

# Thirty years of ISFETOLOGY

## What happened in the past 30 years and what may happen in the next 30 years<sup>☆</sup>

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### Abstract

This paper describes the development of ISFETs in an historical setting, but is not limited to that. Based on the development regarding the theory, the technology, the instrumentation and the experience with many specific applications, also future projects are defined, such as concerning cell acidification, REFET biasing and a complete new range of FET sensors based on local pressure induction by (bio)chemical interaction with immobilised charged molecules (hydrogels). Also the present patent and market position is discussed. It is concluded that in the past 30 years the ISFET research and development made continuous progress on a regular base, but the practical applications stayed behind, especially concerning the dynamic use of ISFETs in combination with an integrated pH actuator. The newly proposed research projects may be good for another 30 years.

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### 1. Introduction

Although the title of this paper might suggest that after 30 years of research, development and application of ISFETs and related devices, the author, who introduced the ISFET concept in the seventies, decided to write a review paper, this will not be the case. More than 600 papers appeared in these 30 years devoted on ISFETs and another 150 on related devices, such as EnzymeFETs (ENFETs), ImmunoFETs (IMFETs), etc. This amount is too large to be listed and commented in a readable paper. It is more realistic to step through the years and focus on the experience of the author with respect to his own research items in the field of ISFETs and relate this to the ideas and discoveries of others, as well as to the follow up in commercialised products. In other words: what was to original idea, did it have any significance in the field of chemical sensors, did it stimulate the development of other sensor systems and last but not least was it taken up by the sensor industry, directly or indirectly? Based on the historical experience during the last 30 years, it will also be interesting whether a prediction can be made with respect to developments to be expected in the next 30 years.

The history concerning the application of silicon for a wide variety of sensors is well reviewed by Middelhoek [1]. We will focus in this paper only on the application of silicon for chemical sensors which explicitly make use of the field effect concept and limited to measurements in an aqueous solution. Due to their small dimensions, these sensors were initially mainly meant for biomedical applications, such as electrophysiological measurements.

The introduction of silicon as a substrate for microelectrodes, specifically meant for electrophysiological measurements (action potentials), started in the late sixties at the Stanford University by Wise et al. [2]. Applying an etching technique for the silicon needles and evaporation of gold for the electrode leads resulted in 5 mm long and 0.2 mm wide electrodes, insulated with silicon dioxide, except for the tip. The needle ended in a silicon part of some square millimetres, which was meant to contain fully integrated buffer amplifiers in a later version. Fig. 1 shows one of the original design drawings.

Reading this, the author of the present paper thought that this approach was not “wise”, because it would not be (and is still not) necessary to integrate a complete amplifier, but only the impedance transforming part of it, which was at that time a junction field effect transistor. This approach was already described in the literature (factually the author’s first

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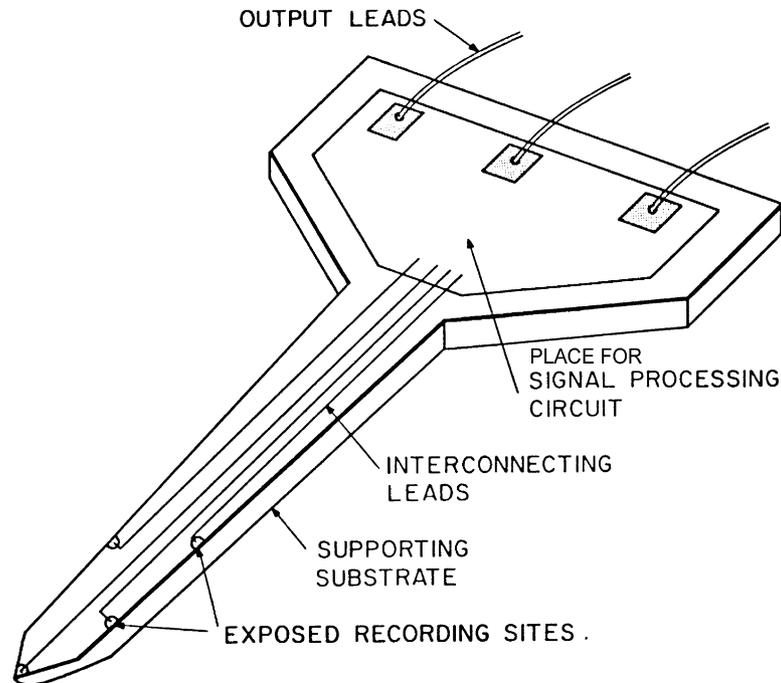


Fig. 1. Original design of silicon needles, with place for integrated buffer amplifiers [2].

paper), describing how with a source and drain follower circuit a field effect transistor can be symmetrically connected with long unshielded wires to a remote electronic circuit with low-ohmic input terminals (see Section 4) [3]. In fact, this paper was anticipating on the already at that time existing idea of the author that the transistor chip itself should form the electrode tip. This should shorten the actual electrode lead to the minimum and moreover abandon even the use of a metal electrode contact in case, instead of a JFET, a MOSFET without gate metallisation should be applied, leaving the bare oxide as the actual contact between the electronic system and the biological environment. Because with this device ion concentrations could be measured beside electrical potentials, this device was already called an ISFET in the first short communication in 1970 [4], in which it was introduced for the measurement of ionic in and effluxes around a nerve. These signals were indeed measured as illustrated in the next full paper in 1972 [5], which paper is often referred to in introductory sections of later papers by many other authors. At the same time, the Japanese student Matsuo was in the group of Angell at the Stanford University independently developing a similar device, which was published in 1974 [6]. After these historical publications, many other groups started to publish concerning their research on ISFETs and related devices, which will be referred to in the following sections as far as it can be seen as an important addition to the original idea in the view of the author. First we will give some comments on the significance of the first papers as mentioned above.

It is striking that all papers, referred to above, describe ISFETs as future tools for electrophysiological measurements and they were therefore published in a biomedical

journal. The author's paper of 1972 shows that specifically with an ISFET monophasic pulses were recorded, which could only be attributed to the accumulation of potassium ions in the small extracellular cavity between the nerve membrane and the ISFET surface, which hypothesis has however never been proven. The only indirect proof at that time was that in case the ISFET was temporarily insensitive for ions, accidentally due to some glue covering the gate area, normal biphasic action potentials were recorded as shown in a later paper [7]. Examples of monophasic as well as biphasic potentials are shown in Fig. 2.

We will come back to the possible significance of the monophasic pulses in Section 6. In addition, it was shown in the last-mentioned paper that with one and the same probe extracellular recordings could be made in a frequency band of 0 Hz to 40 kHz, which is, even up to now, not possible with any other extracellular recording probe. Nevertheless, the new measuring possibilities for electrophysiology, concerning local ion concentrations as well as potentials, were not further exploited by electrophysiologists and the research on ISFETs went in the direction of ion sensing in general, not specifically biomedical. Not until 20 years later ISFETs have been exploited to measure cell metabolism of cultured cells on ISFET arrays, which will be described in Section 6.

The first papers gave hardly any theoretical explanation of the observed ion sensitivity other than that the Nernst equation was inserted in the equation which describes the MOSFET behaviour. However, the origin of the (sub)Nernst response was not understood, but it can easily be imagined that a comparison with the glass membrane of a conventional pH glass electrode was made, especially in the case of an ISFET with the bare silicon dioxide as the gate material. In

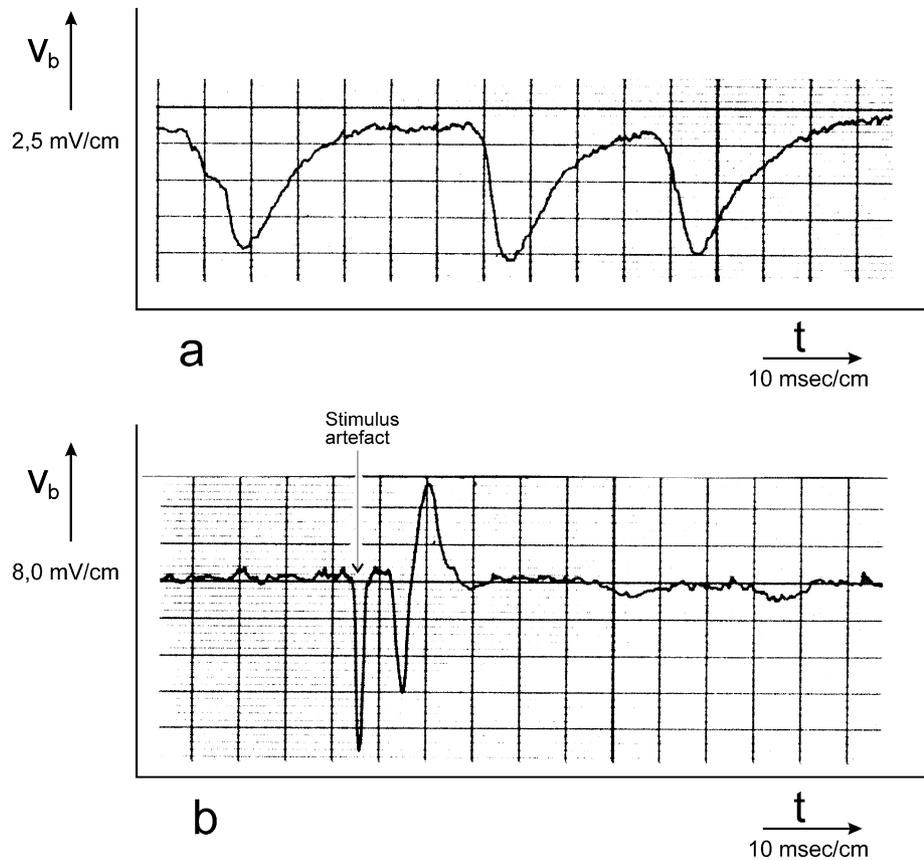


Fig. 2. Monophasic potential, recorded with an ion sensitive field effect device (a) and biphasic recording, originating from a similar device which was (temporarily) not sensitive for ions (b). Both registrations from the flexor tibialis of a Locust.

Section 2, the validity of this comparison will be elucidated in detail. Historically, it is regretful that from the beginning the lack of theoretical insight forced the comparison with the glass electrode, which unfortunately also directed the discussion with respect to useful ISFET applications. ISFETs were not directly meant to replace the conventional pH glass electrodes, but to develop alternatives for measurement circumstances where glass electrodes could not be applied: measuring in ultra small volumes and with an extremely fast response. Examples of these type of measurements will be given in the further sections.

Another regretful detail of the original papers is that it led to a discussion whether or not a reference electrode should be necessary for the application of ISFETs. The enthusiasm of the author about the new approach to ion sensing made that he mixed up several ideas in one and the same paper, which should not have been done. Of course he knew as an electrical engineer that loading a capacitor (with the gate oxide as the dielectric) needs two connections, in this case respectively the silicon and the electrolyte. What was meant with the “no reference electrode” phrase in the first papers was that one could think of alternatives, based on another type of modulation of the flatband potential of a semiconductor, for instance by means of (electro)chemical modulation of the workfunction of a gate material.

This was unfortunately not further elucidated in the first papers, but has recently been shown to be possible, as will be explained in Section 7.

