Advances in chemical sensors, biosensors and microsystems based on ion-sensitive field-effect transistor

V K Khanna*
MEMS and Microsensors, Central Electronics Engineering Research Institute, Pilani 333 031
*Email: vkk@ceeri.ernet.in, vkk_ceeri@yahoo.com
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Introduced as a tool for electrophysiology three and a half decades ago, the ion-sensitive field-effect transistor (ISFET) is a generic electrochemical structure offering a family of analytical devices for applications in agriculture, food processing, medical diagnostics, pharmaceuticals, paper, pulp and chemical industries, and environmental control. Besides its use as a chemical sensor, this device has also served as the electrical transduction element for many enzyme and immunological biosensors. This paper presents the current status of this microelectronic device in terms of the theoretical understanding of its operational mechanism, the techniques used for its fabrication and the measurement circuits used in instrumentation. The state of the art of ISFET as a chemical and biosensor is examined, major problems in this area are discussed, perspectives of recent interesting developments are described and future research directions are indicated. Highlights of the work done on ISFET at our institute are presented.

Keywords: ISFET, Chemical sensor, Biosensor, Microsystem, Analytical instrumentation.

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1 Introduction
During the past few decades, dramatic progress has been experienced in the field of chemical and biosensors. The starting point of this progress was the introduction of ion-sensitive field-effect transistor (ISFET) by Bergveld\textsuperscript{1,2} for neurophysiological measurements in 1970. The ISFET is a metal-oxide-semiconductor field-effect transistor (MOSFET) with the metal gate replaced by the electrolyte and a reference electrode (Fig. 1). Interesting landmarks\textsuperscript{3} achieved by ISFET technology are the development of nanoscale\textsuperscript{4} ISFETs and ISFETs using macroporous silicon\textsuperscript{5} to enhance the sensitivity.

The ISFET offers advantages of small size suited for \textit{in-vivo} analysis such as for \textmu-TAS (micro-total analytical system), laboratory-on-chip, electronic tongues, etc; low cost; instant response (30-50 s); and high input impedance with low output impedance. Environmental monitoring, biomedical analysis, healthcare, food processing, drugs, pharmaceuticals and chemical industries, etc., are attractive application areas of ISFET. This paper provides a comprehensive up-to-date review of ISFET in terms of theory of operation, ISFET-based chemical and biosensors and microsystems, research areas and future outlook. Research work done on ISFET at our institute is briefly described.

2 Physico-chemical and Biological Mechanisms of Operation
2.1 Chemical sensors
When the gate of the ISFET is immersed in the solution whose pH is to be measured (Fig. 2), ions are adsorbed at the gate dielectric/solution interface\textsuperscript{6-14}. Discrete amphoteric surface sites of the form Si-OH
are assumed to be present on the dielectric surface, acting both as proton acceptors and donors. According to the site dissociation model, the SiOH group can be protonated/deprotonated as:

\[
\text{SiOH} + \text{H}^+ \Leftrightarrow \text{SiOH}_2^+ \quad \text{... (1)}
\]

\[
\text{SiOH} \Leftrightarrow \text{SiO}^- + \text{H}^+ \quad \text{... (2)}
\]

In case of silicon nitride, the surface binding sites are SiOH and SiNH$_2$ (primary amine) groups. The SiNH$_2$ groups are protonated as:

\[
\text{SiNH}_2 + \text{H}^+ \Leftrightarrow \text{SiNH}_3 \quad \text{... (3)}
\]

The adsorbed ions, protons (in acidic solution) or hydroxyl ions (in alkaline solution), attract ions of opposite polarity to ensure charge balance. Thus, an electrical double layer, the Helmholtz double layer, is set-up at the interface. Fig. 2 shows the origin of the double layer in HNO$_3$ solution. The double layer is associated with an interfacial potential $\psi$ at the solution/dielectric interface, which forms a component of the threshold voltage of ISFET:

\[
\psi = 2.303 \left( \frac{kT}{q} \right) \frac{\beta}{\beta + 1} (pH_{\text{pec}} - pH) \quad \text{... (4)}
\]

The symbol $pH_{\text{pec}}$, referred to as the $pH$ value at the point of zero charge, is the value of $pH$ at which the dielectric surface is electrically neutral, and $\beta$ is a sensitivity parameter. The change in threshold voltage of the device in accordance with the $pH$ of the solution produces proportional changes in drain-source current. Fig. 3 shows representative $pH$ response characteristics of ISFET. The $pH$ sensitivity depends on the gate dielectric used: 53-55 mV/pH for Si$_3$N$_4$, 54-56 mV/pH for Al$_2$O$_3$ and 56-58 mV/pH for Ta$_2$O$_5$ while ideal response is 59.2 mV/pH according to the Nernst equation.

