

## ISFET, Theory and Practice

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### Introduction

Biomedical engineers exploited as the first the possibilities of the chip technology to develop silicon based sensors, to be incorporated in the tip of a catheter (since about 1970). This technology should provide the clinicians with cheap sensors in analogy with the electronic chips, which would become continuously cheaper and cheaper, even with improved characteristics. Moreover the reproducibility of sensor characteristics should be highly improved compared to the usually piecewise-assembled sensors existing up to that time, due to the replication procedure on which the silicon technology relies. Therefore many of the first papers on silicon sensors appeared in the biomedical engineering literature, for instance with respect to the development of ion sensors. The ISFET sensor is one of the most well-known examples.

In order to be able to judge the value of ISFETs and to understand the operational mechanism, it makes sense to first elucidate the basics of potentiometric sensors in general, of which the pH glass-membrane electrode is the most well-known example.

### Ion sensors for chemical analysis

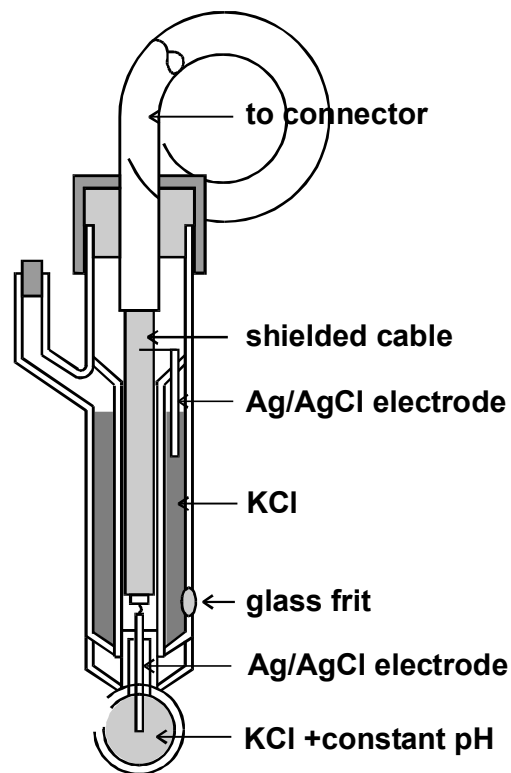
Most ion sensors are potentiometric sensors, which means that the electrical potential difference,  $\Delta\phi$ , at a solid/liquid interface as function of the ion concentration to be determined is measured. This is always according the Nernst equation:

$$\Delta\phi = \frac{RT}{F} \ln a_{i1}/a_{i2} \quad (1)$$

Here R is the gas constant, T the absolute temperature (K) and F the Faraday constant. The sensitivity  $RT/F$  is the same factor as  $kT/q$  as known from solid state physics. Ion concentrations  $c_i$  are noted in terms of activities  $a_i = f_i \cdot c_i$ , with  $f_i$  being the activity coefficient. In diluted electrolytes  $f_i = 1$ . This equation shows that, providing that at one side of the interface the activity  $a_i$  of the ion of interest is kept constant, the electrode potential is a direct logarithmic function of the ion activity on the other side. Thus, for instance, a metal electrode in its "own solution", for instance copper in a copper sulphate solution, will result in a stable defined potential, provided that no interference reaction will occur. In practice this type of ion sensor will hardly appear, especially not in biomedical measurements. A well-known electrode makes use of a membrane of conducting glass, which buffers the ion of interest in a thin surface layer of the membrane. pH, pNa, pK etc. sensitive glass compositions have been developed, which are shaped in a bulb size and melted to a glass shaft. The internal volume of the bulb with its shaft is filled with a liquid of known, constant composition. This means that now in fact a cell is constructed with a constant potential drop at the inner surface of the glass membrane and a "sense potential" at its outer surface, both according to the Nernst equation. This can only be measured by contacting both, the internal

solution as well as the external solution in which an ion concentration has to be measured, by reference electrodes. A reference electrode is in fact nothing else than a contact between a metal wire and an aqueous solution, to determine the electrical potential of this solution. In practice a reference electrode consists of a chloridated silver wire (silver with a coating of insoluble silver chloride) in a potassium chloride solution with a constant concentration. The electrochemical couple thus formed results in a constant potential according to the Nernst equation. The inner solution of a reference electrode makes contact with the solution of which the electrical potential has to be measured by means of a barrier, the so-called frit. Often glass membrane electrodes are combined with reference electrodes to one system.

Various performances of such rather bulky electrode assemblies, both separate pH glass electrodes and reference electrodes, as well as combined systems, are in daily use in laboratories of chemical analysis, process industry etc. A typical performance of a combined pH glass electrode is shown in figure 1.



**Fig.1**

*Cross sectional view of combined pH electrode.*

Note that for this type of potentiometric sensors it is essential that the sense material (the membrane) is conducting, because the circuit of measurement should be "closed". Insulating materials, which are sensitive to ions, can not be used.

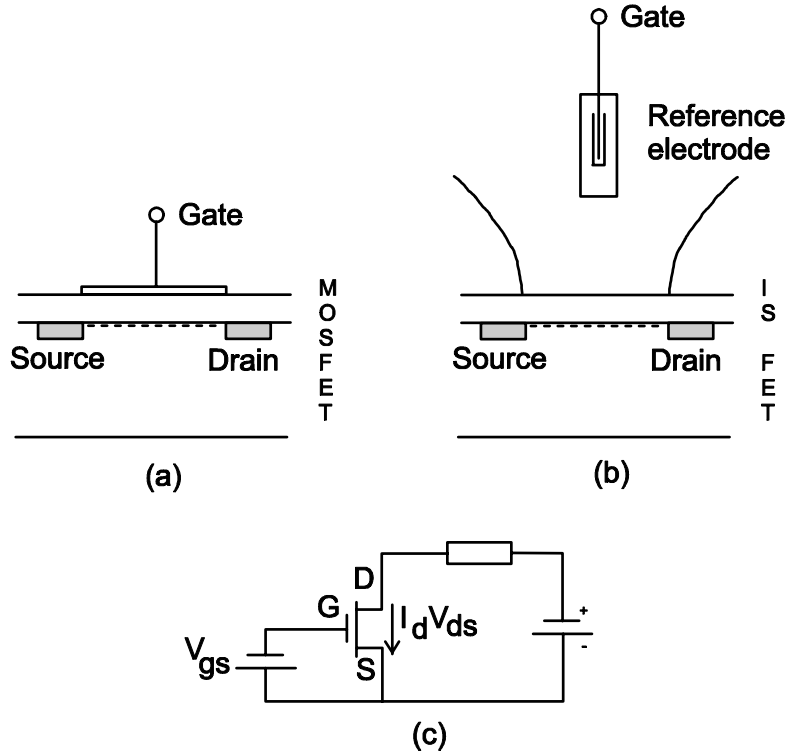
Miniaturized versions of the glass-membrane electrode appear to be less stable and moreover for *in vivo* monitoring these relative fragile electrodes can not be applied. Therefore especially for biomedical applications new concepts had to be developed, of which the ISFET is the first example (research started around 1970 by the author).

### **Ion sensors for biomedical applications**

In the open literature many efforts have been described which are focussed on the conversion of the electrodes as described in the previous section to more rigid devices by applying the glass membranes in a more solid state performance: leaving out the inner solution and replace it by a solid state contact. For instance sputtering the glass directly onto a metal electrode. This idea is a mistake, because the basic recipe should always be to create a cell where all interfaces are thermodynamically well defined and the Nernst equation can be obeyed. In terms of solid state physics: Fermi levels of charged species should be able to become equal throughout the whole system. It is remarkable that solutions to this problem did not come from chemical engineers, but from electrical engineers and physicists, who are most probably more familiar with thermodynamic equilibria, as for instance occurring in electronic components with doped semiconductors, contact potentials etc. Especially the knowledge how to create a stable interface between silicon and silicon dioxide, as in the case of a MOSFET device, is of importance. It couples in a stable way an electric field, penetrating the oxide, to the “electronic world”. In case the outer surface of the oxide is in equilibrium with an ionic solution in contact with the oxide, the resulting interfacial potential will modulate the electric field and thus a perfect and stable contact is provided between the “ionic world” and the “electronic world”. This idea was the first new concept investigated by the author and received the name ISFET technology.

### **The ISFET concept**

The operation of an ISFET can best be described by comparing it with its purely electronic analogue, the MOSFET (Metal Oxide Semiconductor Field Effect Transistor) Figure 2 illustrates the similarities and differences between these two devices.



**Fig. 2**  
 (a) Schematic diagram of a MOSFET; (b) schematic diagram of an ISFET;  
 (c) schematic electrical diagram for both, MOSFET and ISFET.

The metal gate of the MOSFET of figure 2(a) is replaced by the metal of a reference electrode, whilst the liquid in which this electrode is present makes contact with the bare gate insulator (figure 2(b)). Both devices have the same electrical equivalent circuit, which is symbolised in figure 2(c). Mounting of the chips is of course different: a MOSFET can be completely encapsulated, whereas for an ISFET source and drain leads as well as chip edges have to be encapsulated carefully, meanwhile leaving the gate area open for contact with the liquid.