## 2. Theory

The ISFET is in fact nothing else than a MOSFET with the gate connection separated from the chip in the form of a reference electrode inserted in an aqueous solution which is in contact with the gate oxide. Therefore, most of the papers dealing with the ISFET operational mechanism start with the theoretical description of a MOSFET.

The general expression for the drain current of the MOSFET and thus also of the ISFET in the non-saturated mode is

$$I_d = C_{ox}\mu \frac{W}{L} \left[ (V_{gs} - V_t)V_{ds} - \frac{1}{2}V_{ds}^2 \right] \quad (1)$$

with  $C_{ox}$  is the oxide capacity per unit area,  $W$  and  $L$  the width and the length of the channel, respectively, and  $\mu$  is the electron mobility in the channel. The other parameters are illustrated in Fig. 3.

In the saturated region of operation the expression is different, but this is not of any influence for a clear apprehension of the matter.

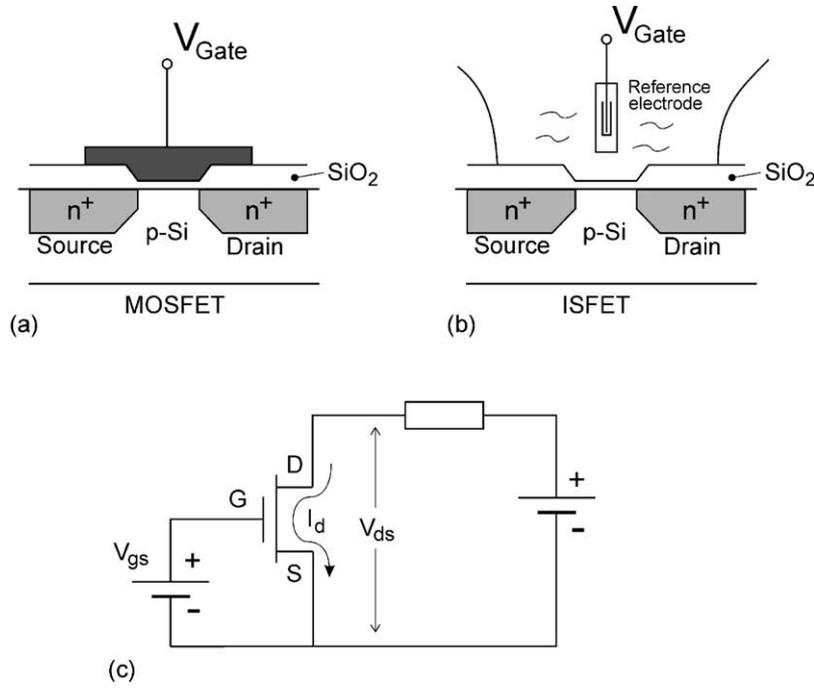


Fig. 3. Schematic representation of MOSFET (a), ISFET (b), and electronic diagram (c).

The schematical representation of a MOSFET and an ISFET is given in Fig. 3, as well as their common electronic diagram.

In both cases, the drain current  $I_d$  is a unique function of the input voltage  $V_{gs}$ , only when the geometric sensitivity parameter  $\beta = \mu C_{ox} W/L$ , as well as the applied drain-source voltage  $V_{ds}$  and the threshold  $V_t$  are constant. The parameter  $\beta$  is a design constant and  $V_{ds}$  is kept constant by the applied electronic circuit. In addition, the fabrication processes for MOSFET devices are so well under control that  $V_t$  is also a constant, which manifests itself only as a certain threshold voltage, hence its name. Thus,  $V_{gs}$  is the only input variable. In case of the ISFET, it was initially debated whether the observed ion sensitivity should be described as an additional input variable in terms of a modification of  $V_{gs}$  or a modification of  $V_t$ . Defining the metal connection of the reference electrode as a remote gate, as shown in Fig. 3b, suggests that any interfacial potential in the input circuit should be described in terms of  $V_t$ . Therefore, the second important MOSFET equation is that of the threshold voltage:

$$V_t = \frac{\Phi_M - \Phi_{Si}}{q} - \frac{Q_{ox} + Q_{ss} + Q_B}{C_{ox}} + 2\phi_f \quad (2)$$

where the first term reflects the difference in workfunction between the gate metal ( $\Phi_M$ ) and the silicon ( $\Phi_{Si}$ ), the second term is due to accumulated charge in the oxide ( $Q_{ox}$ ), at the oxide–silicon interface ( $Q_{ss}$ ) and the depletion charge in the silicon ( $Q_B$ ), whereas the last term determines the onset of inversion depending on the doping level of the silicon. All terms are purely physical in nature.

In case of the ISFET, the same fabrication process is used, resulting in the same constant physical part of the threshold voltage (Eq. (2)). However, in addition to this, two more contributions manifest themselves: the constant potential of the reference electrode,  $E_{ref}$ , and the interfacial potential  $\Psi + \chi^{sol}$  at the solution/oxide interface of which  $\Psi$  is the chemical input parameter, shown to be a function of the solution pH and  $\chi^{sol}$  is the surface dipole potential of the solvent and thus having a constant value.

Hence the expression for the ISFET threshold voltage becomes

$$V_t = E_{ref} - \Psi + \chi^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ox} + Q_{ss} + Q_B}{C_{ox}} + 2\phi_f \quad (3)$$

Note that the parameter  $\Phi_M$  in Eq. (2), being the workfunction of the gate metal seems now to be disappeared, but this is not true, because it is “buried” by definition in the term  $E_{ref}$ .

In case the ISFET is treated as a MOSFET and connected to a curve tracer with the reference electrode connected to the  $V_{gs}$  port,  $I_d/V_{ds}$  curves can be recorded as function of  $V_{gs}$  as is usually done with MOSFETs. However, with the reference electrode connected to the source ( $V_{gs} = 0$ ) similar curves can be achieved by changing the pH of the solution. This is shown in Fig. 4a and b. The effect shown in Fig. 4b is due to the relation  $\Psi = f(\text{pH})$ .

From the experiment described above and with the theoretical description as given in Eqs. (1) and (2) in mind, it may be concluded that an ISFET is electronically identical to a MOSFET and can thus still be seen as an electronic device, with one additional feature: the possibility to chemically

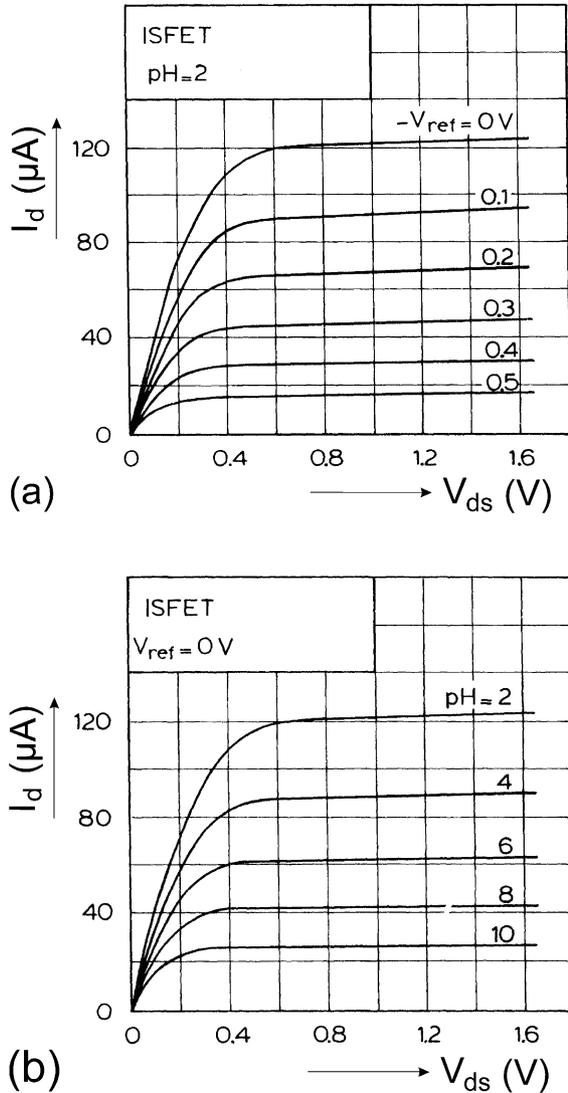


Fig. 4.  $I_d/V_{ds}$  curves of an ISFET with  $V_{gs}$  (a), and pH (b) as a parameter.

modify the threshold voltage via the interfacial potential at the electrolyte/oxide interface. In Section 7 we will show how the threshold voltage of a MOSFET can also be chemically modified by the workfunction of the gate material, leading to an other chemically sensitive FET device. However, for the present moment we will focus on the ISFET and it will not be surprising that all efforts to explain its operational mechanism have thus been devoted completely to the elucidation of electrolyte/oxide interface phenomena.

As mentioned in Section 1, the first ISFET gate material exploited was silicon dioxide, obtained in the conventional MOSFET technology by heating silicon up to 1100 °C in a dry oxygen atmosphere, forcing the comparison with a glass membrane [4,5], of which it is known that it needs a certain amount of hydration of the surface to become pH sensitive. However, Matsuo already used a sandwich of silicon dioxide and silicon nitride [6], although it was known from the latter material that it will not hydrate. This means that one thus has

to consider pure surface phenomena at insulator/electrolyte interfaces. Therefore, the original work of Yates et al. [8] drew the attention, describing the electrical double layers at colloidal oxide–water interfaces with the site-binding model. In this model the oxide surfaces are assumed to be amphoteric, meaning that the surface hydroxyl groups can be neutral, protonized (thus positively charged) or deprotonized (thus negatively charged) depending on the pH of the bulk solution. This basic model was adopted to the electrolyte/insulator/silicon (EIS) structures by Bousse, a Ph.D. student of the author in 1980 [9] and proven to be applicable for ISFET surfaces of  $\text{SiO}_2$  and  $\text{Al}_2\text{O}_3$ . Although in the last part of the seventies much work on ISFETs had been published, including extensions with different membranes to change the sensitivity to other ions, the approach has been empirical rather than theoretical and pH sensitivities below the theoretical Nernstian value could not be explained. However, Bousse developed for the first time a rather simple theory with two parameters, derived from the already existing site-dissociation and double-layer models. These parameters are the  $\text{pH}_{\text{pzc}}$ , being the value of the pH for which the oxide surface is electrically neutral and  $\beta$ , determining the final sensitivity. The resulting equation for the surface potential  $\Psi$  as given in Eq. (3) for relative large values of  $\beta$  is

$$\Psi = 2.3 \frac{kT}{q} \frac{\beta}{\beta + 1} (\text{pH}_{\text{pzc}} - \text{pH}) \quad (4)$$

From Eq. (4) it can be seen that the sub-Nernstian response can now easily be explained by means of a not too large value of  $\beta$  for the oxide under investigation.

The value of  $\beta$  can be expressed in terms of the acidic and basic equilibrium constants of the related surface reactions, for which a parameter  $[\text{H}^+]_s$  has been introduced, which represents the surface concentration of  $\text{H}^+$  ions, being related to the  $[\text{H}^+]$  bulk value by Boltzmann statistics. This parameter has since the introduction of the site-dissociation model served as a mathematical quantity for many further models with different type of surface sites, even including a PSPICE model of the group of Grattarola [10], but the chemico/physical significance was rather unclear.

