

Modification of ISFETs by covalent anchoring of poly(hydroxyethyl methacrylate) hydrogel. Introduction of a thermodynamically defined semiconductor-sensing membrane interface

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ABSTRACT

Silicon dioxide ion-sensitive field effect transistors were modified by silylation with methacryloxypropyltrimethoxysilane (MPTS) and with in situ photopolymerized poly(hydroxyethyl methacrylate). Subsequently, the covalently linked methacrylate was swollen with a buffered potassium chloride solution, prior to the introduction of a hydrophobic sensing membrane. The introduced hydrogel layer effects a significant reduction in the peak-to-peak noise levels and eliminates completely interference from carbon dioxide. The method is compatible with integrated circuit photolithographic techniques and improves the development of potentiometric biosensors and chemical sensors.

In recent years, research on fundamental aspects of microsensor development has become increasingly important. One reason is that improvements in computer hardware and software for automation of process control have proceeded much faster than the development of sensors of the quantitative and selective detection of the different process variables. Another reason is the promising application of biomolecules for the continuous sensing of (bio)substrates in the (bio)medical field, i.e., the development of the biosensor. Although this study is not directed to the development of biosensors but to a system that makes use of synthetic receptor molecules for the specific and accurate detection of ionic species, i.e., a chemical sensor, it is felt that the results are important for research on both types of sensors.

A sensor can be regarded as an integrated device of a sensing and a transducing element. In this study alkali cation sensors were investigated based on a synthetic ionophore containing sensing membranes and ion-sensitive field effect transistors (ISFETs) as a transducer [1,2]. The focus of this paper is on the integration of these two elements. In 1975 Moss et al. [3] described a potassium ion sensor based on the valinomycin ionophore incorporated in plasticized PVC, which was solvent cast on the gate oxide of an ISFET chip. Like the coated-wire electrode (CWE) [4], this modified ISFET lacks a thermodynamically well defined interface between the sensing membrane and the gate oxide. However, the PVC-modified ISFETs did not seem to suffer from the ill-defined inner contact, and acceptable stabilities and drift values have been reported [5–8]. This apparently satisfactory behaviour of the PVC-modified ISFETs was probably the main reason why no

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experimental efforts were made to improve the system, although theoretical concern about the inner interface was expressed by Janata [9]. However, in 1985 Fogt et al. [10] showed that carbon dioxide in the sample solution interferes strongly with the measurements. This was attributed to carbon dioxide that penetrates the sensing membrane and with traces of water forms carbonic acid at the membrane-gate oxide interface. The carbonic acid offsets the potential at the gate oxide surface and, therefore, affects the measured response. A possible means of preventing carbon dioxide interference is to apply an intermediate Ag/AgCl layer on the ISFET gate oxide [11–13], thus blocking the contact with the surface silanol functionalities. However, this generates a new problem with respect to the AgCl membrane interfacial potential. This problem has been solved by the introduction of an additional layer of sodium glass between the sensing membrane and the AgCl layer [14] or by the introduction of poly(vinyl alcohol) between both layers [15]. Subsequently, common ions may be added to the intermediate layer. Another solution was found by applying buffered potassium chloride solution containing dextran [16]. The solution and sensing membrane were fixed and encapsulated on the ISFET by anodic bonding of glass. However, in none of the reported studies the aspect of covalent chemical bonding of the successive layers by integrated circuit(IC)-compatible photolithographic methods was mentioned.

In this paper, results are presented for a chemical system using poly(hydroxyethyl methacrylate) (polyHEMA) as an intermediate layer, which is covalently attached to the ISFET gate oxide and which controls the interfacial thermodynamics at this interface [17]. An important advantage of this chemical system is that it can be patterned photochemically, which renders possible ISFET modification on the wafer scale.

EXPERIMENTAL

Silylation

The oxide surfaces of separate silicon dioxide ISFETs or complete silicon wafers containing the

ISFETs were placed in a solution of 2.5 ml of methacryloxypropyltrimethoxysilane (MPTS) in 22.5 ml of toluene or mesitylene, to which the appropriate amounts of water or diethylamine were added (see Table 1). The solution was kept at 90 °C for 45 min to 4 h. Subsequently, the ISFETs or wafers were taken out and washed with ethyl methyl ketone, followed by ultrasonic cleaning in the same solution. Finally, the silylated oxide was allowed to dry in an argon atmosphere. Gas-phase silylation was done by placing the SiO₂ ISFET chip in the vapour of refluxing MPTS for 3 h.

Deposition of polyHEMA layers

Solvent casting. On a mounted and encapsulated, presilylated ISFET, a small drop of hydroxyethyl methacrylate (HEMA, Janssen Chimica) containing 4% (w/w) of 2,2'-dimethoxyphenylacetophenone (DMPA, Janssen Chimica) was applied via a capillary. Subsequently, the mounted ISFET was placed in a glass tube from which oxygen was excluded by thorough and continuous flushing with argon (oxygen inhibits the free-radical photopolymerization). Subsequently, the surface of the ISFET was exposed to UV light for 2 min in order to complete the photochemical linking and polymerization. In this way a layer thickness ranging from 5 to 15 μm was obtained, as estimated with a microscope. The polyHEMA layers were conditioned for 1 h in 0.1 M potassium chloride solution before the polymer membranes were applied. All modified ISFETs with a polyHEMA layer that were measured were prepared according to this procedure.