For both devices the following equation is valid for the non-saturated region (below pinch-off):

$$I_d = \beta(V_{gs} - V_T - \frac{1}{2}V_{ds}) \cdot V_{ds} \quad (3)$$

in which  $\beta$  is a parameter determined by the mobility  $\mu$  of the electrons in the inversion layer (assuming an n-channel transistor), the gate insulator capacitance per unit area  $C_{ox}$  and the width to length ratio of the channel  $W/L$ , according to:

$$\beta = \mu C_{ox} W / L. \quad (4)$$

The threshold voltage  $V_T$  is given by:

$$V_T = V_{FB} - \frac{Q_B}{C_{ox}} + 2\phi_F \quad (5)$$

where  $V_{FB}$  is the flatband voltage,  $Q_B$  is the depletion charge in the silicon and  $\phi_F$  the Fermi-potential. The flatband voltage is given by:

$$V_{FB} = \frac{\Phi_M - \Phi_{Si}}{q} - \frac{Q_{ss} + Q_{ox}}{C_{ox}} \quad (6)$$

with  $\Phi_{Si}$  the silicon workfunction,  $\Phi_M$  the workfunction of the gate metal,  $Q_{ss}$  the surface state density at the silicon surface and  $Q_{ox}$  the fixed oxide charge.

From equations 5 and 6 it can be seen that the threshold voltage of a MOSFET is determined by material properties such as the workfunction  $\Phi$  and charge accumulation. For a stable operation of a MOSFET it is of importance that the threshold voltage is constant which can be achieved by applying an appropriate MOS process such as ion implantation. Equation 3 shows that the drain current is now only a function of the gate voltage, using a constant drain-source voltage.

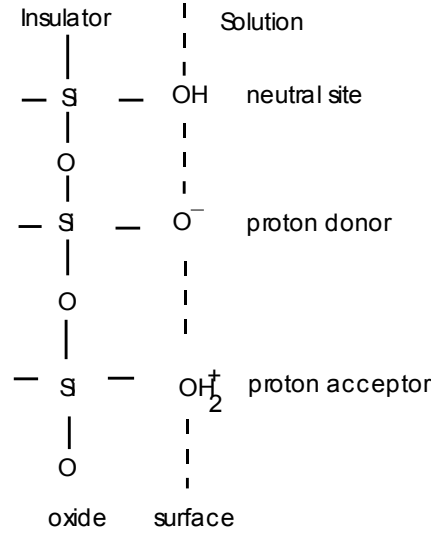
In case of an ISFET the gate voltage is the voltage at the reference electrode, usually 0V (grounded reference electrode), but the threshold voltage contains also terms which reflect the interfaces between the liquid and the gate oxide on the one side and the liquid and the reference electrode at the other side. The latter term is in fact the reference electrode potential relative to vacuum  $E_{ref}$ , which includes  $\Phi_M$ . The interface potential at the gate oxide-electrolyte interface is determined by the surface dipole potential of the solution  $\chi_{sol}$ , which is a constant, and the surface potential  $\psi_o$ , which results from a chemical reaction, usually governed by the dissociation of oxide surface groups. The resulting equation for the flatband voltage of an ISFET is thus given by:

$$V_{FB} = E_{ref} - \Psi_0 + \chi_{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ss} + Q_{ox}}{C_{ox}} \quad (7)$$

Because all terms are constant except  $\psi_o$ , it is this term which makes the ISFET sensitive to the electrolyte pH, which is controlling the dissociation of the oxide surface groups. Designing a pH sensitive ISFET with a maximum sensitivity and selectivity needs therefore a detailed investigation of the oxide-electrolyte interface in order to be able to choose the best oxide, which is not a priori the silicon dioxide as used for MOSFETs.

### The oxide-electrolyte interface

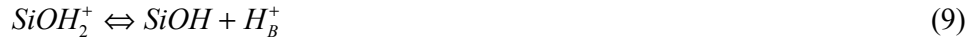
The surface of any metal oxide always contains hydroxyl groups, in the case of silicon dioxide SiOH groups. These groups may donate or accept a proton from the solution, leaving a negatively charged or a positively charged surface group respectively, as schematically represented by figure 3.



**Fig..3**  
Schematic representation of the site-binding model.

In figure 3 is indicated that (equilibrium) reactions can occur between protons in the solution and the hydroxyl groups formed at the SiO<sub>2</sub>-solution interface.

The mechanism responsible for the oxide surface charge can be described by the site-binding model, which describes the equilibrium between the so-called amphoteric SiOH surface sites and the H<sup>+</sup>-ions in the solution. The reactions are



with H<sub>B</sub><sup>+</sup> representing the protons in the bulk of the solution. From these chemical reactions it is clear that an originally neutral surface hydroxyl site can bind a proton from the bulk solution, becoming a positive site as well as donate a proton to the solution, leaving a negative site on the oxide surface. For this reason it is called an amphoteric site.

For these equilibrium reactions, the following set of thermodynamic equations is valid:

$$\mu_{SiOH} = \bar{\mu}_{SiO^-} + \bar{\mu}_{H_B^+} \quad (10)$$

$$\bar{\mu}_{SiOH_2^+} = \mu_{SiOH} + \bar{\mu}_{H_B^+} \quad (11)$$

with  $\mu_i, \bar{\mu}_j$  representing the chemical and electrochemical potential of species i and j, respectively. The electrochemical potentials can be written as

$$\bar{\mu}_{H_B^+} = \mu_{H_B^+} + q\psi_B \quad (12)$$

$$\bar{\mu}_{SiOH_2^+} = \mu_{SiOH_2^+} + q\psi_S \quad (13)$$

$$\bar{\mu}_{SiO^-} = \mu_{SiO^-} - q\psi_S \quad (14)$$

with  $\psi_B$  and  $\psi_S$  the electric potentials in the bulk of the solution and at the oxide surface, respectively. By using the following equation

$$\mu_x = \mu_x^0 + kT \ln a_x \quad (15)$$

where  $\mu_x^0$  is the standard chemical potential of species  $x$  and  $a$  denotes either the dimensionless activity of the bulk protons  $H_b^+$  or the number of sites in moles per unit area of one of the surface groups, in which case  $a_x$  is replaced by  $v_x$ . In combination with eqns. 12-14 and substituting the result in eqns. 10 and 11, we obtain

$$\mu_{SiOH}^0 + kT \ln v_{SiOH} = \mu_{SiO^-}^0 + kT \ln v_{SiO^-} - q\psi_S + \mu_{H_b^+}^0 + kT \ln a_{H_b^+} + q\psi_B \quad (16)$$

$$\mu_{SiOH_2^+}^0 + kT \ln v_{SiOH_2^+} + q\psi_S = \mu_{SiOH}^0 + kT \ln v_{SiOH} + \mu_{H_b^+}^0 + kT \ln a_{H_b^+} + q\psi_B \quad (17)$$

In equation 7,  $\psi_0$  was introduced as the difference between the potential of the oxide surface and the bulk solution:

$$\psi_0 = \psi_S - \psi_B \quad (18)$$

With  $\psi_0$  and the Boltzmann equation, the activity of the bulk protons  $a_{H_b^+}$  can be related to the activity of the protons in the direct vicinity of the oxide surface,  $a_{H_s^+}$ :

$$a_{H_s^+} = a_{H_b^+} \cdot \exp\left(\frac{-q\psi_0}{kT}\right) \quad (19)$$

By the introduction of dimensionless intrinsic dissociation constants

$$K_a = \exp\left(\frac{\mu_{SiOH}^0 - \mu_{SiO^-}^0 - \mu_{H_b^+}^0}{kT}\right) \quad (20)$$

$$K_b = \exp\left(\frac{\mu_{SiOH_2^+}^0 - \mu_{SiOH}^0 - \mu_{H_b^+}^0}{kT}\right) \quad (21)$$

eqns. 16 and 17 can be simplified with eqns. 19-21 to

$$\frac{v_{SiO^-} \cdot a_{H_s^+}}{v_{SiOH}} = K_a \quad \text{and} \quad \frac{v_{SiOH} \cdot a_{H_s^+}}{v_{SiOH_2^+}} = K_b \quad (22)$$

The  $K$ -values are called intrinsic, because they are only valid for the chemical equilibrium at the interface and not for the bulk reaction. As can be concluded from eqns. 20 and 21, these intrinsic dissociation constants are real constants, not depending on the ionisation state of the oxide surface.

