Spatially Controlled Membrane Depositions for Silicon-Based Sensors

David J. Strike*, Philippe Arquint, Nico F. de Rooij, and Milena Koudelka-Hep

Abstract. The membrane deposition technology on silicon-based transducers constitutes the most delicate part of the miniaturized (bio)chemical sensor fabrication. Membrane adhesion to the transducer, reproducibility of the deposition process and its spatial control are the three most important parameters which determine the sensor performance and lifetime.

The fabrication of two sensors is described: *1*) a combined pO_2, pCO_2, pH sensor for which a polyacrylamide gel and a polysiloxane gas-permeable membrane were deposited and patterned at the on-wafer level and *2*) a glucose amperometric enzyme electrode where the glucose oxidase was immobilized electrochemically either in a polypyrrole matrix or co-deposited with bovine serum albumin by electrochemically aided adsorption. The optimization of the deposition procedures allowed reproducible devices with reasonable lifetimes to be obtained.

Introduction

In principle most electrochemical (bio)-sensors rely on a combination of a solid-state transducer with one or more organic membranes. These membranes impart to the sensor sensitivity, selectivity, interference free detection, diffusion limitation, and outer protection. Many significant transducer developments have been realized through the use of silicon microfabrication technology. The technology of the membrane deposition, however, has progressed more slowly and is, generally, the most problematic part of the sensor fabrication.

Although each membrane application makes its own specific demands, some properties such as good adhesion to the transducer, reproducibility of the membrane deposition process and its spatial control are common to all sensor types. In order to illustrate some of the problems associated with the membrane deposition, patterning and adhesion onto silicon-based transducers and to propose some solutions, the fabrication of two sensors will

be described. The first one is a combined pO_2, pCO_2, and pH sensor for blood gas and pH extracorporeal monitoring. Both amperometric (pO_2) and potentiometric (pCO_2, pH) transducers are integrated on one 10 × 10 mm chip and the required membranes, a polyacrylamide hydrogel and a polysiloxane gas-permeable membrane, are deposited and patterned by photopolymerization at the on-wafer level. This on-wafer fabrication of the complete sensor allows the reproducibility to be improved in comparison to the more usual membrane deposition on each individual sensor.

The second sensor is an amperometric glucose electrode consisting of an amperometric transducer and an enzyme layer containing immobilized glucose oxidase (GOx). In our previous work, chemical co-cross-linking of glucose oxidase and bovine serum albumin (BSA) by glutaraldehyde was used for membrane deposition either by casting or by spin coating followed by lift-off [1][2]. In this work an electrochemical deposition by an entrapment in electrochemically grown polypyrrole (PPy) [3–5] and electrochemically aided adsorption [6][7] followed by the
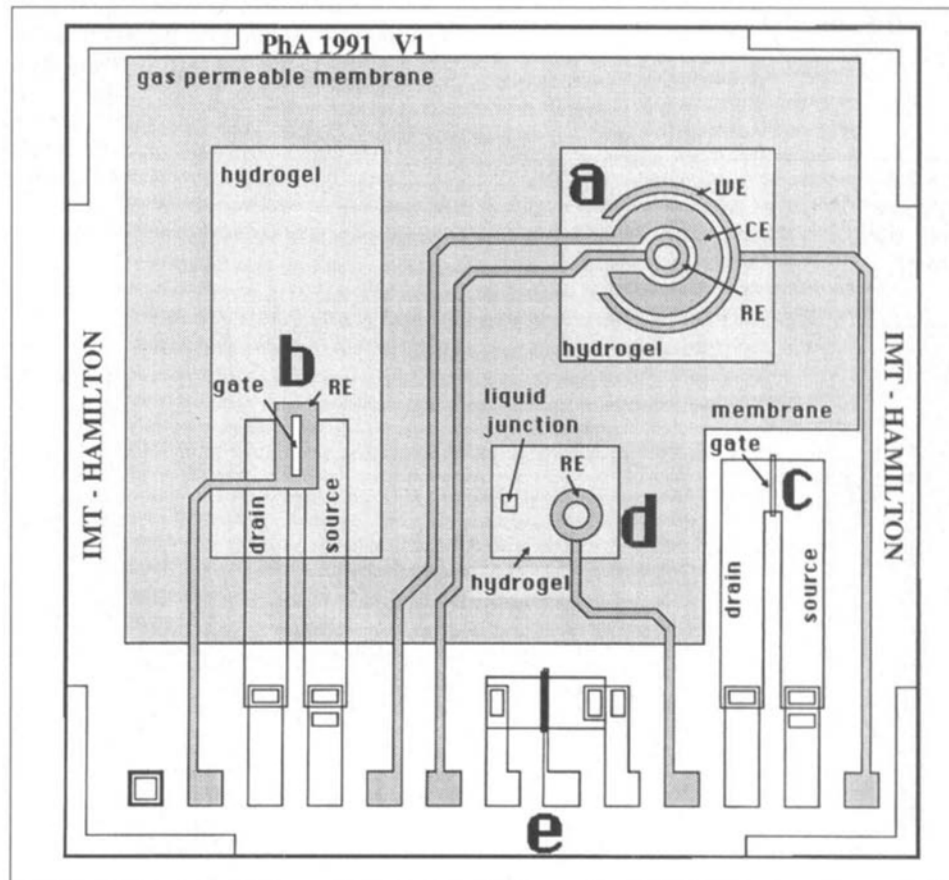

