Reference field effect transistors based on chemically modified ISFETs

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ABSTRACT

Different hydrophobic polymers were used for chemical modification of ion-sensitive field effect transistors (ISFETs) in order to prepare a reference FET (REFET). Chemical attachment of the polymer to the ISFET gate results in a long lifetime of the device. Properties of polyacrylate (polyACE) REFETs are described in detail. The polyACE-REFET is superior to other polymer modified REFETs, showing an excellent pH insensitivity (≤ 1 mV pH⁻¹), a long lifetime and an electrically identical behaviour as an unmodified pH ISFET or a cation-selective PVC-MEMFET (membrane FET). The cation permselectivity of the polymer can be significantly reduced by addition of immobile cations. The applicability of a polyACE-REFET in differential measurements with a pH ISFET and a K⁺ MEMFET is demonstrated.

The ion-sensitive field effect transistor (ISFET) may develop into an important potentiometric transducer because of the wide range of potential applications as a microsize sensor for physiological and microanalytical measurements [1–3]. The small size, the solid-state design, fast response time, in situ impedance transformation and signal amplification, cheap mass fabrication using integrated circuit technology and the possibility of manufacturing multi-sensing microdevices renders the ISFET advantageous over conventional ion-selective electrodes (ISEs).

However, these advantages of the ISFET are virtually eliminated without the availability of a reference electrode with similar electronic characteristics, which is required for the proper operation of the ISFET [3]. The large size of commonly used reference electrodes (Ag/AgCl or saturated calomel electrode) and the necessity for

a liquid junction and different temperature-dependent performances of traditional reference electrodes and the ISFET render the use of both devices in combination unattractive. Therefore, most investigations aimed at solving this problem have been focused on the miniaturization of the classical Ag/AgCl electrode. However, all the constructions have the disadvantage of a liquid-filled internal cavity [4–8].

A better approach to the problem of the reference electrode could be the use of two chemically unequally sensitive ISFETs in a differential mode with a common quasi-reference electrode (QRE) (e.g., a metal wire), which can be easily integrated on the silicon chip [9–11]. For this device, any combination of chemically unequally sensitive ISFETs can be used. In this way temperature and light sensitivity of the ISFETs can in principle be compensated [9–11]. Since the accuracy of differential measurements depends on the difference in the ion sensitivity of both ISFETs, complete insensitivity of one ISFET would be preferred.

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Such a reference FET (REFET) should in an ideal case show insensitivity to all species present in the sample solution.

Originally, the inorganic oxide on the gate surface of an ISFET is pH sensitive, owing to the presence of hydroxyl groups which can dissociate and can be protonated. Recently it was shown that the complete removal of the pH-sensitive groups by chemical monolayer modification cannot be achieved [12]. However, the pH sensitivity can be suppressed by attaching to the gate surface an (ion-blocking) hydrophobic polymeric layer. The most important factor in such a modification to form a REFET is the chemical bonding of a polymer to the gate surface, which results in a long lifetime of the device. For ion-blocking layers, a stable attachment has been realized previously by plasma deposition [13–18]. However, Matsuo and Masashi [18] observed potential variations with electrolyte compositions for such modified REFETs. Moreover, the deposition is limited to very thin polymeric layers because of diminishing electrical sensitivity (transconductance) with increasing insulator thickness [19].

In contrast to ion-blocking polymers, modification of REFETs with ion-unblocking (conductive) polymers would have the advantage of an equal transconductance of the REFET and ISFET, which should work in a differential mode [20,21]. Unfortunately, such ion-unblocking hydrophobic membranes result in a short lifetime of the device if they are not chemically bound to the surface.

Taking this into account, ISFETs have been modified in order to prepare REFETs with polymeric membranes which are covalently anchored to the gate surface. Moreover, with respect to mass fabrication using photolithographic techniques, modifications were preferentially carried out with polymers formed in situ on the ISFET gate by photopolymerization.

EXPERIMENTAL

Reagents

Polybutadiene (PBD), phenyl terminated, 99% unsaturation (25% vinyl, 40% *trans*-1,4), average $M_N = 3400$, and tolylene-2,4-diisocyanate (80%

technical, 20% 2,6-isomer) (TDI) were purchased from Janssen Chimica. ACE and Epocryl DRH 370 resin were donated by Shell and Diacryl 101 by AKZO. Terathane 1000, a polyether glycol, was obtained from DuPont. Didodecyldimethylammonium bromide was purchased from Eastman Kodak. Benzophenone (Merck) and 2,2-dimethoxy-2-phenylacetophenone (Janssen Chimica) were used as initiators for the photopolymerizations. As solvents for the preparation of REFETs, dichloromethane and tetrahydrofuran of HPLC grade (Aldrich) were used. All metal salts and titration reagents were of analytical-reagent grade. Distilled, de-ionized water was constantly used.