Spin coating. In order to obtain a better defined and more uniform layer, a deposition procedure based on the spinning technique was developed. HEMA layers were spun on the presilylated ISFET-containing wafers or on presilylated Si/SiO₂ wafers using a solution of HEMA containing 4% (w/w) DMPA photoinitiator. For the use of the mask-aligner for photo-patterning of the HEMA, a special arrangement was provided. In order to ensure a small spacing between the spun HEMA layer (which is still fluid) and the mask (see Fig. 1), a Viton ring (thickness 3.5 mm, radius 60 mm) was used, leaving a distance of ca. 3 mm between the wafer with the spun layer and the

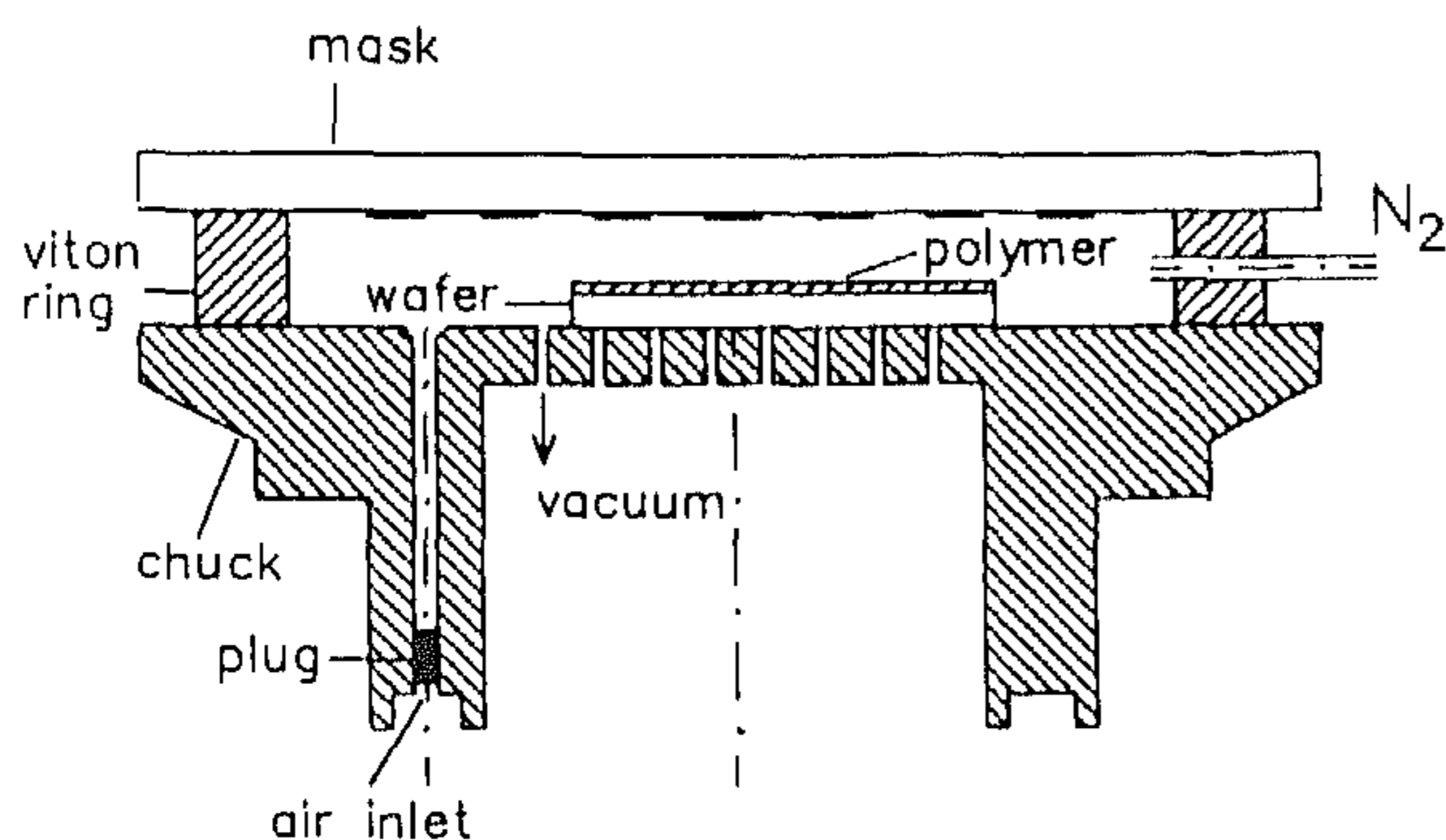


Fig. 1. Schematic representation of the arrangement for photochemical polymerization of spun monoHEMA with a mask aligner.

lower side of the mask. A plug in the air inlet prevented oxygen from entering the compartment above the wafer. The quenching of the photopolymerization process appeared to be very critical, and therefore the polymerization was carried out under strictly oxygen-free conditions. In addition, the HEMA monomer appeared to evaporate very readily owing to its high vapour pressure. As alternatives, (partly) prepolymerized HEMA and a 1:1 (w/w) mixture of HEMA with a solution of polyvinylpyrrolidone (PVP) in 10^{-2} M potassium chloride (1:10, w/w) were used. In particular cases a pH buffer (Titrisol from Merck, citrate-HCl, pH 4) was also added. Both alternatives resulted in more reproducible and more uniform layers. The latter method has the advantage that the conditioning is already carried out before the photopolymerization starts.

