Sensors and Actuators B, 6 (1992) 101–105

Detection of charged proteins by means of impedance measurements

J. Kruise[a], J. G. Rispens[a], P. Bergveld[a], F. J. B. Kremer[b], D. Starmans[b], J. R. Haak[b], J. Feijen[b] and D. N. Reinhoudt[b]

Department of Electrical Engineering[a] and Chemical Technology[b], University of Twente, P.O. Box 217, 7500 AE Enschede (Netherlands)

Abstract

The electrical resistance of a membrane containing charged proteins is a function of both concentration and mobility of the electrolyte ions present in the membrane, and thus it depends on the Donnan effect. We were able to determine the resistance of such a membrane deposited on an ISFET, by means of a simple a.c. impedance measurement. As an example a membrane containing cross-linked lysozyme was used. We found the membrane resistance to be inversely proportional to the fixed charge density, in good agreement with the theoretical description based on the Donnan theory.

1. Introduction

Detection of charged proteins, deposited on the surface of an ISFET, was first introduced in the literature by Schenck [1], who assumed that the presence of charged molecules would directly modulate the drain current. Despite a lot of research effort, up to now the results of these static measurements are disappointing. Nowadays, it is generally accepted that screening of the protein charge by small counterions from the electrolyte prevents successful measurements. Schasfoort *et al.* [2] and Kruise *et al.* [3] stated that static detection of charged proteins is only possible if a Donnan potential arises at the membrane–solution interface. However, only relatively high densities of protein charge cause a Donnan potential. Furthermore, this potential can only be measured using ISFETs with a low pH sensitivity, because simultaneously with the Donnan potential a change in the membrane pH is developed, causing a variation of the ISFET surface potential which counteracts the Donnan potential.

To overcome this compensation problem, we propose to detect the presence of protein charge on an ISFET surface with an a.c. impedance measurement. First, using the Donnan theory, it is shown how the membrane impedance depends on the fixed charge density. Secondly we show how this membrane impedance can easily be measured with an ISFET. As an example experimental results of ISFETs covered with lysozyme membranes are shown, of which the protein charge density is modulated by a variation of the solution pH.

2. Theory

The electrical characteristics of a permeable membrane containing charged proteins can be described by the Donnan theory [4]. According to this theory the presence of fixed charge causes a redistribution of electrolyte ions, until an equilibrium is reached where the following relation must be obeyed.

$$a_+ a_- = \bar{a}_+ \bar{a}_- \tag{1}$$

a_+ and a_- are the activities of postive and negative ions in the solution (bi-ionic), while $\bar{a}_+$ and $\bar{a}_-$ are the activities in the membrane. Furthermore electric neutrality is required in both membrane and solution:

$$c_+ = c_- = c_s \tag{2}$$

$$\bar{c}_- = \bar{c}_+ + C_P \tag{3}$$

where c_s is the electrolyte concentration, which equals the cation and the anion concentration c_+ and c_- in the solution, $\bar{c}_+$ and $\bar{c}_-$ are the concentrations in the membrane and C_P is the protein charge density (equivalents/litre) in the membrane. Combining eqns. (1), (2) and (3) and using the approximation that all activity coefficients are equal to unity leads to the following equations for

0925-4005/92/$5.00

the anion and cation concentration in the membrane:

$$\bar{c}_+ = \tfrac{1}{2}\sqrt{C_P^2 + 4c_s^2} - \tfrac{1}{2}C_P \tag{4}$$

$$\bar{c}_- = \tfrac{1}{2}\sqrt{C_P^2 + 4c_s^2} + \tfrac{1}{2}C_P \tag{5}$$

Since the membranes used are fully permeable for electrolyte ions, a purely resistive membrane impedance is assumed. This membrane resistance is represented by the following equation:

$$R_m = \frac{d_m}{A_m \sigma_m} = \frac{d_m}{FA_m(\bar{u}_+ \bar{c}_+ + \bar{u}_- \bar{c}_-)} \tag{6}$$

where A_m and d_m are the area and thickness of the membrane, σ_m is its specific conductivity, $\bar{u}_+$ and $\bar{u}_-$ are the mobilities of cations and anions in the membrane and F is the Faraday constant. After combining eqns. (4), (5) and (6) and some rearrangements, we obtain:

$$R_m = \frac{2d_m}{FA_m(\bar{u}_+ + \bar{u}_-)\left[\sqrt{C_P^2 + 4c_s^2} - \dfrac{\bar{u}_+ - \bar{u}_-}{\bar{u}_+ + \bar{u}_-} C_P\right]} \tag{7}$$

From eqn. (7) it follows that R_m is inversely proportional to C_P, under the condition that a relatively large Donnan potential is present ($C_P > c_s$).

