Design and properties of a flow-injection analysis cell using potassium-selective ion-sensitive field-effect transistors as detection elements

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Abstract

The combination of flow-injection analysis (FIA) and chemically modified ion-sensitive field-effect transistors (CHEMFETs) is described. In a wall-jet cell, two identical potassium-selective CHEMFETs were used for a differential measurement using a platinum (pseudo-)reference electrode. Silicone-rubber membrane materials, chemically bound to the SiO₂ gate oxide, were used with valinomycin as the ionophore. The optimized FIA system showed a linear response of 56 mV per decade for potassium concentrations above 5×10^{-5} M. Preliminary results of potassium determinations in human serum and urine samples are presented.

Keywords: Flow system; Voltammetry; Ion-sensitive field-effect transistors; Potassium; Serum; Urine

Since the first study by Moss et al. [1] on the modification of the gate surface of an ion-sensitive field-effect transistor (ISFET) with an ion-selective PVC–plasticizer–valinomycin membrane, several groups have published results in this field. Although these CHEMFETs exhibit interesting properties and advantages compared with conventional ion-selective electrodes (ISEs), it soon became clear that these microsensors suffer from several serious problems. Since then some of the problems have been solved. The loosening of the physically adhered membrane from the gate oxide surface can be prevented by mechanical [2] or chemical attachment [3] of the membrane. The effect of carbon dioxide interference on the CHEMFET response can be eliminated by application of a buffered hydrogel interlayer [4,5]. Also,

the necessity to use a stable reference electrode can be circumvented when a reference FET (REFET) and a CHEMFET are used in combination with a pseudo-reference electrode in a differential measurement set-up [6–9]. Despite these stimulating developments, some problems still remain unsolved, including the leaching out of electroactive components from the thin ion-selective membrane, the high drift rate, and the laborious membrane deposition and encapsulation procedures of the ISFETs.

Since its first introduction by Ruzicka and Hansen [10], flow-injection analysis (FIA) has proved to be a versatile measuring technique that can enhance the performance of several detector systems. The advantages of ISEs when used in FIA have been reviewed extensively by Frenzel [11,12]. These advantages are also applicable to CHEMFETs. The most important advantages in relation to CHEMFETs are minimization of the drift by baseline correction before and after the

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peak and by a better conditioning of the membrane, no contamination of the sample by the reference electrode when the latter is positioned downstream, resulting in a lower detection limit, and reduction of the risk of fouling of the membrane by adsorbing species (e.g., proteins) because of the short sample residence time.

The use of ISFETs and CHEMFETs in FIA has been reported [13–17]. In addition to the advantages mentioned for conventional ISEs, there are some additional advantages for the combination of ISFETs and FIA. In FIA systems there is a general need for small and rapidly responding sensors resulting in a simple experimental set-up and the possibility of a high sampling rate. The use of a special cell design brings along new possibilities for measurements with ISFETs, e.g., a flow-through cell in which ISFETs without encapsulation are used has been described [18].

In this paper, a wall-jet cell is described in which two identical potassium-selective CHEMFETs are used in a differential measurement set-up. One of the CHEMFETs functions as a REFET and a platinum tube is used as a pseudo-reference electrode. The CHEMFETs have silicone-rubber membranes, chemically bound to the gate oxide with valinomycin as the ionophore. The performance of the cell is optimized by adjusting the flow-rate, the injection volume and the tube length between the injector and the detector. Preliminary results of potassium measurements in human serum and urine samples are presented.

EXPERIMENTAL

Potassium-selective CHEMFETs

The ISFETs used in this work have a SiO_2 gate oxide, which is thermally grown in an oxygen atmosphere for 10 min at 1150°C (estimated thickness $\approx 700 \text{ \AA}$). The ISFETs with external dimensions of $1.2 \times 3.0 \text{ mm}$ were positioned on polycarbonate blocks, glued to the blocks using beeswax and subsequently wire bonded to copper leads on the blocks.

After encapsulation with silicone-rubber (Dow Corning 3140 RTV), the electroactive material was solvent cast on the gate surface. Two different

types of two-component room-temperature vulcanizing (RTV-2) silicone-rubbers were used as membrane materials. The hydroxyl-terminated polydimethylsiloxane Silopren K-1000 (Fluka) and a hydroxyl-terminated copolymer of cyanopropyl (methyl)dimethylsiloxane were both cross-linked by the addition of Silopren Vernetzer K-11 (Fluka) as described earlier [3]. The synthesis of the copolymer is described below. An amount of 2–3% (w/w) of valinomycin, of which 66 mol-% was present as a complex with potassium tetrakis(4-chlorophenyl)borate (Fluka), was added to the membrane materials.

