ISFET responses on a stepwise change in electrolyte concentration at constant pH

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Abstract

Responses on a stepwise increase of the electrolyte concentration of bare ISFETs can interfere with responses of an ISFET with an affinity membrane deposited on the gate. In this paper the responses of bare ISFETs are studied. Results of experiments and simulations are presented and the mechanism is explained.

Introduction

In 1990 a new measuring method was introduced to detect changes in charge density in a porous membrane deposited on top of an ISFET [1]. Schasfoort *et al.* [1] already showed the possibility to determine aHSA concentrations by using HSA as affinity ligand. At this moment the ISFET-based measuring method is under further investigation aiming at the development of a heparin sensor [2].

The device used in this dynamical measuring method is an ISFET with on top of the gate a porous membrane of polystyrene beads in an agarose gel. Proteins can be immobilized in the membrane via physical adsorption or covalent binding to the polystyrene beads. The titratable groups of the proteins result in a net charge density in the membrane which is pH dependent. If the concentration c_s of the electrolyte (in which the device is immersed) is suddenly changed and the pH is kept constant, the ISFET shows a transient response of which the amplitude is a function of the net charge density in the membrane. The response is mainly caused by a release or uptake of protons by the protein molecules in the membrane which result in a temporarily pH change in the membrane. The origin of the response is investigated by Eijkel *et al.* [3], and a detailed description is in preparation for publication. For a short description we refer to ref. 2.

During experiments we also noticed that a bare ISFET (without membrane) shows a significant response on a stepwise change of the electrolyte concentration, and in some cases this response interfered with the membrane response. For the progress of the research project it was necessary to study this ISFET behaviour. In this paper results are presented of ion-step experiments

with bare ISFETs and the ISFET behaviour will be explained.

Experimental

The measurement setup consists of two vessels with an electrolyte, connected to a flow-through system in such a way that the electrolyte in front of the ISFET can be changed within 200 ms by switching a valve. The two vessels contain solutions of equal pH and different electrolyte concentration c_s1 and c_s2, and are continuously purged with nitrogen to prevent CO_2 to dissolve. The pH in each vessel is monitored by a battery-supplied Radiometer pH meter. Two Metrohm burettes are connected to the vessels to adjust the pH with KOH. The ISFET is mounted in a cell where the liquid flow is perpendicular to the ISFET surface (wall-jet principle). A peristaltic pump ensures a constant flow of 2.7 ml/min and a calomel reference electrode defines the potential.

Results and discussion

Experiments

A typical response of a Ta_2O_5-ISFET on an ion-step from 10 to 50 mM KCl, is shown in Fig. 1, curve 1. In this case the pH was 8.3 (adjusted with KOH), and no buffer was added. The three other curves in Fig. 1 show ion-step responses after addition of increasing amounts of TRIS buffer to both solutions. As can be seen, the amplitude and the duration of the response become smaller with increasing buffer capacity.

In Fig. 2, the amplitudes of ion-step responses (10 to 100 mM KCl) for different types of ISFET gate
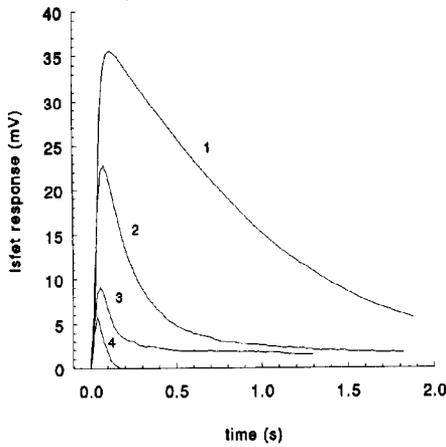
Fig. 1. Ta_2O_5-ISFET response on an ion-step (10 to 50 mM KCl) at pH 8.3. Curve 1: no buffer added; curve 2: 0.1 mM TRIS; curve 3: 0.5 mM TRIS, and curve 4: 1 mM TRIS.

Fig. 3. Amplitude of a Ta_2O_5-ISFET response on an ion-step from c_s1 to $5 \times c_s1$ as function of c_i1.
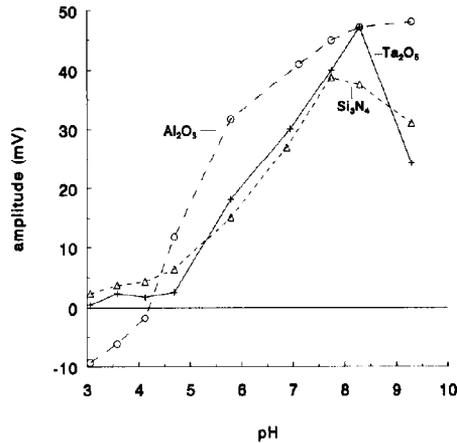

Fig. 2. Amplitude of ISFET-responses on an ion-step of 10 to 100 mM KCl (no buffer added) as a function of the pH.

oxides are shown as a function of the pH. The pH was adjusted with HCl and KOH, and no buffer was added. The curves of the Ta_2O_5 and Si_3N_4-ISFETs reach a maximum around pH 8; only the curve of the Al_2O_3-ISFET intersects the zero axis (around pH 4.2).