Deposition of ion sensitive polymeric layers

Poly(vinyl chloride). Plasticized PVC membranes were made by solvent casting of a mixture of 110 mg of PVC, 225 mg of dioctyl sebacate (DOS) and 5 mg of valinomycin in 3.3 ml of tetrahydrofuran by hand on mounted ISFETs, with and without a conditioned polyHEMA layer, that were encapsulated with epoxy resin (Hysol). The solvent was allowed to evaporate for 24 h before use.

ACE-Epocryl DRH 370. Polyacrylate membranes were made by solvent casting of various ACE-Epocryl mixtures (with ACE:Epocryl ratios of 100:0, 80:20 and 50:50, w/w) with 1.5%

(w/w) valinomycin by hand on mounted ISFETs, with and without a conditioned polyHEMA layer, that were encapsulated with epoxy resin. The ACE-Epocryl mixtures were exposed to UV light for 1 min under a nitrogen atmosphere in the presence of 4% (w/w) DMPA. The different mixtures investigated did not affect the observed V_{pp} values. For the impedance measurements, polyacrylate membranes were made by spinning a 3:1 ACE-Epocryl mixture dissolved in dichloromethane on top of an Si/SiO₂ wafer covered with an Ag/AgCl/polyHEMA layer conditioned in 0.1 M potassium chloride. The ACE and Epocryl materials were kindly provided by Shell (The Netherlands).

Silopren. Ion-sensitive Silopren layers were applied by solvent casting of a solution of 200 mg of K-1000 Silopren (Bayer), 20 mg of KA1 "Vernetzer" and 6 mg of valinomycin in 4 ml of dichloromethane, on mounted silicon dioxide ISFETs with or without a conditioned polyHEMA layer. Because it appeared that Silopren does not adhere very well to the epoxy encapsulant, in this case silicone rubber was used for the encapsulation. After leaving the coated ISFETs for 1 h at room temperature, the thermal polycondensation was completed.

Silicone rubber. Ion-sensitive silicone rubber membranes were applied by solvent casting of a solution of 50 mg of RTV 3140 silicone rubber (Dow Corning) with 1.5 mg of valinomycin dissolved in dichloromethane on mounted silicon dioxide ISFETs with or without a polyHEMA layer, previously encapsulated with the same silicone rubber. To complete the polymerization, the modified ISFETs were kept in contact to the air for at least 24 h at room temperature.

Impedance measurements

Impedance measurements were made with polyacrylate layers ($d = 10\text{--}20 \mu\text{m}$) on Si/SiO₂ wafers with or without an underlying conditioned polyHEMA layer, with polyHEMA ($d = 4 \mu\text{m}$) and with polyHEMA/PVP/ 10^{-2} M potassium chloride ($d = 4.5 \mu\text{m}$) layers. A Solartron 1170 frequency response analyser was used. The amplitude of the applied sinusoidal voltage was 10.0 mV. Platinum electrodes with an estimated area of

0.2 cm² were used to contact the solution in which the membranes were immersed. It was confirmed that in the concentration (1–10⁻⁵ M) and frequency range (10⁴–1 Hz) used, the impedance of the electrodes relative to the membrane impedance could be neglected.

CHEMFET measurements

The modified ISFETs immersed in 0.1 M potassium chloride solution were measured with the source and drain follower type ISFET amplifier in a constant drain-current mode ($I_d = 100 \mu\text{A}$), with a constant drain-source potential ($V_{ds} = 0.5 \text{ V}$). Ten ISFETs were simultaneously monitored and the measured data were collected and controlled with an Apple II computer. MOST switches allowed computer-controlled disconnection of ISFETs with too high a leakage current ($I_l > 50 \text{ nA}$). The amplifier output voltage was recorded with an $X-Y-t$ recorder (Kipp BD90). The cut-off frequency of the ISFET amplifier was 1 kHz and that of the recorder ranged from 2 to 10 Hz, depending on the signal amplitude and the recorder setting. For the experimental conditions, a cut-off frequency of 3 Hz was estimated. This means that the band width of the measuring set-up was limited to frequencies below 3 Hz. Fluctuations of the recorded amplifier output voltage are interpreted as instabilities of the measured CHEMFET. The peak-to-peak voltages of the fluctuations (V_{pp}) are called "noise levels".