Fig. 1. *Layout of the combined pO_2, pCO_2, and pH sensor. a)* pO_2 Sensor with working electrode (WE), counter electrode (CE), and reference electrode (RE), covered by a hydrogel and the gas-permeable membrane. *b)* pCO_2 Sensor consisting of an ISFET and an Ag/AgCl reference electrode (RE), both covered by a hydrogel and the gas-permeable membrane. *c)* pH Sensor with an ISFET. *d)* Reference electrode for the pH sensor, consisting of a Ag/AgCl layer (RE) covered by a hydrogel and the gas-permeable membrane. A small opening in the membrane serves as the liquid junction. *e)* MOSFET for testing.

Correspondence: Dr. D.J. Strike
Institute of Microtechnology
University of Neuchâtel
Breguet 2
CH–2000 Neuchâtel

deposition of the PPy layer have been investigated. In the former, the enzyme is immobilized in an 'active' matrix due to the size exclusion properties of the polypyrrole layer [8]. This is of particular importance for the oxidase-based sensors using hydrogen peroxide detection, where the presence of the polypyrrole layer allows the electroactive interference problem to be reduced. In the latter, a slightly different approach has been studied. Following the electrochemically aided adsorption of the GOx and BSA, a very thin anti-interference layer of PPy was grown.
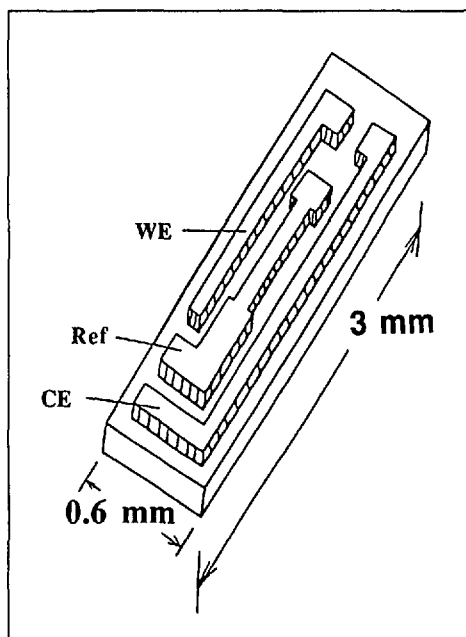

Fig. 2. *Diagram of the transducer showing* (left to right) *the working platinum* (WE), *silver reference* (Ref), *and platinum counter* (CE) *electrodes*

Experimental

Oxygen, carbon dioxide, and pH sensors are realized on one chip using standard silicon technology and photolithographic patterning of polyacrylamide hydrogel and polysiloxane gas permeable membrane (*Fig. 1*).

Oxygen Sensor. The membrane-covered oxygen sensor is an amperometric device *i.e.* current is measured at –0.7 V (*vs.* Ag/AgCl) and is directly proportional to the oxygen partial pressure in the medium investigated:

$$O_2 + 2\,H_2O + 4e \rightarrow 4\,OH^-$$
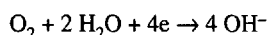
It consists of an amperometric transducer, internal electrolyte hydrogel layer and an outer gas-permeable membrane. The transducer is a three thin-film electrode microcell consisting of two Pt (working and counter) and one Ag/AgCl reference electrode arranged in a concentric configuration. The metals are deposited by electron beam evaporation and patterned by lift-off. The Ag layer is partially chloridized in an $FeCl_3$ soln. to yield the Ag/AgCl part of the reference electrode.