### 2.2 Biosensors

Biosensors$^{3,15}$ unify the advantages of semiconductor technology with the specificity of recognition provided by biological materials and processes. A biosensor is an analytical device incorporating an intimate combination of a biological sensing element (for recognizing a specific biomolecule through a reaction, adsorption or physico-chemical process) with a traditional physical or chemical transducer (for conversion of the recognition into a usable signal).
3 ISFET-based Chemical Sensors: Membrane FETs (MEMFETs) or Chemical FETs (CHEMFETs)

ISFETs with ion-sensitivity and selectivity to different ionic species are fabricated by deposition/attachment of polymeric plastic membranes containing specific receptor molecules termed ionophores on the gate surface. Typically, these sensors are used in a concentration range between approximately 10^{-5} up to 1 M. Common materials for coating ISFET gate include borosilicate glass for Na\(^+\) ion, valinomycin and crown ethers for K\(^+\) ion, and a phosphoric acid derivative such as p-(1,1,3,3, tetramethylbutyl) phenyl phosphoric acid for Ca\(^{2+}\) ion. Sodium and potassium sensors based on siloprene (silicon rubber polymer) membranes have shown linear responses\(^16\).

Nitrite (NO\(_2^-\)) -selective electrochemical sensor based on field-effect transistor as transducer was described by Wróblewski et al\(^17\). The sensing layer, PVC plasticized membrane, contained uranyl salophen as an anion receptor. Wróblewski et al.\(^18\) presented the design of NO\(_2^-\) - and ClO\(_4^-\) -selective CHEMFETs based on PVC plasticized membrane containing: uranyl salophen and thioamide calix arene derivatives as anion-sensitive receptors. A high nitrate (NO\(_3^-\)) selectivity and almost theoretical response were obtained for CHEMFETs based on o-NPOE/PVC membranes containing 1\% of symmetrical tetradodecylationmonium nitrate. Slightly lower nitrate selectivity of CHEMFETs was obtained using silicon rubber polymer (siloprene) membrane. Lipophilic uranyl salophenes derivatives I and II were used as ionophores in membranes of phosphate-selective CHEMFETs. High selectivity for H\(_2\)PO\(_4^-\) over other anions was obtained for these sensors\(^20\).

The ammonium-sensitive sensors utilize silicon rubber polymer (siloprene) and polysiloxane PSX 851 membranes containing non-actine as an ionophore\(^21\). Humenyuk et al\(^22\) have reported the development of pNH\(_4^+\)-ISFET microsensors for water analysis.

CHEMFET technology based on chemically attached poly(2-hydroxyethyl methacrylate) (polyHEMA) hydrogel between a hydrophobic membrane and the gate oxide layer, allows the design of chemical sensors based on polymeric membranes containing molecular receptors. Using this technology, CHEMFETs selective to K\(^+\), Na\(^+\), Ag\(^+\), some transition metals cations (Pb\(^{2+}\), Cd\(^{2+}\)) and some anions (NO\(_3^-\)) have been fabricated. Pradel et al\(^24\) found that the chalcogenide material, an amorphous thin film obtained by RF sputtering of a composite target Cu/Ge\(_{23}\)Sb\(_{12}\)Se\(_{50}\) glass, showed sensitivity to Cu\(^{2+}\) ions. Cadmium sensors based on chalcogenide glasses have also been fabricated\(^25\).

Urea and thiourea compounds substituted with aminochromene groups exhibit high association constants with oxyanions\(^26\). The ionophore mercuracarborand-3, is useful for making a highly selective sensor for chloride\(^26\).