On the oxide surface, there is a fixed number of surface sites per unit area,  $N_s$ :

$$N_s = v_{SiOH} + v_{SiO^-} + v_{SiOH_2^+} \quad (23)$$

Depending on the chemical equilibrium of the surface sites, a surface charge density  $\sigma_0$  [C/m<sup>2</sup>] exists:

$$\sigma_0 = q(v_{SiOH_2^+} - v_{SiO^-}) = -qB \quad (24)$$

where B is the number of negatively charged groups minus the number of positively charged groups in moles per unit area. The combination of eqns. 22-24 yields

$$\sigma_0 = qN_s \left( \frac{a_{H_s^+}^2 - K_a K_b}{K_a K_b + K_b a_{H_s^+} + a_{H_s^+}^2} \right) \quad (25)$$

Equation 25 shows the relation between the activity of the protons at the oxide surface  $a_{H_s^+}$  and the surface charge density  $\sigma_0$  in terms of the total number of available sites  $N_s$  and the intrinsic dissociation constants  $K_a$  and  $K_b$ .

The effect of a small change in the surface proton activity  $a_{H_s^+}$  on the surface charge density,  $\sigma_0$ , can be found from eqn. 24:

$$\frac{\partial \sigma_0}{\partial pH_s} = -q \frac{\partial B}{\partial pH_s} = -q\beta_{int} \quad (26)$$

where the change in  $a_{H_s^+}$  is expressed in its corresponding  $pH_s$ , and the resulting change in  $\sigma_0$  in the change in the net number B of basic groups. By definition,  $\partial B/\partial pH_s$  represents the buffer capacity  $\beta_{int}$  of the oxide surface. It is called intrinsic in this case, because it is only capable of buffering small changes in the surface pH ( $pH_s$ ) and not in the bulk pH ( $pH_b$ ), under normal conditions.

For reasons of charge neutrality the surface charge  $\sigma_0$  is balanced by an equal but opposite charge,  $\sigma_{dl}$ , in the electrolyte. The position of this charge density  $\sigma_{dl}$  defines the so-called double layer and the two opposite charges,  $\sigma_0$  and  $\sigma_{dl}$ , parallel to each other form the integral double-layer capacitance  $C_{dl,i}$ .

The potential difference over the capacitance  $C_{dl,i}$  is equal to the difference between the potential of the oxide surface and the bulk solution, as defined in eqn. 18:  $\psi_0 = \psi_s - \psi_b$ . The relation between  $\sigma_0$ ,  $\sigma_{dl}$ ,  $C_{dl,i}$  and  $\psi_0$  is given by

$$\sigma_{dl} = -\sigma_0 = -C_{dl,i} \psi_0 \quad (27)$$



The integral double-layer capacitance  $C_{dl,i}$  can be calculated by using the Gouy-Chapman-Stern model. In this model, the double-layer capacitance consists of a series network of a Helmholtz-layer capacitance (the Stern capacitance) and a diffuse-layer capacitance. The Helmholtz layer models the effect that the ions in the solution have a finite size and the centres of the ions cannot approach the surface any closer than the ionic radius including a possible water layer which means that there exists a plane of closest approach for the centres of the ions at some distance,  $x_H$ . The diffuse layer, starting from  $x_H$ , contains the same amount of charge (of opposite sign) as the oxide surface charge, because the Helmholtz layer is by definition not containing any charge. The charge in the diffuse layer  $\sigma_{dl}$  is given by

$$\sigma_{dl} = -\sqrt{8kT\varepsilon_r\varepsilon_0n^0} \sinh\left(\frac{zq\psi_1}{2kT}\right) \quad (28)$$

where  $\psi_1$  is the potential at  $x_H$ ,  $n^0$  the concentration of each ion in the bulk solution in number/litre, and  $z$  the valence of the ions. The parameters  $\varepsilon_r$ ,  $\varepsilon_0$ ,  $k$ ,  $q$  and  $T$  have their usual meaning.

The difference between the potential  $\psi_1$  at  $x_H$  and the surface potential  $\psi_0$  is the potential difference across the Stern capacitance. The Stern capacitance has a value of  $\varepsilon_r\varepsilon_0/x_H$  [F/m<sup>2</sup>] and is usually assumed to be constant with a value of 20  $\mu\text{F}/\text{cm}^2$  (with  $\varepsilon_r \approx 11$  in the Helmholtz layer, and  $x_H \approx 5$  Å). The potential  $\psi_1$  can therefore be described by

$$\psi_1 = \psi_0 - \frac{\sigma_0}{C_{Stern}} = \psi_0 - \frac{\sigma_0 x_H}{\varepsilon_r \varepsilon_0} \quad (29)$$

With eqns. 28 and 29 it is now possible to calculate the integral double-layer capacitance as a function of  $\psi_0$  and the electrolyte concentration. The ability of the double layer to store charge in response to a small change in the potential  $\partial\sigma_0/\partial\psi_0$  is defined as the differential double-layer capacitance,  $C_{dif}$  and can be calculated using eqns. 28 and 29:

$$\frac{\partial\sigma_0}{\partial\psi_0} = -\frac{\partial\sigma_{dl}}{\partial\psi_0} = C_{dif} \quad (30)$$

For reasons of simplicity, not the expression for  $C_{dif}$  is stated here, but of its inverse, which also clearly shows that this capacitance is made up of two components in series:

$$\frac{1}{C_{dif}} = \frac{\partial\psi_0}{\partial\sigma_0} = \frac{1}{C_{Stern}} + \frac{1}{\sqrt{\frac{2\varepsilon_r\varepsilon_0z^2q^2n^0}{kT}} \cosh\left(\frac{zq\psi_1}{2kT}\right)} \quad (31)$$

Combining eqns. 26 and 30 shows the effect of a small change in the surface pH ( $pH_S$ ) on the change in the surface potential  $\psi_0$ :

$$\frac{\partial \psi_0}{\partial pH_S} = \frac{\partial \psi_0}{\partial \sigma_0} \cdot \frac{\partial \sigma_0}{\partial pH_S} = \frac{-q\beta_{int}}{C_{dif}} \quad (32)$$

Combining eqn. 32 with the Boltzmann equation:

$$a_{H_S^+} = a_{H_B^+} \cdot \exp\left(\frac{-q\psi_0}{kT}\right) \quad \text{or} \quad (pH_S - pH_B) = \frac{q\psi_0}{kT} \quad (33)$$

results in the general expression for the pH sensitivity of an ISFET:

$$\frac{\partial \psi_0}{\partial pH_B} = -2.3 \frac{kT}{q} \cdot \alpha \quad \text{with} \quad (34)$$

$$\alpha = \frac{1}{\frac{2.3kTC_{dif}}{q^2\beta_{int}} + 1} \quad (35)$$

The parameter  $\alpha$  is a dimensionless sensitivity parameter that varies between 0 and 1, depending on the intrinsic buffer capacity,  $\beta_{int}$ , of the oxide surface and the differential double-layer capacitance  $C_{dif}$ . If  $\alpha=1$ , the ISFET has a so-called Nernstian sensitivity of precisely -59.2 mV/pH at 298K, which is also the maximum achievable sensitivity.

It appears that the usual  $SiO_2$  from the MOSFET process does not fulfil the requirements of a high value of  $\beta_{int}$ . The pH sensitivity is only about 30mV/dec depending also on the electrolyte concentration via  $C_{dif}$ . Therefore other layers have been introduced such as  $Si_3N_4$ ,  $Al_2O_3$  and  $Ta_2O_5$  with increased values of  $\beta_{int}$ . The intrinsic buffer capacity of  $Ta_2O_5$  is even so high that the value of  $C_{dif}$  becomes less important which means that independent of the electrolyte concentration a pH sensitivity of 58 mV/dec can be achieved over a pH range from 1 to 12.

### Biomedical applications

The pH ISFET chip is very suitable to be built-in in a catheter as has been done in the period 1975-1985 by the pacemaker company Cordis in Roden, the Netherlands. The reference electrode was made in the tip of the catheter which contained a polyHEMA plug, a KCl gel and a Ag/AgCl electrode. The  $Al_2O_3$  ISFET was mounted behind a side window near the tip of the 6F catheter

Each ISFET was factory tested *in vitro* before sterilisation, and the essential parameters, which will be dealt with below, are stored in a PROM (Programmable Read Only Memory) which is an integral part of the ISFET connector. Experience obtained from animal as well as clinical experiments has shown that the *in vitro*

characterisation is also valid for the *in vivo* use of the devices. Therefore, the data stored in the PROM connector also have an *in vivo* valuation and are used by the floating signal conditioner to convert the ISFET output signal to the blood pH.

The signal conditioner applies the ISFET in the constant drain current mode, with constant drain-source voltage,  $V_{ds}$ , resulting in a gate-source voltage,  $V_{gs}$ , which directly reflects the pH-sensitive interfacial potential at the gate surface.