The internal electrolyte consists of a polyacrylamide hydrogel layer. In order to improve the adhesion of the gel and also subsequently of the polysiloxane membrane, the wafer was functionalized with a methacrylic functional silane [9]. The silanization was performed at 60° in a toluene soln. containing 10% (trimethoxysilyl)propyl methacrylate (*Aldrich*) and 0.5% water.

The monomer soln. contains 30–50 wt.% acrylamide (*Merck*) and 1.5–10 wt.% N,N'-methylene-diacrylamide (*Merck*) as cross-linking agent in a H_2O/glycerine soln. (volume ratio $^1/_1-^1/_3$) [10]. Following some preliminary tests with different photoinitiators, riboflavin-5'-phosphate was chosen (0.05–0.2 mg/ml, *Merck*) together with N,N,N',N'-tetramethyl-ethylenediamine catalyst (0.5–2 μl/ml, *Fluka*). Depending on the

desired thickness (50–100 μm), a corresponding volume of the monomer soln. is cast on the wafer which is then covered with a *Mylar* sheet to avoid O_2 quenching of the photopolymerization reaction. The UV light exposure times are in the order of 3 min and the unexposed parts are removed in water.

The gas-permeable polysiloxane membrane was deposited from monomer soln. containing 97% dimethyl siloxane and 3% (methacryloxypropyl)methyl siloxane copolymer (*Petrarch*). 0.5% of 2,2-dimethoxy-2-phenyl-acetophenone photoinitiator was then added and the photopolymerization carried out as described for polyacrylamide with the development carried out in xylene.

Carbon Dioxide Sensor. The transducer consists of an Al_2O_3-ISFET (Ion Sensitive Field Effect Transistor) and a thin film Ag/AgCl reference electrode. The ISFET, described in a previous paper [11], is an n-Si, p-well type device with the sensitive gate (16 × 600 μm) consisting of a thermally deposited SiO_2 (1000 Å) and APCVD deposited Al_2O_3 (600 Å) layers.

The polyacrylamide hydrogel and polysiloxane membrane are deposited as described above. The measured pH change of the internal electrolyte (50 mM $NaHCO_3$ + 100 mM KCl) arises from the hydration of CO_2 and the dissociation equilibrium of carbonic acid:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$
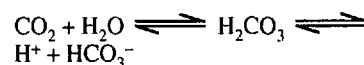
pH Sensor. An Al_2O_3-ISFET in direct contact with the sample soln. and a thin-film Ag/AgCl reference covered by a polyacrylamide hydrogel and a polysiloxane layer with a small opening, serving as a liquid junction, are used.

Glucose Sensor. The amperometric transducer of the glucose sensor is a three thin-film electrode microcell (*Fig. 2*) of overall dimensions 0.6 × 3 × 0.38 mm. The Pt working electrode has the width of 100 μm and the length of 1000 μm. The transducer realization is identical to that described above for the oxygen sensor transducer and is described in details elsewhere [12].