The CHEMFETs were measured in a constant drain-current mode ($I_d = 100 \mu\text{A}$), with a constant drain-source potential ($V_{ds} = 0.5 \text{ V}$). The differential signal from the ISFET amplifier (Electro Medical Instruments, Enschede, The Netherlands) was recorded with an $X-Y-t$ recorder (BD90, Kipp & Zonen); 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. The noise levels, i.e., the peak-to-peak voltages of the fluctuations of the recorded amplifier output voltage, are interpreted as instabilities of the measured CHEMFETs.

Flow-injection system

The flow-injection system is shown schematically in Fig. 1a. A Gilson Minipuls 3 peristaltic pump (1) was used to propel the carrier stream. The sample was injected with a rotary valve (2) (Rheodyne 5020), which could be used with exchangeable sample loops. The volume of each sample loop was measured by filling it with an automatic burette (Metrohm, Dosimat 655). In the absence of an external loop, the internal volume of the valve was determined to be 0.100 ml. The i.d. of the PTFE tubing between the injection valve and the measuring cell was 0.5 mm.

A more detailed drawing of the wall-jet cell is shown in Fig. 1b. The polycarbonate blocks with the ISFETs (3 and 4) are positioned in the measuring cell in such a way that the gate covered with the ion-sensitive material is placed accurately be-

hind a conically shaped (angle = 45°) hole having a smallest diameter of 1 mm. Spring contacts connect the copper leads to the wiring from the ISFET amplifier. The flow stream enters a small chamber opposite the gate surface via a nozzle of 0.15 mm i.d. The distance between the nozzle and CHEMFET surface is 6 mm. Three outlets can be used separately to facilitate the filling of the cell and to prevent the build-up of air bubbles. The output signal of the two CHEMFETs was measured in a differential measurement set-up using a platinum tube as a pseudo-reference electrode (5). The platinum tube was positioned in the central outlet channel.

The FIA cell characteristics were determined by injection of a sample containing 0.1 M KCl and 0.1 M NaCl in a carrier stream containing 1 mM KCl and 0.1 M NaCl. For the peak height versus injection volume, flow-rate and tube length studies, the default settings were an injection volume of 0.217 ml, a flow-rate of 1.4 ml min^{-1} and a tube length of 13 cm. These settings were

also employed for measurements with clinical samples.

Chemicals

Doubly distilled, deionized water was used throughout and all alkali metal salts used were of analytical-reagent grade (Merck-Schuchardt), except sodium chloride (Suprapur). Human serum and urine samples were supplied by the clinical laboratories of hospitals in Nijmegen and Enschede.

The copolymer was synthesized in a slightly modified way as described [19]: 9 g (30 mmol) of octamethylcyclsiloxane (Petrarch Systems) and 1.5 g (ca. 3.5 mmol) of a mixture of the trimer and the tetramer of cyanopropyl(methyl)cyclsiloxane (Petrarch Systems) were slowly added to a vigorously stirred emulsion of 0.45 g (1.38 mmol) of *n*-dodecylbenzenesulphonic acid (Polysciences) (of which 33% was neutralized by the addition of NaOH) in 20 ml of water. After 20 h of stirring at 90°C the polymerization was stopped by neutrali-

Fig. 1. (a) Schematic diagram of the flow-injection system. 1, Peristaltic pump; 2, injection valve; 3 and 4, CHEMFETs; 5, (pseudo-)reference electrode. (b) Detailed structure of the measuring cell.

zation with NaOH. The polymer was recovered from the emulsion by the addition of three volumes of isopropanol. After separation, the precipitated polymer was dissolved in dichloromethane and precipitated by the addition of methanol. The silicone-rubber was separated and the volatile compounds removed in vacuo.

RESULTS AND DISCUSSION

Cell design

In the cell design, some consideration should be given to easy washing out of the sample and the minimization of the diffusion layer. With a wall-jet cell theoretically the minimum thickness of the diffusion layer is established and the response time is minimized. A wall-jet flow cell in combination with a PVC-plasticizer-valinomycin CHEMFET was used by Moody et al. [16]. Unfortunately, no detailed description of the cell design was presented, but the cell performance was fairly good. However, a serious problem was the detachment of the PVC membrane. This demonstrates the need for chemical binding of the membrane to the ISFET surface. The condensation-type RTV-2 silicone-rubbers used in this study are presumed to form a chemical bond with the surface silanol groups of the gate oxide [3].

