Development of potassium-selective ion-sensitive field-effect transistor (ISFET)
by depositing ionophoric crown ether membrane on the gate dielectric, and its
application to the determination of K⁺-ion concentrations in blood serum

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Received 10 October 2005; accepted 2 March 2007

This paper reports the fabrication and characterization of potassium ISFET (ion-sensitive field-effect transistor) by
deposition of a crown ether layer on the gate. This ISFET is referred to as potassium MEMFET (membrane field-effect
transistor) or potassium-selective MEMFET device. Calibration of ISFET in standard KCl solutions has shown that the
sensitivity of crown ether-ISFET towards potassium (2.3 mV/mg/L) was approximately double that of the nitride-gate
ISFET (1 mV/mg/L) in the 100-400 mg/L range. The mechanism of ISFET potassium sensitivity enhancement by the
ionophoric crown ether layer on the gate has been explained. Use of the device for potassium concentration measurements in
human blood serum has been demonstrated. The distribution of potassium ion concentrations (151-210 mg/L) in a series of
human blood serum samples has been determined from the reference value obtained by atomic absorption spectroscopy and
the ISFET sensitivity measured with KCl solutions. These diluted samples showed a constant pH ~ 7.0-7.1, confirming the
maintenance of pH within close tolerance by the human body; therefore any errors due to pH variation amongst the samples
were eliminated. Sensitivity of the ISFET with/without crown ether coating on the gate towards sodium ions (0.34
mV/mg/L) has been found to be comparatively less than that of the crown ether ISFET for potassium, showing that sodium
ions in blood serum do not interfere with the measurements.

IPC Code: C12, C25, G01, H01L21/335

Potentiometric ion sensors based on ion-sensitive field-effect transistors (ISFETs) are attracting
increasing attention. The interest in development of sensors based on ion- sensitive field-effect transistors
is primarily due to their potentialities: their miniaturization benefit, high sensitivity, robustness,
fast response time, low output impedance, multisensing implementations, compatibility with
integrated circuit technology, and suitability for large-scale production at a low unit cost1-3. All these
advantages make the ISFET device appropriate for micro-total analysis system, lab-on-chip and
electronic tongue applications.

For the ISFET, the metal connection of the reference electrode acts as a remote gate. The equation giving the dependence of threshold voltage on the pH of the solution in contact with the
gate is2

\[ V_{Th(ISFET)} = E_{ref} - \Phi_{Si} - \psi + \chi - \frac{Q_s}{C_d} + 2|\phi_p| \]

\[ + \frac{1}{C_d} \sqrt{2\varepsilon_0 \varepsilon_s q N_A (2|\phi_p|)} \]  

\[ \text{... (1)} \]

where \( E_{ref} \) is the constant reference electrode potential, \( \Phi_{Si} \) is the silicon work function, \( \chi \) is surface
dipole potential of the solvent and \( \psi \) is the interfacial electrostatic potential at the solution/dielectric
interface whose sensitivity to changes in bulk pH is expressed by the equation4

\[ \frac{\partial \psi}{\partial \rho H} = -2.303 \left( \frac{RT}{F} \right) \alpha \]

\[ \text{... (2)} \]

\( R \) is universal gas constant, \( T \) is absolute temperature, \( F \) is Faraday’s constant and \( \alpha \) is a dimensionless
sensitivity parameter (0<\( \alpha <1 \)), given by4

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where \( \alpha = \frac{1}{\left(\frac{2.303kT/C}{q^2\beta}\right)+1} \) ... (3)

\( k \) is Boltzmann constant, \( T \) is temperature in Kelvin scale, \( C \) is the differential double layer capacitance at the insulator-electrolyte interface, \( q \) is electronic charge and \( \beta \) is surface proton buffer capacity determining the ability of the gate dielectric surface to absorb or release protons.

As the bare silicon dioxide-silicon nitride, Al2O3, or Ta2O5 gate ISFET is a pH sensor3,5, for detecting a particular ion, its gate has to be modified with a sensing membrane containing an ionophore that specifically selects the ion in presence of other ions present in the solution. The ionophores are molecules that bind to a particular ion to allow specific ions to move across a membrane. An ISFET in which the gate insulator is covered with an ion-selective membrane is known as a Membrane FET or MEMFET. The objective of the present work is MEMFET development for K⁺ ion measurement in blood serum.