If no temperature sensitivity and time drift would occur, the equation handled by the signal conditioner can be simply:

$$pH = pH_{cal} + \frac{V_{gs}}{S} \quad (36)$$

where  $pH_{cal}$  is the pH of a calibration liquid at  $37^{\circ}\text{C}(T_{cal})$ ,  $V_{gs}$  the electrical output signal of the ISFET amplifier circuit and  $S$  the pH sensitivity (mV/pH) of the particular ISFET, stored in the memory. Although the ISFET is roughly applied at the temperature-insensitive bias point, it appears that a temperature sensitivity of  $V_{gs}$  as well as  $S$  still occurs, which has to be corrected. Furthermore it appears that, after an initial drift, the time drift of the  $\text{Al}_2\text{O}_3$ -ISFET can be considered to be linear for a certain time. Therefore a linear time drift correction is necessary. This correction (DC in mV/h) as well as the temperature coefficient TC (in mV/ $^{\circ}\text{C}$ ) and the temperature dependence of  $S$ ,  $dS/dT$  (in mV/pH/ $^{\circ}\text{C}$ ), are also determined *in vitro* and stored in the ISFET-PROM connector. These data are used for the exact pH determination by the signal conditioner according to:

$$pH = pH_{cal} + \frac{\Delta V_{gs} + DC\Delta t + (TC - S * 0.0147)\Delta T}{S + \frac{dS}{dT}\Delta T} \quad (37)$$

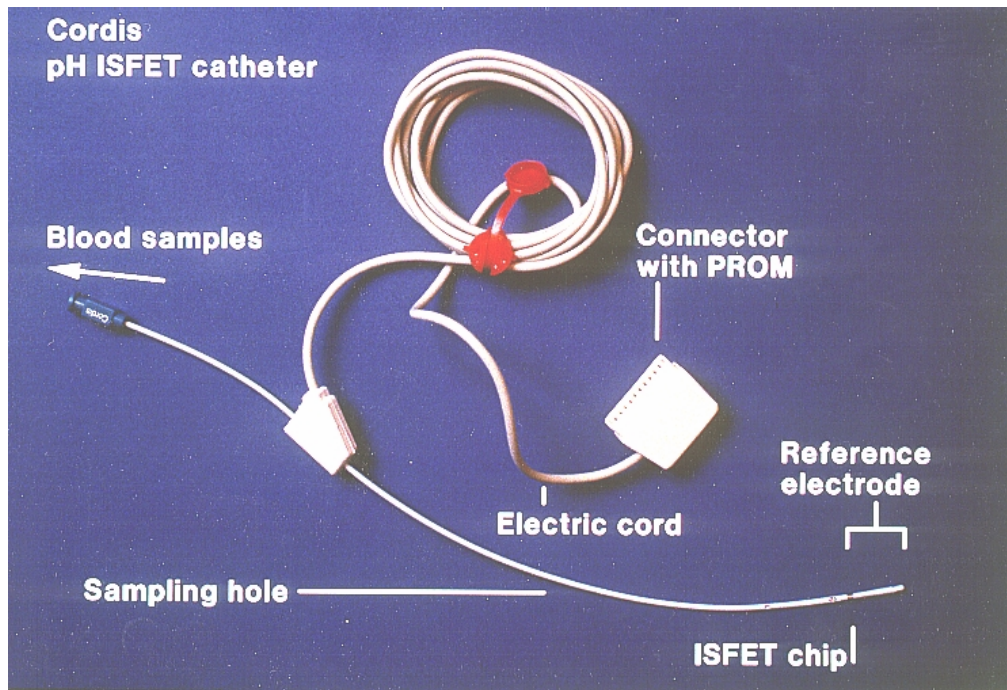
where  $\Delta t$  is the time after calibration with  $pH_{cal}$ , and  $\Delta T$  is the difference of the temperature with respect to  $T_{cal}=37^{\circ}\text{C}$ . In addition a factor  $S*0.0147$ , which is the same for all ISFETs, is taken into account for correction of the temperature sensitivity of the blood pH itself.

In order to measure the actual blood temperature necessary to solve eqn. 37, the ISFET chip contains a temperature-sensitive resistor which is also characterised beforehand; the data from this resistor are also stored in the PROM connector.

Equation 37 expresses the essential procedure as developed and patented by Sentron (formerly Cordis). In this way the present ISFETs can be applied with well-defined accuracy, despite their intrinsic drift phenomenon and temperature dependency. The complete Cordis-ISFET catheter assembly is shown in figure 4.

The sterilised catheters are stored with the ISFET chip in a dry environment, while the tip, containing the reference electrode, is kept in a wet environment. In addition the ISFETs are sufficiently protected against electrostatic damage. When making a recording the ISFET and the reference electrode have to be brought into contact with the calibration liquid or with blood for at least half an hour, before the signal

conditioner can handle eqn. 37 correctly. If the ISFET is in contact with blood during the conditioning period, the calibration is achieved by means of a blood sample, the pH of which has to be determined by pH laboratory equipment.

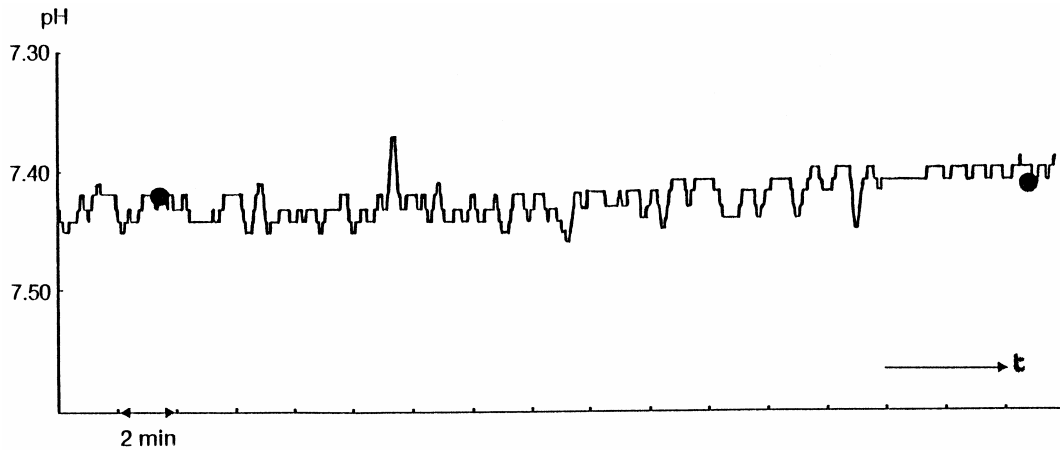


**Fig. 4**

*Complete Cordis ISFET catheter assembly. (Photograph courtesy of Sentron, Roden, The Netherlands.)*

An actual ISFET registration during spontaneous breathing is shown in figure 5. The ISFET was calibrated using a blood sample, after an initial conditioning period of half an hour, with the catheter tip in the iliac artery. The two dots are blood sample pH values for comparison with the ISFET recording.

The registration shows that the ISFET measures with an accuracy within the values that can be obtained with the usual intermittent sampling technique using an off-line blood gas analyser.



**Fig. 5**

*Result of pH monitoring with the Cordis pH ISFET catheter and signal conditioner during spontaneous breathing. (By permission of Sentron, Roden, The Netherlands.)*

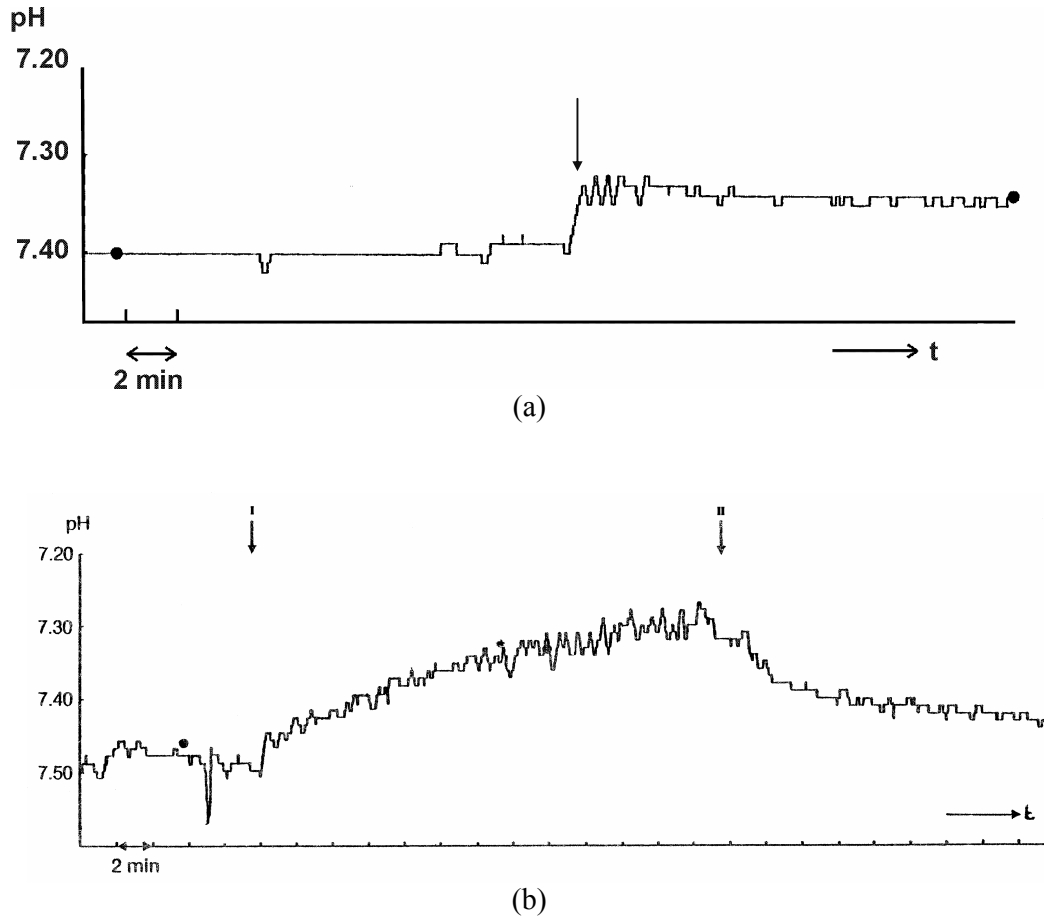
Furthermore, one can conclude from this registration and from others not given here, that during spontaneous breathing the blood pH may vary by at least 0.06 pH units. This indicates that the pH determination of an arbitrary blood sample within an accuracy of 0.001 pH unit, as is possible with modern pH-blood gas analysers, is physiologically insignificant.