A differential measurement set-up with a CHEMFET-REFET combination and a pseudo-reference electrode can be used to circumvent the use of a reference electrode [6–9]. If the CHEMFET and the REFET used in this combination have similar drift properties and respond identical to external disturbances (e.g., light and temperature changes), this measurement set-up might also be useful for the suppression of these effects. In addition to the difficulties of making an ideal REFET (no response to electrolyte changes such as pH and ionic strength), the REFET membrane has by definition a different composition compared to the CHEMFET membrane. This implies a priori that the REFET behaves slightly differently to the CHEMFET. The wall-jet cell described here makes use of two identical CHEMFETs (3 and 4, Fig. 1) in a differential measurement set-up. A platinum tube in the central outlet

channel is used as a pseudo-reference electrode (5). CHEMFET (4) is placed in a stream of constant composition (see Fig. 1). In this way, the membrane potential is fixed at a constant value and therefore the CHEMFET functions as a REFET. In this way two identical CHEMFETs which respond identically to external disturbances can be used.

Cell characteristics

The FIA system was characterized by determining the influence of the injection volume, flow-rate and the tube length between the injection valve and the sensor cell on the peak height. The response time of the system was also determined and the linear range of the optimized system was determined by calibration with KCl solutions.

Figure 2 shows the results of the studies of peak height versus injection volume, flow-rate and tube length between the injector and the CHEMFET. The peaks were recorded for a two-decade increase in concentration. In Fig. 2a the influence

Fig. 2. Effect of different flow system parameters on the peak height, V : (a) injection volume, V_i ; (b) flow-rate, Q ; (c) tube length, L .

of the injection volume is shown. The peak height is maximum when the injection volume (V_i) is 200 μl or more. When $V_i = 100 \mu\text{l}$, the internal valve volume, the peak is slightly lower. In Fig. 2b the influence of the flow rate (Q) is shown. An increase in the flow-rate results in a slight decrease in the peak height. With a flow-rate of 1.4 ml min^{-1} the peak width was reasonably small whereas the peak height was not greatly influenced. Variation of the tube length (L) between the injector and detector had only a minor influence on the peak height (Fig. 2c), but the peak width increased considerably. From these observations, the optimum configuration chosen was $V_i = 217 \mu\text{l}$, $Q = 1.4 \text{ ml min}^{-1}$ and $L = 13 \text{ cm}$. In this configuration the peak height is almost maximum and the peak width is small enough to ensure a high sampling rate ($150\text{--}200 \text{ h}^{-1}$).

It was also observed that a pulsating signal was superimposed on the peaks. The use of pulse suppressors was shown to be beneficial for the elimination of these pulses, but had no influence on the peak heights. This pulsating signal is most likely caused by concentration differences sensed by the CHEMFET originating from the pulsation of the peristaltic pump, as it did not influence the baseline. Further, it was also concluded that the injection valve causes a dispersion of the sample. The sample plug with the smaller injection volumes is dispersed in such a manner that the maximum concentration is slightly lower than the original concentration, and this effect is more pronounced at higher flow-rates. Because of the sample dispersion, response time studies of the CHEMFETs could not be performed in an optimum way. Nevertheless, several experiments were performed in order to estimate the time response of the system. The time to reach full response was about 6 s, whereas 63% of full response (time constant of an exponential function) was reached in ca. 400–500 ms, to an increase in concentration. The real response time of the CHEMFETs is much faster and should be of the same order as the literature values; a CHEMFET with a PVC–plasticizer–valinomycin membrane has been reported to reach 63% of full response in $239 \pm 58 \text{ ms}$ to an increase in concentration and in $486 \pm 128 \text{ ms}$ to a decrease in concentration [13].

Fig. 3. Strip-chart recording of a calibration run. Calibrant: $10^{-6}\text{--}10^{-1} \text{ M KCl}$ in 0.1 M NaCl .

The impulse-response function of the FIA system described here has been determined in an earlier study [20]. From this study it was concluded that the FIA system suffered from coloured noise, caused by the pump. A CHEMFET is more susceptible to coloured (and white) noise than an SiO_2 ISFET without a membrane. It was also concluded that the washing out of the sample needed some improvement.

In Fig. 3 a calibration graph of the FIA system is shown. In a carrier stream of 0.1 M NaCl different solutions with various KCl concentrations in NaCl of constant concentration 0.1 M were injected. A linear response of 56 mV per decade was observed for concentrations of KCl above $5 \times 10^{-5} \text{ M}$. The linear range and the detection limit were extended by ca. one decade compared with normal addition methods (Fig. 4).