To determine the dependence on the absolute electrolyte concentration, ion-step responses of a Ta_2O_5-ISFET were recorded using different electrolyte concentrations c_s1 and c_s2 while keeping the step ratio constant at 5 ($c_s2 = 5c_s1$). In Fig. 3 the amplitude of the ion-step response is plotted as a function of the initial concentration c_s1. The amplitude of the ISFET responses decreases with increasing concentration but remains more or less constant for $c_s1 = 40$ to 160 mM ($c_s2 = 200$ to 800 mM). The duration of the responses did not change.
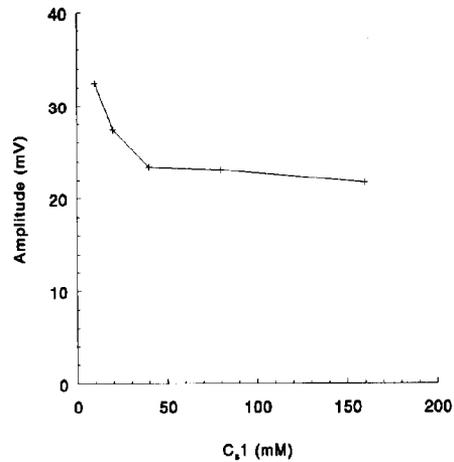
When we used other electrolytes than KCl the ISFET responses did not change on an ion-step. Even the response on an ion-step in TBACl, with the very large cation TBA$^+$, was comparable with the response in KCl.

Modelling

To verify the results of the experiments, the site-binding model of the ISFET was used and a simulation program was made for the dynamical behaviour of the ISFET.

The ISFET is described by the acid/base equilibria at the amphoteric surface of the gate oxide:

$$A\text{-}OH \rightleftharpoons A\text{-}O^- + H_s^+ \tag{1}$$

$$A\text{-}OH + H_s^+ \rightleftharpoons A\text{-}OH_2^+ \tag{2}$$
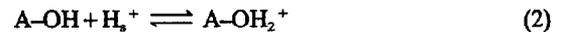
with H_s^+ the protons which stay in the direct vicinity of the surface. For the simulation of a Ta_2O_5-ISFET the pK_s's of the eqns. (1) and (2) are chosen to be 2 and 4, respectively [4].

The resulting charge density σ (in C/cm^2) at the surface is

$$\sigma = q([A\text{-}OH_2^+]\text{-}[A\text{-}O^-]) \text{ with } q = 1.6 \times 10^{-19} \text{ C} \tag{3}$$

The pH where $\sigma = 0$ is called the point-of-zero charge pH$_{pzc}$ (for Ta_2O_5, pH$_{pzc}$ = 3 [4]). The relation between the surface charge σ and the surface potential Ψ is given by:

$$\sigma = \Psi C_{dl} \text{ with } C_{dl} \text{ the double-layer capacity (in F/cm}^2)$$
$$\tag{4}$$

The ISFET measures the surface potential Ψ with respect to the bulk potential which is defined by a

reference electrode. This potential Ψ follows from the Boltzmann equation:

$$[H_s^+] = [H_b^+] \exp(-q\Psi/kT) \text{ or}$$

$$\Psi = 2.3 \, kT/q \, (pH_s - pH_b) \tag{5}$$

with H_b^+ the proton concentration in the bulk solution.

The combination of the large number of surface sites ($10^{15}/cm^2$ for Ta_2O_5), the chemical equilibria and the relative low value of the double-layer capacitance (maximum 20 $\mu F/cm^2$) makes the oxide act as a very good buffer for H_s^+ which results in a (almost) constant pH_s. Consequently the surface potential Ψ changes with -59 mV/pH_b [5].

The chemical equilibria at the ISFET surface are incorporated in a dynamical model of the ISFET with a stagnant layer in which ion transport is caused only by diffusion and migration. The model consists of the Nernst–Planck and Poisson equations which are solved by a finite difference procedure. The variables which are described in the model are the concentration of the water ions OH^- and H_3O^+, the salt ions K^+ and Cl^-, the electrical field and the electrical potential. The variables are considered as functions both of time and place. A detailed description of the model will be published soon.

Simulations and verification

In Fig. 4, simulations are shown of a Ta_2O_5-ISFET response on an ion-step from 10 to 100 mM KCl at $t = 0$ s and subsequently from 100 to 1000 mM KCl at $t = 3$ s (pH 7, not buffered). We have defined a stagnant
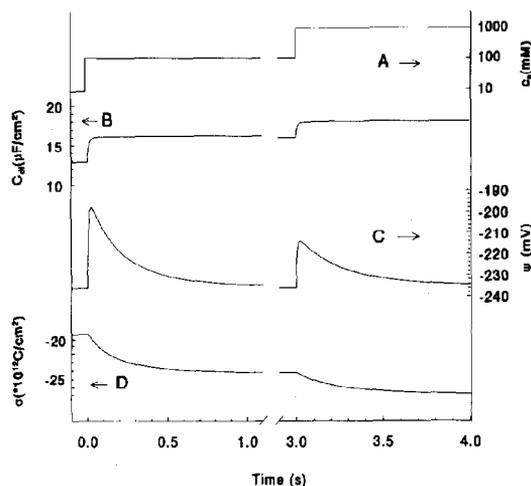