The real value of a continuous on-line pH measurement will be clear from observation of the registration in figure 6(a) and (b). Again the dots refer to the pH value of blood samples, taken for comparison.

Figure 6(a) shows the result of successful weaning after a period of mechanical ventilation. The patient was put on a pH value of 7.4 and took control of his own acid-base balance after starting weaning from the mechanical ventilation (at ↓) within a few minutes at a slightly lower pH value. The registration shown in figure 6(b) shows an unsuccessful weaning at point “I”, which is the reason for the anaesthetist deciding to restart the mechanical ventilation at point “II”.

It will be clear that such a very important and relatively fast phenomenon can hardly be observed with a sampling technique needing further time-consuming off-line analysis and especially not on a routine basis.

Unfortunately, due to biocompatibility problems the catheter tip pH ISFET has been withdrawn from the market.

**Fig. 6**

Result of pH monitoring with the Sentron pH ISFET catheter and signal conditioner in a post-operative patient with (a) successful weaning and (b) unsuccessful weaning from mechanical ventilation. (By permission of Sentron, Roden, The Netherlands.)

### Other ISFET applications

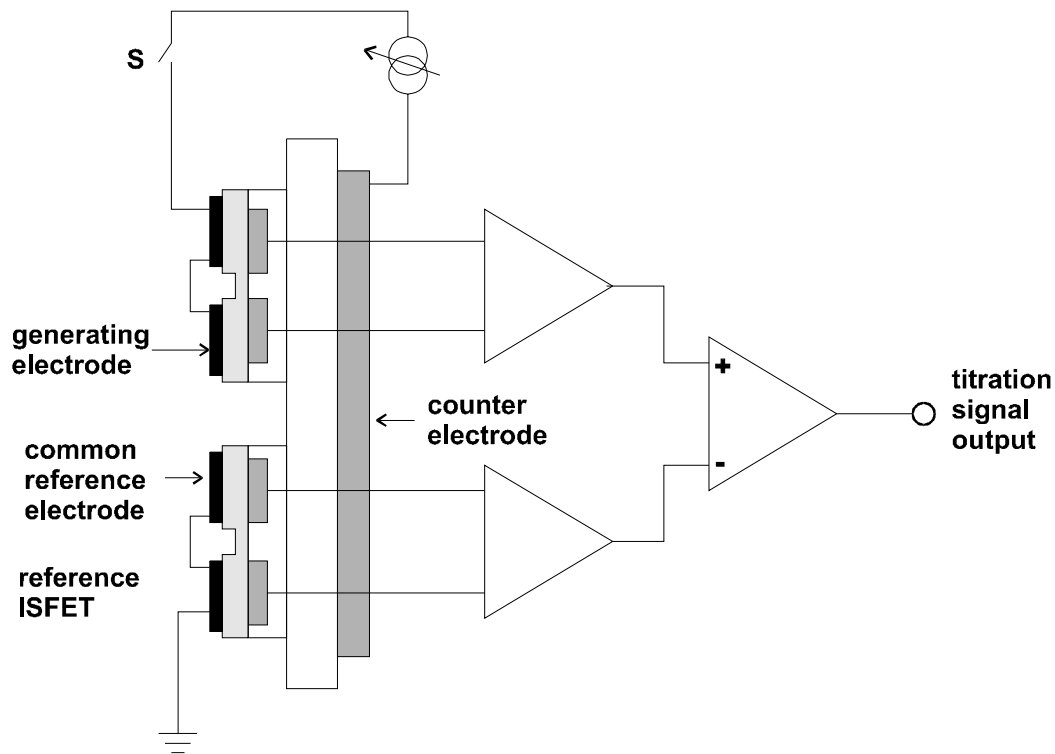
The usual way of coping with the effect of drift in chemical sensors is to carry out calibrations at regular intervals. For pH sensors, the common calibration procedure involves successive immersion in two buffer solutions with a different pH, with which both the offset and the sensitivity can be adjusted. Needless to say, that this is a relatively cumbersome procedure and could not be performed with the *in vivo* use of ISFETs.

Coping the drift of an ISFET was done in the Cordis system described above by predicting the drift beforehand, storing the prediction in a PROM and compensating the effect automatically.

However, there is a different way of controlling the pH of a solution without any handling of liquids: by coulometry. Coulometric generation of  $H^+$  or  $OH^-$  ions by a specific electrochemical reaction at a generating electrode, further referred to as

actuator, enables the control of the pH of a solution. Coulometry is an absolute method of ion generation. Provided that (1) the stoichiometry of the electrode reaction is known, (2) no side reactions occur, and (3) the current efficiency of the electrode reactions is close to 100%, the relation between the number of coulombs applied to the actuator and the amount of generated ions is fixed. Hence, the sensor signal can be adjusted to yield the read-out appropriate to the defined change in concentration by the generated ions. Note that this method of calibrating a pH sensor depends on the buffer capacity of the solution.

An example of such a coulometric sensor-actuator system is the combination of an ISFET pH sensor and a noble metal actuator at which  $H^+$  or  $OH^-$  ions can be generated through the electrolysis of water. With the use of the small, planar, IC-processed ISFET, a high degree of integration can be achieved by the deposition of a thin gold or platinum film electrode, closely spaced around the pH-sensitive gate of the ISFET. Figure 7 shows the basic elements of such a device.



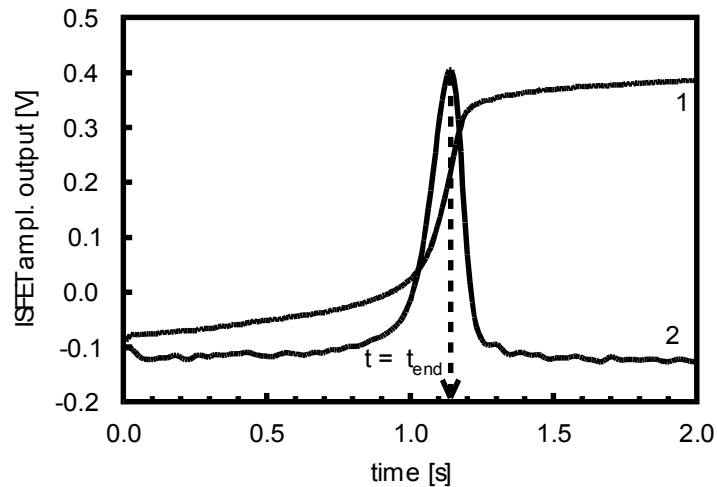
**Fig. 7**

*The basic elements of an ISFET-based sensor-actuator system.*

The ions generated at the actuator can be considered as the titrant, with which it is possible to perform a coulometric titration, thereby using the pH-sensitive ISFET as the indicator electrode for the detection of the end-point in the titration curve.

One of the sensors based on this principle is the acid/base concentration sensor, relying on the mechanism of free diffusion. Some time after the start of the generation of the titrant, the acid or base at the pH-sensitive gate of the ISFET is totally depleted,

which results in a sudden and sharp change in pH, as shown in figure 8 curve 1. This moment is easily detected by the rapidly responding ISFET. The time it takes to reach the end-point in the titration curve after the actuator current source has been switched on, depends, among other things, on the acid or base concentration of the sample solution, and can easily be determined from the first derivative of curve 1 as shown as curve 2.

