Multi-ion Sensing System Based on Glass-encapsulated pH-ISFETs and a Pseudo-REFET

H. H. VAN DEN VLEKKERT and N. F. DE ROOIJ
Institute of Microtechnology, University of Neuchâtel, Rue A. L. Breguet 2, CH-2000 Neuchâtel (Switzerland)
A. VAN DEN BERG and A. GRISEL
Centre Suisse d'Electronique et de Microtechnique SA, Rue de la Maladière 71, CH-2017 Neuchâtel (Switzerland)

Abstract

Since the introduction of CHEMFETs much attention has been paid to the development of multi-ion sensors especially for extra-corporeal continuous patient monitoring. Most of the systems use different ionophores, suspended in inert matrices on top of the gate insulator, to provide multi-ion sensitivity.

A system is developed whereby glass is used to encapsulate the individual sensors thereby providing not only an excellent encapsulation but also a means to immobilize hydrogels and membranes. To avoid the problem of the reference electrode a pH-sensitive ISFET with a retarded pH response was developed, thereby creating the possibility to operate this ISFET as an ion-insensitive FET over short periods of time (pseudo-REFET).

A multi-ion sensor, consisting of a pH-sensitive, a potassium-sensitive and a calcium-sensitive ISFET together with a pseudo-REFET, is developed. It is shown that in the differential measurement set-up, the system exhibits a high precision in combination with a high sample frequency. Furthermore the modelization of the pseudo-REFET permits the estimation of diffusion coefficient of hydrogen ions in different hydrogels.

Introduction

Since the introduction of CHEMFETs much attention has been paid to the development of (multi-)ion sensors small enough for in vivo (i.e. intravascular) measurements [1–4]. However, problems such as recalibration due to insufficient accuracy, sterilization and undesirable in vivo use of a potentially thrombogenic device have prevented the introduction of such devices for routine patient monitoring so far. An alternative approach, on-line monitoring, embodies the important advantages of both in vivo and in vitro monitoring and avoids the above-mentioned problems; with this approach the sensor is connected directly to the subject in a non-invasive manner and to the appropriate instrumentation so as to provide real-time, physiological data [4, 5]. Several systems based on this principle using ISFETs have been reported in the literature [5–12].

Most of the systems, for other than hydrogen ions, use different types of ionophores, dissolved in inert matrices which are placed on top of the gate insulator to provide multi-ion sensitivity. In order to obtain a reliable system, problems such as encapsulation (leakage current), loss of membrane adhesion and loss of sensitivity due to the leaching out of the ionophore have to be solved. Furthermore when using pH-sensitive ISFETs as sensor unit, the use of an intermediate hydrogel is necessary in order to avoid interference of, for example, carbon dioxide [13–15]. Although the existing encapsulation techniques for flow-through cells are rather adequate [5–12], the fabrication of such an intermediate hydrogel layer is not completely compatible with these encapsulation techniques.

Glass encapsulation, based on the anodic bonding technique [16], can be a solution for the above-mentioned problems. Glass is known to be highly resistant to chemical attack, and the holes, drilled through the glass in order to have access to the sensing area, provide an excellent physical fixation for both hydrogel and ion-sensitive membrane. In addition, modification of the polymer matrix can result in an improved adhesion of the membrane to the glass substrate, thereby increasing the lifetime of the sensor unit considerably [17]. Moreover, leaching out of the ionophore can be prevented by chemical bonding of the ionophore to the polymer matrix [15].

Not only the reliability but also the accuracy of the system should be very high. In this respect a kind of flow injection analysis system, whereby 100% response is achieved, can guarantee a high precision over extended periods of time despite long-term baseline signal drift. Another essential part of the multi-ion sensor, which is often neglected, is the reference electrode. Normally traditional reference electrodes are used, preventing the development of a complete system based on solid-
state technology. The use of a REFET with a differential measurement set-up could end this discrepancy. Although much effort has been put into the development of such an ion-insensitive FET [15, 18, 19], a perfect REFET does not exist.

To circumvent this problem, and using the analyte chopping technique, a pseudo-REFET can be used. This is a pH-sensitive ISFET having a retarded response. The fact that in this case both ISFETs have the same properties, effects of, for example, drift and temperature sensitivity are more or less cancelled which increases the accuracy of the system.