Prior to the deposition, the electrode was pretreated by cyclic voltammetry in 0.5M H_2SO_4 at 50 mV/s. The electrochemical deposition of glucose oxidase was performed using either a galvanostatic deposition of the GOx and BSA or an entrapment of the GOx in a polypyrrole matrix. In the former, the enzymatic layer was deposited at a constant current of 5 mA/cm² from phosphate-saline buffer soln. (PBS) containing 5 wt.% GOx (type VII, 100–200 U/mg, *Sigma*) and 5 wt.% BSA (fraction V, *Calbiochem*). After the deposition the electrode was rinsed in deionized water and then cross-linked with glutaraldehyde 2.5% in PBS for 30 min or 25% for 4 min. The polypyrrole anti-interference layer was then deposited in two stages. Firstly, the Pt electrode was platinized at –0.2 V *vs.* SCE in a 10 mM potassium acetate, 50 mM KCl soln. containing 1 mM K_2PtCl_4. The total charge passed corresponds to c. 10 μg/cm² Pt. Secondly, a polypyrrole layer was deposited out of 0.1M potassium phosphate buffer soln. containing 0.1M KCl and 0.15M pyrrole (*Fluka*). The pyrrole was distilled at atmospheric pressure prior to each experiment. The polymer was deposited by 5 cyclic voltammetry sweeps in the potential range 0.1 to 1 V *vs.* SCE
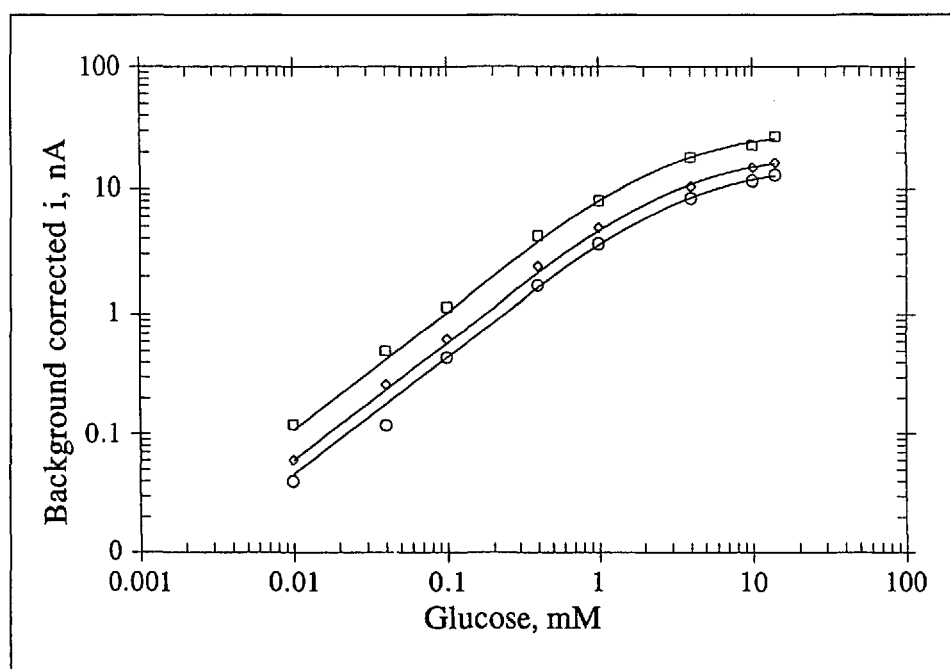

Fig. 3. *Glucose response curves of polypyrrole/GOD electrodes*. The electrodes were deposited, as described in the text, from solutions containing, O 180, □ 350, and ◇ 680 Uml⁻¹ of GOD. The thickness of each electrode corresponds to 68 mCcm⁻². The responses were recorded in unstirred phosphate buffer, 37° and the electrodes were biased at 650 mV (*vs.* SCE).

with the fourth scan held for 5 min at the anodic limit.

The enzyme entrapment in the polypyrrole layer was performed as follows: GOx (2.4 mg/ml) was added to the 0.1M pyrrole (*Fluka*), 0.01M sodium perchlorate and 0.1M potassium phosphate soln. The deposition was performed at 0.8 V *vs.* SCE.

Experimental Set-up. The amperometric glucose measurements were performed at 0.650 V *vs.* SCE in a phosphate-saline buffer soln., pH 7.2. An *EG&G* Potentiostat/Galvanostat model *273A* or an *IBM EC 225* Voltammetric Analyzer were used.

The flow injection system was composed of a peristaltic pump (*Ismatec*) a rotary injection valve (*Instrument Gesellschaft*) and a perspex flow through cell (on loan from *Ciba-Geigy*).

The combined pO_2, pCO_2, and pH sensor was first characterized in aq. solns. and subsequently also in a blood intended for transfusion (Hôpital des Cadolles, Neuchâtel). All measurements were performed in a flow-through cell using an in-house-built potentiostat and a specially designed electronic control system for ISFET operation.

Results and Discussion

Combined pO_2, pCO_2, pH Sensor. The main characteristics of the combined pO_2, pCO_2, and pH sensor are given in the *Table*. At present, the operational lifetime of two weeks could be obtained with a reasonably low drift (pCO_2 and pH) or good stability (pO_2).

These good performances (especially with respect to the life-time and stability) can be ascribed mainly to the greatly improved adhesion of the polyacrylamide and polysiloxane membrane due to the chemical pretreatment of the wafer surface. This functionalization, resulting in a covalent attachment of the membrane to the transducer surface, thus allows more than two weeks of operational life-time while in its absence, the life-time is limited to a few hours.