RESULTS AND DISCUSSION

Prior to the covalent chemical anchoring of the polyHEMA hydrogel, the silicon dioxide gate oxide of the ISFET was silylated using MPTS. In this way the gate oxide is covered with methacryl functionalities (Fig. 2). In an earlier study [18] it was found that the coverage of silica gel with organofunctional silanes at the surface is ca. 1.5 functionalities per nm², resulting in ca. 3.5 residual silanol groups per nm² of surface. It is assumed that in silylated ISFETs a similar amount of silanol groups is present. These silanol sites are a prerequisite for proper functioning of the final

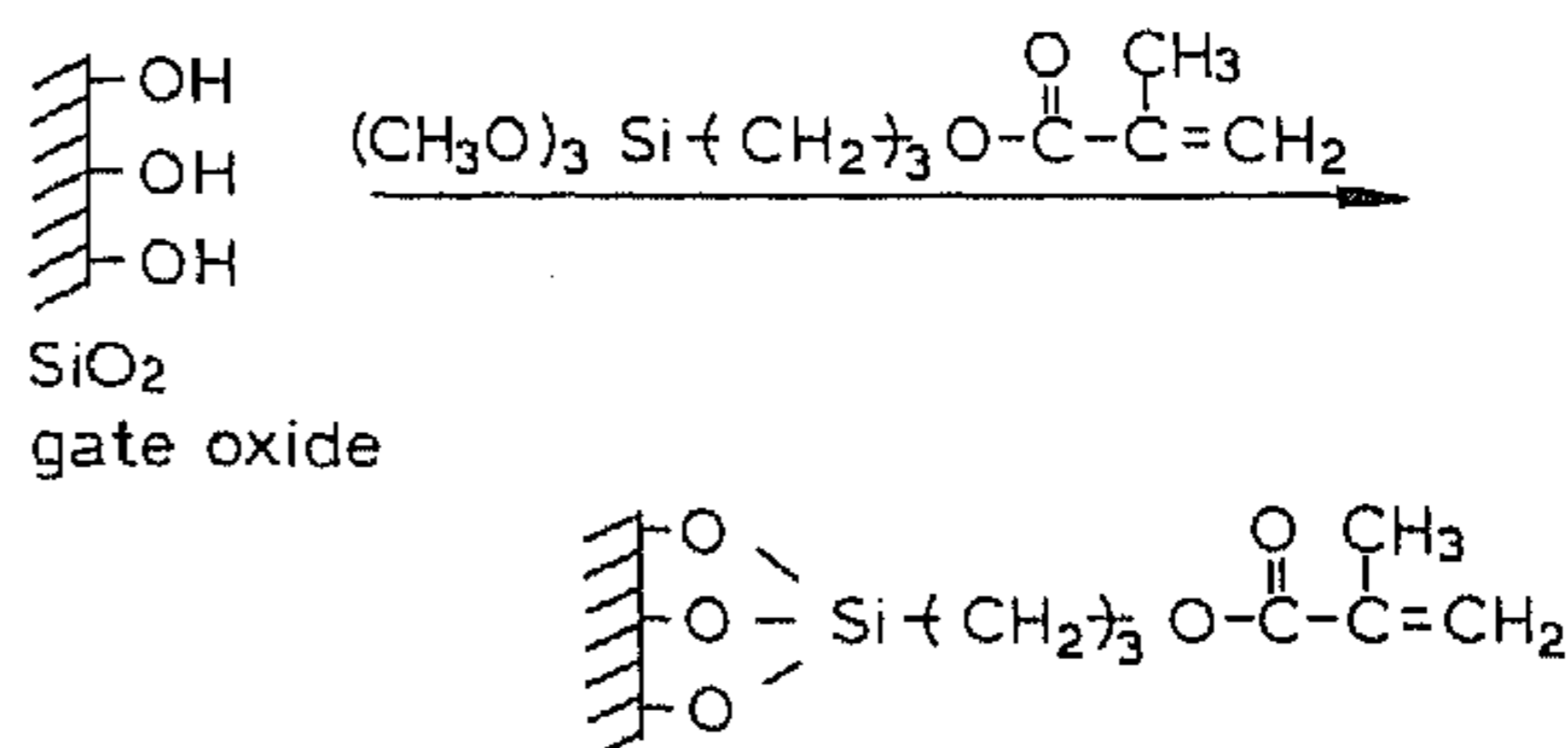


Fig. 2. Surface silylation of silicon dioxide ISFETs with MPTS.

sensor, as they will fix the surface potential of the ISFET, if the local pH is kept constant.

Subsequently, monomeric hydroxyethyl methacrylate (monoHEMA) was deposited on the silylated gate oxide in the presence of a photoinitiator and polymerized. This resulted in polyHEMA covalently linked to the gate oxide of the ISFET chip (Fig. 3). Layer thicknesses in the range 5–15 μm were observed, depending on the deposition method (see Experimental). Using light microscopy, the homogeneity of the patterns of polymerized HEMA can clearly be visualized (Fig. 4). Different silylation procedures were applied in order to investigate the effect on the durability of the covalent linkage of the anchored polyHEMA (Table 1).

Without silylation, the polymerized HEMA is easily removed from the gate oxide. Solution silylation in toluene or mesitylene clearly shows better results than silylation in the gas phase. Both water and diethylamine function well as catalysts in the surface silylation reaction. With diethylamine as a catalyst at 90 °C, the surface modification reaction is complete after 45 min.