Fig. 4. Simulation of the double-layer capacitance C_{dl} (curve B), the surface potential Ψ (curve C) and the surface charge σ (curve D) as a result of an ion-step in c_s of 10 to 100 mM KCl, applied at $t = 0$ s and an ion-step of 100 to 1000 mM at $t = 3$ s (curve A).

layer of 8 μm in front of the ISFET. The ion-step is simulated by a stepwise increase of the concentration at the outside of the stagnant layer.

The electrolyte concentration c_s, the double-layer capacitance C_{dl}, the surface potential Ψ and the surface charge density σ are plotted as a function of time. The relation between the last three parameters is given by eqn. (4).

In the equilibrium situations, $t < 0$, $1 < t < 3$ and $t > 4$ s, Ψ (and thus σ/C_{dl}) has the same value (from 10 mM to 1 M KCl, the difference in Ψ is only 1.5 mV). This means that the static pH response of a Ta_2O_5-ISFET should barely be influenced by the electrolyte concentration. This is in agreement with earlier experiments.

For the non equilibrium situations the mechanism is as follows. After an ion-step, C_{dl} increases very fast due to a sudden increase in the diffuse capacitance. As a result the surface potential Ψ will decrease (become less negative) according to eqn. (4). According to the Boltzmann equation, the H_s^+ concentration will tend to decrease because of the decreased Ψ. However, the oxide surface acts as a very good buffer for H_s^+ and will keep the H_s^+ concentration constant by dissociating A–OH groups. This will change σ and a new equilibrium is reached when σ/C_{dl} ($= \Psi$) has the same value as before the ion-step. The time constant of adapting σ by dissociating A–OH groups is determined by the diffusion of the H^+ ions and the buffer capacity of the electrolyte. A thick stagnant layer and/or a low buffer capacity will delay the establishment of a new equilibrium. The dependence on the buffer capacity was already observed in the experiments which are shown in Fig. 1; for higher buffer capacities the response is faster.

The simulated response on an ion-step from 100 to 1000 mM ($t = 3$ s) is smaller than the response on a step from 10 to 100 mM ($t = 0$ s) because the change in C_{dl} is smaller as can be seen in Fig. 4. Comparable results were found in the experiments shown in Fig. 3.

If the double-layer capacitance changes from C_{dl1} to C_{dl2}, the theoretical maximum change in Ψ directly after the ion-step (assuming σ is not changing yet) is:

$$\Delta\Psi_{max} = \Psi_1 - \Psi_2 = \frac{\sigma}{C_{dl1}} - \frac{\sigma}{C_{dl2}} = \Psi_1 \left(1 - \frac{C_{dl1}}{C_{dl2}}\right) \tag{6}$$

For the example given in Fig. 4, this gives a $\Delta\Psi_{max}$ of 49 mV at $t = 0$ s and 26 mV at $t = 3$ s. The simulated values in Fig. 4 are 38 and 22 mV, respectively. The reason for this discrepancy is an insufficient time separation of the changes in C_{dl} and σ which is caused by the stagnant layer. Sufficient separation can only be realized if we choose a smaller stagnant layer which

is not realistic. Therefore, submaximal responses are to be expected in experiments.

The results of Fig. 2 where the amplitude of ion-step responses for different types of ISFETs is plotted as a function of the pH, can now be explained. The Al_2O_3-ISFET which was used for the experiment apparently has a point-of-zero charge at pH 4.2. The other ISFETs do not intersect the x-axis and therefore the points-of-zero charge must be found at lower values of the pH. If the difference between the pH and the pH_{pzc} increases, then Ψ increases and the amplitude of the response increases. Because the buffer capacity of the (unbuffered) solution reaches a minimum at pH 7, a maximum amplitude at a pH higher than 7 can be expected (due to the still increasing Ψ) which is observed in Fig. 2.

Conclusions

The ion-step method seems a very simple and direct method to detect the point-of-zero charge of an ISFET gate oxide. However, if this point is below pH = 3 or above pH = 11, the buffer capacity of the solution will suppress the response. Apparently, the pH_{pzc} of the Al_2O_3-ISFET we used is 4.2. In the literature, values of 4.8 to 9 are reported [6]. However, more experiments are needed to compare our results with other determinations of the pH_{pzc}.

The mechanism of the bare ISFET response being known, the consequences for the ion-step measuring method applied to membrane-covered devices can be considered. If there is an interlayer (with low buffer capacity) between the ISFET and the membrane of polystyrene beads with immobilized proteins, the ISFET response can interfere with the membrane response. However, when the protein layer is deposited directly on the gate surface, the effect of the proton release of the gate oxide will be negligible if the protein layer has a sufficient buffer capacity. We will consider this point in the further development of the heparin sensor [2].

Acknowledgements

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