K⁺ ion is a fundamental ion in the human body6 with well-documented physiological and clinical roles. ISFETs are used for potassium ion measurement in medical applications such as for on-line monitoring of K⁺ ion concentration during heart surgery; this helps in the detection of myocardial ischemia. Besides their medical use, potassium ISFETs also find wide applications in agriculture and biotechnology7-11. To make the ISFET selective towards alkali metal ions, especially potassium, the gate is coated with ionophoric layers like crown ether layer12,13. The ionophore can recognize and bind the desired ion. Complex formations of the ionophore with the ion introduce a charge on the gate thereby altering the gate potential. However, a systematic study of the ISFET characteristics without and with the ionophoric layer is lacking.

In this paper, the fabrication of the ISFET structure has been described. The results of experimental characterization of the ion-sensitive field-effect transistors, with and without the crown ether layer, as a function of K⁺ ion concentration have been presented, and interpretation of the increase in potassium sensitivity of ISFET device by crown ether membrane has been offered. The developed potassium MEMFET has been applied to estimation of potassium ion concentrations in human blood serum. As the blood serum samples had a narrow spread of pH range, influence of pH variations on the measurements was insignificant. Cross-sensitivity of crown ether membrane FET towards sodium ions has also been investigated and found to exert negligible effect.

**Experimental Procedure**

**ISFET device fabrication and packaging**

The developed dual dielectric \( \text{SiO}_2-\text{Si}_3\text{N}_4 \) gate ISFET (Fig. 1) has a channel length \( L = 12 \) microns and channel width \( W = 4800 \) microns. The designed threshold voltage of the equivalent metal gate device is 1 V. Threshold voltage measured in \( \text{pH} = 7 \) solution using Ag/AgCl reference electrode at \( V_{DS} = 2 \) V has been found to be \( V_{GS} = 2.4 \) V for \( I_{DS} = 50 \) µA and \( V_{GS} = 2.8 \) V for \( I_{DS} = 100 \) µA.

The device has been fabricated on \( P \)-type Czochralski silicon wafers of resistivity 15-20 \( \Omega \cdot \text{cm} \) (7.4 ×10¹⁴ \( \text{cm}^{-3} \)) and orientation <100>. Fabrication technology of the ISFET, based on the NMOSFET technology comprised the following processing steps: (i) Field oxidation (1100°C, 30 min. dry \( \text{O}_2 \) + 120 min. wet \( \text{O}_2 \) + 30 min. dry \( \text{O}_2 \)) giving field oxide thickness = 0.9 µm. (ii) First photolithography for source/drain \( N^+ \) diffusion, and oxide etching. (iii) Phosphorous diffusion (1050°C, 30 min.). (iv) Second photolithography for gate window, and oxide etching. (v) Gate oxidation (trichloroethylene ambient), 1000°C, 120 min, dry \( \text{O}_2 \), oxygen flow rate 2 L/min, a little TCE vapour was carried down the tube by a slow bleed of \( \text{N}_2 \) through TCE bubbler at 25°C, \( t_{ox}=140 \) nm. (vi) Nitridation (LPCVD), 780°C, 25 min, initial pressure = 0.02 torr, deposition pressure of dichlorosilane and ammonia gas mixture = 0.2 torr, dichlorosilane=20 cc, ammonia=200 cc, gas ratio=1:10, \( t_{nitride}=100 \) nm; annealed at 900°C for 30 min in \( \text{N}_2 \). (vii) Third

![Fig. 1 — Schematic cross-section of potassium ISFET.](image-url)
photolithography for contact holes, and dielectric etching. (viii) Sputtering of chromium (50 nm) and gold (500 nm). (ix) Fourth photolithography for metal pattern delineation, and metal etching. (x) Metal sintering, (xi) Wafer scribing, chip sorting and mounting on ceramic substrate. (xii) Wire bonding. (xiii) Protecting the metal pads and wires by insulating epoxy (Epotek H 70E/H74, cured at 120°C, 30 min.) with soldering pads protected by RTV compound. The gate region has been left exposed.