The evaluation of the effect of the polyHEMA intermediate layer on the sensor characteristics is

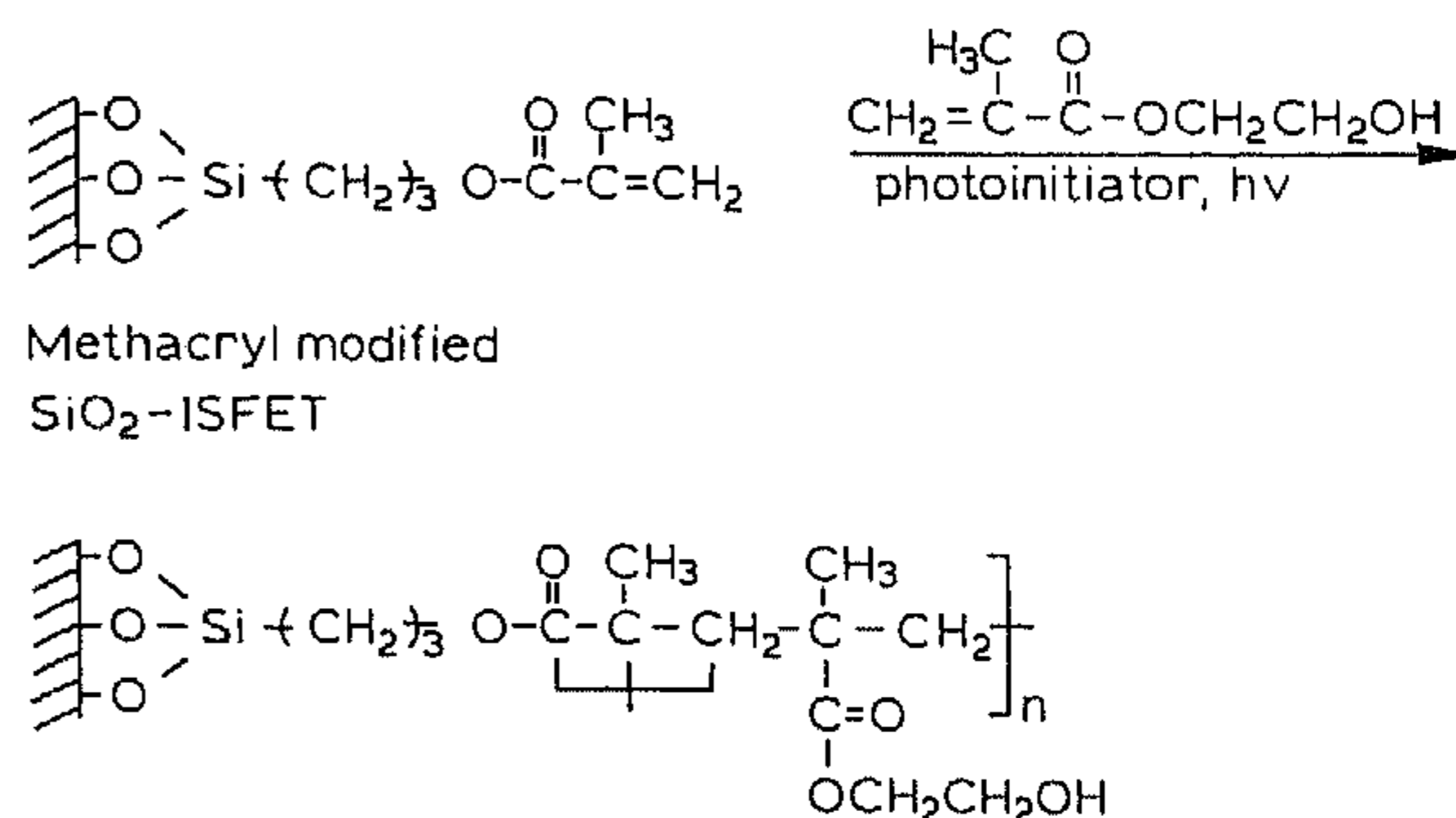


Fig. 3. Covalent linking of polyHEMA on methacryl-modified ISFETs by photopolymerization.

TABLE 1

Effect of silylation conditions on the durability of covalently linked polyHEMA

Silylation conditions ^a	Stimulated ageing conditions ^b	Observation ^c
Not silylated	0.1 M aq. NaBr, 100 °C	Hydrogel removed after 18 h
Not silylated	EtOH, 23 °C	Hydrogel partly removed after 1 h
Vapour phase, 3 h, 120 °C	EtOH, 23 °C	Hydrogel partly removed after 24 h
Toluene, +0.5% (v/v) H ₂ O 4 h, 90 °C	0.1 M aq. NaBr, 100 °C EtOH, 23 °C	Hydrogel present after 6 days Hydrogel present after 2 h
Mesitylene, +5% (v/v) Et ₂ NH, 3 h, 90 °C	0.1 M aq. NaBr, 100 °C EtOH, 23 °C	Hydrogel present after 3 days Hydrogel present after 3 days
Mesitylene, +1 eq. Et ₂ NH, 45 min, 90 °C	0.1 M aq. NaBr, 100 °C	Hydrogel present after 4 days
Mesitylene, +1 eq. Et ₂ NH, 3 h, 90 °C	0.1 M aq. NaBr, 100 °C	Hydrogel present after 4 days

^a See Experimental. ^b The modified chips were kept in the indicated solution at the given temperature. ^c Observations made using a light microscope.

not straightforward. Here the determination of the peak-to-peak voltages (V_{pp}) of the fluctuations of the output signal was chosen (see Experimental). These noise levels were determined when the poly-HEMA layer is covered with the appropriate hydrophobic membrane, after equilibration of the intermediate layer with a buffered potassium chloride solution (Table 2). From the results in Table 2 it is obvious that for all the investigated hydrophobic polymers the peak-to-peak noise level is decreased significantly. The most pronounced effect was observed for the ACE-Epocryl polyacrylate membranes. In the absence of a poly-HEMA intermediate layer the observed large instabilities are probably due to a very low uptake of water molecules by these materials, resulting in a low proton (or hydroxide ion) exchange current at the membrane-gate oxide interface. Attempts to measure the charge-transfer resistance by impedance measurements failed. Only scattering was observed at impedance values of about $10^9 \Omega$. In the presence of a polyHEMA intermediate layer it

was possible to perform impedance measurements (Fig. 5).