4 ISFET-based Biosensors: Gate Sensitization for Biosensor Realization

Enzyme-immobilized ISFETs are also called ENFETs\(^27-31\). Matrix entrapment, membrane confinement, covalent binding and adsorption methods are used for immobilization of the enzyme on the ISFET gate. Enzymes are frequently entrapped in polymers, e.g., polyacrylamide hydrogels, polyurethane, etc. Membranes like nafion or polyvinylpyridine extend the dynamic range or increase the selectivity of the layer. Typically, the lifetimes of ENFETs range from a few weeks to a few months.

Notable reported ISFET-based biosensors include those for glucose\(^32-46\), urea\(^47-57\), penicillin\(^58-64\), proteins\(^65-70\), creatinine\(^71-73\), ascorbic aid\(^74\), acetylcholine\(^75-77\), and cyanide\(^78\); these also include immunological biosensors\(^65-66\). ISFET glucose sensor determines the quantity of glucose in a specimen through pH variation:

\[
\begin{align*}
2\text{C}_6\text{H}_1\text{O}_6\text{H}_2\text{O} + 2\text{H}_2\text{O} + 3\text{O}_2 & \rightarrow \beta\text{-D-Glucose Oxidase (GOD)} \rightarrow 2\text{C}_6\text{H}_1\text{O}_6\text{H}_2\text{O} + 4\text{H}_2\text{O}_2 \\
(\beta\text{-D-glucose}) & \rightarrow \text{C}_6\text{H}_{12}\text{O}_7 \\
\text{C}_6\text{H}_{10}\text{O}_6 + \text{H}_2\text{O} & \rightarrow \text{C}_6\text{H}_{12}\text{O}_7 \\
(\text{D-glucono-\(\delta\)-lactone}) & \rightarrow (\text{D-glucolic acid})
\end{align*}
\]

An approach that has aroused considerable interest is the incorporation of nanoparticles\(^46\) such as silica microspheres (or MnO\(_2\) particles\(^45\)), in the enzyme layer. Because of their large specific surface area and high surface free energy, these nanoparticles significantly improve the enzyme immobilization capacity.
For triglyceride, the lipolysis reaction is:

$$\text{Triglyceride} + 3\text{H}_2\text{O} \overset{\text{Lipase}}{\longrightarrow} \text{Glycerol} + 3\text{Fatty acids} \quad \ldots \ (6)$$

The production of fatty acids results in decrease in pH due to H\(^+\) ion liberation.

Urea is hydrolyzed in the presence of enzyme urease into ammonium and bicarbonate ions according to the reaction:

$$\text{NH}_2\text{CONH}_2 + 2\text{H}_2\text{O} + \text{H}^+ \overset{\text{Urease}}{\longrightarrow} 2\text{NH}_4^+ + \text{HCO}_3^- \quad \ldots \ (7)$$

Consumption of H\(^+\) ions from the solution results in an increase in its pH.

Creatinine hydrolysis is described by the equation:

$$\text{Creatinine} + \text{H}_2\text{O} \overset{\text{Creatinine deiminase (CD-ase)}}{\longrightarrow} \text{N-methylhydantoin} + \text{NH}_3 \quad \ldots \ (8)$$

Because of the alkaline chemical species (ammonia), there is an increase in pH.

Similarly, acetylcholine is hydrolyzed in the presence of the enzyme acetylcholine esterase (AcChE)

$$\text{Cl}^- \quad \text{CH}_3\text{CO(CH}_2)_2\text{N}^+(\text{CH}_3)_3 + 2\text{H}_2\text{O} \overset{\text{AcChE}}{\longrightarrow} \text{Cl}^- \quad \text{CH}_3\text{COO}^- + \text{3H}^+ + \text{HO(CH}_2)_2\text{N}^+(\text{CH}_3)_3 \quad \ldots \ (9)$$

5 Fabrication Technology and Instrumentation of ISFET

Commonly employed geometrical layouts of the ISFET chip are shown in Fig. 4. The ISFET is fabricated\(^{86-85}\) on the p-type silicon wafers; generally the ratio of channel width and length is high ~ 20-50. Typical ISFET fabrication process follows standard NMOS/CMOS technology. Packaging is done by epoxy to protect the wire bonds and contact regions.

