)	Corr. Coefficient	Sensitivity rate, SR (mm^{-2}) ($S_{H_2O_2}/S_{glucose}/A$)
Sensor implemented in silicon						
Hydrogen peroxide	Phosphate buffer	0–13.4	134.2	Full tested range	0.998	–
Glucose	Phosphate buffer + GOx at concentration 132 U/ml	0–41.8	16	0–27	0.998	4.9
Glucose	Phosphate buffer + GOx at concentration 22 U/ml	0–18.8	7.3	Full tested range	0.999	10.6
Sensor made in Perspex						
Glucose	Phosphate buffer + GOx at concentration 132 U/ml	0–35.8	179	0–25	0.992	2.4

was asymmetrical with respect to the dialysis tube. Therefore, the hydrogen peroxide produced in the enzymatic reaction can diffuse from some regions of the reaction cell through the dialysis tubing and then be transported downstream with the carrier solution, before reaching the working electrode located on the opposite side of the dialysis capillary. In the sensors made in Perspex, the working electrode surrounds the dialysis capillary. This highly symmetrical configuration reduced loss of the hydrogen peroxide particles from the reaction cell. Summarising, performances of both sensors are very good, nevertheless the spiral-like working electrode based sensor exhibits better parameters (in particular a very high sensitivity) than the silicon sensor with the planar electrode. On the other hand the later one can be easily fabricated using a standard photolithographic process, which is an important aspect of sensor evaluation for integration in micro-analytical systems.

Finally, the glucose microsensor was tested in an integrated microdialysis system consisting of a sensor array, a microdialysis probe and calibration facilities. The glucose sensor response as function of the sampling time for a glucose concentration of 12.5 mM and a flow rate of 2 μl/min is shown in Fig. 3.

As can be seen, the output signal from the glucose sensor depends on the sampling time. The current peaks increase in amplitude with a longer sampling time (0.85 nA/s). The sampling was performed at a very low flow rate, low enough to assume that the concentration of glucose in the plug formed via the microdialysis probe is homogenous in its entire volume. This is a result of comparable values for a

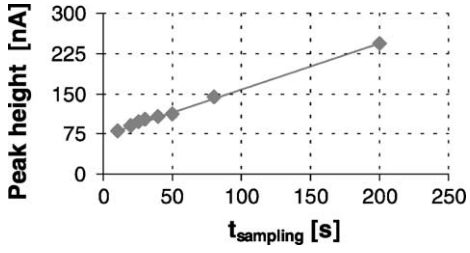

Fig. 3. Sensor response as a function of the sampling time via a microdialysis probe for 12.5 mM glucose concentration and a flow rate of 2 μl/min.

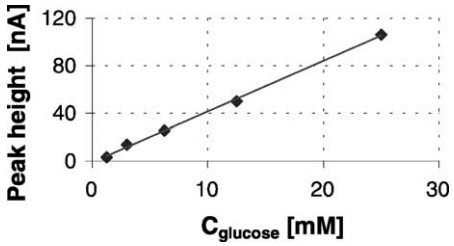

Fig. 4. Sensor response as a function of the glucose concentration at a constant sampling time via an integrated microdialysis probe (25 s) and a flow rate of 2 μl/min.

diffusion time for glucose molecules (diffusion coefficient for glucose 6.7×10^{-6} cm^2/s) for the diffusion path through the dialysis membrane and the perfusate layer in the microdialysis probe (50 and 25 μm, respectively), yielding of ca. 8.4 s and a residence time of the plug in the microdialysis probe, which is of about 8.8 s for the effective probe length 20 mm. On the other hand, for the longer sampling time, the volume of the plug formed via the microdialysis probe is larger so that, according to the FIA theory [22], the travel time of the plug through the sensor area was longer, resulting in the higher output signal peak.

An example of the sensor response to glucose sampled via a microdialysis probe for fixed sampling time (25 s) and a constant flow rate of 2 μl/min is shown in Fig. 4, showing a sensitivity of 4.3 nA/mM.

Since the sampling time strongly affects sensor response, reproducible results can only be obtained under a precise sampling regime.

4. Conclusions

In conclusion, this paper presents the fabrication and initial characterisation of a micro flow-through glucose sensor implemented in silicon. A high sensitivity of 16 nA/mM was obtained for a high glucose oxidase concentration in the reaction cell. The performance of the amperometric sensor implemented in silicon corresponds to those obtained with the previously tested model made in Perspex block. The difference in the sensors performances

can be explained by the geometry difference of the working electrodes for both sensors.

The main advantage of the silicon sensor over Perspex device is easy fabrication process with use a standard photolithographic process, being an important factor when integration into microsystem is considered.

The glucose microsensor was successfully integrated in a micromachined microdialysis system consisting of a sensor array and a microdialysis probe. The microsystem has been fabricated with use of a monolithic technology. Since the sampling time via the integrated microdialysis probe strongly affects the sensor response, reproducible results can only be obtained under a precise sampling regime.

Further research will concern calibration methods for the glucose sensor, operating in the integrated microdialysis system, with use of the built-in calibration facilities.