Another important parameter is the adhesion of the gas-permeable membrane (polysiloxane) to the polyacrylamide hydrogel. This is provided by the binding of the methacryloxypropyl groups of the polysiloxane cross-linker to the acrylamide groups. The quality of this interface depends on several parameters: polymerization grade of the hydrogel prior to the deposition of the membrane, the polymerization rate of hydrogel and membrane and the acrylamide content of the hydrogel. By adjusting these parameters a good hydrogel/membrane interface could be obtained.

Preliminary testing of this sensor using intermittent flow-through measurements in phosphate buffer solutions and blood for transfusion gave reproducible results, with the sensitivities similar to those obtained in aqueous solution. The next step
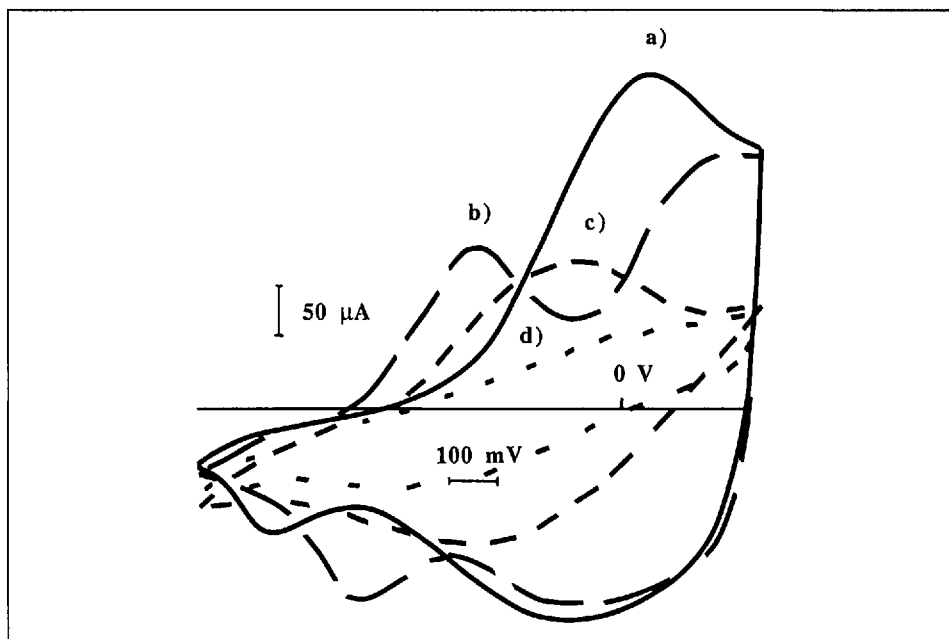

Fig. 4. *Effect of overoxidation on the cyclic voltammogram of a polypyrrole coated electrode.* The polypyrrole film corresponds to a deposition charge of 700 mCcm^{-2} and was grown in 0.1M KCl. The cyclic voltammograms were recorded at a scan speed of 50 mVs^{-1} in N_2 saturated 0.1M KCl, at r.t. Curve *a)* was recorded immediately after the electrode was prepared, *b)* after 16 min at 0.6 V (*vs.* SCE), *c)* 70 min. and *d)* after 104 min.
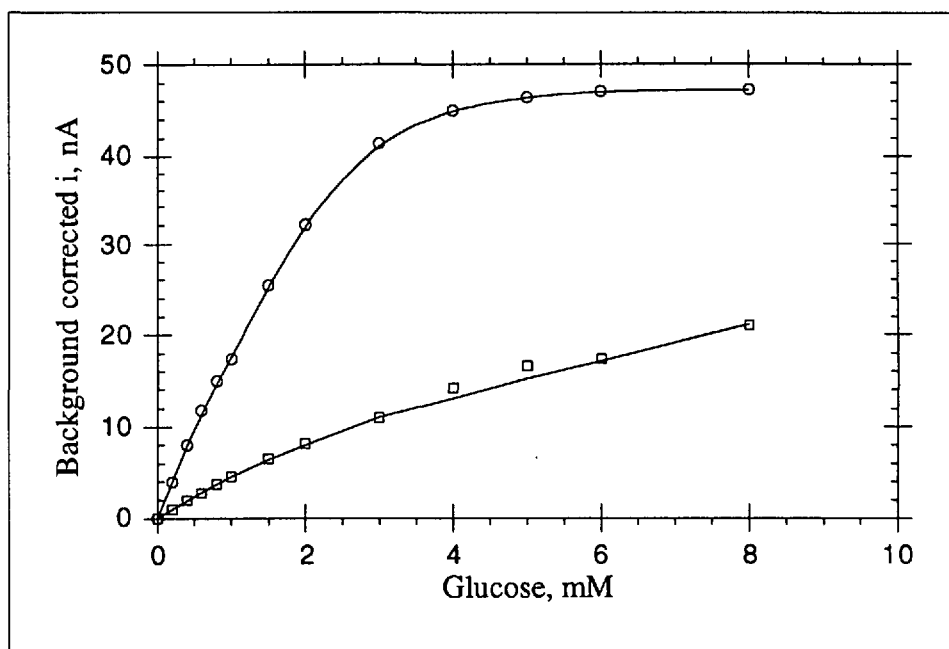

