

Thin-Film Microbiosensors for Glucose–Lactate Monitoring

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A miniaturized device for simultaneous measurement of glucose and lactate levels was produced by means of photopatterning of enzyme-containing photosensitive membrane precursors. This device shows no cross-talk and a lifetime for both the glucose and the lactate sensors of more than 2 weeks when continuously operated in undiluted bovine serum. Linear response ranges of up to 40 mM for glucose and 25 mM for L-lactate, in combination with 95% response times of <30 s, were realized. The devices are mass produced by means of thin-film technology on flexible carriers to give catheter-type multisensing devices for in vivo applications. Ex vivo experiments, performed with human volunteers, where the device was continuously operated in an extracorporeal, undiluted, heparinized blood stream for 6 h, gave a correlation of $r > 0.98$ with respect to laboratory techniques. Subcutaneous measurements of glucose levels in pigs were close to the corresponding blood levels obtained without in vivo calibration.

There is a strong demand, especially in the intensive care unit and the operation theater, for miniaturized multianalyte sensing devices that are able to measure simultaneously a set of physiological parameters in vivo or in vitro and that are fast, accurate, cheap, and, of course, reliable.^{1,2} These devices might find additional applications in the field of metabolic monitoring, bedside analysis, and clinical analyzers.

Most of the glucose sensing devices intended for in vivo use reported in the literature suffer from the serious drawback that the production processes are rather delicate and therefore difficult to automate. This not only increases the production costs but may also cause serious problems with the reliability of such devices. Therefore, much effort is being focused on the development of biosensors employing established mass production technologies. Screen printing³ and thin-film⁴ technology have already shown success in the manufacture of reliable disposable biosensors.

Furthermore, to create multianalyte devices, the technology employed has to allow the spatially controlled formation of different functional membranes on the corresponding electrodes. Different approaches for the spatially controlled deposition of functional membranes have been reported, including drop-on techniques,⁵ ink-jet printing,⁶ spray techniques,⁷ electropolymerization,⁸ lift-off techniques,⁹ screen printing technology,¹⁰ enzyme membranes deposited by electrodeposition,¹¹ and photolithographically patterned enzyme membranes.^{12,13}

A straightforward approach is to use membranes that can be directly patterned by photolithography. Of particular advantage is the UV-initiated free radical cross-linking of the polymer directly on the substrate, which allows design of membranes with different physicochemical properties simply by altering the composition of the UV-sensitive membrane precursor or the UV exposure time. To date, however, such attempts have provided a low measuring range^{12,13} and, in the case of multienzyme sensors, also suffer from the danger of enzyme mixing.

Finally, for monitoring applications, these devices have to be continuously operated in undiluted whole blood without unacceptable loss in sensitivity.

A thin-film process is presented in this paper that successfully overcomes the aforementioned challenges by immobilizing the enzymes glucose oxidase, lactate oxidase, and catalase in pHEMA-hydrogel membranes and by modification of the platinum anode.

A catalase membrane was created as the topmost layer of this multimembrane setup to prevent cross-talk and the release of the cytotoxic agent hydrogen peroxide¹⁴ to the bulk. The catalase membrane is separated from the oxidase membrane by an enzyme-free diffusion-limiting pHEMA membrane. All mem-

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(1) Astrup, J.; Symon, L.; Branston, N.; Lassen, N. *Stroke* **1977**, *8*, 51.

(2) Strang, R. H. C.; Bachelard, H. S. *J. Neurochem.* **1973**, *20*, 987.

(3) Matthews, D.; Holman, R.; Sreemson, J.; Watson, A.; Hughes, S. *Lancet* **1987**, *778*.

(4) Erickson, K. A.; Wilding, P. *Clin. Chem.* **1993**, *39* (2), 283–287.

(5) Hintsche, R.; Dransfeld, I.; Scheller, F.; Pham, M.; Hoffmann, W.; Hueller, J.; Moritz, W. *Biosens. Bioelectron.* **1990**, *5*, 327–334.

(6) Kimura, J.; Kawana, Y.; Kuriyama, T. *Biosensors* **1988**, *4*, 41–52.

(7) Yokoyama, K.; Sode, K.; Tamiya, E.; Karube, I. *Anal. Chim. Acta* **1989**, *218*, 137–142.

(8) Tamiya, E.; Karube, I.; Hattori, S.; Suzuki, M.; Yokoyama, K. *Sens. Actuators B* **1989**, *18*, 297–307.

(9) Murakami, T.; Nakamoto, S.; Kimura, J.; Kuriyama, T.; Karube, I. *Anal. Lett.* **1986**, *19*, 1973–1986.

(10) Bilitewski, U. In *Trends in electrochemical Biosensors*; Costa, G.; Miertus, S., Eds.; World Scientific: Singapore, 1992; pp 59–68.

(11) Mastrototaro, J.; Johnson, K.; Morff, R.; Lipson, D.; Andrew, C.; Allen, D. *Sens. Actuators B* **1991**, *1–4*, 139–145.