Polyurethane prepolymer

A 10-g amount of Terathane 1000 dissolved in 70 ml of dry toluene was dried in a Dean–Stark apparatus for 3 h. Then an appropriate amount of the resulting solution containing 1 g (1 mmol) of Terathane and diluted to a concentration of 0.15 mmol ml⁻¹ was added to previously carefully degassed TDI (0.35 g, 2 mmol). The mixture was stirred at 80 °C under nitrogen for 24 h. The resulting prepolymer solution was used for ISFET modification.

REFETs

The SiO₂ ISFETs were silylated with 3-(trimethoxysilyl)propyl methacrylate (Janssen Chimica) or 3-(triethoxysilyl)propylamine (Merck) according to the literature [12]. With the exception of polybisacryl-REFET and polyurethane-REFET, the modified ISFETs were mounted on custom-made printed circuit boards and encapsulated manually using an epoxy resin (Hysol) for insulation from the aqueous solutions before the deposition of the polymer.

VE-REFET

VE-REFETs were prepared by spray coating of SiO₂ ISFETs with a commercially available plastic insulator, Vernis pour l-Electronique (Siceront, France). A layer thickness of 1–3 μm was obtained as measured by interferometry.

Polybisacryl-REFET

A solution of Diacryl 101 (10%, w/w) in tetrahydrofuran containing 4% (w/w) of 2,2-di-

methoxy-2-phenylacetophenone was deposited by spin coating on the surface of ISFETs, presilylated with 3-(trimethoxysilyl)propyl methacrylate. Subsequently the layer was photopolymerized for 1 s with UV light from a Mask Aligner (Karl Süss) under strictly oxygen-free conditions. Layer thicknesses of 0.4–1.0 μm were obtained as measured by interferometry.

PolyACE-REFET

Neat ACE or mixture of ACE and Epocryl DRH 370 containing 20, 50, 80 or 100% (w/w) of Epocryl in dichloromethane with 4% (w/w) of photoinitiator (2,2-dimethoxy-2-phenylacetophenone) were deposited on polyHEMA-ISFETs by solvent casting. The photopolymerization was carried out by UV irradiation under nitrogen for 2–4 min. The layer thicknesses varied between 2 and 5 μm (interferometry).

PBD-REFET

Polybutadiene [10–20% (w/w) solutions in tetrahydrofuran] containing 4% (w/w) of benzophenone was deposited on a presilylated SiO_2 ISFET by solvent casting. Photopolymerization was carried out for 15 min followed by thermal curing at 80 °C for 18 h. Layer thicknesses of 7–15 μm were found (interferometry).

PU-REFET

A 1-ml volume of the polyurethane prepolymer solution was diluted with 1 or 2 ml of dry toluene and a small droplet of the mixture was deposited on ISFETs presilylated with 3-(triethoxysilyl)propylamine. The ISFETs were kept at 80 °C for 1 h, then dipped in butan-1-ol and conditioned at 100 °C for 24 h.

K⁺ ISFET

For the preparation of a K^+ -sensitive ISFET, a typical procedure was applied using PVC (for Ion-Selective Electrode, Fluka) and di-n-butyl phthalate (BDH) in a ratio of 1 : 2 (w/w) with 3% (w/w) of valinomycin (Fluka) as ionophore.

Measurements of pH response curves

The pH response curves were recorded automatically using a standard pH electrode, which

was calibrated before measurements with commercially available buffers (Merck). The REFETs were placed in the stirred sample solution (10 ml), containing 0.01 M citric acid, 0.01 M phosphoric acid, 0.02 M boric acid and 0.11 M tetraethylammonium chloride. Titrations were carried out using 0.15 M tetraethylammonium hydroxide between pH 2 and 10 for 10 min. The potential differences were measured by a source and drain follower circuit as described [25], with a drain current of 100 μA and a constant drain-source potential of 0.5 V. All experiments were performed in a dark and grounded metal box to eliminate static electricity interferences and photosensitivity of the ISFETs.

RESULTS AND DISCUSSION

Insensitivity to pH

In order to obtain a pH-insensitive surface of the ISFET gate (REFET), the original number of hydroxyl groups has to be reduced to less than 0.01% [23]. Previously, it was shown that the chemical modification of the gate of ISFETs via silylation is not effective enough [12].

In order to obtain a pH-insensitive REFET, the gate surface has to be covered by a hydrophobic polymer that has no functional groups which can be protonated or deprotonated on pH changes of the analyte solution. In addition, this polymer should have high chemical and mechanical resistance. As possible polymeric materials polybutadiene (PBD), polyacrylates, polymethacrylates and polyurethanes (PU) were investigated. For chemical attachment to the gate surface, the SiO_2 surface of the ISFETs was first silylated with 3-(trimethoxysilyl)propyl methacrylate. In this way the surface was modified with reactive groups that are able to copolymerize with vinyl monomers [bisacryl, lauryl methacrylate, ACE (1), Epocryl DRH 370 (2) and mixtures thereof] and vinyl prepolymers (unsaturated polybutadiene). Alternatively, for the deposition of prepolymers of polyurethane bearing isocyanato groups, the surface was first reacted with 3-(triethoxysilyl)propylamine. For comparison experiments were also

performed using Vernis pour l'Electronique (VE), which is physically bound to the ISFET gate oxide.