It took another 15 years before the significance of  $[\text{H}^+]_s$  could be elucidated, based on the comparison with the acid–base behaviour of protein molecules [11]. The new model was introduced by the Ph.D. students van Hal and Eijkel in the research group of the author [12,13] and is in fact nothing else than the, for electrical engineers, well-known equation for capacitors  $Q = CV$ , where  $Q$  is the surface charge in the form of protonized ( $\text{OH}_2^+$ ) or deprotonized ( $\text{O}^-$ ) OH groups of the oxide surface,  $C$  is the double-layer capacitance at the interface and  $V$  is the resulting surface potential, denoted as  $\Psi$  in Eqs. (3) and (4). The surface charge acts as a source or sink for protons, whereas the load of this source is the double-layer capacitance. The surface proton buffer capacity as well as the value of the double-layer capacity determine together the final value of the potential  $\Psi$  which

can be expressed in a sensitivity factor  $\alpha$ , resulting in the simple equation:

$$\Delta\Psi = -2.3\alpha \frac{RT}{F} \Delta\text{pH}_{\text{bulk}} \quad (5)$$

with

$$\alpha = \frac{1}{(2.3kT/q^2)(C_s/\beta_s) + 1} \quad (6)$$

where  $\beta_s$  symbolises the surface buffer capacity, e.g. the ability of the oxide surface to deliver or take up protons, and  $C_s$  is the differential double-layer capacitance, of which the value is mainly determined by the ion concentration of the bulk solution via the corresponding Debye length.

Eq. (5) shows that only in case  $\alpha$  approaches 1, the maximum Nernstian sensitivity of 58.2 mV per decade (at 20 °C) can be obtained, whereas for oxides having a value  $\alpha < 1$  a sub-Nernstian response can be expected. Eq. (6) shows that  $\alpha = 1$  is reached for oxides with a large value of the surface buffer capacity  $\beta_s$  and a low value of the double-layer capacity  $C_s$ . Fig. 5a shows the measurement results of four typical oxides in use for ISFETs, from which it can be concluded that  $\text{Ta}_2\text{O}_5$  has clearly the largest surface buffer capacity, even so large that variation in the value of the

double-layer capacity has hardly any influence, as proven with the experiment shown in Fig. 5b.

Now also the significance of the parameter  $[\text{H}^+]_s$  is clear: maximum sensitivity can only be achieved in case the surface proton concentration is constant, independent of pH and ion concentration variations, which is the same as having a surface with a large buffer capacity. Retrospectively this is a logical conclusion because the original Nernst equation already predicts that a maximum sensitivity over a membrane will only be achieved in case the concentration of the ions to be measured is kept constant at the interface side not in contact with the analyte. Now also the design rules for chemFETs, meant for the measurement of a variety of ions in general, can be formulated: add a membrane to the ISFET, of which the internal chemical composition buffers the type of ion to be measured.

Summarising this section on the theoretical explanation of the pH sensitivity of ISFETs, it should be concluded that an ISFET is an electronic component, similar to the MOSFET, but with an modulation possibility of the threshold voltage by means of the oxide/solution interface potential. The relation between this interface potential and the pH is determined by the buffer capacity of the oxide surface.

Note that due to the introduction of the field effect concept in the sensor field, now also insulating materials can be used for ion sensing, contrary to the conducting materials which are required for the conventional ion sensor concept, such as the glass membrane. The only prerequisite for these insulating materials is that their surface buffers the ion of interest.

### 3. Technology

The technology used for the processing of ISFETs has always strongly been related to the various (C)MOS technologies. Most of the ISFET devices therefore consist of planar devices with the source and drain connections at the same side as the gate. Because the actual sensitivity and selectivity is determined by the chosen gate insulator (see Section 2), it will be clear that the most essential difference between a MOSFET and an ISFET is the choice of the outer gate insulator, which is usually deposited on top of the original silicon dioxide. Not being informed about the theory as elucidated in Section 2, it is logical that in the beginning, maybe extending over a period of 1.5 decades, the application of a particular gate covering layer was merely determined by the availability of a certain deposition facility for a certain material. In that sense it is not surprising that well-known materials in the MOS technology, like  $\text{Si}_3\text{N}_4$  and  $\text{Al}_2\text{O}_3$  have been investigated as gate materials. However, many other materials have been used, most probably due to their availability, mostly inorganic oxides, but also clays and even Japanese laquers. In order to influence the selectivity in addition many mixtures have been used as well as ion implantation of existing oxides. All of these investigations are more or less trial-and-error experiments. One will always

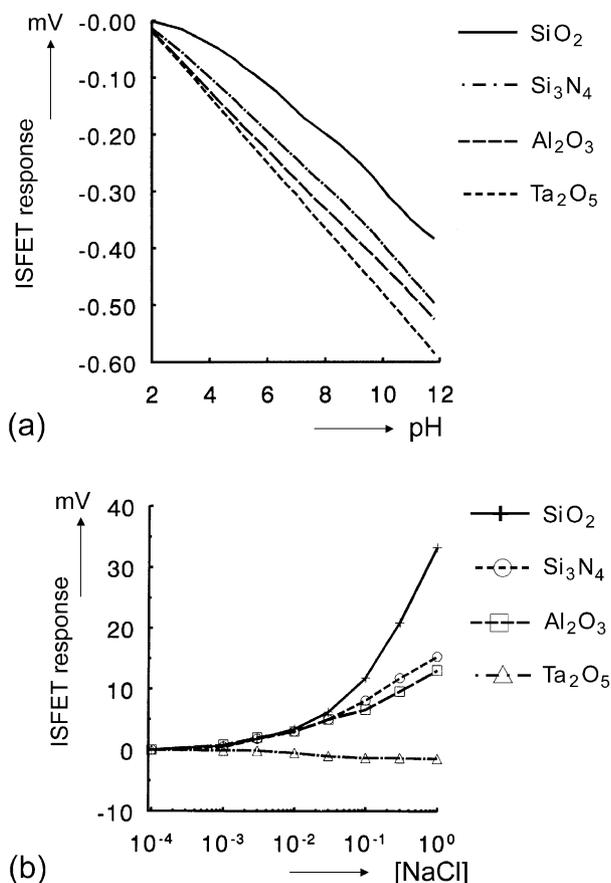


Fig. 5. ISFET responses (a) to electrolyte pH at constant TBACl concentration of 0.1 M and ISFET responses (b) to NaCl electrolyte concentration at constant pH (5.8).

find something, but without the theoretical background as given in Section 2 the experimental results could not be explained.

A further difference between an ISFET and a MOSFET, made with the same technology, is that the metalisation pads for the source and drain connections are made at some distance from the gate area in order to provide for an easy encapsulation procedure of the metal parts and the bonding wires, usually with an epoxy. This means that in one or another way relative long diffusion areas have to be made for the source and drain. Regarded as an electronic device, this means that source and drain series resistances have been introduced, which leads to an effectively smaller mutual conductance, usually indicated by  $g_m$ , which effect can, however, be compensated for by applying the ISFET in a feedback circuit (see Section 4). Besides the problem of encapsulation of the metal parts (pads and bonding wires) also the bare edges of the chip should carefully be encapsulated. Therefore, most of the modifications of ISFET designs are directed towards a solution for the encapsulation problems.

The first ISFET design fully devoted to the solution of the encapsulation problem was published by Esashi and Matsuo [14], who made use of the etching technology as introduced by Wise et al. [2], leading to silicon needles, of which the edges were completely covered by silicon dioxide. The flat side of the needles contained two ISFETs, having the gate area covered with silicon nitride and aluminosilicate, respectively, resulting in sensitivities towards pH and pNa. The source and drain connections were made at the blunt end of the needle and could therefore easily be encapsulated.

Later on also the silicon on sapphire (SOS) and silicon on insulator (SOI) technologies have been applied for ISFET processes, resulting in completely isolated subchips with source and drain connections on one side and the gate area on the other side of the structure [15]. This is schematically shown in Fig. 6.

It was the group of de Rooij in Neuchâtel which introduced ultimately the backside contact ISFET design [16] with a relatively cheap process, using double side polished silicon wafers. The “standard” ISFET fabrication process is used, with one exception, namely the location of the source and drain contacts. Under the intended source and drain regions, holes are etched from the backside, leaving a membrane of about 10  $\mu\text{m}$  thickness. A high phosphorus diffusion at the side walls of these holes and through these

membranes, covered with aluminium, establishes the electrical contacts to the source and the drain from the back-side of the chip. Later on Van den Vlekkert used this design for flow-through sensors with a rubber O-ring on the channel side and spring-loaded contacts on the backside [17], but in principle the edges of the chips with back-side contacts still need epoxy encapsulation, when used for instance as dip-stick probes.

As an alternative approach, also attempts have been made to solve the encapsulation problem with electronics in such a way that the silicon of the substrate can continuously be in contact with the electrolyte, but is electrically insulated by a reverse biased diode formed by source-drain versus substrate [18]. This approach has, however, never been evaluated in practical situations as far as we know. The same yields for our own design of a mesaISFET, with the actual ISFET on the top of an etched mesa (small hillock) with the source and drain contacts at a lower level of the chip surrounding the mesa [19]. The contacts were made by means of pushing the mesa through a hole in  $z$ -axis conductive tape and making in one and the same temperature treatment the ohmic contacts as well as the polymer attachment to the mesa side walls.

The previous paragraph was completely devoted to protecting the ISFET against undesired leakage currents. Being a very sensitive field effect device it should however also be protected against high external electric fields, in order to prevent the electrostatic damage of the gate oxide. It is striking that this item is to the best of our knowledge not treated in the open literature. The reason may be that the problem does not manifest itself so dramatically in research projects, as our own experience is indeed. Grounding of the sample solution with the reference electrode prior to inserting the ISFET or inserting them both at the same time may form enough protection. However, a practical situation will usually differ from such a cautious treatment and this is the reason that Sentron patented and always uses ISFETs with added protection against electrostatic discharge [20]. The ISFET chip is provided with an additional electrode around the gate area, which is connected to a highly boron doped diffusion, located in the source phosphorus diffusion, thus forming a zener or avalanche diode, which conducts above a predetermined breakdown voltage.

#### 4. Instrumentation

From the beginning of the sensor developments in silicon it was suggested that, as one of the advantages, now easily a complete signal modifier could be integrated, even with an on-board signal processor. To mention a few, distributed over the last decade: in 1989 Wong and White reported on a CMOS-integrated operational amplifier [21] and in 1999 Bausells et al. published how ISFETs can be made in a common CMOS technology [22]. The first one focuses on the instrumentation as such: an operational amplifier with an ISFET input and a feedback loop to a MOSFET, made in the

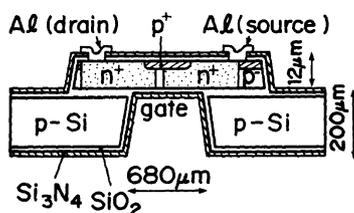


Fig. 6. Cross-sectional view of silicon-insulator-silicon structure for ISFET design [15].

same process. In this way (common) temperature effects could be reduced. Secondly Wong and White showed how a differential measurement between two ISFETs with different gate oxides, both as part of an ISFET operational amplifier, made it possible to apply a platinum electrode as (pseudo)-reference electrode. The second publication focuses on the application of an unmodified commercial CMOS process for ISFET fabrication, making use of the passivation oxynitride layer as the pH-sensitive layer, be it on top of a floating electrode consisting of polysilicon plus two metal layers. Both publications as well as others, not mentioned here, are more or less demonstrations that it is possible to integrate electronic and sensing functions, but no proof has been given that the ultimate result is better than can be obtained with separate functional devices, of which the fabrication process can be optimised for that specific function. This may be the reason that up to now no ISFETs with integrated amplifiers have been commercialised (to the best of our knowledge).

