Technology and Health Care, 1 (1994) 215–218
0928-7329/94/$07.00

Miniaturized integrated biosensors

G. Urban *, G. Jobst, F. Keplinger, E. Aschauer, R. Fasching, P. Svasek

Institut für allgemeine Elektrotechnik und Elektronik, L. Boltzmann-Institut für biomedizinische Mikrotechnik and Hirnkreislaufforschung. Technical University Vienna, Gußhausstr. 27, 1040 Vienna, Austria

(Received 11 June, 1993; Accepted 2 September 1993)

Abstract

Miniaturized integrated thin-film biosensors were developed for use in clinical analyzers and for in vivo applications. A glucose and a lactate sensor were integrated with a pH-sensor on a flexible substrate. Both enzyme sensors are based on the electrochemical measurement of H_2O_2 produced by the enzymes glucoseoxidase and lactateoxidase respectively. The solid state pH-sensor uses a neutral carrier membrane. The intended application of this device is the monitoring of metabolic parameters in the intensive care unit and the operation theater and the use as a sensor module in clinical analyzers. The glucose-, lactate- and pH-sensor was tested in buffer solutions and undiluted serum showing excellent performance.

Key words: Glucose sensor; Lactate sensor; pH-sensor; Integrated biosensor; Thin-film technology

1. Introduction

Miniaturized enzymatic biosensor devices were investigated extensively. Not only inexpensive, reproducible and small sensor devices for clinical analyzers but also small and therefore less invasive sensors for in vivo applications are required. A variety of different parameters have to be measured by a clinical analyzer as well as for continuous monitoring of metabolic activities either in vivo or ex vivo [1–6]. For good clinical performance and cheap production, miniaturized *and* integrated biosensors are required. This target can be reached by thin film technology. An on-chip integrated thin film electrochemical device is presented in this paper exhibiting a pH-sensor and electrochemical transducers for glucose and lactate measurement. A miniaturized flexible carrier was chosen to prevent tissue damage in the case of in vivo applications. Tests were performed in standard buffer solutions and undiluted serum to characterize the devices.

2. Experimental procedures

2.1. Hydrogenperoxide electrodes

The electrochemical arrangement consists of two Pt working electrodes, a common counter electrode and an electrode for the potentiometric pH-electrode. The working electrodes have an

* Corresponding author, Tel.: + +43-1-58801-3672, Fax: + +43-1-5042477.

SSDI 0928-7329(93)E0033-O

area of 0.4 mm^2 each and the counter electrode has an area of 1 mm^2. As reference electrode an external double-chambered Ag/AgCl-electrode was used.

The electrochemical oxidation of H_2O_2 was performed on each Pt working electrode at +500 mV vs. an Ag/AgCl reference electrode.

To suppress electrochemical interferences an electropolymerized semipermeable membrane was deposited upon the working electrodes [7]. This semipermeable membrane consisted of an electropolymerized di-amino-benzene that was polymerized in phosphate buffer with saline (pH = 7). The polymerization was done by cycling the potential between 200 mV and +800 mV for 2 hours.

2.2. Enzyme electrodes

The well-known glucoseoxidase (GOD)/H_2O_2 system [3] was used for glucose and the lactate-oxidase(LOD)/H_2O_2 for lactate detection. An easy to handle thin film process was developed for immobilizing the enzymes. A modified hydrogel layer was structured by photolithography and placed selectively on the two working electrodes [6]. An uppermost photostructurable membrane was introduced containing catalase that recycles excess H_2O_2 into O_2. This extends the linear range of the sensor. In addition this membrane prevents electrode fouling by blood components allowing measurements in undiluted media.

2.3. pH-sensor

Potentiometric pH-measurement using a neutral carrier membrane [8] placed on a conducting polymer was employed.

Conducting polymer coatings of 10 μm thickness were deposited by electropolymerization from an aniline/perchloric acid or a pyrrole/acetonitrile solution. Finally a drop of a solution of a standard neutral carrier membrane with Tri-n-dodecylamine was put on the electrode to create a pH-sensor [6].