In this paper the fabrication of the basic structure (pH-sensitive ISFET), suitable for anodic bonding will be presented together with its characteristics. Furthermore design criteria, based on theoretical modelling for the pseudo-REFET and its realization will be discussed. Finally we will present differential measurements which show the efficiency of this system for hydrogen, potassium and calcium ion determination.

Theory

The pH-sensitive ISFET can be considered to be a MAOSFET in which the metal gate is replaced by a reference electrode, a solution with unknown pH and a pH-sensitive insulator, in our case aluminum oxide. Combination of standard MOS theory [20] with the site-binding model for the reaction of hydrogen ions with the insulator layer [21] yields the following expression for the measured quantity \( V_{gs} \), the reference electrode to source potential of the ISFET, when it is operated in the constant current mode

\[
V_{gs}^{PH} = \frac{I_D}{W} + \frac{E_{ref} - \Psi_0^{PH} + \chi^{PH} - V_{ss}}{\mu_n L C_{ins} V_D}
\]

where \( I_D \) is the drain current, \( V_D \) the drain to source voltage, \( \mu_n \) the mobility of electrons in the inversion layer, \( W \) the channel width and \( L \) the channel length. \( E_{ref} \) is the reference electrode potential relative to vacuum, \( \Psi_0 \) the electrical potential difference between the insulator surface and the bulk of the solution and \( \chi \) the dipole potential at the insulator/solution interface. \( V_{ss} \) is the solid state part and can be described by

\[
V_{ss} = \left( \frac{\phi_S}{q} + \frac{Q_{ins} + Q_{inv}}{C_{ins}} - 2\phi_F - \frac{1}{2} V_D \right)
\]

with \( \phi_S \) the silicon work function, \( Q_{ins} \) the charge inside the insulator per unit area (assumed to be located at the insulator/silicon interface) and \( C_{ins} \) the insulator capacitance per unit area. \( Q_{inv} \) is the charge at the silicon surface at inversion per unit area and \( \phi_F \) the Fermi potential.

For \( Al_2O_3 \), \( \Psi_0 \) can be described by

\[
\Psi_0 = 2.303 \frac{kT}{q} \frac{\beta}{\beta + 1} (pH_{pec} - pH)
\]

where \( \beta \) is a dimensionless sensitivity parameter and \( pH_{pec} \) the pH at the point of zero charge of the oxide material. The constants of the surface reactions and of the electrical double layer are included in \( \beta \). For alumina typical values are: \( \beta \approx 5 \) and \( pH_{pec} \approx 8 \) [21].

For a pH-sensitive ISFET covered with a hydrogel layer and an ion-sensitive membrane a similar expression can be found

\[
V_{gs}^{i} = \frac{I_D}{W} + \frac{E_{ref} - \Psi_0^{iH} + \chi^{iH} - V_{ss}}{\mu_n L C_{ins} V_D}
\]

\[
+ \frac{kT}{zq} \ln \frac{a_i}{a_0} - V_{ss}
\]

with \( z \) the charge of ion \( a \), \( a_i \) the activity of ion \( i \) in the outer solution and \( pH_0 \) and \( a_0 \) the pH and activity of ion \( a \) in the hydrogel. Furthermore the membrane is considered to operate in its linear response region.

When the pH of the test solution is constant, both sensors can be used in a differential measurement set-up whereby effects such as drift, temperature sensitivity and light sensitivity are strongly reduced and ideally \( AV_{gs} \) becomes only a function of \( a_i \), independent of the stability of the reference electrode and the properties of the ISFET. However the pH is not constant and therefore a pseudo-REFET has to be used.

When the pH of the test solution is constant, both sensors can be used in a differential measurement set-up whereby effects such as drift, temperature sensitivity and light sensitivity are strongly reduced and ideally \( AV_{gs} \) becomes only a function of \( a_i \), independent of the stability of the reference electrode and the properties of the ISFET. However the pH is not constant and therefore a pseudo-REFET has to be used.