Fig. 5. *Response curves for glucose.* The electrodes were modified with protein films deposited out of phosphate buffer containing, ☐ 5% GOX, ○ 5% GOx/5% BSA, during a 2-min period. The responses were recorded in unstirred phosphate buffer, 37° and the electrodes were biased at 650 mV (*vs.* SCE).

of the sensor characterization will be performed with whole blood.

Glucose Sensor. Fig. 3 shows the glucose response of three different electrodes modified with PPy/GOx films deposited from solutions containing different GOx activity. As it can be seen, the enzyme activity of 350 U/ml seems to give the optimum results with respect to the glucose response. The thickness of the PPy/GOx layer can be easily controlled *via* the charge. It was found that the glucose response increases as the thickness of the

layer decreases which is in agreement with previous reports [13]. However, with decreasing thickness the stirring effect on the sensor response increases resulting in a noisy signal. The deposits corresponding to 68 mC/cm^2 (*ca.* 300 nm thick) were found to be the best compromise between these effects.

An interesting feature of these films is the so-called break-in period observed when the electrode is used for the first time. This period is characterized by a high, although rapidly decaying, background current and a greatly reduced glu-

Table. *Characteristics of the Combined* pO_2, pCO_2, *and pH Sensor*

	pO$_2$ Sensor	pCO$_2$ Sensor	pH Sensor
Sensitivity	37 nA/kPa range: 0–27 kPa	–52 mV/dec range: 0–20 kPa	55 mV/pH range: pH 1–13
Required range	4–40 kPa	1.3–16 kPa	pH 6.8–7.8
Response time t_{95}	1 min[a]) 10 min[b])	2 min[c]) 5 min[b])	< 10 s
Temp. dependence	1%/°	0.7 mV/°	0.8 mV/°
Flow dependence	3% (double speed)	no dependence	no dependence
Drift/stability	± 2%	1–2 mV/d	1–2 mV/d

[a]) Measured in aq. soln. with a flow of 5 ml/min.
[b]) Measured in blood for transfusion with a flow of 0.4 ml/min.
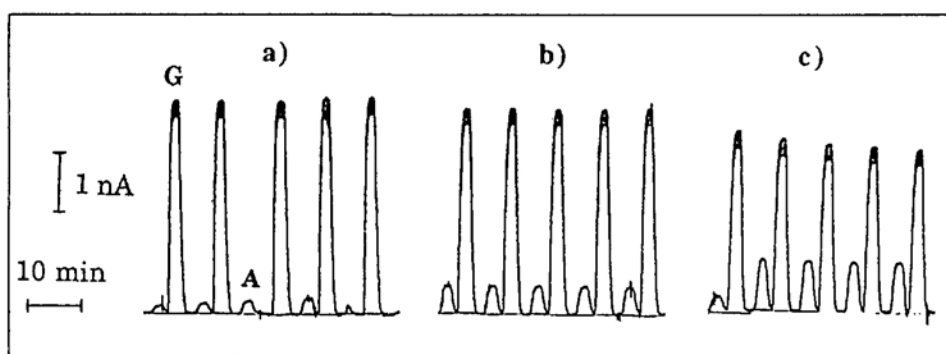[c]) Measured in aq. soln. (unstirred).