A schematic drawing and a cross-section of the whole sensor is shown in Fig. 1.

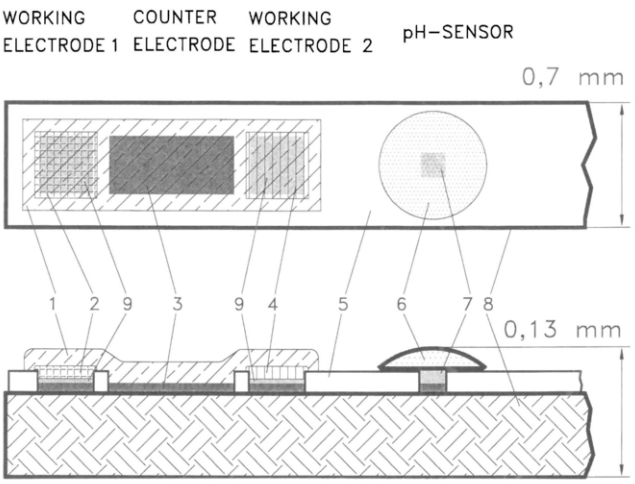

Fig. 1. Schematic cross section and view of the used sensor device, working electrode 1 represents the glucose sensor and working electrode 2 the lactate sensor. 1..Catalase membrane, 2..GOD-membrane, 3..Ti-Pt-electrodes, 4..LOD-membrane, 5..polyimide insulating layer, 6..pH-neutral carrier membrane, 7..polypyrrole as conducting polymer, 8..polyimide carrier, 9..semipermeable membrane.

2.4. Technological procedures

The in vivo sensor substrate consists of a 0.1 mm thick flexible polymer carrier (Upilex^R) 60 mm long and 0.7 mm wide. This sensor substrate is highly flexible, exhibits fairly good insulation properties for up to one month on average, and therefore allows potentiometric pH-measurements. The small size of the sensor probe seems well suited for less invasive in vivo applications, for example in an arterial vessel.

Titanium-platinum sandwich layers evaporated in high vacuum on 0.1 mm thick polyimide substrates act as conducting lines and titanium as an adhesion layer. The 40 μm wide conducting lines exhibit a thickness of 0.1 μm and were structured by standard thin-film technology through a lift off process. Areas to be insulated were covered by a 2 μm polyimide film that was structured by a photochemical process.

The electropolymerisation was performed on a Pt electrode on chip, then the active GOD layer and subsequently the LOD layer was crosslinked on top of the working electrodes. The catalase layer was structured upon both enzyme electrodes and the counter electrode. Finally, a drop of neu-

tral carrier membrane was placed on the conductive polymer through a micro dispensing unit. There were no cross sensitivities of the glucose and lactate sensors.

3. Results

The low residual current of < 1 nA/mm^2 of the biosensor device allows one point calibration. The 95% response time is 25 sec for increasing and decreasing glucose and respectively lactate contents either. The glucose sensor responds linearly to changes of glucose concentrations up to a range of 40 mmol/l with a correlation coefficient of 0.992. The lactate sensor exhibits a linear range of up to 15 mmol/l (Fig. 2).

The drift of the glucose sensor at 30°C is less than 0.2%/h of continuous use at a fixed glucose concentration of 10 mmol/l during two weeks. The flow dependence is dramatically reduced by the catalase membrane.

The sensor was tested in undiluted serum exhibiting full sensor response. Fig. 3 shows the response of a lactate, glucose and pH-sensor in undiluted serum by changing the glucose, lactate and pH alternatively. There is no crosstalk between the different integrated sensors, the disturbance of signal stems from the addition of different solution and is caused by electrical noise.