(12) Hanazato, Y.; Nakako, M.; Maeda, M.; Shiono, S. *Anal. Chim. Acta* **1987**, *193*, 87–96.

(13) Wilke, D.; Müller, H. In *Trends in electrochemical Biosensors*; Costa, G., Miertus, S., Eds.; World Scientific: Singapore, 1992; pp 155–162.

(14) Zhang, Y.; Bindra, D. S.; Barrau, M. B.; Wilson, S. *Biosens. Bioelectron.* **1991**, *6*, 653–661.

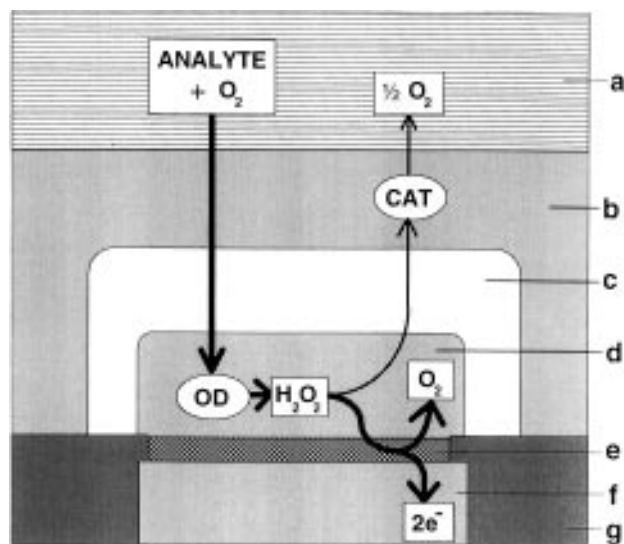


Figure 1. Schematic drawing of sensor buildup and the dominant reaction and transport pathways: a, bulk solution; b, catalase membrane; c, spacer membrane; d, oxidase membrane; e, electropolymerized permselective membrane; f, platinum anode; g, insulation.

branes were patterned by photolithography. Figure 1 gives a schematic view of the sensor construction and the main reaction and transport pathways.

A miniaturized flexible carrier was chosen to prevent tissue damage in case such a sensor is intended for in vivo measurements. The platinum anode was modified by the deposition of an electropolymerized semipermeable membrane to suppress electrochemical interferences.

EXPERIMENTAL SECTION

Apparatus. A Süss MJB3 mask aligner was used for device preparation and photopatterning of the hydrogels. The electrochemical measurement setup consisted of a home-made SMD bipotentiostat operated in the three-electrode mode, linked to a PC-based data acquisition and actuating program written in Asyst 4.0 (Asyst Technologies Inc.) via an PCL818 ADC board (Advantech). Measurements were performed either in a flow-through cell with 1 mm² diameter of the flow channel or by simply inserting the sensor into a magnetically stirred solution in a beaker.

Reagents. Upilex substrates were from ICI (Vienna, Austria); Ti, Pt, and Ag were obtained from Balzers (Liechtenstein); the enzymes glucose oxidase (GOx, EC 1.1.3.4, *Aspergillus niger*, Biozyme GO3A, 360 units/mg protein) and catalase (EC 1.11.1.6, *Aspergillus niger*, Biozyme CATANIF, 3000 units/mg protein) were kindly provided from Biozyme UK; lactate oxidase (LOD, EC 1.1.3.2, *Aerococcus viridans*, Asahi Chem. Ltd., LOD II) was kindly made available by Genzyme UK. Polyimide photoresists are a product of OCG Switzerland. The photoinitiator ω,ω' -dimethoxy- ω -phenylacetophenone was purchased from Aldrich. Hydroxyethyl methacrylate (HEMA, >95%) and tetraethylene glycol dimethacrylate (TEGDMA) (75–85%) were from Fluka, pHEMA was from Polyscience, and all other chemicals were of p.a. grade.

A typical hydrogel precursor consisted of 28% pHEMA as polymeric binder, 28% HEMA as reactive monomer, 3% TEGDMA as cross-linker, 40% ethylene glycol as plasticizer, and 1% ω,ω' -dimethoxy- ω -phenylacetophenone as photoinitiator. Dissolution of all compounds gave a clear, colorless solution, which was finally

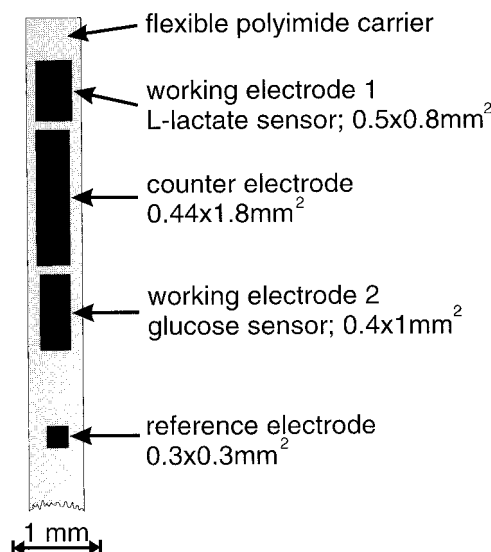


Figure 2. Schematic drawing of the integrated glucose-lactate device. Conducting lines are not shown.