The modified ISFETs were evaluated in terms of the reduced pH response. In Fig. 1 the pH response of SiO₂ ISFETs chemically modified with different vinyl polymers and polyurethane (curve e) when a relatively fast pH scan was applied (in 10 min from pH 2 to 10) is shown. For comparison, a modified ISFET with a physically attached plastic insulator, deposited by spray coating on an ISFET gate that was not presilylated, is included (curve f).

All ISFETs exhibit a very small pH response in the pH range 2–10 except the the polyurethane

(insensitive in the pH range 2–5.5) and polybisacryl. The latter polymer shows an S-type response curve, which suggests the presence of pH-sensitive sites in the polymer or at the surface of the polymer.

Since the polyurethane-REFETs show some pH sensitivity above pH 5.5, in the range particularly of interest for physiological measurements and where the polymer deposition cannot be achieved by photopolymerization, further investigations with such modified REFETs were not performed.

The lifetime of the REFETs was investigated by continuously exposing the electrode to an aqueous solution at room temperature and recording the pH response of the devices at different time intervals. The effect of chemical attachment of the polymer on the durability of the REFET is shown in Fig. 2. Whereas REFETs with chemically attached PBD still show complete pH in-

Fig. 1. Reduced pH response of REFETs obtained by modification with different hydrophobic polymers: (a) polyACE; (b) Vernis pour l'electronique (VE); (c) polybutadiene (PBD); (d) polybisacryl; (e) polyurethane (PU); (f) poly(lauryl methacrylate)-ethylene glycol dimethacrylate (95:5). Scan speed, 10 min from pH 2 to 10. The curves have been offset along the vertical axis for clarity.

Fig. 2. Influence of immersion time on the pH response of SiO₂ REFETs modified with physically bound membrane of VE and chemically bound membrane of PBD. The curves have been offset along the vertical axis for clarity.

Fig. 3. REFET response of (1) polyACE and (2) poly[ACE-Epocryl (80:20)] modified ISFETs. (A) Time course before the pH scan; (B) pH scan; (C) time course after the pH scan. The curves have been offset along the vertical axis for clarity.

sensitivity even after storage for 90 days in water, the physically attached layer of VE exhibits pH sensitivity even after 1 day.

Although REFETs based on PBD, VE and polyACE show a very low pH sensitivity when a relatively fast pH scan was applied (10 min), with much slower scans some pH response was observed for PBD- and VE-REFETs. This suggests that in both layers the H^+ or OH^- diffusion does take place to a considerable extent, which restricts the application of such devices. In contrast, polyACE-REFETs do not exhibit pH sensitivity in slow scan measurements. The results are shown in Fig. 3. Some initial drift of the polyACE-REFETs was observed (≤ 1 h), but measurements carried out at pH 5 for 40 h (Fig. 3A), followed by titration to pH 9 (Fig. 3B) and subsequent stability tests at pH 9 for 40 h (Fig. 3C), confirm the pH insensitivity of polyACE-based REFETs.

Ion-blocking versus ion-unblocking REFETs

Despite this pH insensitivity, the deposited polymeric films may show a different response towards other ions present in the solution. Two types of REFET structures can be distinguished with respect to the penetration of ions into the polymeric layer, resulting in two different mechanisms of the REFET operation.

In a non-ion-blocking REFET structure there will be ion exchange between the solution and the polymer. As a result of a certain ion concentration and ion mobility in the membrane, the polymer has a conductive character. Consequently, a thermodynamic equilibrium between ions in the solution and in the polymer is achieved and the measured electrical potential is a membrane potential. In an ion-blocking REFET structure, ion exchange between the solution and the polymeric layer is negligible and in this case the electrical

potential measured is a surface potential resulting from reversible ion-complexation reactions at the surface of the polymer.

In order to estimate what types of REFETs were obtained, the electrical relaxation time (related to the time needed to redistribute the established charge and potential profiles in the polymer after application of an external electrical field) was measured by means of transconductance measurements, bulk modulation and light pulse response. The results that have been published in detail elsewhere [24] indicate that VE and PBD behave like non-ion-blocking polymers whereas polyBA has an ion-blocking character. The non-ion-blocking character of polybutadiene was confirmed by secondary ion mass spectrometric (SIMS) measurements. After immersion of the PBD sample in 10^{-3} M potassium chloride solution, a substantial uptake of K^+ ions in the polymer was observed. In contrast, for polybisacryl, no significant change in K^+ signal was found after storage of the polymer sample in the potassium chloride solution [24].