Although integration of a sensor and the necessary electronic circuitry may be an advantage, for instance to obtain an improved signal to noise ratio, this is definitely not the case for an ISFET as already stated in the introduction. The ISFET is in fact a perfect impedance transformer at the spot of the measurement and can therefore easily be connected remotely to a specific signal modifier.

A general design criterium for ISFET and amplifier is that electrolytic breakdown of the gate oxide has to be prevented. ISFETs are basically polar devices, like electrolytic capacitors. Therefore, care should be taken that the silicon is always positive with respect to the electrolyte, or at least greater than  $-1.3$  V. This requirement can most easily be realised by using  $n$ -channel devices in a “normally-on” condition.

Assuming that the sample solution can in practice accidentally be grounded, the best is to ground it anyway by means of a grounded reference electrode. The amplifier design is then forced to consist of a system where the source/bulk connection is positively biased with a protection for high voltages and the drain is slightly higher in potential. This is an other reason that the source/drain follower system as already described in the introduction is by far the best approach [3] and presently incorporated in all commercially available systems as far as we know.

The original design [3] made use of separate components but was followed by a system which uses the instrumentation amplifier concept [23]. In this system an additional feature is that with a floating sinusoidal signal source, connected between the source and the bulk, the temperature sensitivity of the connected ISFET can continuously be monitored, which signal can be used for automatic temperature compensation. However, this approach, patented by Philips, has never been applied in commercial products as far as we know. A later, more simple, version of the source and drain follower circuit is shown in Fig. 7.

The operational amplifier keeps its input voltage difference at zero ( $U_+ = U_-$ ), thus  $U_{R_1} = U_{R_3}$  and  $V_{ds} = U_{R_2}$ .

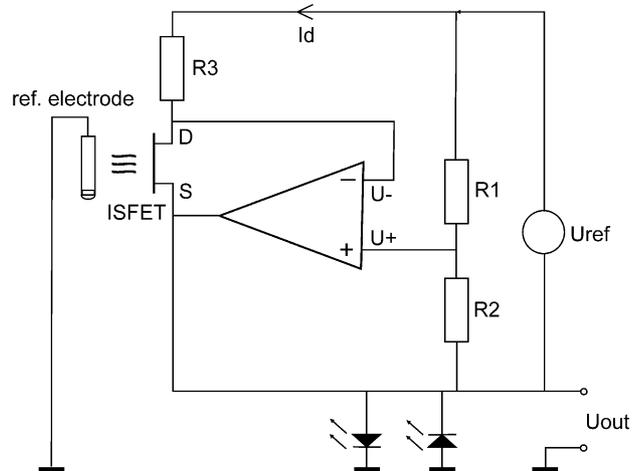


Fig. 7. The basic circuit diagram of a source and drain follower.

Because  $U_{R_2}$  is constant (part of constant  $U_{ref}$ ),  $V_{ds}$  is also constant, whereas the drain current  $I_d = U_{R_3}/R_3$  is also constant, because  $U_{R_3} = U_{R_1}$  is constant (other part of constant  $U_{ref}$ ). The result of these circuit settings is that  $V_{gs}$  is kept constant by automatic adaptation of the source potential with respect to ground to any changes in the ISFET threshold voltage  $V_t$ . Changes in the oxide surface potential are thus very accurately reproduced by the circuit output voltage  $U_{out}$ . Practical bias values are  $I_d = 100 \mu A$ ,  $V_{ds} = 0.5$  V, resulting in  $U_{out} = -1$  V at pH = 7. The diodes at the output serve as a limitation of the output voltage and thus the source voltage with respect to ground in the range of  $-1.3$  to  $+3.0$  V. In a practical realisation, these diodes are LEDs which light up in case the system is out of range.

In literature many other amplifier systems have been published but they mostly do not fulfil the requirements of failproof. For instance, the application of a feedback system to the reference electrode is not practical, because it forces the sample liquid to a certain potential, which is often in conflict with accidental grounding. Moreover, multi-ISFET measurements with one reference electrode can not be performed in this way. Also a differential ISFET/REFET (see Section 5) pair in a conventional differential amplifier concept is hardly an option, because the actual amplification of the input signal can not be controlled.

Although many papers deal with the problem of temperature sensitivity, light sensitivity and drift, no general solution for prevention, reduction or compensation of these effects exists. The temperature insensitive point of the MOSFET  $I_{ds}/V_{gs}$  curves does not exist for an ISFET, due to additional temperature effects of the surface potential and the reference electrode. Therefore, the only way out is to characterise the temperature behaviour beforehand, to simultaneously measure the temperature during an actual application and compensate for it with respect to the sensitivity as well as the offset. The same yields for the drift behaviour. A predetermined drift of, say 1 mV/h, can be compensated for by a programmed amplifier drift of  $-1$  mV/h.

Both compensation techniques have been developed and performed by Sentron, formerly Cordis R&D group [24]. The solution for the light sensitivity is simple: measuring in a (local) dark environment, sometimes partly made by adding a screen electrode to the ISFET chip.

In conclusion, most of the practical ISFET measurements make use of a remotely placed source and drain follower circuit and one can live with possible interferences of temperature, light and long-term drift, which approach does not differ, by the way, from measurements with conventional ISEs.

## 5. The REFET

One of the frustrations in the field of solid-state chemical potentiometric as well as amperometric sensors is the lack of a solid-state reference electrode. Always the small and rigid chip sensors have to be combined with a conventional liquid filled reference electrode. Miniaturisation of such an electrode, possibly with a gelled internal electrolyte, is also not an option, because due to out-diffusion of KCl and indiffusion of ions from the sample solution, the stable lifetime of these electrodes is limited. So, in practice, usually a small sensor probe is operated in combination with a rather large reference electrode, which contradicts the possibility of measuring in extreme small sample volumes. It has long been thought that due to the development of ISFETs the reference electrode problem could be solved, by applying a differential measurement between an ISFET and an identical FET which does not react on the ion concentration to be measured. Such a device is called a REFET. Grounding of the sample solution can in this case be performed by a metal wire, for instance platinum, of which the unknown and unstable electrode potential manifests itself as a common signal in the differential system and is thus suppressed by a factor equal to the so-called common-mode-rejection-ratio (CMRR) of the system. A block diagram of such a differential system is given in Fig. 8.

For both the ISFET and the REFET amplifier, the source and drain follower circuit as shown in Fig. 7 can be used, of which the output voltages are connected to an additional differential amplifier. The platinum electrode is usually

called a pseudo-reference electrode and may consist simply of an evaporated Pt layer as an integral part of the ISFET, the REFET or the substrate on which the sensors are mounted. In this way, a totally solid-state sensing probe can be constructed, provided that indeed well-defined REFETs can be made. Therefore, much attention is paid throughout the years to decrease the pH sensitivity of an ISFET to ultimately zero, delivering a perfect REFET.

Most of the attempts to create a REFET are based on covering the gate oxide of an ISFET with an additional ion insensitive membrane. The first publication concerning this approach is from Matsuo, who deposited a parylene film on the  $\text{Si}_3\text{N}_4$  gate of an ISFET [25]. Although the pH sensitivity decreased, the stability was not as expected, most probably due to pinholes in this very thin layer. Therefore other membranes have also been deposited, usually thicker layers, such as Teflon [26]. The key parameter for the choice of the material was initially the hydrophobicity, but later on one discovered that this is not the only criterion for chemical inertness as required for a REFET. At the present moment, with the theoretical knowledge as given in Section 2 in mind, it is clear that in fact the sensitivity factor  $\alpha$  as defined in Eq. (6), prescribes that a pH insensitive surface should have a very low buffer capacity, preferably leading to  $\alpha = 0$ . This might also have been the reason, by intuition, that one also tried to develop REFETs based on chemical modification of the original gate oxide surface sites as for instance in depth investigated by the group of Ecole Central de Lyon [27]. Their method concerns the careful oxide surface preparation, including a sulphochromic treatment, followed by the condensation of aminosilanes on the surface hydroxyl groups. The result is a dense monolayer of long-alkyl-chain silanes. The reduction in pH sensitivity of thus grafted silica surfaces appears to depend on the chain length, but also on the grafting completeness, which may differ from sample to sample and is also a function of the contact time with an electrolyte. So, with respect to stability also this type of REFET is not yet suitable as a real reference.

It is striking that during the continuing attempts to develop a stable REFET, focused on the realisation of a complete inert gate surface, nobody paid attention to the early observation of Matsuo, already published in 1984. Matsuo showed that also the silicon substrate is involved in the proper operation of a REFET [28]. The reason may be that the theoretical explanation was difficult to understand at that time and the years after. Nowadays, with the new theory as given in Section 2 in mind, it is clear that the sensitivity factor  $\alpha$  (Eq. (6)) shows that also the differential double-layer capacitance,  $C_s$ , is determining the ultimate (ion)sensitivity of an ISFET and thus also of a REFET, regarded as an ISFET with a low value of its surface buffer capacity  $\beta_s$ . Because  $C_s$  is a non-selective function of the ion concentration through the Debye length, it may not be a surprise that REFETs respond to ion concentrations in general. Moreover, even in the case that a REFET's buffer capacity will be zero and thus the influence of  $C_s$  will be neglectable through the sensitivity factor  $\alpha$ , there will still be a diffuse charge layer in

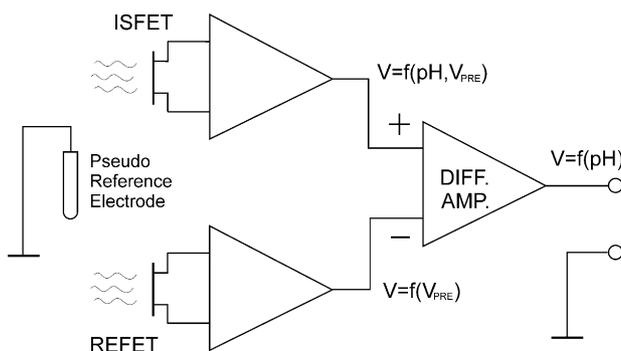


Fig. 8. Block diagram of a differential ISFET/REFET measuring system.

the solution at the gate surface, because the REFET (as well as the ISFET) will always be electrically biased to a certain voltage, usually in the order of several volts. In Fig. 8, it can be seen that the electrolyte is grounded and the source potential will be positive. Unfortunately this dc bias voltage will be different for the ISFET and the REFET, respectively, because both devices are electrically not identical as soon as the REFET has an additional ion blocking surface layer. This voltage is divided over the diffuse charge layer in the solution and the gate insulator and any change in this division, due to a concentration change, will result in a differential ISFET/REFET signal. The only way out is to bias the REFET in such way that it behaves electrically identical to the ISFET, for instance by means of applying a certain bulk-source potential. In the latter case the resulting depletion charge in the silicon compensates, at least partially, for the effect of the diffuse charge in the solution. In fact, this conclusion is in agreement with the never recognised observation of Matsuo in 1984 that the REFET behaviour can electrically be tuned by biasing the silicon substrate [28]. However, usually bulk and source connections are short circuited for practical reasons and biasing is thus less easy to perform, although it is remarkable that the first papers on the development of ISFETs make explicitly use of drain current control by means of bulk feedback [4,5].