**Deposition of crown ether membrane on the ISFET gate**

Dibenzo 18-crown-6 (DB18C6) ether layer has been deposited on the ISFET gate. A small quantity = 200-500 mg of crown ether was dissolved in chloroform solvent forming a paste. The paste was coated as a monolayer on the gate. On exposure to air at room temperature, chloroform immediately evaporated leaving behind the ionophore.

**Measurement procedure**

Measurements have been carried out using an in-house assembled signal conditioning circuit for direct reading of pH\(^5\)\(^,\)\(^14\). Figure 2 shows the block diagram of the measurement circuit. An Ag/AgCl reference electrode has been used. This circuit gives an output voltage equal to the pH of the solution in which the ISFET is immersed. The circuit including the ISFET has an overall voltage gain of 20. It comprises three amplifier stages, one AD8627 JFET amplifier and two BJT 741 operational amplifiers. The first stage is an impedance matching stage, the second stage is the buffer stage and the third stage is for signal gain. The circuit has been calibrated by immersing the ISFET in standard buffer solutions of known pH and adjusting the output voltage. Satisfactory performance has been achieved from pH = 4 to 10. Detailed operation of the circuit has been reported earlier\(^5\)\(^,\)\(^14\). The measurements have been performed before and after crown ether layer deposition.

**Results and Discussion**

**Characterization of ISFET without and with crown ether layer in the blood serum range**

Figure 3 shows the measured calibration characteristics of the ISFET with respect to KCl concentration from 100 mg/L to 400 mg/L, the range of interest for human blood serum analysis. The amplified output potential from the signal conditioning circuit for direct readout of pH by the ISFET\(^14\) has been plotted in increasing order of potential with respect to concentration, taking zero potential at the lowest concentration. On coating the crown ether layer, the slope of the curve increased indicating that this layer improved the sensitivity of the device. A comparison of the gradients of the curves before and after crown ether coating has been made. It has been observed that average potassium sensitivity of amplified potential without crown ether was 1 mV/mg/L and that with crown ether was 2.3 mV/mg/L, in the range of interest for blood serum analysis. Thus sensitivity of ISFET with crown ether was 2.3 times that without crown ether.

When the potentials in Fig. 3 were plotted with respect to logarithm of concentrations in mol/L, the resulting plots were linear, as shown in Fig. 4. On dividing the slopes by the circuit gain (20), it has been estimated that the potassium sensitivity of the potential created on the gate without crown ether was 36.7 mV/decade of potassium concentration, which

![Fig. 3 — ISFET response characteristics (a) without and (b) with crown ether layer on the gate; measurements have been carried out in standard KCl solutions with concentrations in human blood serum range.](image-url)
rose to 52.8 mV/decade after crown ether coating. These plots signify that the potassium sensitivity of ISFET increases and approaches towards the ideal Nernstian response (59.2 mV/decade) on applying the crown ether film. According to the Nernst equation\textsuperscript{15}, which is the fundamental principle of all potentiometric transducers, potential changes are logarithmically proportional to the specific ion activity. The Nernst equation is written as\textsuperscript{15}

\[
E = E^0 - \left( \frac{RT}{nF} \right) \ln \left( \frac{a_{\text{red}}}{a_{\text{ox}}} \right) \quad \ldots (4)
\]

where \( E \) is the electrode potential, \( E^0 \) is the standard electrode potential, \( R \) is the universal gas constant, \( T \) is the temperature in Kelvin, \( F \) is the Faraday constant, \( n \) is the number of electrons transferred in the half-reaction, \( a_{\text{red}} \) and \( a_{\text{ox}} \) are the activities of the reduced and oxidized species respectively. Combining the numerical values of constants at 25°C (298.15 K) and converting from base \( e \) to base 10 logarithm, gives the simpler form of the Nernst equation for a solution at this standard temperature:

\[
E = E^0 - \left( \frac{0.05915}{n} \right) \log \left( \frac{[C_{\text{red}}]}{[C_{\text{ox}}]} \right) \quad \ldots (5)
\]

where \([C_{\text{red}}]\) and \([C_{\text{ox}}]\) are the concentrations of reducing and oxidizing species respectively. For the univalent potassium ion, \( n=1 \) and therefore the slope of \( E \) versus \( \log ([C_{\text{red}}]/[C_{\text{ox}}]) \) plot can achieve a maximum value of \( 0.05915/1 \) V or \( \sim 59.2 \) mV/decade in the ideal case represented by the Nernst equation.