At the low-frequency side of the spectrum the impedance is determined by the silicon dioxide gate oxide. If it is assumed that the bulk proper-

TABLE 2

Peak-to-peak voltages (V_{pp}) of different modified ISFETs, in the presence of 0.1 M KCl, measured with a source and drain follower

Hydrophobic membrane	V_{pp} (mV)	
	Without polyHEMA (± 0.01)	With poly-HEMA ^a (± 0.005)
PVC ^b	0.10	0.030
ACE-Epocryl ^c	> 10	0.020
Silopren ^d	0.07	0.030
Silicone rubber ^e	0.10	0.040

^a Conditioned with 0.1 M aq. KCl. ^b PVC-dioctyl sebacate-valinomycin. ^c Polyacrylate membranes obtained from Shell, containing valinomycin. ^d Obtained from Bayer, containing valinomycin. ^e Dow Corning RTV 3140, containing valinomycin.

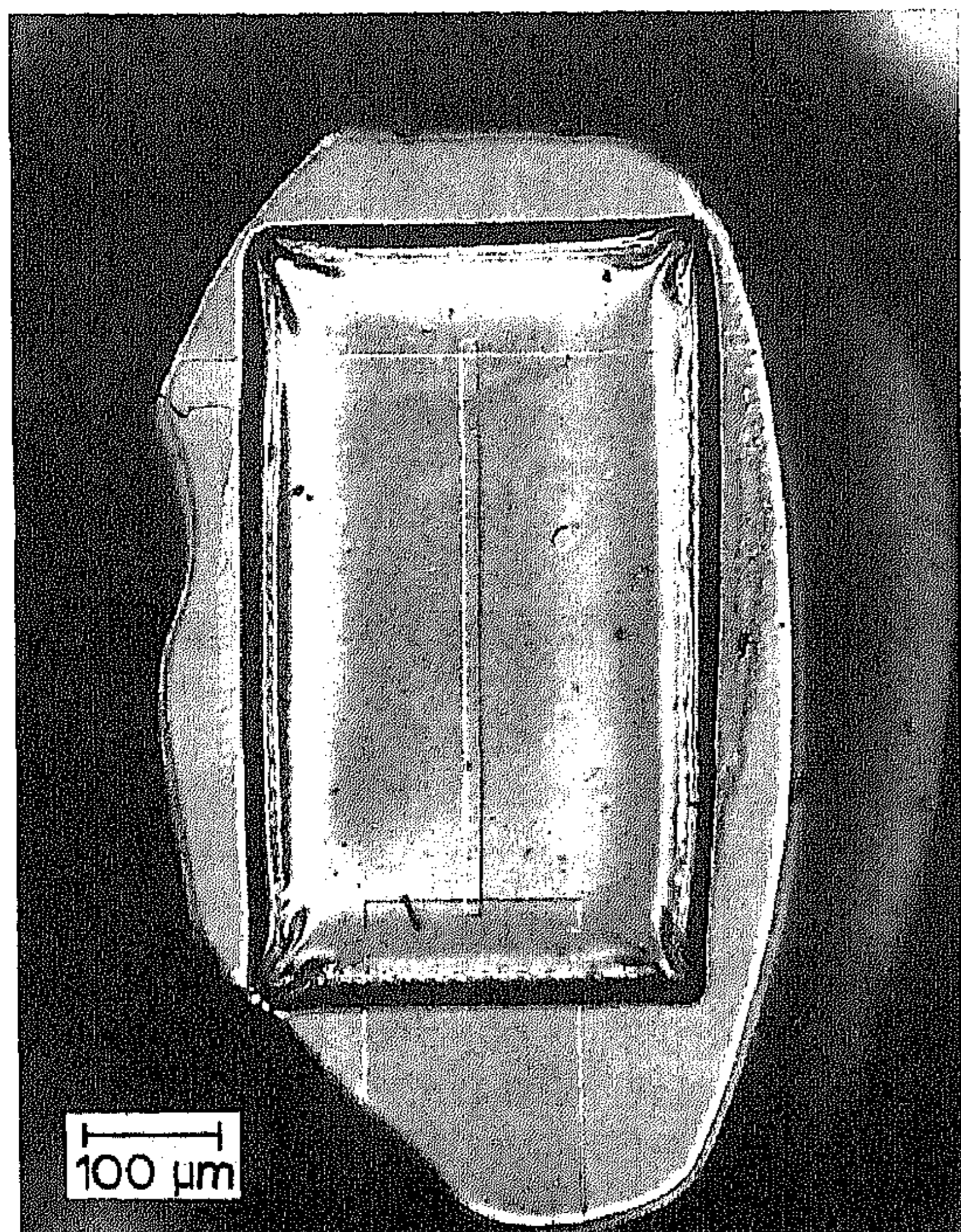


Fig. 4. Light microscopic picture of covalently linked polyHEMA on an encapsulated ISFET.