The conclusion is that the ISFET/REFET differential system can only be successful in case both devices are electrically identical, resulting in exactly the same dc bias. In that sense a REFET based on a CHEMFET may be more realistic for a differential CHEMFET/REFET system. A CHEMFET is in fact an ISFET with additional membranes of which the outer membrane contains a buffer component for the ion to be measured, in agreement with the theory as given in Section 2. For the electrically identical REFET, the same membrane should then not contain such a buffer component. The ion concentration in the membrane should just “follow” the concentration changes in the sample solution. Such a REFET is developed by Chudy et al. [29], but not yet fully characterised.

An other approach is to apply an ISFET in a differential set-up (Fig. 8) with another (identical) ISFET of which only the response is retarded by adding a diffusion barrier. Such a system is described by Van den Vlekkert in a flow-through arrangement [30]. The pseudo-REFET was covered with a 200  $\mu\text{m}$  pHEMA hydrogel, resulting in a non-response time of up to 10 min. Using a relatively high sample frequency, fitted to the response time of the integrated ISFETs and CHEMFETs, the pseudo-REFET reacts slow enough to act as a reference sensor during passage of the sample. It will be clear that this approach is limited to the application of ISFET/REFET pairs in a flow-through system.

## 6. Typical ISFET applications

As stated in the introduction, initially ISFETs were developed to serve as new probes for electrophysiological

experiments, but this challenge has not been taken up by the field. Only recently publications appear concerning the monitoring of cell metabolism [31,32] in which not electro-physiological signals are measured but physiological, mainly focused on the extracellular acidification rate of a cell culture. In this type of experiments the ISFETs form, with other sensors, like temperature sensors (diodes), conductivity sensors (interdigitated electrodes) and oxygen sensors (amperometric), all integrated in a silicon wafer, the bottom of a cell culture chamber. With a fluid perfusion system for cell culture media the cells can be kept alive or be stimulated by added drugs. It will be clear that for the expected pH changes of 0.1 pH/min in a chamber volume of 10  $\mu\text{l}$ , the application of an ISFET as pH sensor is advantageous.

It can be expected that in the future more silicon chamber bottom plates with integrated ISFET arrays will be applied for the study of cell metabolism, for instance to elucidate the behaviour of tumour cells.

### 6.1. Catheter tip sensors

Beside the new field of physiological research, mentioned above, from the beginning also the possibility for measurement of in vivo physiological parameters drew the attention of clinicians. In line with the development of catheter tip blood pressure sensors, also ISFET catheter tip sensors were developed. The fact that the sensor chips are so small and rigid made this development obvious. The R&D group of Cordis in Roden, The Netherlands, developed a catheter tip pH sensor, based on an  $\text{Al}_2\text{O}_3$  ISFET of which the temperature and drift behaviour could be predicted by testing MOSFETs made on the same wafers as the ISFETs. These predicted parameters were stored in a PROM, which was an integral part of the connector of the catheter and could be read out by the signal processing unit, resulting in an accuracy of 0.02 pH units over a period of 10 h [24]. It was the first time that it could be shown that blood pH changes periodically with the lung ventilation and that after stopping artificial ventilation during an operation a patient is not always able to take control of his acid–base balance. Unfortunately, problems with the biocompatibility of the catheter, especially at the place of the window opening for the ISFET contact, made it impossible to launch the product ultimately.

### 6.2. Flow-through cells

To face the problem of biocompatibility, but yet make use of the ISFET advantages of being able to measure in very small volumes with a fast response, micro systems were developed in which a small “bloodline” was connecting the patient to the extracorporeally placed ISFETs. Sibbald [33] was one of the first who showed the possibilities and based on his research results Thorn EMI developed a prototype cartridge, containing Sibbald’s four-function ISFET chip

(pH, pNa, pK and pCa), as well as rinsing and calibration facilities, controlled by a microprocessor [34]. The cartridge contained enough calibrant solution to perform 200 bedside measurements, using 18 ml/h unheparinized blood from the patient, resulting in a semi-continuous monitoring of a patient's condition over 24 h.

Although not yet developed as a clinical instrument ready for use, a similar approach has been followed by the group of de Rooij in Neuchâtel for the measurement of pH, pCO<sub>2</sub> and pO<sub>2</sub> in a flow-through cell arrangement [35]. The first parameters are measured with ISFET-based sensors, the last one with an amperometric sensor. The liquid channel containing the sensors is 18 mm in length and 2 mm in width, resulting in an internal volume of 5 µl. In total, nine sensors have been integrated to achieve redundancy for reliability increase.

Especially focused on the calibration of the extracorporeally placed sensors, Gumbrecht et al. developed a flow-through cell, containing the sensors, which is connected to the patient by means of a double lumen needle [36]. The outer lumen serves for the injection of a calibration liquid, the inner lumen for the take up of blood or calibration liquid, depending on the speed of the two motors which drive the two corresponding pumps. The system can thus automatically “decide” whether calibration liquid or blood is in contact with the incorporated ISFET or other sensor.

Note that all these examples, as well as many similar ones, not described here, make explicit use of the fact that the sensor chips can so easily be incorporated in a narrow channel and measure relatively fast in the resulting small sample volumes, similar to FIA measurements as known from analytical chemistry. However, none of the systems has at present been commercialised as far as we know, most probably again because measurements in whole blood are more difficult than expected. Moreover, the measurement approaches are in principle conventional in character. Only miniaturisation is obtained. Therefore, one of our approaches has been to show that the application of ISFETs has, beside miniaturisation also other advantages, especially the possibility to integrate actuators and develop in this way feedback systems. This might open completely new concepts of measurement, explicitly exploring the fact that the ISFET gate has such small dimensions, typically in the µm range.

### 6.3. Sensor/actuator systems

Especially with respect to a pH sensitive ISFET, the integration with a pH actuator is relatively easy: deposit a noble metal electrode around the gate area and generate coulometrically protons or hydroxyl ions by means of a current pulse applied to the actuator electrode with respect to a remotely placed counter electrode. The electrolysis of water will result in a pH change in the direct vicinity of the gate, which will thus be measured by the ISFET with only a small delay, determined by the diffusion of protons from actuator to gate.

The first experiments with a pH ISFET sensor/actuator system were performed by Van der Schoot as a Ph.D. student of the author [37]. He developed a flow channel in silicon with an array of 10 ISFETs and four actuators and showed that with a stop-flow mode, as well as with a continuous flow, titration curves of an acid or base could be performed within some seconds, applying current pulses of some microamperes. Factually this coulometric titrator can be seen as the first device in the field of what later has been called µTAS. A later version of such a device is the dipstick titrator, applying two identical ISFETs, both with a noble metal electrode around the gate [38]. One of these electrodes serves as the pH actuator, the other as a pseudo-reference electrode as mentioned in Section 5 (Fig. 8). The counter electrode for the actuator system was deposited on the backside of the substrate on which the ISFETs were mounted. A cross-sectional view of this device is shown in Fig. 9a.

Note that in fact a pH change is measured between the sensor/actuator ISFET and the other ISFET, which is situated at a place where no pH changes occur. This sensor can thus be seen as a perfect REFET, however not because of any modification of its surface, but because it is situated at a place with constant pH. This concept may in general be a solution to the remaining problem of ISFET/REFET pairs as described in Section 4. It should also be noted that with the differential set-up, no longer the static ISFET amplifier output voltage is measured, nor of interest, but the time delay between the onset of the actuator current pulse and the deflection point in the recorded titration curve, which can easily be determined by means of differentiating the titration curve. A typical recording is shown in Fig. 9b. Note that applying this measurement concept no problems due to ISFET drift are influencing the measurement. Therefore the system, being in fact the first and only complete solid-state ISFET-based sensor, is very suitable for long-time measurements without the need for regular recalibration. This was also shown by Van der Schoot in the form of a CO<sub>2</sub> sensor, where a liquid in equilibrium with CO<sub>2</sub> through a Teflon membrane, was continuously titrated forwards and backwards, resulting in an accuracy of 10% in concentration over a period of 7 weeks [39].

In later versions of the dipstick titrator also other materials have been used for the actuator, such as WO<sub>3</sub> and Ir<sub>2</sub>O<sub>3</sub> in order to force a reaction with protons, preventing possible interference with other ions [40].

### 6.4. Feedback systems

Layers of immobilised enzymes are often used to convert a substance for which no sensor is available into a substance for which a chemical sensor exists. A well-known example is the urea sensor which makes use of immobilised urease which converts urea into ammonia ions, carboxyl ions and hydroxyl ions. As one of the options, the resulting pH in the membrane containing the enzyme can serve as the indirect measurand for urea. It will not be surprising that

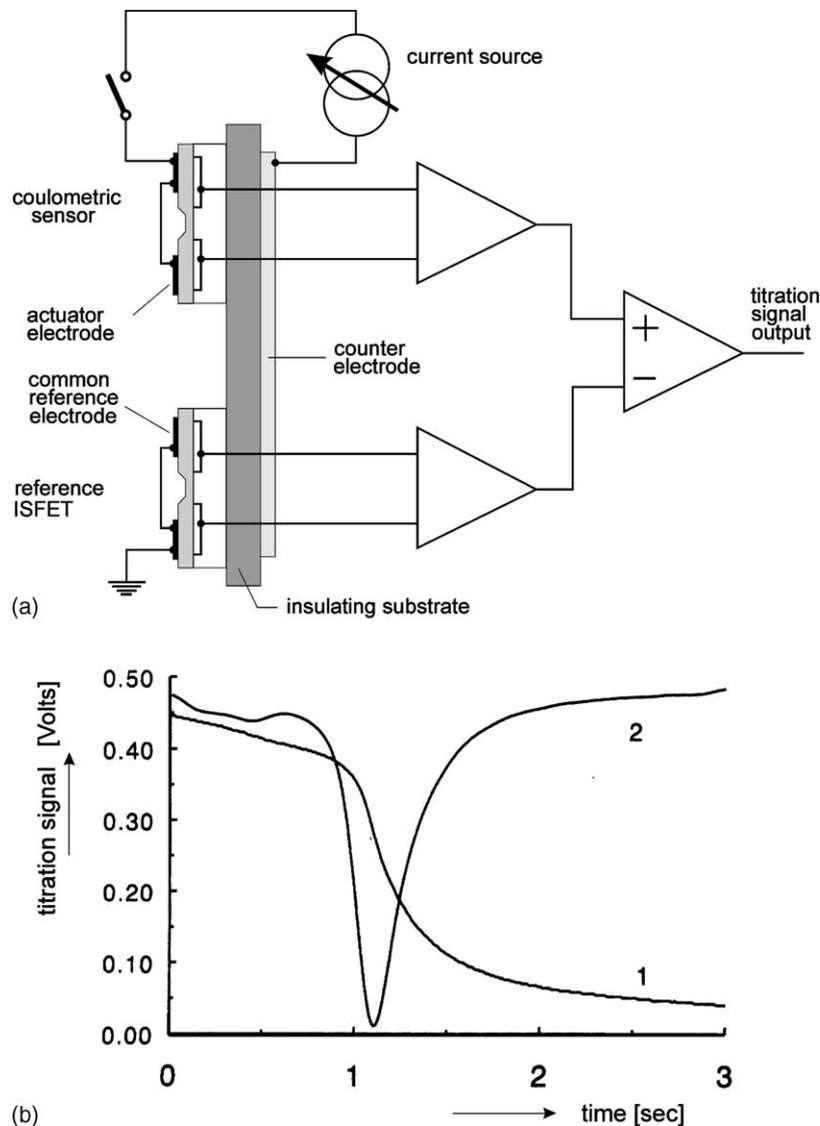


Fig. 9. (a) Cross-section of dipstick titrator and block diagram of electronic system, and (b) typical titration curve of 2 mM lactic acid, obtained with a current pulse of 10  $\mu$ A, with its derivative for detection of the equivalence point.