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References

[1] A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: a novel concept for chemical sensing, Sens. Actuators B: Chem. 1 (1993) 244–248.
[2] H. Hinkers, C. Sundermeier, K. Cammann, F. Walfort, M. Knoll, Amperometric microelectrode array in containment technology, Sens. Actuators B: Chem. 26–27 (1995) 398–400.
[3] R. Steinkuhl, C. Dumschat, C. Sundermeier, H. Hinkers, R. Renneberg, K. Cammann, M. Knoll, Micromachined glucose sensor, Biosens. Bioelec. 11 (1–2) (1996) 187–190.
[4] J. Perdomo, H. Hinkers, C. Sundermeier, W. Seifert, O.M. Morell, M. Knoll, Miniaturized real-time monitoring system for L-lactate and glucose using microfabricated multi-enzyme sensors, Biosens. Bioelec. 15 (2000) 515–522.
[5] F. Mizutani, S. Yabuki, Flow injection analysis for glucose using an amperometric enzyme electrode based on lipid-modified glucose oxidase as the detector, Biosens. Bioelec. 9 (1994) 411–414.
[6] J. Schneider, D. Gründig, R. Renneberg, K. Cammann, M.B. Madaras, R.P. Buck, K.-D. Verlop, Hydrogel matrix for three enzyme entrapment in creatine/creatinine amperometric biosensing, Anal. Chim. Acta 325 (1996) 161–167.
[7] E.F. Pfeiffer, C. Meyerhoff, F. Bischof, F.C. Keck, W. Kerner, On-line continuous monitoring of subcutaneous tissue glucose is feasible by combining portable glucosensor with microdialysis, Horm. Metab. Res. 25 (1993) 121–124.
[8] F. Palmisano, D. Centonze, M. Quinto, P.G. Zambonin, A microdialysis fiber based sampler for flow injection analysis: determination of L-lactate in biofluids by an electrochemically synthesized bilayer membrane based biosensor, Biosens. Bioelec. 11 (1996) 419–425.
[9] P.N. Bartlett, P. Tebbutt, C.H. Tyrrell, Electrochemical immobilization of enzymes. 3. Immobilization of glucose oxidase in thin films of electrochemically polymerized phenols, Anal. Chem. 64 (1992) 138–142.
[10] P.N. Bartlett, R.G. Whitaker, Electrochemical immobilization of enzymes, J. Electroanal. Chem. 224 (1987) 27–35.
[11] S. L Xie, E. Wilkins, Rechargeable glucose electrodes for long-term implantation, J. Biomed. Eng. 13 (5) (1991) 375–378.
[12] D.J. Strike, N.F. de Rooji, M. Koudelka-Hep, Electrodeposition of glucose oxidase for the fabrication of miniature sensors, Sens. Actuators B: Chem. 13–14 (1993) 61–64.
[13] B.C. Towe, V.B. Pizziconi, A microflow amperometric glucose biosensor, Biosens. Bioelec. 12 (1997) 893–899.
[14] T. Yao, Amperometric determination of glucose in blood serum with a chemically modified enzyme membrane electrode in a continuous flow system, Anal. Chim. Acta 153 1983 (1983) 175–180.
[15] T. Laurell, J. Drott, L. Rosengren, Silicon wafer integrated enzyme reactors, Biosens. Bioelec. 10 (1995) 289–299.
[16] T. Laurell, J. Drott, L. Rosengren, K. Lindström, Enhanced enzyme activity in silicon integrated enzyme reactors utilizing porous silicon as the coupling matrix, Sens. Actuators B: Chem. 31 (1996) 161–166.
[17] J. de Boer, J. Korf, H. Plijter-Groendijk, In vivo monitoring of lactate and glucose with microdialysis and enzyme reactors in intensive care medicine, The Int. J. Art. Org. 17 (3) (1994) 163–170.
[18] D.J. Strike, P. Thiebaud, A.C. van der Sluis, M. Koudelka-Hep, N.F. de Rooji, Glucose measurement using a micromachined open tubular heterogeneous enzyme reactor (MOTHER), Microsys. Technol. 1 (1994) 48–50.
[19] M. Son, F. Peddie, D. Mulcahy, D. Davey, M.R. Haskard, A microbioreactor based on interfacial polymerization and application to flow injection analysis of glucose, Sens. Actuators B: Chem. 34 (1996) 422–428.
[20] S. Böhm, D. Pijanowska, W. Olthuis, P. Bergveld, A flow-through amperometric sensor base on dialysis tubing and free enzyme reactor, Biosens. Bioelec. 16 (2001) 391–397.
[21] A.J. Sprenkels, W. Olthuis, P. Bergveld, On the integration of a microdialysis-based μTAS with calibration facility on a silicon-glass sandwich, in: Proceedings of the 1st Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine & Biology, 12–14 October, Lyon, France, 2000, pp. 326–330.
[22] J. Ruzicka, E.H. Hansen, Flow Injection Analysis, Wiley, NY, 1981.

Biographies

Dorota G. Pijanowska received the MSc degree in biomedical engineering from the Faculty of Precision Mechanic in the Warsaw University of Technology. She completed her PhD thesis on analysis of factors determining parameters of ion sensitive field effect transistors as the sensors of biochemical quantities in 1996. Her research interests include fabrication and characterization of (bio)chemical sensors and micro-analytical systems, and their applications in biomedical diagnosis and environmental monitoring.

Ad J. Sprenkels received his MSc and PhD degrees in electrical engineering from the University of Twente, Enschede, The Netherlands, in 1983 and 1988, respectively. During his PhD research his work was focused on silicon acoustic sensors with the necessary dedicated electronics. In 1988, he joined the R&D department of Microtel (a Siemens company) and was involved in the development of various hearing-aid transducers. From 1994 on he is working as a part-time consultant on microsystem technology and analog electronics. In 1998, he joined the Laboratory of Biosensors, part of the MESA+ Research Institute, University of Twente.