3. Measurement principle

A schematic representation of an ISFET with reference electrode is shown in Fig. 1(a). The d.c. drain current I_D of an ISFET in the unsaturated mode is given by the following equation:

$$I_D = \mu C_{ox} \frac{W}{L}[(V_{GS} - V_T)V_{DS} - \tfrac{1}{2}V_{DS}^2] \tag{8}$$

where μ is the electron mobility, C_{ox} is the ISFET oxide capacitance, W and L are the width and length of the gate area, V_{GS} and V_{DS} are the gate-source and the drain-source voltage and V_T is the pH-dependent threshold voltage. In the a.c. mode the current i_d can be found by differentiating (8) to V_{GS}:

$$i_d = \frac{\delta I_D}{\delta V_{GS}} = \mu C_{ox} \frac{W}{L} V_{DS} v_{gs} = g_m v_{gs} \tag{9}$$

where g_m is the transconductance of the ISFET. Note that i_d is independent of V_T and thus independent of the pH. Using the simplified electrical input circuit of the ISFET as shown in Fig. 1(b), it is easily seen that v_{gs} is the output signal of a simple RC network, consisting of the ISFET oxide capacitance C_{ox} and the combined resistance of the reference electrode and the solution between the reference electrode and the ISFET gate, R_{tot}. Therefore, the complete transfer function from v_i to i_d is given by:

$$i_d = g_m \frac{1}{1 + j\omega R_{tot} C_{ox}} v_i \tag{10}$$

where v_i is the a.c. input voltage applied to the reference electrode. The system has a cut-off frequency $f_c = 1/(2\pi R_{tot} C_{ox})$. As a result of the geometry of the device, a large part of the resistance R_{tot} is determined by a relatively small solution compartment close to the oxide surface. Therefore, if a membrane is deposited on the ISFET gate, it may be expected than its resistance R_m will have a relatively large effect on the total resistance R_{tot} [5]. The above-described situation can be modeled as shown in Fig. 2(a). The volume between the reference electrode and the ISFET is divided into small compartments with thickness d, separated from each other by equipotential surfaces A_i. The value of A_i is very small for compartments close to the gate and large for compartments further away in the solution. The electrical equivalent is

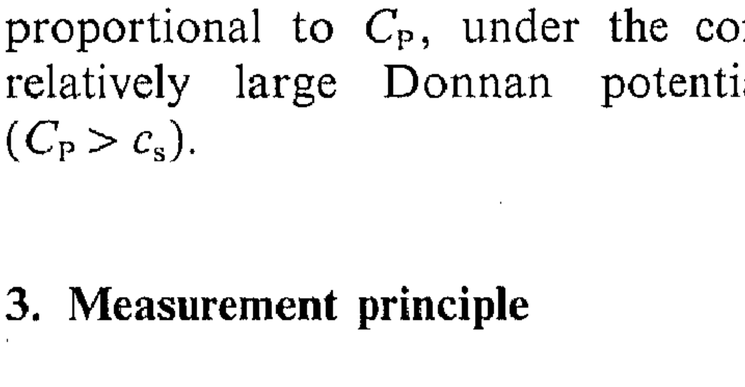

Fig. 1. (a) Schematic representation of an ISFET with reference electrode. (b) A simple electrical equivalent of (a).

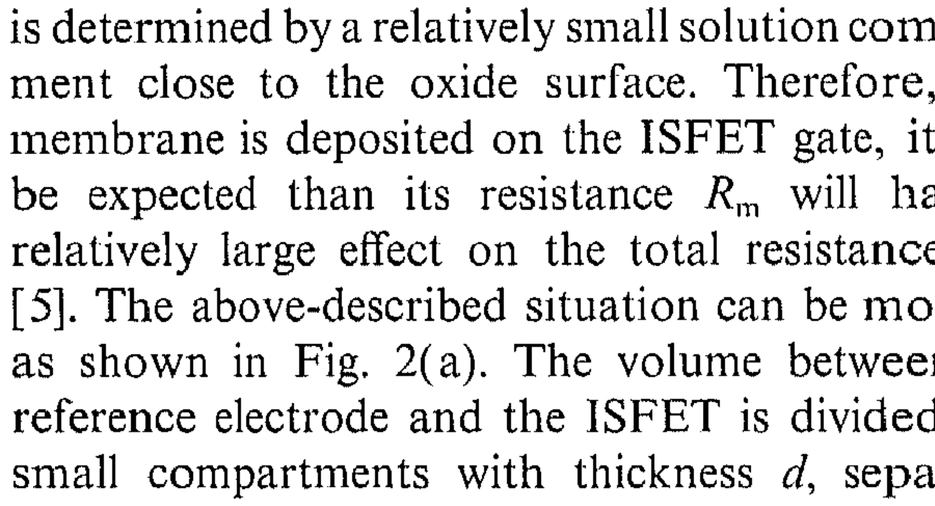

Fig. 2. (a) Schematic representation of an ISFET compartment model. (b) Electrical equivalent of the model.