ISFETs have been used to measure pH, because it is so easy to deposit membranes with incorporated enzymes onto the flat ISFET surface. A serious drawback of such a device, called ENFET, is that the response is very non-linear, caused by the fact that the induced change in pH decreases the enzyme activity drastically. Moreover, the response greatly depends on the buffer capacity of the sample in which the urea concentration has to be measured: the higher the buffer capacity, the lower the pH change. A solution to this problem is the application of feedback by means of the coulometric pH actuator, as described above in this section [41]. Even the same dipstick set-up as illustrated in Fig. 9 can be used. Both ISFETs, including their integrated actuators are provided with a membrane with immobilised urease, which is made inactive by UV illumination at the pseudo-REFET device. The differential output voltage is compared with a predetermined setpoint

(actually the desired pH inside the membrane of the active ENFET) and the difference is led to a controller which determines the continuous current which should flow through the coulometric actuator in order to control the membrane pH to the desired value. Now the value of this current is the measurand which is a function of the urea concentration [U] according to:

$$I = -\frac{E}{A}[U] \quad (7)$$

where  $E$  is the enzymatic sensitivity in  $[\text{OH}^-]/[\text{U}]$  and  $A$  the sensitivity parameter of the sensor/actuator ISFET in  $\text{mol/l A}$ . Note that this equation shows a very linear relation, which is due to the feedback action and that the dependence on buffer capacity is vanished. This is because the nominator as well as the denominator of Eq. (7) both contain the buffer capacity and thus cancel out each other.

It is striking, or maybe disappointing, that the application of ISFETs with an integrated actuator has hardly triggered other research groups to further exploit the unique possibilities of such a system. As far as we know, only the Korean group of Byung-Ki Sohn has made advantage of the possibilities, by developing an ISFET-based glucose sensor with improved sensitivity [42]. Up to that time the ISFET-based glucose sensors were all based on the detection of gluconic acid as a product of the enzymatic conversion of  $\beta$ -D-glucose, which, however, delivers only one proton per glucose molecule. However, a by-product of this reaction is hydrogen peroxide, which deteriorates the enzyme. The Koreans came up with the smart idea to oxidise this hydrogen peroxide by means of an integrated actuator with the advantages of getting rid of the hydrogen peroxide and at the same time generating two additional protons per glucose molecule and moreover generating at the same time oxygen which is necessary for the glucose conversion. The sensitivity was increased by a factor of 5. At present the sensor is further investigated with respect to the response time, lifetime, etc.

In general it can be stated that for all sensors which make use of the conversion of a certain substrate into a weak acid or base, the sensitivity can be improved by not using the ISFET for the measurement of the free protons, but by using an ISFET/actuator system for titration of the reaction product(s).

### 6.5. Dynamic measurements

It should be noted that in the previous sub-section partly the concept of static measurements has been changed to dynamic measurements, thus eliminating the drift problem of the applied sensor, in our case an ISFET. For the titrator systems not the static ISFET amplifier output voltage was the measurand, but a certain (short) lapse of time, which is a dynamic parameter. This dynamic concept can also be applied in the field of protein research.

Due to the many different titrable groups in the side chains of the polypeptides of a protein molecule, as well as possible specific interactions between the groups, proteins show many different pKs and thus several maxima of their buffer capacity. In general, the buffer capacity of an acid or base can be derived from the slopes of the titration curve, which is, however, a difficult procedure in the case of proteins. Direct determination of the different slopes of a very non-linear curve of an electronic device or function is usually achieved by means of applying a small sinusoidal signal and measuring the amplitude of the converted signal around a slowly varying bias point. This procedure can also be followed in the case of an ISFET/actuator system. We therefore developed an ISFET with a porous gold actuator above and around the gate area, 100  $\mu\text{m}$  thick and 1  $\mu\text{m}$  away from the gate oxide [43]. Proteins can be adsorbed inside the pores of the actuator or move freely as constituents of the sample solution. A 1 Hz sinusoidal actuator current of 10  $\mu\text{A}$  is applied to the actuator electrode, resulting in

sinusoidal pH variations inside the pores. This procedure is shown in Fig. 10a. The ISFET measures these pH variations and the reciprocal of the RMS value of the ISFET amplifier output voltage is recorded as function of the pH of the sample solution (the bias point). This value reflects directly the protein buffer capacity as function of the pH. As an example we measured the “buffer fingerprint” of a solution containing 5 g/l ribonuclease and 5 g/l lysozyme, respectively, as shown in Fig. 10b.

Up to now we only focused on the actuation of protons, which is of course because such an actuator is so easily

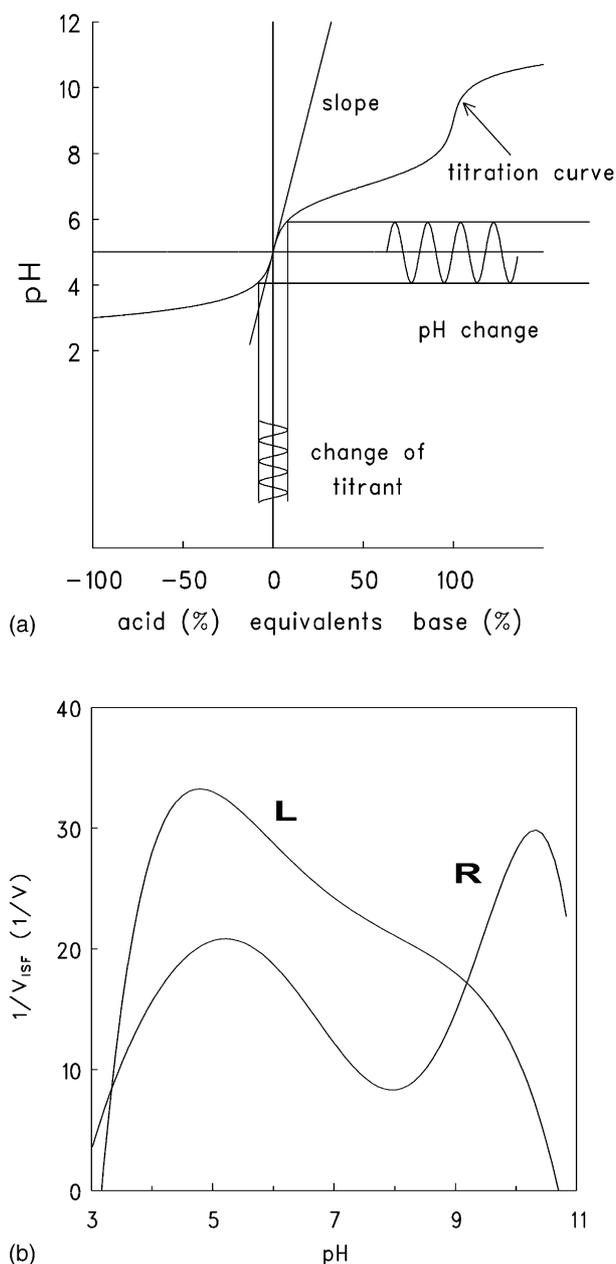


Fig. 10. (a) Illustration of the concept of a buffer capacity measurement by means of an applied sinusoidal perturbation of the titrant, (b) buffer capacity of 5 g/l ribonuclease (R) and lysozyme (L), respectively, both as function of the pH, corrected for the buffer capacity of the blank solution.

available. It is more difficult to imagine the existence of actuators for other substances in combination with sensors for that specific substance. On the other hand, the role of protons is often underestimated in many physiological, biophysical and biotechnological processes. For instance, the net charge of protein molecules is a direct function of the pH, similar to the net surface charge of an ISFET gate oxide [44]. Especially because of the latter similarity it is interesting to investigate the combination of proteins and ISFETs. This has been a wish from the beginning of the ISFET era, especially concerning the development of immuno sensors, IMFETs in this case. However, it has been argued in many papers that a static measurement concerning the presence of a protein layer on an electrode is difficult, because the charged groups are of course neutralised by surrounding counter ions [45]. The resulting double layer extends only over a distance of the Debye length and it can be argued that this will never overlap with the double layer at the surface of an ISFET. Furthermore, the density of the protein layer will determine greatly whether or not it results in a measurable signal. The successes or disappointments of IMFET measurements will therefore mostly depend on the specific conditions of the protein adsorption, which are often not really controlled. Therefore, we developed an alternative, based on our experience with the dynamic measurements as described above.

In an equilibrium state, protein molecules are surrounded by a double layer, with charge  $Q$ , capacitance  $C$  and potential difference  $V$  over the double layer, related to each other by  $Q = CV$ .

The potential difference  $V$  is a function of pH and is zero at  $\text{pH}_{\text{iep}}$ , the isoelectric point and positive or negative around this point depending on the bulk pH. The double-layer capacitance is a function of the ionic strength and the value of  $Q$  will thus adapt. In case a sudden change in the ionic strength is made by an actuator at constant pH, the value of  $C$  will directly change: larger for ionic strength increase and smaller for ionic strength decrease. Because  $V$  will remain constant, being determined by the pH, the value of  $Q$  should adapt. This is realised by a release or uptake of protons, depending on at which side of  $\text{pH}_{\text{iep}}$  the experiment is carried out. The protons will diffuse out or in the double layer over distances beyond the Debye length, where they can be measured by a pH electrode. In this way proteins can be characterised by their natural label: their charged condition.