0.2 μ m filtrated. The desired enzymes were added to this precursor solution to give the enzyme membrane precursors, containing up to 5 wt % of the proteins.

Device Fabrication. The thin-film fabrication process of the flexible electrochemical transducers is described elsewhere.¹⁵ One wafer with 60 devices, each of 60 \times 0.7 mm² size, comprising two platinum working electrodes of 0.4 mm² area, one platinum counter electrode, and an Ag/AgCl pseudoreference electrode, insulated by a 1 μ m thick photoimageable polyimide dielectricum, is produced on top of a 0.1 mm thick, highly flexible polymer carrier (Upilex). Figure 2 gives a schematic view of this device. The semipermeable membrane was formed by electropolymerization of 1,3-diaminobenzene (3 mM) in phosphate-buffered (pH 7) aqueous solution according to the procedure described by Geise et al.¹⁶ The electropolymerization was performed for at least 6 h on the wafer stage with all working electrodes electrically interconnected. The membrane precursors were applied to the wafers by a spin-on technique without special procedures in any way, identical to that used with common photoresist. The solvent was allowed to evaporate from the formed layers for 0.5 h at ambient temperature. The resulting films were exposed to UV light on the mask aligner through a photomask for typically 30 s up to 2 min in proximity mode. Oxygen exclusion during exposure was realized by argon flushing. The exposed layers were subsequently developed in ethylene glycol/water 1:1 (w/w) with ultrasonic assistance for typically 3 min and rinsed with water.

The multimembrane arrangement was realized by repetition of this basic process with different photomasks and membrane precursors. Finally, the wafer was diced with a circle saw to singularize the devices and bonded to a printed circuit board. The devices may also be sterilized with a γ -radiation dose of 25 kGy from a ⁶⁰Co source.

In Vitro Measurements. Electrochemical oxidation of hydrogen peroxide was employed as transducing principle. Oxidation of H₂O₂ was performed on both Pt working electrodes at +500

(15) Urban, G.; Jobst, G.; Keplinger, F.; Aschauer, E.; Tilado, O.; Fasching, R.; Kohl, F. *Biosens. Bioelectron.* **1992**, 7, 733–739.

(16) Geise, R.; Adams, J. M.; Barone, N. J.; Yacynych, A. M. *Biosens. Bioelectron.* **1991**, 6, 151–160.

mV versus the internal Ag/AgCl pseudoreference electrode. In vitro measurements were carried out in 0.1 M phosphate buffer with 0.1 M NaCl (pH 7.4) and undiluted bovine serum. Different glucose and lactate concentrations were realized by addition of the desired amount of 1 M glucose or lithium L-lactate stock solutions, respectively. Oxygen concentrations were decreased by addition of sodium dithionite, while oxygen tension was monitored simultaneously with a home-made thin-film Clark-type oxygen sensing device.¹⁷ Measurements at very low oxygen concentrations were realized by addition of a surplus of dithionite and monitoring while diffusion of oxygen from the surrounding air increased the oxygen level of the test solution.

Ex Vivo and in Vivo Experiments. For ex vivo measurements, the flow cell was combined with a SMD bipotentiostat within one metal housing of 85 × 25 × 25 mm³ to minimize electromagnetic interferences. Ex vivo blood monitoring with human subjects was performed by placing the flow-through cell into the sampling line of a so-called continuous ambulatory blood sampler.¹⁸ The sampling method is based on a double-lumen catheter for extracorporeal blood heparinization and an automatic fluid sampler. The double-lumen catheter (18 gauge × 45 mm, B. Braun Melsungen AG, Melsungen, Germany) was placed in a peripheral vein. The inner lumen of the double-lumen catheter was connected to a 10 mL syringe containing heparin solution (2000 IU Novo Nordisk, Bagsvaerd, Denmark). Heparin was infused at a rate of 7 μ L/min by a piston pump. The outer lumen of the double-lumen catheter was connected to a roller pump. A needle movable in two directions supplied 56 cooled vacuum tubes with the collected blood. The roller pump rate was adjusted to 200 μ L/min to realize a sampling volume of 1.5 mL/vacuum tube within 7.5 min.

The ex vivo experiment was performed with a healthy male subject (age 25 years, 96 kg weight). Informed consent was given by the volunteer. After a 12 h overnight fast, the subject assumed a supine position over 6.5 h, and an intravenous glucose tolerance test (IVGTT), followed by an oral glucose tolerance test (OGTT), was then performed. The duration of the experiment was limited to 6.5 h for the convenience of the volunteer. After 30 min rest, at time -15 min, the double-lumen catheter was inserted in an antecubital vein and connected to the blood sampling device. A butterfly (22 gauge) needle was inserted in a dorsal hand vein for the injection of glucose and insulin. Then, 250 mg/kg glucose was injected as 40% solution at time zero, and 0.02 units/kg regular insulin (Novo Nordisk) was injected 20 min later. At time 180 min, 150 g of glucose was given orally. Whole blood glucose was measured from the continuously sampled vacuum tubes (Cobas Mira, Switzerland). L-Lactate determinations were done with the LDH method. Before and after the measurement, the glucose-lactate device was one-point calibrated with a protein-free buffer solution containing 5 mM glucose and 2 mM L-lactate. The measured sensitivities before the experiment were applied to the sensor currents of the experiments for calculation of glucose and lactate levels.