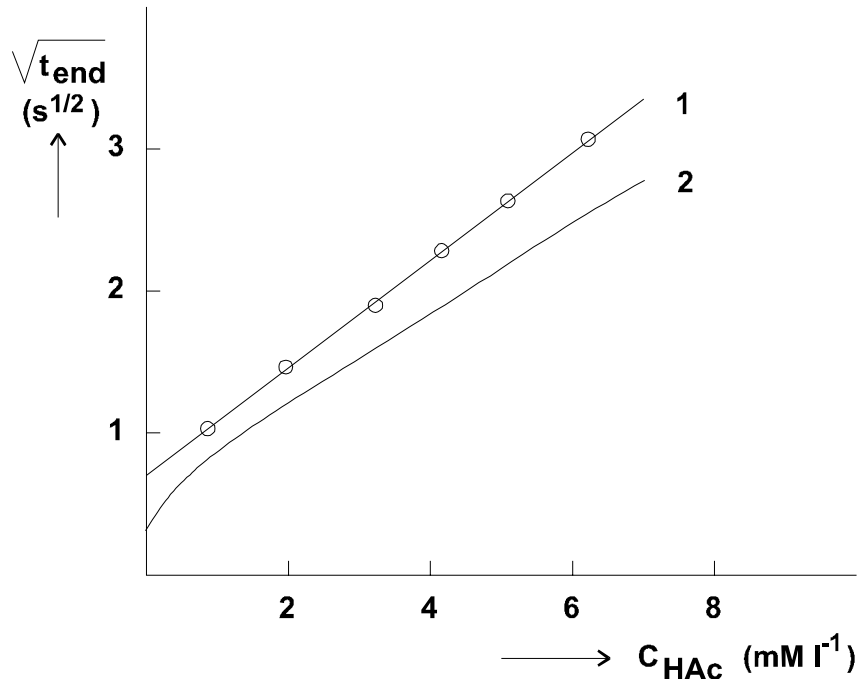


**Fig. 8**

*Typical measured coulometric titration (curve 1) and its first derivative (curve 2).*

The end point is easily determined from the first derivative of the titration curve by calculating its maximum. A series of measurements with the sensor-actuator device as shown in figure 7 were performed to determine  $t_{\text{end}}$  in solutions with a different acetic acid concentration. The results are presented in figure 9, together with the calculated results of the analytical model description.





**Fig. 9**

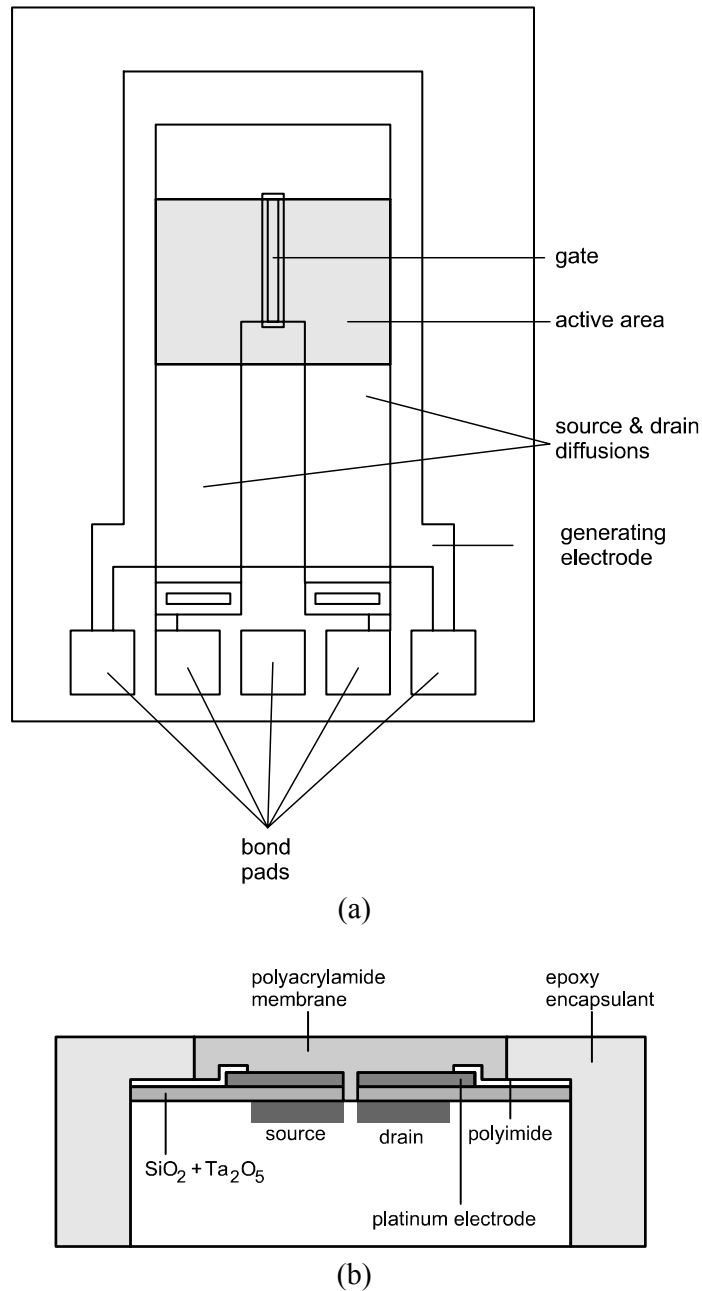
Results of the coulometric titration of HAc; curve 1: measurement results; curve 2: results of the analytical model,  $j_c=10\mu\text{A}/\text{mm}^2$ ,  $D_{\text{HAc}}=1.21\cdot 10^{-9}$ ,  $D_{\text{H}}=9.33\cdot 10^{-9}$  and  $D_{\text{OH}}=5.27\cdot 10^{-9}$  m<sup>2</sup>/s.

It is clear from figure 9 that the square root of the time needed to reach the end point depends linearly on the acid concentration, except for very low concentrations.

### ISFET-based enzyme sensor

The ISFET belongs to the class of chemically sensitive electronic devices, which means that the device is in the first place a field effect transistor. The so-called threshold voltage of the transistor is a function of the solution surrounding the gate. The operational mechanism of the ISFET originates from the pH sensitivity of the inorganic gate oxide such as SiO<sub>2</sub> or Ta<sub>2</sub>O<sub>5</sub>, as discussed in the previous section. By applying an enzyme-entrapping membrane on top of the ISFET-gate, it behaves as an enzyme sensor.

The layout of an enzyme modified ISFET chip is shown in figure 10(a) and (b). It contains a single ISFET fabricated with NMOS technology. Ta<sub>2</sub>O<sub>5</sub> is used as the pH-sensitive gate insulator. The generating electrode is constituted by a thin film of gold or platinum that closely surrounds the gate of the ISFET. The active area of the noble metal electrode measures 1 mm<sup>2</sup> and is defined by a patterned polyimide layer.



**Fig. 10**

(a) Layout of the enzyme-modified sensor-actuator chip.

(b) Cross-section of the chip. The height of the epoxy encapsulant determines the thickness of the membrane, which amounts to approximately 200  $\mu\text{m}$ .

The excellent stability and low-cost of urease make it an ideal enzyme-sensitive membrane material. The urease is immobilised in a polyacrylamide membrane which is formed in the epoxy as shown in figure 10(b). The membrane contains 10% of the polymer and 2.5% of protein, with some addition of bovine albumin for improved stability.

However the practical application of enzyme sensors based on the measurement of pH changes in an enzymatic membrane is limited by several factors. These factors that complicate the response are the buffer capacity of the sample, which itself is also a function of pH, the pH-dependent enzyme kinetics and the fact that the products of the enzyme reaction may be weak protolytes so that the amount of  $H^+$  or  $OH^-$  ions produced per mole of converted substrate also depends on pH. On the other hand, the pH of more alkaline solutions will be decreased by the same molecules. As a result of these factors, the response of ISFET-based enzyme sensors is strongly non-linear and the dynamic range depends on the composition of the sample solution. The general conclusion must be that an elaborate calibration procedure is required and that the practical value of these sensors is limited.

In subsequent research it was demonstrated that by coulometric control of the pH inside the enzymatic membrane, it is possible to overcome these problems. The pH-static enzyme sensor measures the pH inside the membrane with an ISFET and controls it through the generation of protons or hydroxyl ions at a noble metal electrode, spaced closely around the ISFET gate area as shown in figure 10(a). The acidic or alkaline products of the enzyme reaction are thus continuously neutralised. The generating current needed to maintain the pH at a constant level now becomes the output signal of the sensor. It is linearly related to the substrate concentration and independent of the buffer capacity of the sample. In fact, an electrochemical pH-actuating mechanism, the current-controlled electrolysis of water, is used to compensate the biochemical pH actuator, i.e. the enzyme, converting substrate molecules and thus generating pH change.