From bulk modulation and light pulse response measurements carried out with polyACE-modified REFETs, electrical relaxation times of 0.35 and 0.40 s, respectively were found (polyACE was deposited on the top of conducting polyHEMA hydrogel, which was found not to influence the relaxation time). This indicates the non-ion-blocking behaviour of polyACE-REFETs [24].

Permselectivity of polyACE membranes

The main features of polyACE-REFETs are the pH insensitivity, stable chemical attachment of the polymer to the gate oxide, in situ photopolymerization of the monomers and the transconductance, which is similar to that of the pH ISFET for the frequencies of interest. Hence the polyACE-RE-

Fig. 4. Effect of addition of didodecyldimethylammonium bromide (DDMAB) on the permselectivity of polyACE-REFET toward K^+ ions: (1) K^+ response of the REFET without DDMAB added; (2) with 2×10^{-5} mol DDMAB g^{-1} ACE; (3) with 9×10^{-5} mol DDMAB g^{-1} ACE. The curves have been offsetted along the vertical axis for clarity.

FET seems to fulfil all requirements for a properly operating device. However, because of the non-ion-blocking nature of polyACE the polymer still exhibits an undesirable cation permselectivity which in practice results in a response variation in ionic strength. The permselective behaviour, which is a property of many polymeric membranes and also of conventional reference electrodes, may have two different origins. First, a much lower mobility of anions than that of cations in the polymer leads to the formation of a diffusion potential. Second, the presence of immobile anionic sites in the membrane influences the concentrations of exchangeable cations in the membrane, resulting in the formation of a boundary potential. In order to eliminate the cation permselectivity of the polymer, the influence of the addition of lipophilic cations of low mobility was studied.

Various amounts of didodecyldimethylammonium bromide (DDMAB) were added to ACE before the photopolymerization was carried out and the K^+ response of the resulting REFETs was measured.

As shown in Fig. 4, an initially very high permselectivity of polyACE membrane without DDMAB added (curve 1) changes with addition of an appropriate amount of the salt (2×10^{-5} mol DDMAB g^{-1} ACE) to give complete insensitivity towards K^+ concentration (curve 2). Too

high concentrations of DDMAB (e.g., 9×10^{-5} mol DDMAB g^{-1} ACE) are undesirable because this, changes the REFET into an anion-sensitive ISFET, with DDMAB working as anionic ion exchanger (curve 3). However, after a few days of storage of such a modified REFET in an aqueous solution, the permselectivity was restored owing to the leakage of DDMAB into the aqueous phase. Only complete immobilization of the cationic sites in the polymeric backbone will provide the ultimate long-term solution to this problem.

Differential measurements

Both ISFETs and REFETs require contact with the analyte solution via a quasi-reference electrode (QRE). Although a bare metal such as Ag, Au or Pt has a low impedance contact with the electrolyte, the unstable interfacial potential results in an unstable reference signal towards the ISFET or REFET. When both the ISFET and REFET are combined in a differential set-up via such a QRE, this unstable electrode potential is common to both devices and will be eliminated if the transconductance properties of the ISFET and REFET for the frequencies of interest (viz., 10 Hz) are similar. The use of a polyACE-REFET in differential measurements in combination with a pH-sensitive Al_2O_3 ISFET using Pt as a QRE was recently demonstrated [24]. Although both individual devices show an unstable response on pH changes with respect to Pt when measured separately, a differential pH scan exhibits a constant slope of 53 mV pH^{-1} in the pH range 3–11 [24].

Fig. 5. Differential measurements of potassium concentrations using a MEMFET-REFET-Pt setup.

icability of a polyACE-REFET in with a K^+ -selective ISFET, based on in plasticized PVC, is shown in Fig. RE shows a more stable signal toward valinomycin-PVC-ISFET and poly-T in the electrolyte measured, than in measurements in the electrolyte with

observed instabilities are completely in the differential measurement, resultant slope of $55\text{--}57\text{ mV (pK}^+)^{-1}$ in $10^{-4}\text{--}10^{-1}\text{ M KCl}$ solution. Moreover, of a small amount of acid after of the pK^+ scan does not influence ($\leq 2\text{ mV}$) the differential signal, although change had shifted the Pt electrode out 140 mV.

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have been prepared and characterized. chemical anchoring of the acrylate poly- on the ISFET gate oxide results in a H-insensitive system, having a lifetime in 90 days. From electrical relaxation gations, the non-ion-blocking nature of membrane was demonstrated. Cation ity can be suppressed by the incorporation of the lipophilic didodecyldimethylammonium salt. The REFET is especially differential measurements using pH-sensors and/or ion-selective ISFETs.

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