The response of the pH-sensor device follows Nernstian law with a slope of 56 mV/pH at 20°C

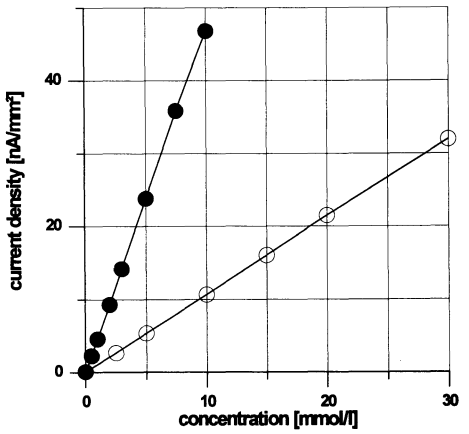

Fig. 2. Linear response of the glucose and lactate sensor (o..glucose, •..lactate).

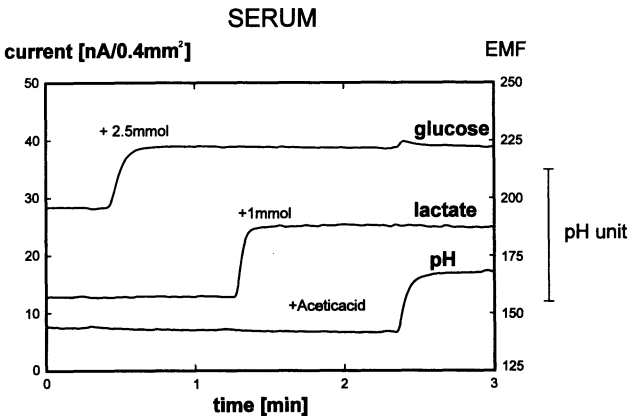

Fig. 3. Response of the integrated glucose-, lactate- and pH-sensor in human serum after adding 2.5 mmol/l glucose, 1 mmol/l lactate and 1 mmol/l acetic acid respectively.

and a response time of less than 1 second. The potential drift is about 250 μV/d corresponding to a pH drift of 5/1000 pH units per day [6].

4. Discussion

These sensor devices were designed to obtain precise, reliable, integrated sensors for in vivo application produced by means of standard miniaturization technology. Different enzymes can be immobilized into photostructurable membranes and additionally potentiometric electrolyte sensors can be integrated. The multi-enzyme membrane approach enhances the linearity of the sensors compared with single-enzyme layered devices [5]. The highly miniaturized and flexible design of the sensor probe is well suited for in vivo applications with less invasive properties. Additionally such devices could be used as sensor chips for miniaturized clinical analyzers. Both applications can easily be accomplished by miniaturized integrated thin film biosensors. Additionally measurements in undiluted biological media can be performed by the multi-enzyme membrane approach.

Acknowledgments

This work was supported by the Austrian Science Foundation Grant S49/07 and the Oesterreichischen Nationalbank Grant 4099.

References

1 Astrup J., Symon L., Branston N., Lassen Na. (1977) Stroke 8,51–56.
2 Strang R.H.C., Bachelard H.S., (1973) J. Neurochem. 20,987–995.
3 Turner A., Karube I., Wilson S., (1987) Biosensors: Fundamentals and Applications, Oxford University Press.
4 Lambrechts M., Sansen W., (1992) Biosensors: Microelectrochemical Devices, Inst. of Physics.
5 Hanazato, Y., Nakako, M., Satorus, S., Mitsuo, M., (1989) Integrated Multi-Biosensors based on Ion-sensitive Field-Effect Transitor Using Photolithographic Techniques IEEE Trans. Electron. Dev. 36:7;1303–1310.
6 Urban, G., Jobst, G., Keplinger, F., Aschauer, E., Jachimowicz, A., Kohl, F., (1992) Biosens. Bioelectron. 7:733–739.
7 Gleise R.J., Adams, Z.M., Byrone, N.J., YACYNYCH, A.M., (1991) Biosens. Bioelectron. 6:151–160.
8 Ammann D., Morf W., Anker P., Meier P., Pretsch E., Simon W., (1983) Ion-selective electrode review 5:3–92.