Considering the hole in the glass, which is the access for the sample solution to the gate, as a porous liquid plug with length \( l \) and \( H^+ \) concentration of \( C_{int} \), the variation of \( C_{int} \) at the interface at time \( t \) after continuous exposure to the sample with \( H^+ \) concentration \( C_{out} \) can be given by

\[
C_{int}(t) = C_{ext}(0) + \frac{4}{\pi} \left[ C_{ext}(0) - C_{int}(0) \right] \times \sum_{n=1}^{\infty} \frac{(-1)^n}{(2n-1)} \exp(-2n-1)(2n-1)D_{eff}t/4l^2
\]

where \( D_{eff} \) is the effective diffusion coefficient of \( H^+ \) and \( C_{ext} \) is constant over time period \( t \). For the long-term response only the first term of the summation has to be considered. From eqn. (5) it can also be deduced that the non-response time of the system depends on \( D_{eff}/l^2 \). In a differential measurement set-up the minimum sample time
will be determined by the response time of the ISFET and the maximum sample time by the non-response time of the pseudo-REFET. The response time of the pH-ISFET will be 10 s for a 98% response with \( D_{\text{eff}} = 9 \times 10^{-5} \text{cm}^2 \text{s}^{-1} \), \( \Delta \phi = 1 \) and \( l = 200 \mu \text{m} \). The non-response time will be around 0.4 s. In order to have a pseudo-REFET with a non-response time of 10 s with the same \( D_{\text{eff}} \) \( l \) should be 1 mm. However to have only one fabrication process \( l \) should be equal for all ISFETs and therefore the variable \( D_{\text{eff}} \) has to be changed. \( D_{\text{eff}} \) can be changed by filling the hole with, for example, a hydrogel. Besides this lowering of \( D_{\text{eff}} \) the porous plus material has to be also biocompatible. Poly(2-hydroxyethylmethacrylate) is known to be suitable both for its diffusion properties and its biocompatibility [23]. Furthermore it can be polymerized in situ by either UV radiation, pressure or temperature depending on the initiator. Values of \( D_{\text{eff}} = 4 \times 10^{-8} \text{cm}^2 \text{s}^{-1} \) have been reported for chloride ions in pHEMA [22, 23] (in water \( 2 \times 10^{-5} \)) which would result in a non-response time of 200 s. Combining eqns. (1), (4) and (5) expressions can be found for the response of the differential measurement set-up at time \( t \) after changing the solution.

For the pH-ISFET

\[
\Delta V(t) = \Delta V(0) + S (\phi - \log C_{\text{int}}(t))
\]  

(6)

and for the membrane covered ISFETs

\[
\Delta V(t) = \Delta V(0) + S \log C_{\text{int}}(t) \\
+ S_i (\log \alpha_i - \log \alpha_{\text{at}})
\]

(7)

with \( \Delta V(t) \) the output of the system at time \( t \), \( \Delta V(0) \) the output of the system at time 0 with the calibration solution \( \phi \) equal to the \( \phi \) of the pseudo-REFET and ion activity \( \alpha_{\text{at}} \). \( S \) the sensitivity of the pH sensors (from eqn. (3)) and \( S_i \) the sensitivity of the membrane for ion \( i \) (from eqn. (4)). All other parameters are considered constant and are included in \( \Delta V \).

Experimental

Sensor Layout

For most applications the simultaneous measurement of three different ions is sufficient, therefore we opted for three pH-ISFETs and one pseudo-REFET as a basic unit. One of the ISFETs was transformed into a potassium-sensitive ISFET and one into a calcium-sensitive ISFET, for which the membranes were based on valinomycin resp. ETHZ 1001 [24]. A photograph of the total cell is shown in Fig. 1.

In order to have a high yield of completed multi-ion sensors, the individual ISFETs were anodically bonded one by one to the glass. This method suppressed the necessity of placing the ISFETs in a p-well and reduced the possibility of incomplete bonding due to dust particles. As a cover a second glass plate can be used to which connections can be glued or the sensor unit can be mounted directly into a plastic tube. With the glass plate a sample volume of only 12 \( \mu \text{l} \) is necessary.

ISFET Fabrication

In an earlier study it was demonstrated how to protect a solid-state chemical sensor against the harsh conditions of anodic bonding: high temperature and high voltage [16]. It was shown that two protections have to be provided in order to maintain the quality of the ISFET.

1. The part covered with the glass has to be protected by a grounded polysilicon layer.
2. The sensitive area, not covered by the glass, has to be screened from the electrical field developed in the hole. This can be accomplished.
by covering this area with a metal layer which is connected to a conductor. When this conductor is n-type in a p-type substrate it is under normal conditions a reverse biased diode, however during the anodic bonding it will become conductive and can thereby discharge the metal.