ties of the ACE-Epocryl membrane remain mainly unchanged on interposing the polyHEMA layer, it must be concluded that the difference in the observed spectra originates from a reduction in the charge-transfer resistance (R_{CT}) at the membrane inner interface. Since the bulk resistance (R_{bulk}) of polyHEMA is at least a factor of 50 lower than the impedances indicated in Fig. 5, from this spectrum values of $R_{bulk} \approx 10^6 \Omega$ and $R_{CT} \approx 2 \cdot 10^6 \Omega$ were estimated.

Finally, the effect of carbon dioxide interference on membrane-modified ISFETs containing an intermediate layer of polyHEMA was investigated. When the polyHEMA layer was equilibrated with a buffered (pH 4) 0.1 M potassium chloride solution [15,16], and subsequently covered with dioctyl sebacate (DOS)-plasticized PVC as the hydrophobic membrane, complete elimination of carbon dioxide interference was observed (Fig. 6, curve 1). In the absence of the intermediate layer, introduction of carbon dioxide into the sample solution gave rise to a fast response of the output signal (Fig. 6, curve 2).

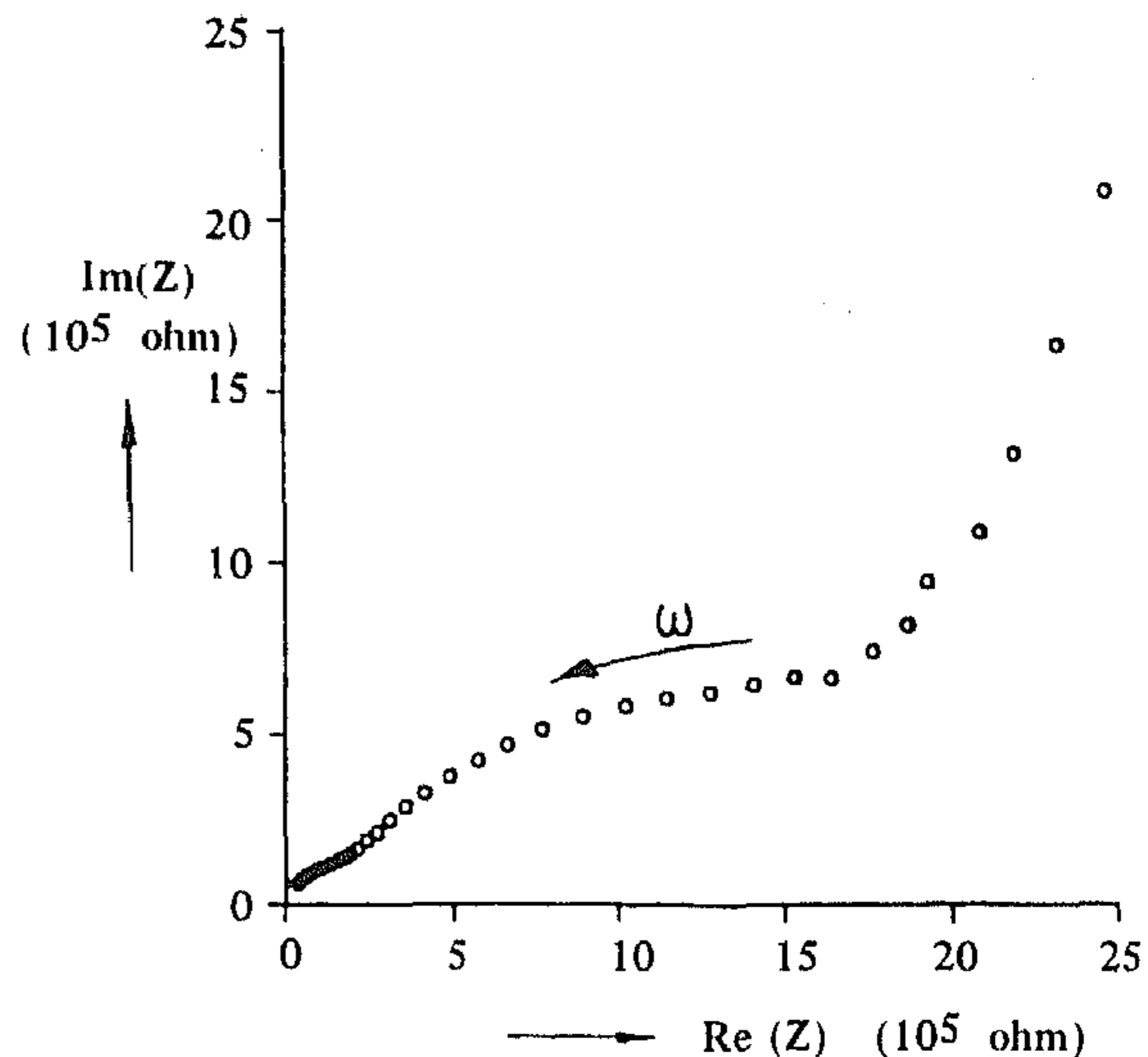


Fig. 5. Impedance measurements of ACE-Epocryl (3:1) on polyHEMA-AgCl-Ag modified silicon dioxide ISFETs.