Venous blood monitoring in animal experiments was done with a glucose-lactate device mounted, together with a 0.3 mm o.d. stainless steel cannula, in an 18 gauge catheter (Braun Melsungen). Heparin solution (1000 IU/mL) was perfused through the steel cannula at a flow rate of 0.3 μ L/min, while venous blood was pumped through the catheter at a flow rate of 10 μ L/min. Heparin supply and blood withdrawal were done with a roller pump equipped with two tubes of different inner diameter in order to realize the desired flow rate ratio of 100:3. Again, one-point precalibration was used in this experiment. Since the sensors were at body temperature during the experiment but were calibrated at ambient temperature, the calibration factors were multiplied by a factor of 1.4, which corresponds to a temperature difference of 15 K and a temperature coefficient of 2.3%/K.

The experiment was performed with a female pig of 15 kg weight. The animal was anesthetized with thiopental/halothane. A catheter placed in an ear vein was used to obtain blood samples for subsequent analysis and also for intravenous administration of glucose and heparin. The monitoring catheter was placed in the vena jugularis and immediately after application was equipped with the sensor and the inner tube for heparin supply.

A second glucose/lactate device was placed subcutaneously near the sternum. Application was done by inserting an 18 gauge steel cannula through the fatty tissue over a distance of 4 cm, inserting the device into the front end of the cannula, and removing the cannula. At time 65 min, 10 mL of 33% glucose solution (220 mg/kg) was administered intravenously. At time 140 min, a dose of 2.5 IU/kg regular insulin (Novo Nordisk) was injected intravenously.

Analysis of the blood samples was done immediately after withdrawal. Glucose analysis was performed with Companion2 (Medisense) and HemoCue B glucose (HemoCue, Sweden). Analysis for L-lactate was done with the Biosen5020L (Analytical Medical Instruments, Vienna, Austria).

RESULTS AND DISCUSSION

Fabrication Technology. PolyHEMA is advantageous as a membrane material because of its well-known blood compatibility,^{19,20} its high mechanical strength in the swollen state, and the fact that various photoinitiators are available for the photoinduced cross-linking of methacrylic systems. Additionally, photopatternable precursor solutions can be made with enzyme-compatible solvent mixtures. Furthermore, physicochemical membrane properties can be varied over a broad range by varying of precursor composition and cross-linking conditions.

The handling procedure of the enzymatic and nonenzymatic hydrogel precursors is compatible to a large extent with common thin-film technology processes. Nevertheless, there are two drawbacks of this system with respect to compatibility with thin-film technology:

First, oxygen has to be excluded during UV exposure, since molecular oxygen inhibits the free radical-initiated polymerization and cross-linking process. This can easily be achieved by nitrogen flushing of the wafer during UV exposure.

Second, the membrane precursor is not solid after evaporation of the solvent, but it is highly viscous because of the presence of monomer and plasticizer. The presence of plasticizer during the

(17) Jobst, G.; Urban, G.; Jachimowicz, A.; Kohl, F.; Tilado, O.; Lettenbichler, I.; Nauer, G. *Biosens. Bioelectron.* **1993**, *8*, 123–128.

(18) Trajanoski, Z.; Jobst, G.; Wach, P.; Urban, G.; Ellmerer, M.; Kotanko, P.; Skrabal, F. *Proceedings of the 16th Annual International Conference of the IEEE Engineering in Medicine and Biology Society*; IEEE: Piscataway, NJ, 1994; Vol. 2, pp 814–815.

(19) Margules, G. S.; Hunter, C. M.; MacGregor, D. C. *Med. Biol. Eng. Comput.* **1983**, *21*, 1–8.

(20) Jeyanthi, R.; Rao Panduranga R. *Biomaterials* **1990**, *11*, 238–243.

photo-cross-linking process allows the cross-linking to be performed above the glass transition temperature of the formed gel, which ensures high mobility of the reaction partners. This is advantageous in order to get high process speed and sufficient cross-linking.

But because of the liquid nature of the layers, a contact exposure, which is common in thin-film technology, where the substrate is pressed against the photomask, can only be done by separating the photomask from the substrate by some UV-transparent foils.²¹ Since the application of such sheets causes some problems with the reproducibility and reliability of the whole process, we decided to employ proximity exposure, where the photomask is separated by a few hundred micrometers from the substrate.

A resolution of $\sim 10\ \mu\text{m}$ can be obtained in hydrogel membranes of $4\ \mu\text{m}$ thickness.

Membrane thickness is easily varied between 1 and $10\ \mu\text{m}$ by proper adjustment of the membrane precursor viscosity. Typically, membranes with a thickness of $4\text{--}6\ \mu\text{m}$ are employed in this work. Electropolymerization of the semipermeable membrane can be done at the wafer stage and therefore presents no bottleneck for the mass production of these thin-film devices.