Several attempts have been made to fabricate small-size solid-state reference electrodes\(^{86-94}\) such as by employing a thin film Ti/Pd/Ag/AgCl multilayered structure in which the Ti layer improves the adhesion to the surface of the FET and the Pd layer protects the Ti layer from oxidation and corrosion effects. To improve the endurance of this electrode against the effects of test solutions and provide a stable and constant reference potential irrespective of Cl\(^-\) ion concentration changes, an agarose-supported KCl-gel membrane\(^{94}\) bridging ionically between the reference electrolyte and test solution is employed as a protection of the electrode from the solution.

The ISFET is usually operated in the constant drain current mode\(^{95-100}\) (Fig. 5a). An ISFET readout circuit is a feedback circuit based on the source and drain follower to maintain the drain voltage constant by varying the gate voltage applied through a reference electrode. The operational amplifier maintains the voltage difference between its inverting and non-inverting inputs at zero volts, i.e., \(V_C = V\). Hence, \(V_{R2} = V_{R3}\) and \(V_{DS} = V_{R1}\). Being part of constant voltage \(V_{\text{ref}}\), \(V_{R1}\) is constant and so is \(V_{DS}\). Therefore, drain-source current \(I_{DS} = V_{R3}/R_3\) is constant because \(V_{R3} = V_{R2}\) does not vary being the other part of \(V_{\text{ref}}\). Thus \(V_{GS}\) is maintained at a fixed value by the automatic adaptation of source voltage with respect to ground to any change in the threshold voltage of ISFET. The overall result is that changes in the interfacial potential are reflected in the output voltage \(V_{out}\). Therefore, the sensitivity of an ISFET is usually
expressed as the gate voltage change per decade of the hydrogen ion concentration \( p_H \). A maximum Nernstian sensitivity of 59.2 mV/decade can be obtained. Typical operating voltages/currents and specifications of ISFETs are: applied drain-source voltage \( V_{DS} = 0.5-2V \), Drain-source current \( I_{DS} = 100 \mu A \) to 1 mA, transconductance > 0.5 mA/V and sensitivity ≥ 55 mV/pH.

To avoid the use of reference electrode like Ag/AgCl electrode, a differential measurement set-up (Fig. 5b) is used in which the ISFET response is determined with respect to an identical reference field-effect transistor (REFET), which does not respond to the ionic concentration being measured. A Pt strip fabricated on the ISFET chip grounds the analyte potential; this electrode is the, pseudo-reference, or ‘quasi-reference’ electrode. The difference between the output voltages of the transistor with sensitized gate and the reference FET is proportional to the concentration of the ionic species. Effects of parasitic elements like temperature and light intensity cancel out.

6 Drawbacks of ISFET and Technologies of ISFET Production

The main limitations of ISFET are instability and drift\(^{101-107}\). Hydration of silicon dioxide changes its dielectric constant and thickness. The hydration problem is overcome by covering the SiO\(_2\) layer with Si\(_3\)N\(_4\), Ta\(_2\)O\(_5\) or Al\(_2\)O\(_3\). Casans et al\(^{107}\), have compensated hysteresis, thermal and long-term drifts in ISFETs using a proper hardware and qualified virtual instrumentation software. Intermittent sampling as in flow injection analysis restricts the exposure of ISFET to contamination avoiding the drift.

Early ISFET sensors were sub-Nernstian in \( p_H \) response\(^{108}\). This problem has been solved by using the more suitable Ta\(_2\)O\(_5\) along with Al\(_2\)O\(_3\) in place of silicon dioxide or silicon nitride as the gate membrane material. Another major problem was the reliable encapsulation of ISFET sensor chips. Reliable ISFET packaging has been achieved by encapsulation using prefabricated housings, or embedding of chips in a male mould, or by photolithographic structuring and curing, or by scaling around the chemically sensitive gate area with the help of elastomeric materials.

7 ISFET-based Microsystems

Several versions of ISFET-based microsystems are reported in the literature\(^{109-110}\). ISFETs are not convenient for on-line monitoring because of drift problems. Therefore, dynamic methods such as flow-injection analysis are used. The approach most commonly followed is to use a micro flow cell containing the ISFET along with the reference electrode. Fig. 6 shows a block diagram of a generic fluidic microsystem useful for biomedical or clinical on-line monitoring.

An example of ISFET microsystem\(^{110}\) is a die consisting of twelve ISFETs, two temperature sensors, and one conductivity sensor designed to monitor the metabolic activity of cellular populations.