**Mechanism of improvement of potassium sensitivity of the ISFET by crown ether layer**

Crown ethers, discovered in the 1960’s by C J Pedersen\textsuperscript{16-18} are macrocyclic polyethers. Structurally, they are cyclic polyethers derived from repeating \( \text{–OCHOCH}_2\text{CHCH}_2\text{–} \) units. The structure contains hydrogen, carbon and oxygen atoms. Each oxygen atom is confined between two carbon atoms and exhibits a conformation with a hole (accordingly called "crown"). The main characteristic of crown ethers is the complexation of oxygen atoms with various ionic species. If metallic elements pass through the centre of the hole, they fasten to oxygen atoms forming stable complexes. The crown compound is then termed "host-guest" chemistry with crown ether acting as the "host" taking ionic species as its "guest".

The cavities and clefts in the crown ether are complementary to the size and charge of potassium ion (Fig. 5). Due to the accurate matching of the size of the cavity of crown ether and the ionic diameter of \( K^+ \), very selective interactions are obtained. Dibenzo-18-crown-6 used here has a circular cavity of diameter 2.6-3.2 Å, which fits the exact size of potassium ion of 2.66 Å and makes it an exceptional choice to be a sensing material for potassium ions. These interactions are responsible for the superior potassium ion sensitivity of ISFET with crown ether.

It must be further noted that the method by which the ionophore was made accessible on the ISFET gate was comparatively easier than the other methods practiced earlier. It did not involve any complicated procedure or use of polymers, expensive solvents and complicated steps to make the ionophore as a working electrode. The response time of ionophore was 30-60 s and the shelf life of the sensor either on use or idle was found to be a three months period. The gate surface can be recoated and used. The method is economically very promising because each fresh monolayer requires < 500 µg of the crown ether.

![Fig. 4 — Plots of the potentials relative to logarithm of potassium ion concentration obtained from the ISFET characteristics shown in Fig. 3](image)
Procedure for the study of potassium ion concentration in blood serum samples

The given 1 mL blood serum samples have been diluted to 50 mL because the 1 mL solution was insufficient for dipping ISFET along with the reference electrode. First the pH of the samples was measured using ISFET without crown ether. The pH was found to be ~ 7 as shown in Fig. 6. The pH level in the human body is strictly regulated\(^1\), and the normal range is 7.36-7.44. The drop in pH was caused by the dilution of the samples with water (pH = 7). The trivial variation of pH amongst the samples eliminates any possibility of errors due to pH variation.

As observed from the steep bottom portion of the curve in Fig. 3, the ISFET sensitivity increases with decrease in concentration. Therefore, the ISFET was recalibrated in the lower concentration range. The calibration characteristics in this range are plotted in Fig. 7. As expected, the average potassium sensitivity of amplified potential without crown ether was 82.2 mV/mg/L; this was 82.2 times the sensitivity without crown ether in Fig. 3. Sensitivity with crown ether was 144.4 mV/mg/L, which was 62.8 fold the sensitivity with crown ether in Fig. 3. Also, in the new concentration range, sensitivity with crown ether was 1.76 times that without crown ether. The Nernst plots for the characteristics given in Fig. 7 are shown in Fig. 8, and their slopes are also indicated. Inspection of the Nernst plots in Figs 4 and 8 clearly reveals that the device sensitivity increases appreciably at lower potassium concentrations.

Now the crown ether-ISFET was successively immersed in different blood serum samples and the output potential was recorded. This data is shown in Fig. 9. When blood serum samples were tested for potassium concentration using ISFET, they showed a distribution of potentials about a mean value, which varied according as whether the samples were fresh or stored or they were subjected to any chemical treatment. The graph shows that the maximum number of patients (here 8) gives a particular output voltage (5.6 V), corresponding to a specific value of potassium ion concentration. Further, the K\(^+\) ion concentration of the remaining patients is distributed about this value.