Device Performance. Oxidase-based biosensors without a hydrogen peroxide decomposing top membrane emit hydrogen peroxide to the bulk solution, where it accumulates, depending on the exchange rate of the bulk solution. This accumulation induces according to Fick's first law an increase in sensor current. Simultaneously, substrate and oxygen are depleted from the bulk solution, decreasing the signal. Therefore, sensor reading is influenced by the flow speed of the bulk solution. One way to circumvent this problem is to balance these two processes by proper choice of membrane materials and improved sensor design.²² Such devices have been shown to display a stir rate-independent reading, and even signal stability for stopped flow is reported.²³

However, one should keep in mind that this feature is the result of two balanced processes and that the relative rates of these processes are dependent on the transport (diffusion, active transport in tissue) and reaction (catalase activity of tissue) properties of the bulk. This means that a device with virtually no flow dependency in a beaker may respond with a different sensitivity when operated in tissue.

This analyte-dependent behavior may partly explain the numerous reports in the literature where significant differences between *in vitro* and *in vivo* sensitivities are described.²⁴

Our approach to minimize this problem is to eliminate the hydrogen peroxide accumulation by top-coating the device with a catalase membrane and to keep the analyte consumption low by proper diffusion limitation.

However, since a minimal sensitivity is necessary for reliable measurements, it is highly desirable to optimize the sensitivity versus analyte consumption ratio. This ratio, which is called efficiency, is expressed as the ratio of charge passing the electrode to the total charge which is nominally converted in the enzymatic

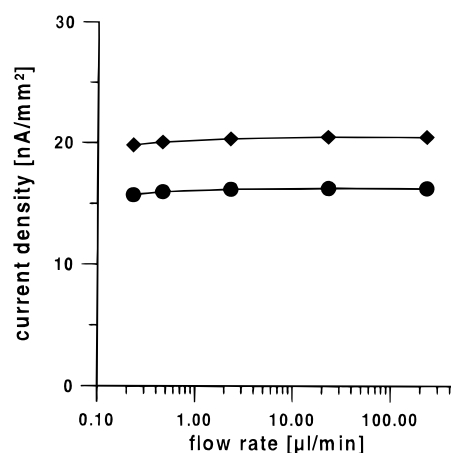


Figure 3. Flow sensitivity of integrated glucose–lactate device: \blacklozenge , L-lactate sensor; \bullet , glucose sensor. The measurement was done in phosphate-buffered saline containing 5 mM glucose and 2 mM L-lactate.

reaction. Since efficiency is considerably decreased when an oxidase membrane is directly coated with a catalase membrane, we introduced the pHEMA–hydrogel spacer membrane between them to increase the diffusion resistance of hydrogen peroxide toward the catalase membrane.

The flow sensitivity of a glucose–lactate device with such a three-membrane setup is shown in Figure 3. Since the measurement was done in a $1\ \text{mm}^2$ cross-sectional area flow channel, the flow rates indicated correspond to flow velocities of millimeters per minute. The current of both sensors is decreased by only 3% when the flow rate is reduced 3 orders of magnitude from $240\ \mu\text{L}/\text{min}$ to $0.24\ \mu\text{L}/\text{min}$. The sensitivities of the sensors can be varied over wide ranges ($2\text{--}10\ \text{nA}\ \text{mM}^{-1}\ \text{mm}^{-2}$ for glucose and $5\text{--}30\ \text{nA}\ \text{mM}^{-1}\ \text{mm}^{-2}$ for L-lactate) by proper choice of membrane thickness and by alteration of the hydrogel precursor composition. For example, L-lactate sensor sensitivity is increased 3-fold and glucose sensor sensitivity is increased 2-fold upon reducing exposure time from 2 to 1 min and increasing the plasticizer content of the prepolymer mixture from 40% to 60%. Relative standard deviations of the sensitivities of flexible devices which were made in the same batch were calculated to be 3.1% ($n = 10$) for the glucose sensors and 3.2% ($n = 10$) for the lactate sensors. Hydration of the device proceeds at a faster rate for thin membranes. More than 95% of the final steady state current, caused by the endogenous glucose and L-lactate of the serum, is reached within 5 min (see Figure 4). This figure also illustrates the absence of cross-talking and the fast response of the device. Base current declines below $1\ \text{nA}\ \text{mm}^{-2}$ within 30 min, which enables one-point calibration.

The influence of oxygen concentration on glucose reading is shown in Figure 5 for different glucose concentrations. At an oxygen partial pressure of 20 mmHg, which represents the lower physiological level in tissue, the linear measuring range still covers the range of physiological glucose levels. The ratio of oxygen versus glucose sensitivity derived from this measurement is 750.

In view of the general lack of widely accepted parameters for the expression of biosensor performance, we suggest the use of this parameter for the characterization of conventional oxidase-based biosensors whose linear working range is limited by an oxygen deficit. It gives a definite relationship between saturation currents, linear range, and oxygen concentration, and it is nondimensional.