Two other design criteria have to be kept in mind besides those mentioned above.

1. Step heights of more than 1000 Å have to be avoided.

2. The reference electrode to source voltage has to be larger than −0.5 V, in order to prevent influences of the protection diode, which is in direct contact with the solution during operation.

Apart from the p-well, the fabrication was similar to the fabrication process reported [16] and was carried out in a CMOS production line, with exception of course of the fabrication of the pH-sensitive aluminum oxide layer. After dicing the wafer anodic bonding was carried out at 800 V at 450 °C.

Glass Fabrication
Micromachining of the two glass plates was carried out by ultrasonic drilling (Bullen Ultrasonics Inc.). The glass was Pyrex 7740 double side polished, however, to achieve complete bonding the glass had to be polished again after the drilling process.

Materials

**pHEMA hydrogel for pseudo REFET**
A mixture of HEMA (hydroxyethylmethacrylate) and 4% photoinitiator (dimethoxyphenylecetophenone) was placed in the glass hole with a micropipette and exposed to UV light for 35 s. By adding polyvinylpyrolidon (PVP) the swelling degree of the pHEMA can be changed and thereby the diffusion coefficient of hydrogen ions.

**Hydrogel for ion-sensitive membranes**
Different hydrogels were tested for their applicability with the glass encapsulated ISFETs. It was established that, although it is not a real hydrogel, a solution containing 20% (wt./wt.) Dextran (Fluka purum, MW 110 000) was very suitable for filling the hole in the glass. Different solutions were then prepared for the inner electrolyte: 20% Dextran in buffer pH 4 (Merck) with either 0.1 M KCl or 0.1 M CaCl$_2$.

**Membrane Compositions**

**Potassium-sensitive membrane.** The solution for solvent casting consisted of 1400 mg DOS (bis(2-ethylhexyl)sebacate, Fluka, purum p.a.), 600 mg PVC (poly(vinyl chloride), Fluka, purum p.a.), 20 mg valinomycin (Fluka, purum p.a.) and 8 mg KtpClpB (potassium tetrakis(4-chlorophenyl)-borate, Fluka, purum p.a.) dissolved in 8 ml tetrahydrofuran (p.a.).

**Calcium-sensitive membrane.** The solution for solvent casting consisted of 650 mg o-NPOE (2-nitrophenyloctylether, Fluka, purum p.a.), 320 mg PVC (poly(vinyl chloride), Fluka, purum p.a.), 50 mg calcium ionophore I (C$_{38}$H$_{72}$N$_2$O$_8$, Fluka, Selectophore) and 7.2 mg KtpClpB (potassium tetrakis(4-chlorophenyl)borate, Fluka, purum p.a.) dissolved in 8 ml tetrahydrofuran (p.a.).

**Solvent Casting Procedure**
The holes in the glass were filled with the hydrogel and the surface was allowed to become tacky (5 to 10 min). Then the sensor was covered with the membrane solution and allowed to dry for 5 min, after which another coating was carried out. After 15 min drying in air, the sensors were stored in a buffer before testing. The estimated membrane thickness was about 50 μm, determined optically with as reference the depth of the hole in the glass. It was noted that if the hydrogel surface was not tacky enough, the membrane took up a lot of water and no homogeneous membranes could be formed.

**Test Solutions (mM/l)**

<table>
<thead>
<tr>
<th>Solution 1</th>
<th>Solution 2</th>
<th>Solution 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>0.3</td>
<td>3</td>
</tr>
<tr>
<td>NaCl</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Tris-HCl</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Tris</td>
<td>100</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>(pH = 8.22)</td>
<td>(pH = 7.68)</td>
</tr>
</tbody>
</table>

**Test Procedure**
The pH sensitive ISFETs, encapsulated with glass, were first tested for pH response and drift rate. After these tests they were rinsed with water and isopropanol and dried at room temperature.

After coating, the sensors, together with a platinum wire, were mounted in a tube and conditioned in test solution 2. The pH, K$^+$ and Ca$^{2+}$ sensors were connected to a special designed differential amplifier together with the pseudo-REFET. The differential signals were registrated on a three-channel recorder. Concentration changes were induced by pumping either solution 1 or 3 through the tube.