The response of an enzyme-modified ISFET is generally pH dependent. However, if the pH is kept constant, the sensitivity may also be considered to be constant. This sensitivity, the change in the steady-state ISFET output voltage  $V_{out}$  per change in substrate concentration  $[S]$ , is given by

$$\partial V_{out} / \partial [S] = EB / \beta \quad (38)$$

where  $B$  is the sensitivity of the ISFET,  $\beta$  is the buffer capacity of the sample and  $E$  is the enzymatic sensitivity parameter representing the change in equivalents of  $H^+$  or  $OH^-$  in the membrane as a function of the substrate concentration.  $E$  is determined by the enzyme load of the membrane, the enzyme kinetics, the diffusion constants of the relevant species in the membrane and the ratio in which protons or hydroxyl ions are produced per mole of substrate. For reactions that produce acidic products the value of  $E$  is positive and reactions that produce  $OH^-$  yield a negative value of  $E$ . At constant pH, the value of  $E$  can be considered to be constant provided that the substrate concentration  $[S]$  is considerably smaller than  $K_m$ , the Michaelis-Menten constant of the enzyme, or when the response of the sensor is limited by diffusion of the substrate instead of by the enzyme kinetics.

A similar consideration holds for the electrochemical actuator. For small changes in pH, the steady-state response of the output voltage on a current  $I$  through the generating electrode may also be considered to be linear and is given by

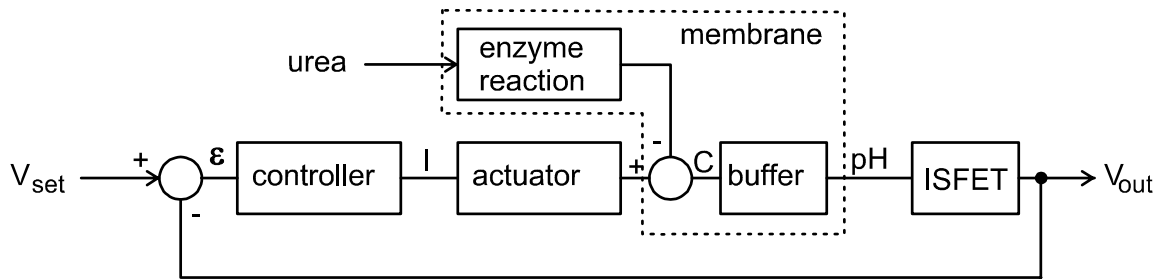
$$\partial V_{out} / \partial I = AB / \beta \quad (39)$$

where  $A$  is the sensitivity parameter of the chemical sensor-actuator system and is determined by the area of the generating electrode (and thus the current density) and the diffusion speeds in the membrane.

The generating current is used to compensate the pH changes produced by the enzyme and hence  $\partial V_{out,current} = -\partial V_{out,enzyme}$ . Combination of Eqns. 38 and 39 shows that the sensitivity of the pH-static enzyme sensor is given by

$$\partial I / \partial [S] = -E/A \quad (\text{A mol}^{-1} \text{ l}) \quad (40)$$

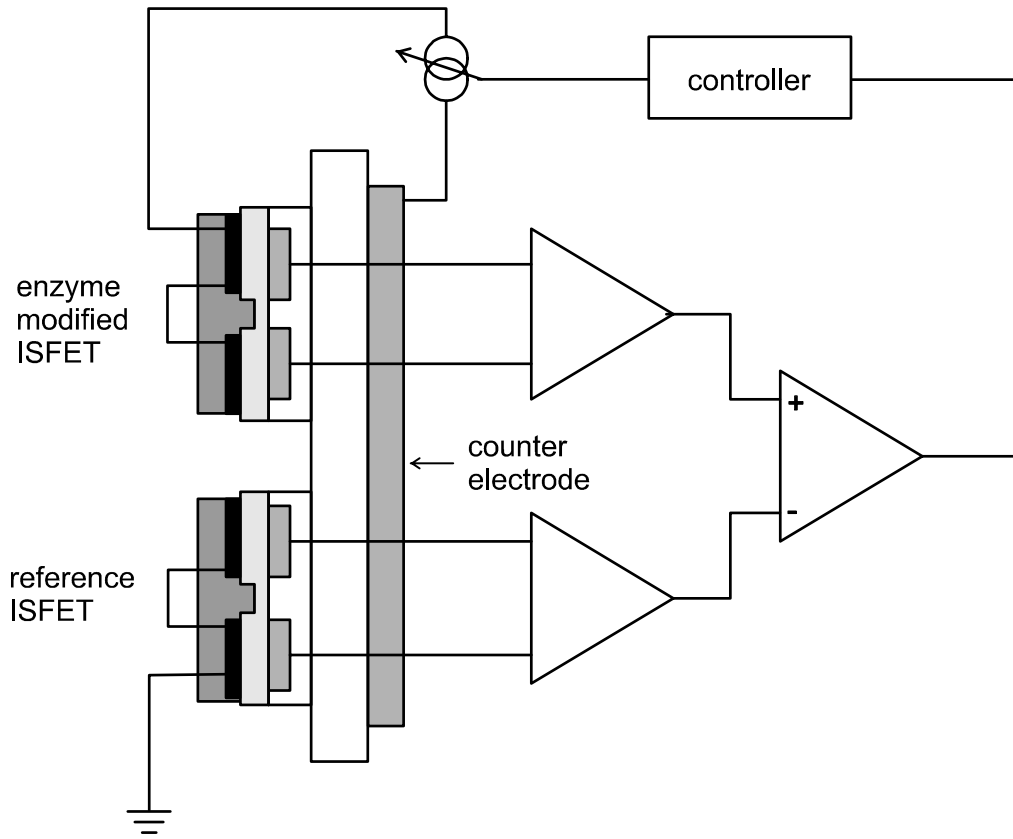
and is independent of the buffer capacity  $\beta$ . Provided that  $E$  remains constant, the response is linearly on the substrate concentration.



**Fig. 11**

*Block diagram of the control system for the pH-static enzyme sensor.*

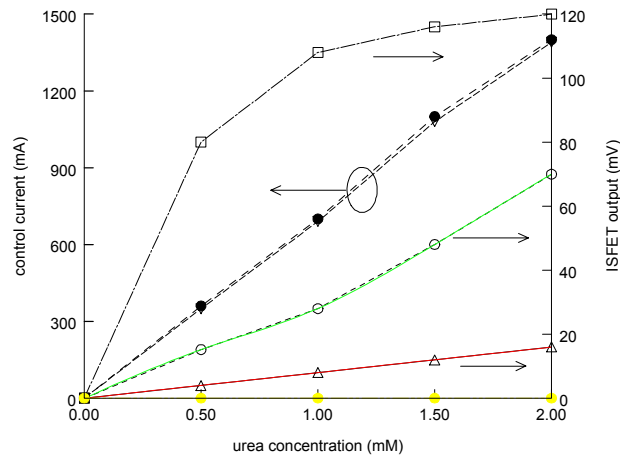
Figure 11 shows the control loop that represents the operation of the pH-static enzyme sensor. The pH inside the enzymatic membrane is measured with an ISFET, resulting in an output voltage  $V_{out}$ .  $V_{set}$  is the desired output voltage that for instance reflects the original pH of the sample. The error signal  $\epsilon = V_{set} - V_{out}$  is transformed to a generating current  $I$  by the controller. The current  $I$  delivers  $C$  equivalents of either  $H^+$  or  $OH^-$  ions to the membrane, which in turn changes the pH of the membrane in a ratio depending on its internal buffer capacity. It is obvious from this figure that the effect of the enzyme reaction is no longer dependent on the buffer. The enzymatic reaction products are neutralised at the central summing point in the figure, before the buffer can assert its influence.



**Fig. 12**  
*Measurement set-up for the pH-static enzyme sensor*

Figure 12 shows the general measurement set-up that is used for the pH-static enzyme sensor. The system uses two pH-sensitive ISFETs with an integrated actuator electrode, one for the actual enzyme sensor and a second as a reference that measures the background pH of the sample solution. The second chip is identical with the first, except that its membrane does not contain the enzyme. Both ISFETs measure with respect to a common (quasi-) reference electrode which is actually the platinum electrode on the reference ISFET. The ISFETs are operated with a constant source-drain voltage and a constant drain current. Their differential signal reflects the substrate-dependent pH change induced in the membrane of the first ISFET.

Figure 13 compares the output of a classical ISFET-based enzyme sensor with that of the pH-static sensor. The lines with solid symbols show the measured pH change as a function of the urea concentration at pH 7 in phosphate buffers of 50, 10 and 2 mM. The lines with open symbols show the control current used by a pH-static sensor as measured simultaneously with the first three curves. As can be seen, the sensitivity and dynamic range of the “normal” enzyme sensor depends strongly on the buffer capacity. In a weak buffer, the response saturates quickly as the pH in the membrane approaches 9. In a strong buffer, the maximum concentration that can be measured is much larger but the sensitivity is reduced.