8 Commercial ISFETs and their Typical Applications

ISFET is in the market place\(^{110}\) (e.g. Orion, Orion Research, Boston, MA; Corning, New York, Sentron Integrated Sensor Technology, Roden, The Netherlands). ISFETs are used for non-invasive \( p_H \) measurements in the fields of ophthalmology, dermatology, gynaecology and dentistry. A gastroesophageal tract \( p_H \) sensor\(^{111}\) consists of an H\(^+\) ISFET, an Ag/AgCl reference electrode and a temperature sensor. Multi-sensor silicon needles including two ISFETs for \( p_H \) and potassium ion (using valinomycin ionophore) concentration measurement fabricated by deep reactive ion etching have been reported\(^{112}\) for monitoring myocardial ischemia.

ISFETs have been adapted to the detection of L Acidophilus bacterial activity by developing PDMS (poly-dimethylsiloxane) closed micro-tanks\(^{113}\). A \( p_H/CO_2 \) sensor system using two ion-sensitive field
effect transistors (ISFETs) has been used to evaluate the metabolic activity of cultured cells\textsuperscript{114}.

Butyrylcholinesterase (BuChE) biosensors based on pH ISFETs have been applied to the detection of potato glycoalkaloids\textsuperscript{115}. Glycoalkaloids biosensor is based on the principle:

\[
\text{Butyryl cholinesterase} (C_3H_7COO^- + H_2O) \\
\text{HO} \rightarrow (C_3H_7COO^- + H^+)}
\]

resulting in proton generation. … (10)

9 R & D at CEERI, Pilani

At our laboratory, a high-transconductance ISFET chip with an aspect ratio of 400 has been developed using interdigitated drain-source geometry. The device has been fabricated using NMOS process and packaged on a PCB strip with the wire bonds and pads protected with insulating epoxy. The set-up for ISFET characterization (Fig. 1) contains two power supplies, viz., drain-source and gate-source supplies $V_{DS}$, $V_{GS}$ for ISFET biasing. After immersing the ISFET along with the reference electrode in a given analyte, the proper biasing conditions are established and the drain-source current $I_{DS}$ is measured. If now the device is withdrawn from this analyte and dipped in a different analyte after cleaning, the drain-source current changes. This current is restored to its previous value by adjustment of gate-source voltage $V_{GS}$. The change of gate-source voltage $\Delta V_{GS}$ corresponds to the pH difference between the two analytes. The pH sensitivity factor of the device has been measured as 53 mV/pH using standard pH=4, 7 and 10 buffer solutions. Features/specifications of the developed sensor are:

(i) A high aspect ratio (channel width/length) ~ 400; (ii) silicon nitride sensing layer; (iii) operating voltages; $V_{DS}$ (drain-source voltage)= 2V; $V_{GS}$ (Gate-source voltage applied through Ag/AgCl reference electrode) = 1.5V; (iv) response time < 1 s.

Signal conditioning circuit for direct readout of pH has been developed leading to development of pHmeter. The circuit comprises three amplifier stages: an impedance matching stage, a buffer stage and a stage providing signal gain. The first stage is a JFET operational amplifier while the second and third stages are BJT operational amplifiers. The overall result is the production of an output voltage equivalent to the pH of the analyte, thus displaying the pH digitally. Gate functionalization of ISFET has been carried out and detailed characterization has been done for potassium and calcium ion sensors as well as biosensors for glucose, triglyceride and cholesterol under the CSIR network program in collaboration with participating laboratories. Fig. 7 shows the glucose response characteristics of the sensor. For fabrication of this sensor, glucose oxidase enzyme has been immobilized on the gate dielectric using bovine serum albumin and glutaraldehyde. Some of the work done at CEERI is reported in references\textsuperscript{116-120}.

10 Summary and Conclusions

The ISFET has established itself as a replacement of the bulky ion-sensitive and enzyme electrodes\textsuperscript{121} of chemical and bio-instrumentation. Impedance transformation property and simplicity of readout electronics have contributed to its popularity. Although extensive work has been carried out worldwide on various aspects of ISFET, much remains to be done regarding proper storage, handling, cleaning and reusage, drift and instability, etc., for biomedical applications. Then only the potential of biosensors allowing easy use by non-specialist personnel for accurate measurements can be
gainfully exploited. Low-priced, disposable devices appear to be the remedy to the ISFET problems.

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References

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