shown in Fig. 2(b). According to this model, the total resistance R_{tot} can be written as:

$$R_{tot} = R_m + \sum_{i=1}^{N} \frac{d}{\sigma_s A_i} + R_{ref} \qquad (11)$$

where σ_s is the specific conductivity of the solution and R_{ref} is the resistance of the reference electrode. We expect the membrane resistance to become smaller as a result of the Donnan effect, as explained in Section 2. This will substantially decrease R_{tot} and, therefore, increase the cut-off frequency f_c.

4. Experimental

The ISFETs were fabricated following the standard NMOS processing steps, with an added tantalum oxide gate dielectric on top of the silicon oxide [6]. After mounting the ISFET chips on a piece of printed circuit board, they were encapsulated with Hysol epoxy. In order to investigate the effect of the shape and size of the aperture above the gate area on R_{tot}, two types of encapsulation were used. ISFETs of type a were encapsulated by hand, leaving a relatively large funnel-shaped aperture above the gate area, whereas ISFETs of type b were encapsulated using an alternative method, leaving a small, rectangular aperture in the epoxy with a fixed size of $600 \times 100 \times 100$ μm.

Before protein deposition the devices were first silanized with 3-aminopropyletoxysilane (APS). After that they were placed in a 2.5% glutaraldehyde solution (pH = 7.4) for half an hour. Lysozyme (10 mg; Sigma, L2879) was dissolved in 2.5 ml phosphate buffer (pH = 7.4), after which 3 μl of this solution were deposited over the gate surface of the encapsulated ISFET. Then the devices were dried for 15 min, after which they were rinsed with demineralized water. To the remaining lysozyme solution 0.5 ml of the glutaraldehyde solution was added. Then ~1 μl of this mixture was deposited over the ISFET surface. After 2 h the cross-linking reaction was assumed to be completed. Using this method close-packed membranes of cross-linked lysozyme were obtained, with a thickness varying from 50–200 μm. Before use the lysozyme covered ISFETs were thoroughly rinsed in demineralized water for several hours.

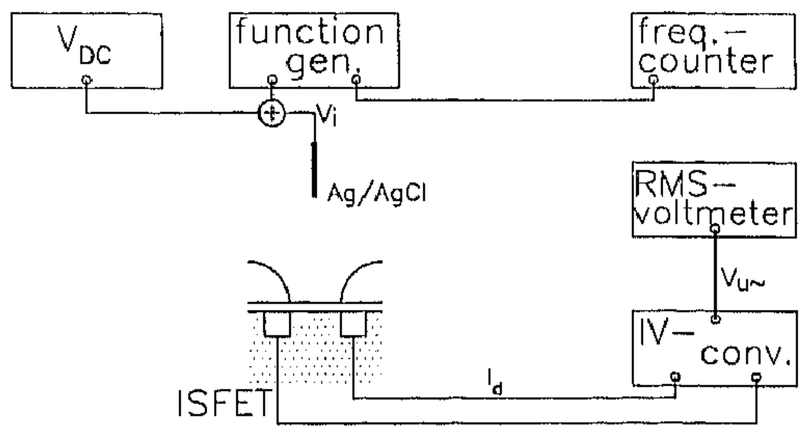

Fig. 3. The measurement setup for the frequency response measurements.

The measurement setup is schematically shown in Fig. 3. A d.c. and an a.c. input voltage are summed and applied to the reference electrode (Ag/AgCl), which has a low impedance in a wide frequency range. The a.c. drain current i_d is converted to a voltage $v_{u\sim}$, which is then converted to an r.m.s. voltage $v_{u=}$. The cut-off frequency was obtained by measuring $v_{u=}$ at 1 kHz, after which the frequency was increased until an output voltate of $1/\sqrt{2}$ times the original value was found.