In practice, experiments have been carried out by our group, where membranes with immobilised protein molecules have been deposited on ISFETs or where the protein molecules have been adsorbed directly onto the ISFET surface. For the ionic strength actuation, continuous flow regimes have been applied, for instance with a wall-jet, where valves served for the sudden change in the  $\text{KNO}_3$  concentration at constant pH and KCl concentration. As an example of such a stimulus-response experiment, Fig. 11 shows the result of the measurement of heparin, after bind-

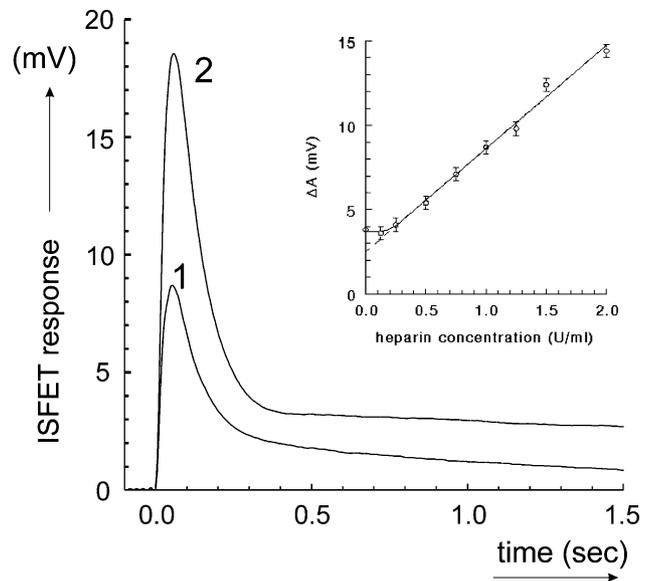


Fig. 11. Typical result of a stimulus-response measurement of a protamine coated ISFET (curve 1) and after 2 min incubation in a PBS solution containing 0.7 units/ml heparin (curve 2). Inset: difference of pulse amplitudes after 2 min incubation in normal plasma as function of the heparin concentration.

ing to an ISFET which was provided with a monolayer of protamine [46]. In fact the protamine, having a positive charge, modifies the initial negative ISFET oxide surface charge to be less negative. The result is a less positive response than a bare ISFET should give. After 2 min of incubation with a heparin solution, the added negative charge of the heparin molecules results in a more positive response. The amplitude of this pulse is a function of the heparin concentration, as shown in the inset of Fig. 11. The sensor could be regenerated in a 5 M NaCl solution and be used again for further heparin detection.

Note that the response of the protein molecules due to the temporarily disturbance of their equilibria delivers a monophasic pulse, being the registration of a proton release or take-up. It is interesting to combine this observation with the recording of monophasic pulses around a nerve as described in the introduction and shown in Fig. 2a. At the time of those registrations it was suggested that it might be the result of a potassium efflux. However, in view of the stimulus-response measurements as described above and knowing that around and in the nerve membrane many protein molecules are present, the monophasic membrane pulses may also be the result of a stimulus-response experiment. The sodium influx and potassium efflux will most probably result in a temporary local change in ionic strength, thus inducing a proton release or take-up by the present protein molecules. If this would be the case, the ISFET in the original registrations should then have measured a local pH change. This new hypothesis should be investigated in more detail, especially regarding the significance of local proton fluctuations in neurophysiology, possibly also in relation with

the experiments concerning the extracellular acidification rate of cell cultures, as described in the first paragraph of this section.

### 7. Other ways to modulate the threshold voltage

At the end of the introduction it was already mentioned that also other parameters than the oxide/solution interface potential  $\Psi$  (see Eq. (3)) in the case of an ISFET may be used to chemically modulate the threshold voltage. Eq. (2) already shows that with a normal MOSFET the difference in workfunction between the gate material and silicon ( $\Phi_M - \Phi_{Si}$ ) is such a parameter. For instance, the threshold voltage of two identical MOSFETs, however having different gate metals, differ with the contact potentials between these metals. This means also that, in case the gate electrode consists of a material of which the workfunction can reversibly be modulated by a chemical reaction from outside, the FET will show a threshold voltage which is controlled by that reaction [47]. In case this material is an electronic conductor, the device is actually a MOSFET and with the gate connected to ground and the MOSFET connected to the

source-drain follower circuit as shown in Fig. 7, the amplifier output voltage will show the corresponding value of the threshold voltage. This circuit diagram is schematically given in Fig. 12a.

In practice, redox active materials like iridium oxide, Prussian Blue, redox polymers, etc. can be used as gate materials, for instance deposited on top of a platinum electrode which is shaped around the gate area as in the case of the actuator electrodes described in the previous section. In contact with an electrolyte containing an oxidising agent, like for instance hydrogen peroxide, the gate material will oxidise (often also observable as a change in its colour) and consequently the value of  $V_t$ , being the output voltage of the amplifier as shown in Fig. 12a, will change [48]. In order to prevent an ongoing oxidation, we usually apply a small reducing current through the gate with respect to a counter electrode, placed somewhere in the sample solution, which balances the oxidation reaction. With the value of this help current one can tune the sensitivity region of the device. Note that for the actual measurement of  $V_t$  with the grounded gate electrode no reference electrode is necessary in the electrolyte. The electrolyte is only contacting the gate and can react with the gate material, but is not a

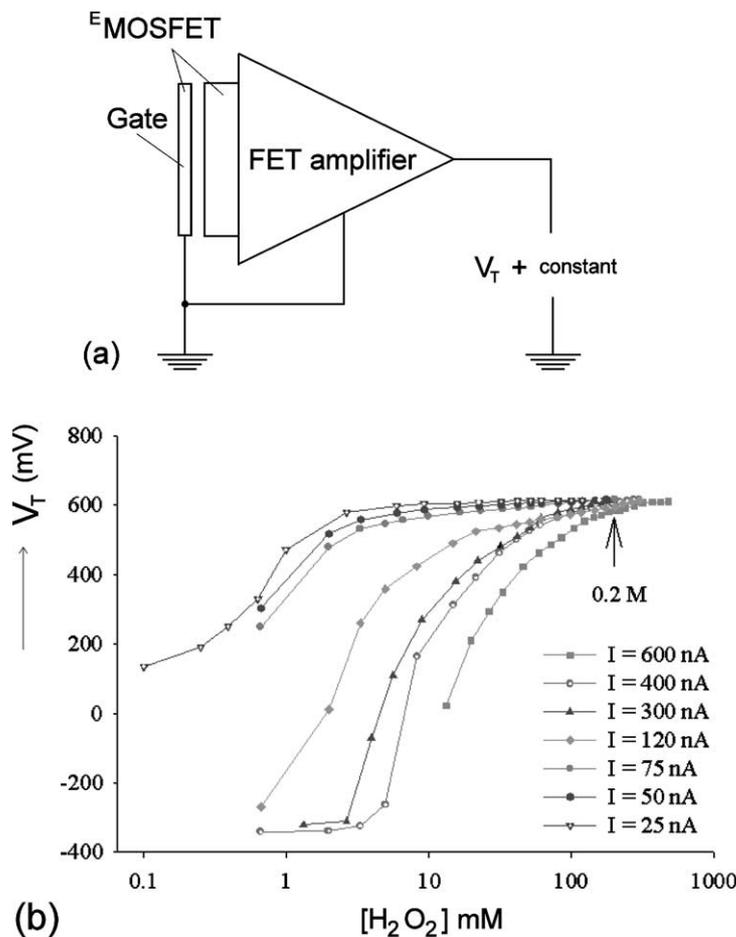


Fig. 12. (a) Block diagram of MOSFET and amplifier with grounded gate electrode. (b) The response on  $H_2O_2$  of an  $IrO_x$  gate  $E^M$ MOSFET with the help current as a parameter.

part of the actual chain of measurement. We therefore suggested as a name for this type of FET sensor the <sup>E</sup>MOSFET. Fig. 12b shows a typical result for the measurement of hydrogen peroxide with an iridium oxide gate electrode and different values of the reducing bias current. Note that the sensitivity is not determined by the Nernst law, because it is not an interfacial potential which is measured, but a difference in workfunction of the gate material, of which it can be proven that relatively high sensitivities can be expected, up to a change in  $V_1$  of 600 mV per decade. A disadvantage may be that workfunctions are bulk properties and thus the whole bulk is involved in the oxidation/reduction phenomena, which may reduce the response time due to diffusion times of the species involved. Therefore, ultra thin gate electrodes should be preferred.

It should be noted that the modulation of a gate's workfunction has also been demonstrated for gasses, especially hydrogen bearing gasses, already in the very beginning of the ISFET development, but in this paper we only refer to measurements with FET devices in electrolytes. Furthermore, in fact the <sup>E</sup>MOSFET is not at all an ISFET, as this is by definition a device where the interface potential with the electrolyte is the actual input variable, resulting in a maximum sensitivity of 59 mV per decade (Nernst). The <sup>E</sup>MOSFET is described here only because of the reference electrode discussion as mentioned in Section 1.

## 8. Patents and products

Around 1970 it was not usual at the relative young University of Twente (founded in 1963) to apply for a patent, especially not in the case of an invention which was subjected to heavy discussions. It was the time that the electronic industry struggled with the hermetic encapsulation of electronic chips, especially concerning the prevention of moisture penetration. So it was doubtful whether or not a chip, like the ISFET, could ever exist, notably in direct contact with an electrolyte. Therefore, it was a more established university, the University of Utah, that applied the first patent in this field [49]. This patent has been granted in 1977, regardless of the existence of the first ISFET papers in the open literature, because the Utah patent does not limit itself to ISFETs with a bare oxide, but introduces the term membranes, a much more general ion sensitive layer, as well as the further possibilities of ISFETs concerning the application of enzymes and antibodies. For later patents as well as manufacturing of ISFETs, often this first patent has been tempted. Legal departments have often used a small announcement, never refereed in the ISFET literature, published already in 1967, in the idea journal "Neues aus der Technik" [50]. This journal published ideas from industry which were not worth patenting at that time, but worth to prevent that others should patent such an idea. Under announcement 773 (authors were not mentioned) it was stated that a MOSFET with a Pt gate, immersed in an

electrolyte, could serve as a hydrogen electrode and when covered by a layer of ion sensitive glass as an ion sensor. In the latter case the Pt layer could also be left out. In fact, in this way the ISFET is described. Note that the announcement appeared in 1967, the time that the author already discussed his ideas concerning ISFETs with members of the Philips Research Laboratories, where he performed his MSc project on FET amplifiers in co-operation with the University of Eindhoven in 1965. It is known that Philips made use of announcements in *Neues aus der Technik*, so perhaps, although a little speculative, the announcement originates from Philips. More interested in electronics than in sensors, Philips patented later on the idea of the source and drain follower circuit with additional facilities to sense simultaneously the sensor temperature [23]. Nevertheless Philips never got engaged with the chemical sensor market.

Since the first patent many other patents have been filed, as far as we could trace, up to 158, distributed over the years as well as the continents, as shown in Fig. 13. This figure shows only the "pure" ISFET/CHEMFET patents and not the related patents concerning all kind of FET-based sensors, counted only once, regardless the number of countries where the patent is granted. The distribution shows some peaks, which we are not able to explain. It may be related to successes or disappointments in commercialisation or to important needs of specific markets in certain years.

With respect to the ISFET manufacturers, or at least the R&D groups of the companies, it is striking that some of the early ones, like Johnson & Johnson, Siemens and Thorn EMI have discontinued their initiatives to commercialise ISFETs, whereas others still exist, like Shindengen, or are still active but using an other name, like Sentron, the former Cordis R&D group. The companies that stopped their activities in the field of ISFETs were all involved in the biomedical market and we believe that it was not the difficulty of producing and packaging ISFETs as such which forced them to decide to stop, but the difficulty of the specific product requirements, especially regarding biocompatibility.