Membrane composition

As stated above, in flow systems the attachment of the membrane to the ISFET surface is often a problem and this effect is more pronounced when high flow-rates are used. CHEMFETs, chemically modified with Silopren membranes, exhibit no problems related to the loosening of the membrane. Further, the polysiloxane membrane is an elastomer without the addition of an external plasticizer, and this reduces the number of membrane components that can leach out of the membrane. The final goal of this research is

Fig. 4. Response of K^+ CHEMFET; "static" calibration (Δ); "dynamic" calibration-FIA ($\bullet$); both experiments with KCl in 0.1 M NaCl.

a membrane composition with all membrane components covalently bound to the membrane matrix which is subsequently covalently bound to the ISFET gate oxide.

A disadvantage of the use of silicone-rubber is the high membrane resistance, which results in a high noise level of the signal (see Experimental for the definition of noise level in this paper). Although the resistance can be lowered by the addition of lipophilic salts and the use of very thin membranes, the high noise level is still a problem, especially when a differential measurement set-up is used. In the measurement set-up used here, the noise level of the differential signal varies between 0.6 and 1.5 mV depending on the membrane thickness. From the literature [21,22], it is known that a modification of polydimethylsiloxane with cyano groups should give an increase in the dielectric constant and a reduction in the electrical membrane resistance. Therefore, a hydroxyl-terminated copolymer of 90 mol-% dimethyl- and 10 mol-% cyanopropyl(methyl)siloxane was synthesized. This copolymer was cross-linked using Silopren Vernetzer K-11. The dielectric constant of this membrane material was estimated to be $\epsilon = 6$ (the estimated dielectric constant of Silopren was $\epsilon = 2.5-3$). CHEMFETs chemically modified with this membrane material, and containing valinomycin and potassium tetrakis(4-chloro-

phenyl)borate performed very well in the FIA system described here. The noise level of the differential signal was less than 0.2 mV, and the selectivity was maintained. A drift experiment with these modified ISFETs under flow conditions (a constant flow-rate of 1.4 ml min^{-1} of 0.1 M KCl solution) showed a drift of the differential signal of only 6 mV over a period of 12 h.

Determination of K^+ in the clinical samples

The FIA cell was applied to the determination of potassium activities in human serum and urine samples. The results were compared with results obtained by flame photometry in the clinical laboratories. The potentiometric determination of potassium activities in these samples is extremely troublesome because the samples are of unknown ionic strength and viscosity and contain unknown amounts of proteins. The FIA measurement system was used with the optimized values for the flow-rate, injection volume and tubing length. In these experiments the samples were injected three times and the measuring system was calibrated before and after the sample injections with mixtures of potassium and sodium chloride. After the first series of experiments some difficulties were noticed; first, the unknown ionic strength and the presence of proteins in the samples had an influence on the membrane potential, and second, the samples caused a distortion of the peak signal. The latter effect is caused by viscosity differences and partial clogging of the nozzle, probably by protein particles. Without an intermediate washing step the nozzle became completely clogged after 5–10 triplicate sample injections. The first effect should not be a problem as it is normal practice in clinical measurements to calibrate potassium-selective electrodes with standards of known pK and pH values having a typical protein content and viscosity close to those of the unknown samples to be assayed. Despite the distortion of the signal peak, the peak height is not influenced. In a second series of measurements the system was also calibrated with a clinical standard. The serum samples were injected undiluted whereas the urine samples were diluted fourfold with 0.1 M NaCl. The results of the measurements are shown in Fig. 5.

Fig. 5. Correlation between K⁺ determinations with the FIA system and with flame photometry (FP). (a) Serum samples; (b) urine samples.

These results show that the K⁺ concentrations measured by the FIA-CHEMFET combination, agree fairly well with the values obtained by flame photometry. These preliminary results are encouraging but when a high sampling rate is needed the clogging of the nozzle is unacceptable. Filtering of the sample or a larger nozzle diameter should be considered for this application.

Conclusions

The incorporation of CHEMFETs in flow systems and especially flow-injection analysis offers new possibilities for the application of these sensors. In the wall-jet cell described, two identical CHEMFETs are used in a differential measurement set-up with a platinum (pseudo)-reference electrode. By using two streams, one of the CHEMFETs can be regarded as a REFET and the other as the sensing CHEMFET.

The performance of the FIA system is good; a linear response from 5×10^{-5} M K⁺ was observed with a slope of 56 mV per decade. The injection volume of ca. 200 μ l can probably be reduced by using a better injection valve. The chemically bound Silopren membranes perform well in flow systems but the noise level of CHEMFETs with these membranes is a problem in accurate measurements. The noise of the signal can be

greatly diminished when polydimethylsiloxane modified with cyanopropyl groups is used as the membrane material. The noise level of the differential signal was reduced from about 1 mV to less than 0.2 mV.

The determination of potassium in human serum and urine samples showed good results when the normal correction procedure was applied. Although these results are encouraging, clogging of the nozzle is a serious problem that must be solved before the measuring system can be further developed for practical use.

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