(21) Arquint, Ph.; van den Berg, A.; van der Schoot, B. H.; de Rooij, N. F. *Sens. Actuators B* **1993**, 13–14, 340–344.

(22) Moussy, F.; Harrison, D. J.; O'Brien, D. W.; Rajotte, R. V. *Anal. Chem.* **1993**, 65, 2072–2077.

(23) Koudelka-Hep, M.; Strike, D. J.; de Rooij, N. F. *Anal. Chim. Acta* **1993**, 281, 461–466.

(24) Moatti-Sirat, D.; Capron, F.; Poitout, V.; Reach, G.; Bindra, D. S.; Zhang, Y.; Wilson, G. S.; Thevenot, D. R. *Diabetologia* **1992**, 35, 224–230.

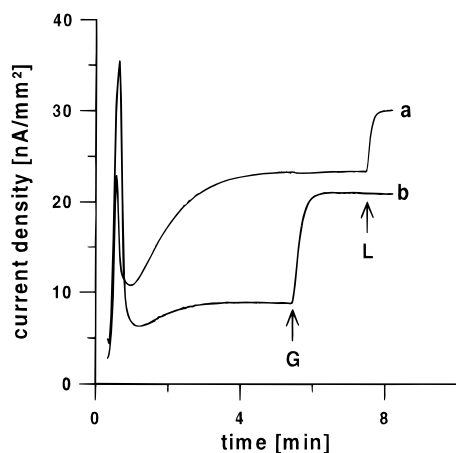


Figure 4. Run-in behavior of an integrated glucose–lactate device which was stored dry in ambient atmosphere. At time zero, the device was inserted into undiluted bovine serum. G, Increase of glucose concentration by 5 mM; L, increase of L-lactate concentration by 1 mM; line a, L-lactate sensor; line b, glucose sensor.

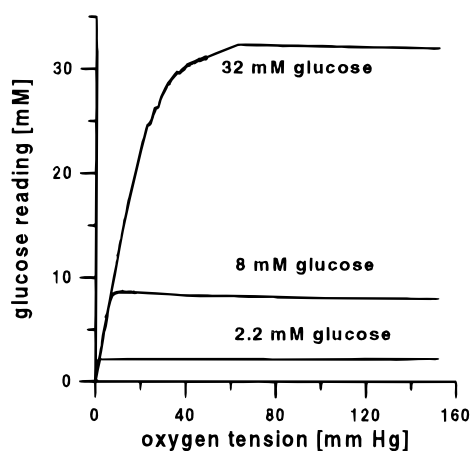


Figure 5. Oxygen dependency of glucose reading.

The influence of interferences was tested with a device that was already continuously operated for 2 days in undiluted bovine serum prior to taking measurements. The concentrations of the interferences tested reflect the upper physiological level. Only paracetamol at the toxic level of 2 mM gave a pronounced increase in sensor reading. Nevertheless, the error in reading is less than 0.5 mM glucose and 0.2 mM L-lactate, respectively, as can be seen in Figure 6. Of particular interest is the very slow response toward paracetamol. The response time of ~5 min can also be observed with electrodes covered only by the semipermeable membrane. The interference becomes negligible when measurement is carried out with these electrodes in batch mode in a clinical analyzer. The temperature coefficient of both the glucose and the lactate sensors is 2.3%/K. This value matches the value of the temperature dependence of diffusion. This is expected since the device works under conditions of mass transfer limitation within the sensor membranes. Therefore, this value should be independent of the nature of the test solution.

Changes in device parameters with continuous operation in undiluted bovine serum for 3 weeks at ambient temperature are shown in Table 1. The useful operational lifetime of the devices is typically more than 2 weeks.

Ex Vivo and in Vivo Monitoring. The well-established continuous blood withdrawal technique by means of a double-

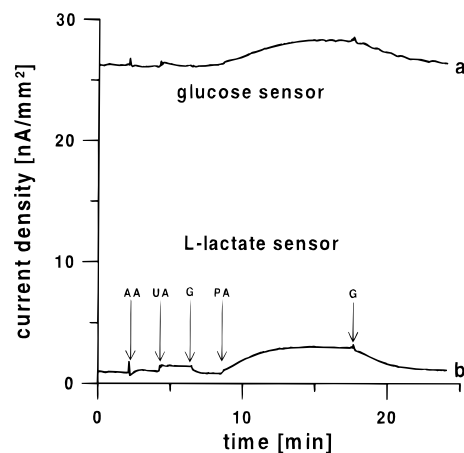


Figure 6. Influence of interferences on sensor reading. The integrated glucose–L-lactate sensor was exposed to 5 mM glucose solutions spiked with different interferences: AA, 0.2 mM ascorbic acid; UA, 0.5 mM uric acid; G, no interferences; PA, 2.0 (!) mM paracetamol; line a, glucose sensor; line b, L-lactate sensor.