At present ISFETs are commercialised especially for markets where the vulnerability of glass membrane electrodes are a problem, like in the food industry. We counted 21 companies (see Table 1), advertising ISFETs at the Internet

Table 1  
Present ISFET suppliers/companies

Beckman Coulter	Lazar Res. Labs.
Belswan	Mettler Toledo
Bioanalytical Systems	Nisso
Delta TRAK	Orion
Denver	Radiometer
Elf Aquitaine	Sanwa Tsusho
Elec	Sentron
Fisher	Sera
Honeywell	Spectrum
Horiba	Team Labs.
IQ Instruments	

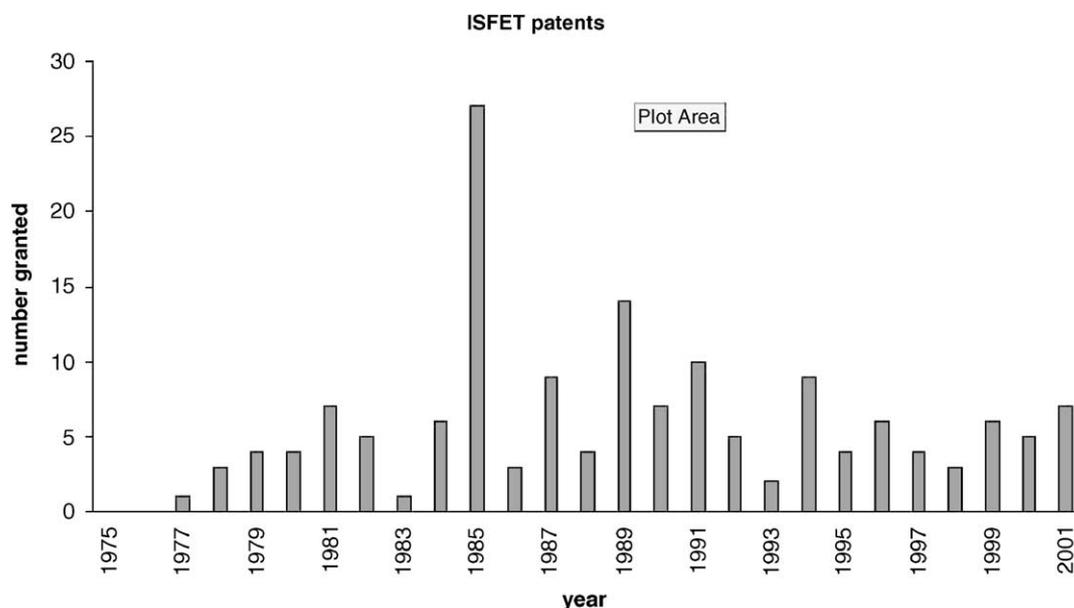


Fig. 13. Distribution of granted ISFET/CHEMFET patents, counted only once, regardless the number of countries where it is granted.

and elsewhere with announcements like: virtually unbreakable, direct insertion in sample at test, fast response, dry storage, easy brush cleaning, measure in viscous, even semi-solid samples like cheese, meat, etc. Terms like non-glass pHuture (Orion) and Durafet (Honeywell) reflect what is essential with respect to the intended ISFET application. The product lines contain benchtop, portable, waterproof and pocket instruments with modern sophisticated data handling and menu driven measurement protocols, including auto buffer recognition, direct 4–20 mA output, etc.

Some of the companies have their own silicon technology facilities (clean room) and can thus easily tune the ISFET properties to specific needs, whereas others make use of a silicon foundry elsewhere and are thus limited to the application of the standard processes of the foundry. It is expected that in the future especially in-line monitoring of industrial processes will become an important market for the application of ISFETs, especially in the food industry.

## 9. Possibilities for the next 30 years

In the previous sections more or less the history has been described of the research, development, application and commercialisation of a new device, based on an original idea. It is striking that such developments can take so long, but it is not an exception for mainly university work, which is more and more dependent on budgets of which the focus may change over the years. Many groups have been involved in the research on ISFETs throughout the years but have changed their subjects, because most probably financial support could more easily be attracted for other areas of research. Moreover, especially in the case of ISFETs, it has always been a question whether or not the traditional

markets of pH electrodes should take up the new device or that new markets should be explored, usually initiated by new small enterprises instead of the “big players”, which was what actually happened. And just these new small companies are usually not able to spend much money and time to improve the technology, to develop specific instruments, etc. So it may not be a surprise that the developments took such a long time.

Nevertheless the FET approach in the sensor era is still alive after 30 years: conference sessions are devoted to it, full papers appear in the sensor literature on a regular base and even the “big players” offer ISFET sensors in their present catalogues. The question is whether or not also new FET sensing devices maybe expected to become into existence in the next 30 years. The driving force may be the impedance transformation on the spot of measurement and the rather simple electronics needed for the read-out. However, it is our conviction that for chemical sensors the reference electrode problem should first be solved. As an example of a possible solution, in [Section 7](#) the concept of workfunction modulation, with a grounded-gate MOSFET, has already been introduced, but this may not be a general approach. Therefore, we introduce here a much more general complete new concept of FET based chemical sensors as a fantasy for the next 30 years, but of course not yet provided with measurement results.

The idea is based on a combination of two of our present Ph.D. projects.

The first project is based on the exploitation of the swelling/shrinking properties of hydrogels upon a chemical stimulus. For instance, a pH sensitive hydrogel in a bicarbonate solution, clamped in between the membrane of a piezo-resistive pressure sensor and a fixed gas-permeable membrane, makes a CO<sub>2</sub> sensor. Such a chemically sensitive pressure sensor does not need a reference electrode.

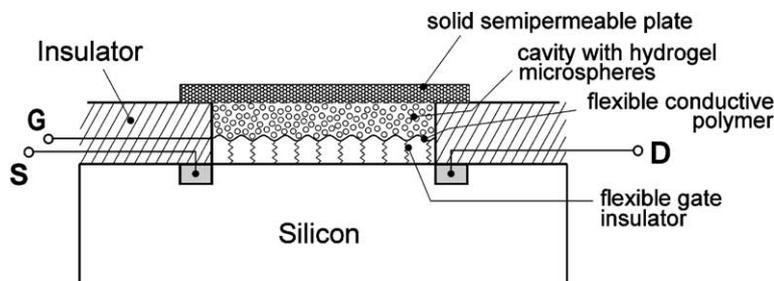


Fig. 14. Design of a chemically sensitive PRESSFET.

The second project concerns the direct coupling of organic monolayers on silicon surfaces [51]. By means of CV measurements we could prove that monolayers of alkynes and/or alkenes are very dense and stable, showing the field effect without the presence of an oxide layer in a very pronounced way. Specific functionalisation of the monolayer may lead to ion selective ISFETs with a very large signal/noise ratio.

In case the organic insulating monolayer of an ISFET, as described above, is not in contact with a liquid, but bound to an organic conducting layer, like polypyrrole, a very sensitive MOSFET is created. Assuming that both layers, the insulating as well as the conducting, are to a certain extent flexible, the MOSFET will be sensitive to pressure, resulting in a PRESSFET. Referring to Eq. (1), now the parameter  $C_{ox}$  is the input variable of the device. Combining this type of pressure sensor with the hydrogel concept as mentioned above, will lead to a chemically sensitive PRESSFET as schematically shown in Fig. 14.

In principle, the hydrogel spheres serve as a matrix for the immobilisation of charged groups and the swelling properties are, among others, due to the induced osmotic pressure according to the Donnan equilibrium. This means that in fact also other immobilisation techniques may be applied. The charged groups may directly be bound to the flexible gate of the PRESSFET. Also enzymes or antibodies may be incorporated in the pressure chamber, all modulating the availability of fixed charge. Any specific (bio)-chemical reaction may compensate the amount of fixed charge and thus the pressure, which is measured by the PRESSFET. Note that this complete new type of chemical sensor does not need a reference electrode. It is a stand-alone measuring system. Maybe except for the placing of the rigid upper plate, which serves as the backplate for the pressure chamber, meanwhile having selective transport properties, all layers may be deposited in a sequence of organic chemistry processes.

Although the concept, the design and the processing of this type of chemical sensors may look rather simple, we realise that the development of a whole new family of this type of sensors will take another 30 years. Nevertheless, we hope that the challenge will be taken up by the young researchers who are active in the interesting field of sensor development.

## 10. Conclusions and discussion

In the previous sections we have focused on those historic events in the field of ISFET developments which were more or less milestones as it was experienced by us. Partly this concerns the theoretical insights and partly the exploitation of ISFETs in various measurement conditions, especially with respect to the combination with pH actuators. However, this review should not be considered only as an historical overview. It serves also as an introduction in possible future research projects, based on experiences in the past.

The first conclusion is that the FET concept opened anyway the possibility to apply insulating materials in the field of ion sensing. Originally introduced on the basis of trial-and-error, the theory concerning the buffer capacity of an insulating oxide in contact with an electrolyte is now a well-understood design criterion.

Based on a combination of observed phenomena, a new aspect came up with respect to the possible significance of proton release of proteins in the vicinity of cell membranes and the corresponding signal propagation along nerves, in relation to the observed acidification of cultured cells. This provisional conclusion should be investigated in detail in the future. The application of ISFET arrays may be important in such a proposed research project.

With respect to the right of existence of REFETs, it was concluded that the dc bias of a REFET is up to now not recognised as one of the key parameters of a REFET's operational mechanism. Detailed investigation of this phenomenon may result in a new view on the development of REFETs.

The application of ISFETs has been taken up by the traditional market mainly because of the robustness of the sensor, which makes it among other advantages, possible to clean them easily, if necessary. Furthermore, the ability to store them dry is an advantage if compared with traditional glass membrane electrodes.

It is striking that the proven ability of local titration of microvolumes has not achieved any practical application up to now. Any (indirect) measurement of a weak acidic or basic reaction product will deliver a larger sensitivity if the product is titrated than if only the free protons, thus the pH, is measured. This might especially be of importance in the field of food processing. Moreover, this type of measurement is dynamic instead of static, thus getting around the sensor

drift problem and the necessity of a conventional reference electrode. Furthermore, the actuator electrode, necessary for the coulometric titration, can also serve the reduction of light sensitivity and the sensitivity for external electromagnetic fields. It is remarkable that these items have been addressed separately, even in commercially available products, but not as a combination, which is the most plausible inference.

The FET concept has up to now mainly been exploited for the measurement of an oxide/electrolyte interface potential, leading to pH sensitive ISFETs, or as a basis for membrane covered ISFETs: the CHEMFETs. Studying the basic FET equations it is clear that also other device parameters can be applied as sensing variables, such as the gate material workfunction and the input capacitance. We recommend to investigate these possibilities in detail in order to enlarge the FET applications. It may influence the market positively, because more basic FET chips will be needed, manufactured by a silicon foundry, whereas different sensor companies may independently add a specific sensor function to the chip, depending on the applied sensing concept and the related market. Note that independent of the function of the FET sensor, always the same electronic circuitry can be applied, which is definitely an advantage.

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