Fig. 6. *Glucose oxidase/BSA electrode with a polypyrrole layer tested at a flow rate of 0.5* cm^3 *min*$^{-1}$, *with alternate 1 ml injections of 1.0 mM glucose* (G) *and 1.0 mM ascorbate* (A). Results are shown after *a*) 25, *b*) 120, and *c*) 250 injections. Carrier PBS, recording made at 650 mV *vs.* SCE, and r.t.

cose response. It is believed to result from an electrochemical and chemical overoxidation of the PPy film, a necessary step before glucose measurements can be performed [14]. This effect is illustrated in *Fig. 4* which shows the gradual loss of the PPy electrochemistry as the potential is held above the stable range. Exposing the film to H$_2$O$_2$, a natural product of the GOx reaction, leads to a similar change in the voltammetric characteristics of the PPy layer. Although as is well known, the overoxidation destroys the polymer conductivity [14], it can still function as a stable matrix for immobilizing the enzyme as well as an anti-interference layer (see below).

An alternative approach to entrapment in electrochemically grown polymers is the direct galvanostatic deposition of the proteins on the electrode surface. *Fig. 5* shows the glucose response of two electrodes (GOx and GOx+BSA) prepared in this way, showing the enhancement of the response in the presence of BSA. The electrodes are stable under intermittent testing for more than 80 d.

As we have previously found the pro-tein depositions can be closely controlled both spatially and for glucose response. By selecting a suitable current it was possible to modify electrodes of widths between 100 and 5 μm. The mechanism of the deposition in not yet completely clear, however, the most probable is an electro-phoretic migration of the proteins to the vicinity of the electrode followed by a concentration induced precipitation.

Both entrapment in PPy and direct galvanostatic deposition have been found to be useful for spatially controlled protein deposition. The later technique gave larger responses although, as described below, for measurements in complex media an additional anti-interference layer was required.

A general problem of enzymatic electrodes based on the hydrogen peroxide detection is that of interference from other oxidizable substances present in the solution. Polypyrrole was found to show anti-interference properties with respect to for example ascorbate [8] which is often considered as a model interferent for glucose electrodes. In consequence, polypyrrole can act as both enzyme immobilization matrix and an anti-interference barrier [15]. For example, the steady state current of the GOx+BSA electrode in the presence of 1 mM ascorbate is about 200 nA. This value decreases to *ca.* 0.3 nA upon the deposition of a very thin PPy layer following that of the proteins. After a break-in period, the electrodes are usable for over 250 measurements (*Fig. 6*) showing only a slight increase in the ascorbate interference signal over this period. This result suggests that such PPy/GOx+BSA electrodes could be used in a more complex media. This was investigated using Complex Yeast Medium (*Ciba*) where the polypyrrole layer considerably extended the lifetime of the electrode by reducing poisoning which otherwise causes rapid deterioration of the electrode performance, *i.e.* dereasing the response and increasing the response time.

[1] M. Koudelka, S. Gernet, N.F. de Rooij, *Sensors Actuators* **1989**, *18*, 157.
[2] S. Gernet, M. Koudelka, N.F. de Rooij, *Sensors Actuators* **1989**, *17*, 537.
[3] N.C. Foulds, C.R. Lowe, *J. Chem. Soc., Faraday Trans.* **1986**, *82*, 1259.
[4] P.N. Bartlett, R.G. Whitaker, *J. Electroanal. Chem.* **1987**, *224*, 37.
[5] M. Umana, J. Waller, *Anal. Chem.* **1986**, *58*, 2979.
[6] M.F. Suaud-Chagny, F.G. Gonon, *Anal. Chem.* **1986**, *58*, 412.
[7] K.W. Johnson, *Sensors Actuators* **1991**, *5*, 85.
[8] W. Schuhmann, *Sensors Actuators* **1991**, *4*, 41.
[9] E.J.R. Sudholter, P.D. van der Waal, M. Skowronska-Ptasinska, A. van den Berg, D.N. Reinhoudt, *Sensors Actuators* **1989**, *17*, 189.
[10] G.G. Guibault, 'Analytical Uses of Immobilized Enzymes', Marcel Dekker, New York, 1984.
[11] L. Bousse, N.F. de Rooij, P. Bergveld, *IEEE Trans. Electron Devices* **1983**, *30*, 1263.
[12] S. Gernet, M. Koudelka, N.F. de Rooij, *Sensors Actuators* **1989**, *18*, 1989.
[13] G. Fortier, E. Brassard, D. Bélanger, *Biosensors Bioelectronics* **1990**, *5*, 473.
[14] D. Bélanger, J. Nadreau, G. Fortier, *J. Electroanal. Chem.* **1989**, *274*, 143.
[15] D. Centonze, A. Guerrieri, C. Malitesta, F. Palmisano, P.G. Zambonin, *Fresenius J. Anal. Chem.* **1992**, *342*, 729.