Table 1. Results of Long-Term Experiment

day	sensitivity to substance (nA mM ⁻¹ mm ⁻²)				upper limit of linear range ^a (mM)		saturation current (nA mm ⁻²)	
	glucose	lactate	uric acid	paracetamol	glucose	lactate	glucose	lactate
0	3.95	11.2	<0.1	<0.1	>40	29	342	318
7	4.58	12.1	1.2	1.5	>40	29	337	325
14	5.18	14.0	1.2	1.0	>40	26	380	328
21	5.03	13.4	4.0	1.2	>40	20	407	290

^a Deviation from linearity > 10%.

lumen catheter and on-line dilution of whole blood with heparin²⁵ has the serious drawback that the real dilution factor is dependent on hematocrit and therefore requires a calibration with an independent method or hematocrit determination. To prevent such errors, we used a small flow of concentrated heparin solution, which causes a maximum dilution of 3%.

The result of an IVGTT/OGTT experiment is shown in Figure 7. The sensor reading closely follows the laboratory results throughout the duration of the experiment. Sensitivities measured before and after the experiment according to the procedure given in the Experimental Section were 4.55 and 4.40 nA mM⁻¹ mm⁻² for glucose and 30.7 and 29.1 nA mM⁻¹ mm⁻² for L-lactate. Error grid analysis²⁶ of the glucose values shows that all values, except the one immediately after the intravenous administration of glucose, lie in zone A (clinically accurate, Figure 8). The correlation coefficient of the linear least-squares fit of this data set is $r = 0.98$. Lactate sensor reading and laboratory results of this experiment are shown in Figure 9. Supported by the results of such IVGTT/OGTT experiments with ex vivo flow-through devices,²⁷ we assume that the deviation between sensor reading and laboratory results is caused by an error in the off-line lactate determination.

Since the high blood withdrawal rates used in this experiments are not acceptable for monitoring applications, in an animal

(25) Pfeiffer, E. F. *Verh. Dtsch. Ges. Inn. Med.* **1989**, *95*, 725–736.

(26) Clarke W. L.; Cox, D.; Gonder-Frederick, L. A.; Carter, W.; Pohl, S. L. *Diabetes Care* **1984**, *5*, 622–627.

(27) Varahram, M.; Jobst, G.; Moser, I.; Svasek, P.; Aschauer, E.; Urban, G.; Trajanoski, Z.; Wach, P.; Gfrerer, R.; Kotanko, P.; Skrabal, F. *Biomed. Tech.* **1995**, *40* (2), 99–101.

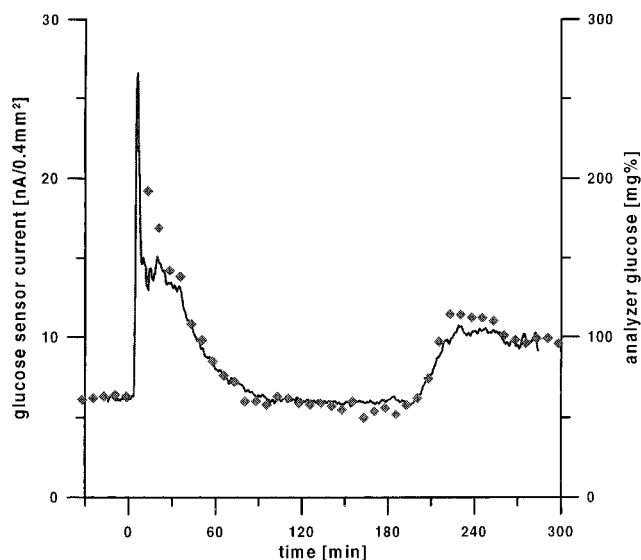


Figure 7. IVGTT-OGTT experiment with human volunteer: —, glucose sensor current; ♦, laboratory glucose. For experimental details, see text.

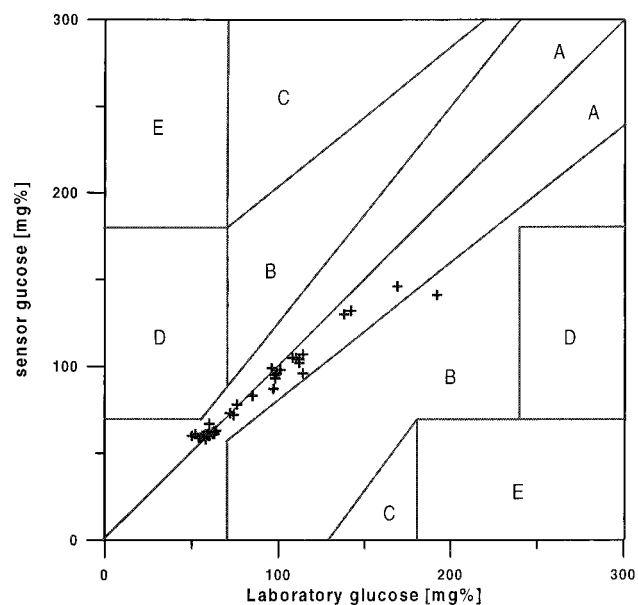


Figure 8. Error grid analysis of data from experiment shown in Figure 7. Total number of points, 39; points in zone A, 38; points in zone B, 1.

experiment we evaluated the feasibility of a monitoring scheme with the sensor mounted in a catheter, which allows reduction of the flow rate to 14 mL/day without an unacceptable delay. Additionally, a device was placed subcutaneously in the pig, which is the most accepted application site for hypoglycemia home monitoring.