**Results and Discussion**
All glass encapsulated ISFETs were tested before deposition of the membranes and hydrogel and showed a pH sensitivity of 54–56 mV/pH at
25 °C and had a drift during the first exposure to the solution of less than 0.5 mV/h similar to that reported before [16].

A typical differential response of the pseudo-REFET (fabricated with 10% PVP) and a pH-ISFET is shown in Fig. 2. In this case a SCE reference electrode was used to determine the absolute response of each sensor. Based on the dimensions of the glass hole (500 μm diameter and 200 μm depth) and the pH of the solutions $D_{ef}$ can be calculated by fitting the data with help of eqns. (5) and (6): $D_{ef} = 0.9 \times 10^{-7}$ cm² s⁻¹. The calculated non-response time of about 40 s agrees well with the measured one.

In principle $D_{ef}$ is sufficiently low, but the method of sampling can influence the response time of the pH-ISFET as is shown in Fig. 3, where at a flow rate of 6 ml/min and a sampling time of 60 s the sample solution was either directly in contact with the calibration solution or with an air bubble inbetween. It can be clearly seen that in the case of the air bubble the 98% response time of the pH-ISFET is in the order of 15 s, but that interdiffusion has taken place when both sample and calibration solution are in direct contact. The amount of interdiffusion depends on contact time which in this case was 60 s. Therefore to obtain a 100% response this system was critical.

In order to improve the non-response time of the pseudo-REFET the amount of PVP can be reduced. In the case when no PVP was added to the HEMA mixture a non-response time of about 10 min was found which means that $D_{ef}$ is in the order of $6 \times 10^{-8}$ cm² s⁻¹ which is in agreement with the change in $D_{ef}$ mentioned elsewhere [22, 23].

Another important parameter of a flow-through system is the sample frequency. In principle the sample concentration is calculated from the voltage shift from base line to the maximum of the peak, however when the sample frequency is increased, the output signal will not have reached its base line value the moment the new sample is measured and therefore the calculation has to be based on the last known stable base line. Because our systems have a differential drift rate, after conditioning, of about 0.1 mV/h, the accuracy depends on the pseudo-REFET. For the first pHEMA with 10% PVP the sample frequency could be increased to 30 samples per hour, using only one base line value, with the same accuracy as for a sample frequency of 12 samples per hour when for every sample a stable base line just prior to sampling was used (sample time 60 s).

The efficiency of the flow-through system for simultaneous measurement of the potassium-, calcium- and pH-sensitive ISFETs is shown in Fig. 4. Calculating the concentration of solution 3 with the two point calibration of solutions 1 and 2 and comparing this value with the real concen-

![Fig. 2](image1.png)  
**Fig. 2.** Differential output of the system pH-ISFET/pseudo-REFET: (1) going from pH 7.68 to 8.22, (2) going from pH 8.22 to 7.68; ● measurement, – – – simulation.

![Fig. 3](image2.png)  
**Fig. 3.** Differential output of the system pH-ISFET/pseudo-REFET: (1) pH jump with sample and calibration solution in direct contact, (2) pH jump with sample separated from calibration solution by an air bubble (vertical lines: interruption of output signal).

![Fig. 4](image3.png)  
**Fig. 4.** Differential output of the multi-ion flow-through cell with solution 2 as calibration solution and the sample solutions 1 and 3 (with air bubbles in between).
itation the accuracy of the system was 100% ± 3. The lifetime of the flow-through cell was determined by the ion-sensitive membranes which exhibit decreasing sensitivities after several days of continuous operation.

Conclusions

Glass encapsulation of ISFETs has proven to be a convenient method for the fabrication of flow-through cells, especially when solvent polymeric membranes, with an intermediate hydrogel, are employed to fabricate multi-ion sensors. The concept of the pseudo-REFET has proven to be successful and offers the advantages of one fabrication and encapsulation technology. With this system a high accuracy can be obtained, together with a high sampling rate.

It should be noted, however, that the fabrication process of the sensor is complicated and that high demands are placed on the smoothness of the glass.

Acknowledgements

The authors wish to acknowledge the financial support of the FSRM (Swiss Foundation for Microtechnology Research). The help of F. Ess (CSEM) for the polishing of the glass was greatly appreciated.

References