**Fig. 13**

*Response of a classical ISFET-based enzyme sensor (open symbols) compared with that of the pH-static sensor (filled symbols) in sample solutions with different buffer capacity.*

► : low buffer; ► : medium buffer; Δ: high buffer

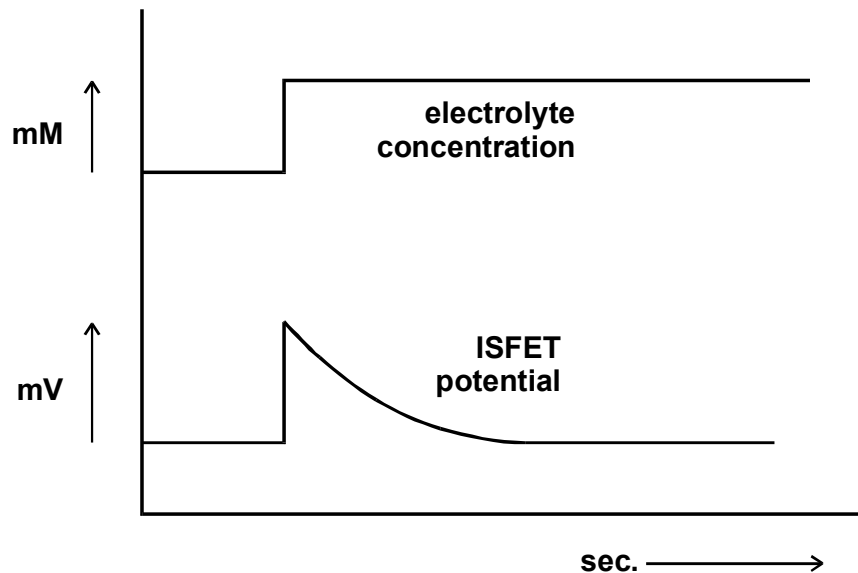
Because the pH-static sensor operates at a constant pH, the enzyme activity and the influence of the reaction products on the pH remain constant. The results with the pH-static sensor show also that the response is fairly linear over the concentration range used. As can be seen, the sensitivity of the sensor is of the order of 700 nA l mmol<sup>-1</sup>.

### Dynamic use of the ISFET in the ion-step method

Fairly soon after the introduction of the ISFET, researchers all over the world started looking for other applications than the determination of pH only. As the potential over the oxide-solution interface of the ISFET determines its output, scientists tried to modify this potential by applying charged molecules on the oxide surface. Doing so, it was thought to be possible to monitor immuno-reactions. By immobilising antibodies on the ISFET surface a certain surface charge is created that might change noticeably after a reaction with antigens. This seemed to be the basis of a whole new family of biosensors. The results of these experiments, however, were very disappointing. Also in the biosensor-group of the University of Twente the possibilities of such an immunosensor were explored. It was concluded, however, that from a theoretical point of view, it is almost impossible to detect a layer of charged biomolecules on an ISFET in a situation of thermodynamical equilibrium. This resulted in the introduction of a new measuring concept: the ion-step method.

## Operational mechanism

Using the ion-step method it appeared to be possible to detect the presence of a layer of charged biomolecules (e.g. proteins) on the surface of an ISFET. The essential difference between this new method and many others is the fact that the ion-step method is a dynamic measuring principle in contrast to most of the other methods, being static and relying on thermodynamical equilibrium. In a dynamical method of measuring the equilibrium is disturbed by a stimulus. The response on this stimulus contains the required information. After some time the system regains equilibrium again, mostly being identical to the situation before the stimulus. The stimulus in the ion-step measuring method consists of a sudden, stepwise change in the ion concentration of the solution surrounding the ISFET. In practice, this is accomplished by a flow-system, in which the solution flowing over the ISFET is suddenly changed with a solution having a higher ion-concentration than the original one. The ion-step measuring method is schematically depicted in figure 14.



**Fig. 14**

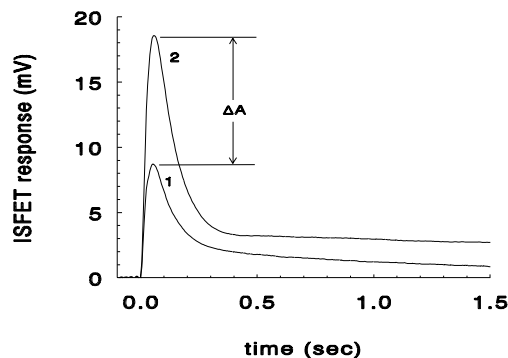
*Schematic representation of the main feature of the ion-step measuring method: the chemical stimulus and the resulting chemo-electrical response.*

In case the ISFET surface is provided with a layer of, e.g., proteins, the response of the ISFET on an ion-step can be a peak in the potential as indicated in figure 14. The amplitude and the time-constant of this peak potential contain information concerning the charge density of the protein layer. In practice, only the amplitude is used.

## Heparin sensor

As a first application of the ion-step measuring method a heparin sensor is developed. Heparin is a highly negatively charged polysaccharide, which is used clinically to delay clotting of blood. The relation between the dosing of heparin and the resulting biological activity is poorly understood and differs between individual patients. Therefore, the heparin treatment must be carefully monitored. Nowadays, this is a cumbersome and time-consuming procedure. At the biosensor group of the University of Twente, a sensor is developed, capable of the determination of heparin concentration in blood plasma.

An ISFET is provided with a layer of protamine, immobilised on the gate oxide. Protamine is a small, highly positively charged protein, being used as protamine sulphate to neutralise heparin, already present in the blood circulation. The interaction between heparin and protamine is only an electrostatic interaction: the negatively charged heparin binds to the positively charged protamine. The protamine, immobilised on the ISFET surface acts as an affinity ligand. In other words, the moment the ISFET is immersed in a blood sample, mainly heparin binds to the ISFET surface. If a fixed time for the incubation is used, then the amount of bound heparin is a measure for its concentration in the blood sample. In practice, a certain amount of a-specific binding of other components of the blood plasma will occur. It is of course of importance to keep this a-specific binding to a minimum.



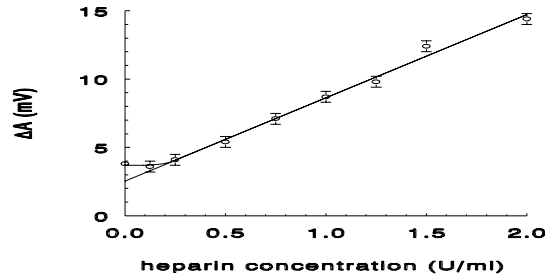
**Fig. 15**

*Two typical responses on an ion-step. Curve 1: Ion-step response of an ISFET with a layer of protamine; curve 2: response of the same ISFET after 2-minute incubation in blood plasma with 0.9 U/ml heparin.*

In figure 15 two typical responses on an ion-step are shown. Curve 1 is the response of an ISFET with a layer of protamine and curve 2 is the response of the same ISFET, but after incubation in a sample of blood plasma to which a certain amount of heparin was added (internationally, heparin concentrations are expressed in standardised Units per millilitre [U/ml]). The difference between the two curves is a measure for the amount of bound heparin. In practice, this difference is used as the relevant parameter, in figure 15 indicated as  $\Delta A$ . After each measurement, the sensor can be regenerated by rinsing the ISFET in 4M NaCl. Then all bound heparin is removed from the ISFET surface, including the a-specifically bound molecules, without removing the



protamine itself. If after this treatment the response on a ion-step is determined, the result is again identical to curve 1 of figure 15. This curve can therefore be considered as a calibration curve. Now, the sensor can be used to determine the heparin concentration of a subsequent sample.



**Fig. 16**

*The measured change in amplitude of the ion-step response,  $\Delta A$ , before and after incubation for 2 minutes in blood plasma as a function of the heparin concentration.*

In figure 16 the parameter  $\Delta A$  is plotted as a function of the heparin concentration, measured in plasma samples with added quantities of heparin. As is clear from this figure, a linear relation exists between the concentration of heparin and the difference in amplitude of the ion-step before and after incubation (being  $\Delta A$ ). The measurement result in plasma without added heparin (0 U/ml) corresponds to the amount of a-specifically bound components from the plasma.

### Present products

In the previous paragraphs most attention has been paid to the exploitation of the specific ISFET properties of being small and rigid, good for biomedical applications and having a very fast response, good for dynamic measurements.

At the present moment the biomedical application is hardly developed. This is mainly due to biocompatibility problems, which is however not related to the ISFET performance as such, but which is a general problem for all *in vivo* sensors.

Due to the fact that the ISFET is the only potentiometric sensor which does not need water for proper operation, ISFETs can very well be used for pH measurements in food, like cheese and meat. This is one of the present markets, supplied by about 20 companies at the present moment. Among them the company Sentron in Roden, the Netherlands, which is based on the R&D group of Cordis, the company which started as one of the first an ISFET production line (see Fig.4).

Application of the excellent dynamic properties of ISFETs is exploited in the relative new product of the company Thermo Orion: the “flash titrator”, of which the basic operation is shown in Fig.7.

## References

Since the first papers of the author in 1970 and 1972, about 600 papers appeared in the literature. These are discussed and partly listed in a recent review paper, to which the reader is referred:

P.Bergveld, Thirty years of ISFETOLOGY, What happened in the past 30 years and what may happen in the next 30 years. Sensors and Actuators B 88 (2003) 1-20.