The results of this animal experiment are shown in Figure 10. Again, a close correlation between venous sensor reading and off-line values can be seen. Error grid analysis gives 100% zone A at $n = 7$. After a 30 min run, the subcutaneous sensor reading approaches venous glucose values. The sensor responded very fast to the intravenous glucose load. There was no time lag relative to the venous sensor reading. Sensitivities before and after the experiment were identical within the limits of experimental error. The correlation coefficient of venous versus subcutaneous sensor readings, starting at time 30 min, is $r = 0.95$.

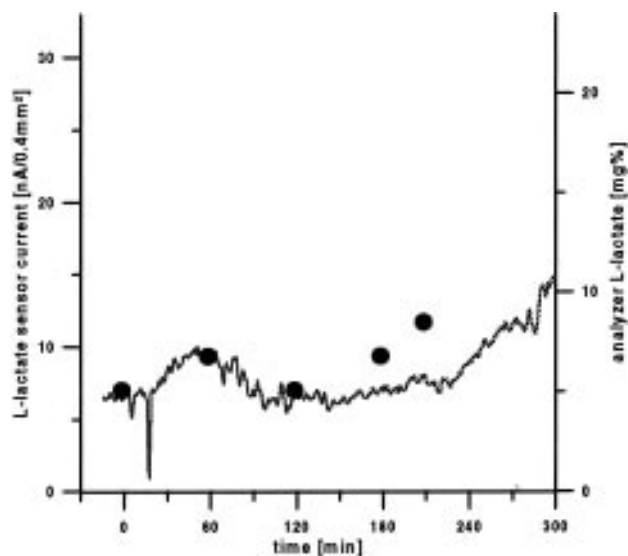


Figure 9. Same experiment as shown in Figure 7: —, lactate sensor current; ●, laboratory lactate.

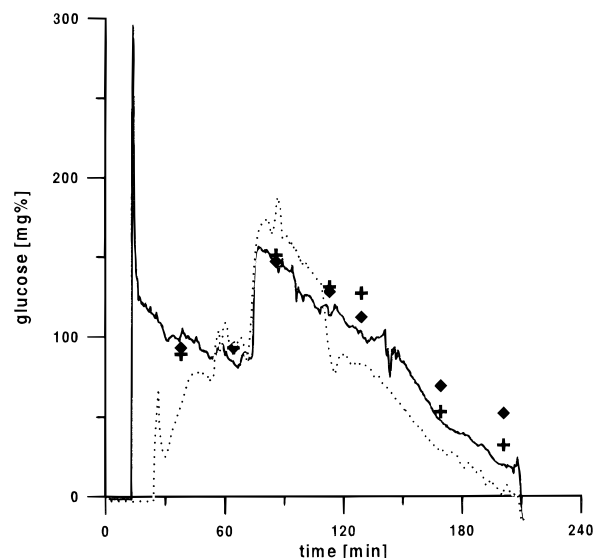


Figure 10. In vivo experiment performed on a pig: —, venous glucose sensor reading; ···, subcutaneous glucose sensor reading; +, Companion2 glucose values; ♦, Hemocue B glucose values. For experimental details, see text.

The sharp decrease of its sensitivity at time 110 min may be caused by a change in the position of the sensor in the subcutaneous tissue. An encouraging observation made in this preliminary experiment is that the in vivo sensitivity of the glucose device was of the order of the in vitro sensitivity. Clinical studies to evaluate the possibility of precalibration for short-term subcutaneous glucose sensing are underway and will be published in a forthcoming paper.

Sensors with the same membrane buildup but with an unmodified platinum anode showed the well-known behavior of a steady decrease in sensitivity when operated in real samples.¹⁵ Most often, this decrease in sensitivity is attributed to improper biocompatibility of the sensor surface.²⁸ The fact that oxygen transducing devices employing gas-permeable membranes to protect the electrodes show excellent stability even in whole

(28) Ammon, H. P. T.; Ege, W.; Oppermann, M.; Göpel, W.; Eisele, S. *Anal. Chem.* **1995**, *67*, 466–471.

blood,²⁹ and our finding that stability can be obtained by modification of the hydrogen peroxide transducing electrode, both indicate that short-term stability is not generally a problem of biocompatibility but is simply a transducer problem.

CONCLUSION

This work presents a biosensing thin-film device, where both the enzymatic and the covering membranes are photopatterned. This total photopatterning process allows the fabrication of miniaturized multianalyte devices. Furthermore, these devices show an excellent short time stability in undiluted human whole blood. This unique combination of device performance and mass production technology could make good some of the promises made for biosensors.

(29) Baker, D. A.; Gough, D. A. *Anal. Chem.* **1995**, *67*, 1536–1540.

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