Conclusion

It has been demonstrated that introduction of a polyHEMA layer, equilibrated with a buffered 0.1 M potassium chloride solution, between the silicon dioxide gate oxide of an ISFET chip and a hydrophobic membrane, gives a significant reduction in the peak-to-peak noise levels and complete

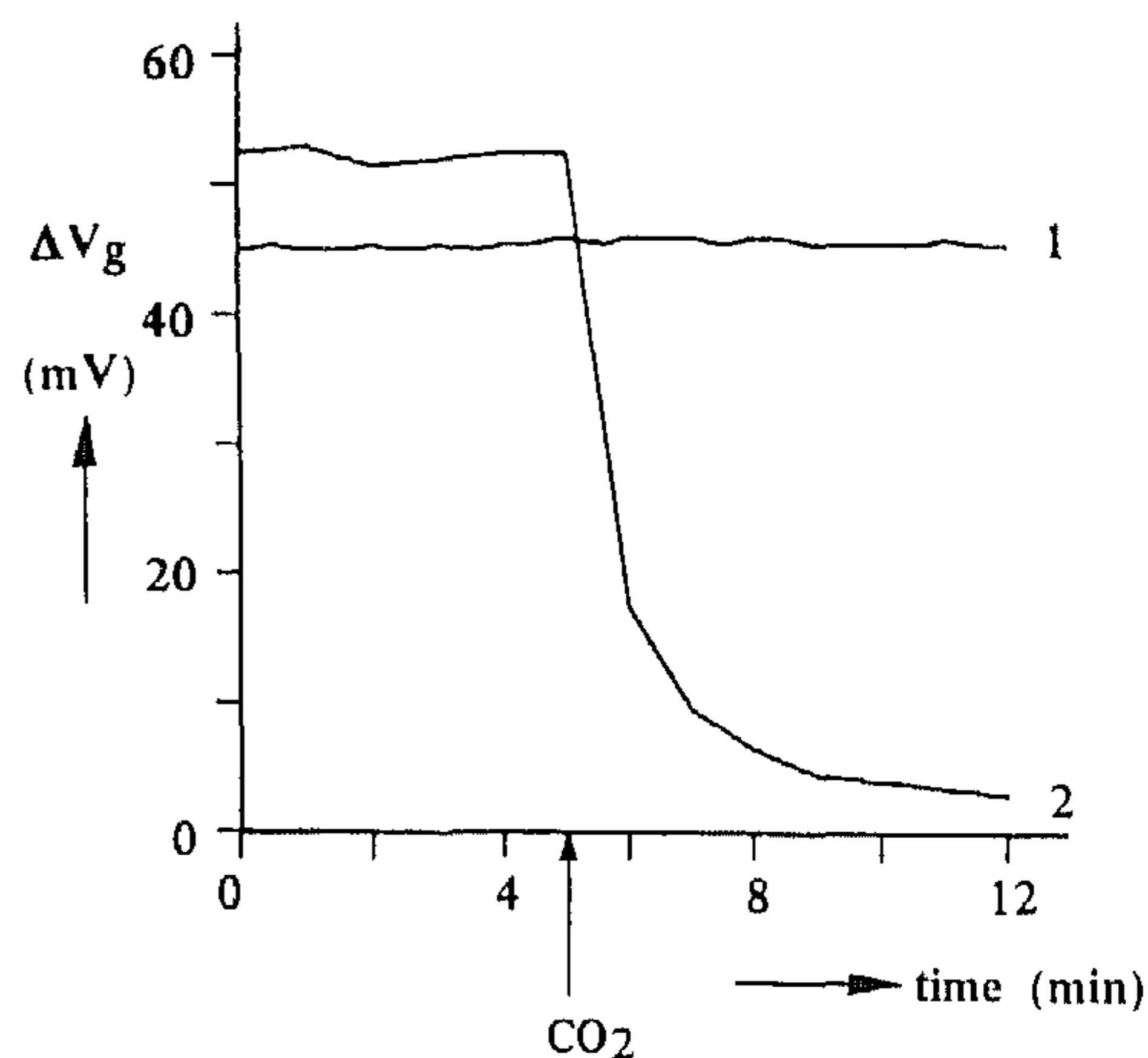


Fig. 6. Amplifier output voltage (ΔV_{gs}) using PVC-modified silicon dioxide ISFETs on saturation of a 0.1 M KCl solution with CO_2 . Curve 1 with a buffered (pH 4.0) 0.1 M KCl polyHEMA intermediate layer; curve 2 PVC membrane deposited directly on the SiO_2 gate oxide.

elimination of carbon dioxide interference. The durability of the hydrogel layer was improved considerably by covalent anchoring of the material to the silicon dioxide gate oxide, using methacryloxypropylsilane coupling agents. Since the hydrogel is formed in situ on the device by photopolymerization, this technique is compatible with IC technologies and therefore has advantages over previously published methods. The surface of the hydrogel contains hydroxyethyl functional groups, which can be easily chemically modified to render possible covalent anchoring of the hydrophobic membrane to the hydrogel.

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