As the ISFET calibration has been done in KCl standard solutions, the potassium ion concentration in the sample giving output voltage of 5.6 V has been measured by atomic absorption spectroscopy. The

Fig. 6 — Study of pH distribution of blood serum samples.

Fig. 7 — ISFET response characteristics at very low KCl concentrations (a) without and (b) with crown ether layer on the gate.

Fig. 8 — Semi-logarithmic plots for the ISFET characteristics of Fig. 7.

Fig. 9 — Distribution of K\(^+\) ion concentration of blood samples in the range of 0 to 10 mg/L.
mean value obtained for this sample by atomic absorption spectroscopy was found to be 151.25 mg/L. Taking this mean value, the K⁺ ion concentrations for the different patients have been determined in accordance with the shift in their output potential relative to 5.6 V, employing the ISFET sensitivity of amplified potential with crown ether (340 mV/mg/L in the concentration range of interest here, i.e., between 3 to 4 mg/L corresponding to 50 times dilution of samples), and upscaling the difference in concentration with respect to mean value by a factor of 50. In Fig. 10, the distribution of potassium ion concentration in human blood serum is shown. This agreed with potassium ion concentration in human blood: 3.5-5.1 mmol/L (137 to 200 mg/L)\textsuperscript{19,20}.

Investigation of cross-sensitivity of ISFET towards sodium ions

To check the influence of sodium ion in blood serum, the calibration characteristics of ISFETs with and without crown ether were measured over the concentration range covering the range of interest for blood serum analysis: 133-144 mmol/L = 3072.3-3326.4 mg/L = 307.2-332.6 mg/dL (Fig. 11). The sodium sensitivity of ISFETs with/without crown ether was found to be in the range of 3.42-3.48 mV/mg/dL ~ 0.34 mV/mg/L, which being much less than potassium sensitivity of crown ether ISFET (340 mV/mg/L), did not vitiate the measurements.

The sensitivities of ISFET with/without the crown ether layer on the gate towards potassium and sodium ions in various concentration ranges studied are compiled in Table 1.

![Fig. 9 — Distribution of ISFET potential with number of patients.](image1)

![Fig. 10 — Distribution of potassium ion concentration with the number of patients.](image2)

![Fig. 11 — Plots of potential versus NaCl concentration for](image3)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ion studied</th>
<th>Concentration range examined</th>
<th>Average sensitivity of readout circuit without crown ether (mV/mg/L)</th>
<th>Gate potential sensitivity from the Nernst plot (mV/decade)</th>
<th>Average sensitivity of readout circuit with crown ether (mV/mg/L)</th>
<th>Gate potential sensitivity from the Nernst plot (mV/decade)</th>
</tr>
</thead>
<tbody>
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<td>Potassium</td>
<td>100-400 mg/L</td>
<td>1</td>
<td>36.7</td>
<td>2.3</td>
<td>52.8</td>
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<tr>
<td>2</td>
<td>Sodium</td>
<td>10-1000 mg/dL</td>
<td>82.2</td>
<td>41.67</td>
<td>144.4</td>
<td>56</td>
</tr>
</tbody>
</table>

Notes: (i) Average sensitivity of readout circuit is the amplified potential sensitivity.

(ii) Gate potential sensitivity has been obtained by dividing the amplified potential sensitivity by circuit gain, i.e., 20.
ISFETs, without and with crown ether layer on the gate.

**Conclusions**

It has been demonstrated that the potassium ion sensitivity of ISFET is significantly increased on coating the gate insulator with crown ether. The device has been applied to potassium concentration measurements and the distribution of potassium ion in human blood serum samples has been estimated using the ISFET sensitivity determined from KCl standard solutions and the mean value of the reference potential sample obtained from atomic absorption spectroscopy. For routine practice, calibration of the ISFET in a standard blood serum sample of known K⁺ ion concentration will be helpful in setting a reference point for such measurements. Sensitivity of crown ether ISFET for sodium ion was shown to be negligibly small.

**Acknowledgements**

This work has been performed under the CSIR network project on MEMS and Sensors. The authors wish to thank the Director, CEERI, and semiconductor fabrication facility members. They are also thankful to Mr A Kumar, Mr G R Naik, Mr G S Negi and Mr D K Agrawal.

**References**