5. Results and discussion

First we tested the validity of the model proposed in Section 3. If a small volume close to the gate determines the value of R_{tot}, then the cut-off frequency should be independent of the distance, l, between ISFET and reference electrode. Therefore using devices of both encapsulation types, we examined the effect of changes in l on the value of R_{tot}. In these experiments 'bare' ISFETs without membranes were used. The measurement solution contained 2×10^{-4} M KCl and 1×10^{-3} M KNO_3 (pH ≈ 6, conductivity 169 μs/cm). Characteristic results of these experiments are shown in Fig. 4. Both $1/f_c$ and R_{tot} are plotted on the y axis as a function of the distance l. The value of R_{tot} is obtained using a calculated value for C_{ox} of 2.8 pF. As shown, R_{tot} depends least on the distance l in the case of the type b device. At $l = 200$ μm, R_{tot} is still equal to 90% of its maximum value in the case of the type b device, wherease R_{tot} decreased to 75% of its maximum in the case of the type a device. It can be concluded that for both ISFET types a large part of R_{tot} is determined by a relatively small solution compartment close to the gate, though type b devices give

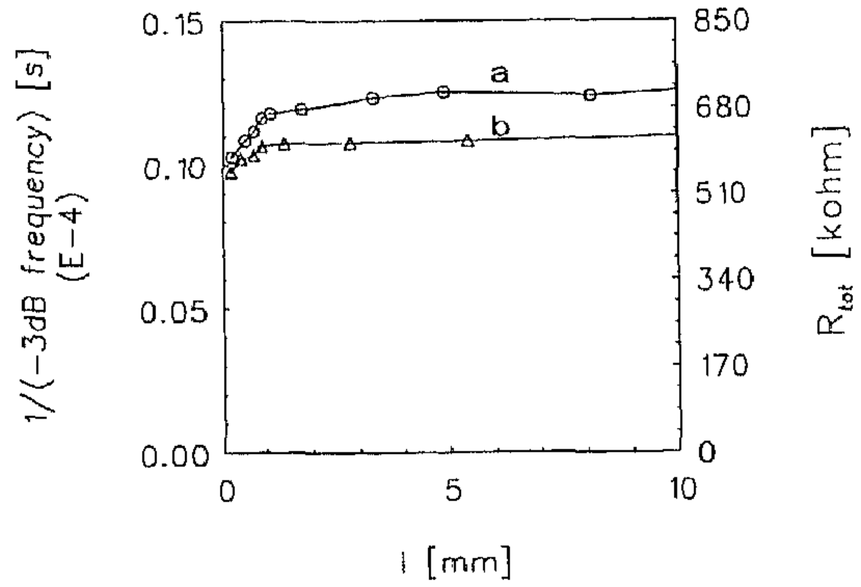

Fig. 4. $1/f_c$ and R_{tot} as a function of the distance l, between ISFET and reference electrode for two 'bare' ISFETs: curve a, funnel-shaped aperture; curve b, rectangular aperture, 100 × 100 × 600 μm.

the best results. Therefore, only type b devices were used for further measurements.

After lysozyme membranes were deposited ($d_m \approx 50$ μm), again f_c was determined. The same measurement solution was used, but now the distance l was kept constant at a value of 2.2 mm. The results of two type b devices are given in Table 1. Both devices show a large increase of f_c due to the membrane deposition, resulting in a decrease of R_{tot}. The low value of R_{tot} is ascribed to a high specific conductivity in the membrane, due to the Donnan effect. Although we do not know the exact fixed charge density, we know that the membranes contain positive fixed charge

TABLE 1. Determined values of f_c and R_{tot} for two ISFETs of type b before and after membrane deposition (pH = 6.2, solution conductivity 169 μs/cm)

Device	f_c (kHz)	R_{tot} (kΩ)
No. 1, bare	92.8	612
No. 1 + membrane	147.6	385
No. 2, bare	147.7	385
No. 2 + membrane	253.3	225

TABLE 2. Determined values of f_c and R_{tot} for two membrane covered ISFETs of type b after a change in pH to 9.4 (solution conductivity 179 μs/cm)

Device	f_c (kHz)	R_{tot} (kΩ)
No. 1	79.6	714
No. 2	139.3	408

at pH ≈ 6. Qualitatively, the results of the two devices agree well with each other, but there is a large difference between the absolute values of R_{tot}. This is ascribed to a difference in the encapsulation.

Finally, we monitored the change in the value of R_{tot} by increasing the pH of the measurement solution to pH = 9.4, a value closer to the isoelectric point of lysozyme (pH = 11 in solution). We noted that it took at least one hour before a new equilibrium situation was reached. This is ascribed to slow titration processes in the membrane, during the developement of a new Donnan equilibrium. Also this experiment was performed at a fixed distance $l = 2.2$ mm. The added base increased the solution conductivity to 179 μs/cm, but should also strongly decrease the fixed charge density of the lysozyme in the membrane. Therefore, the increased values of R_{tot}, shown in Table 2, are in good agreement with our expectations. However, the absolute values of R_{tot} become higher than in the case of the 'bare' ISFETs (Table 1), which cannot be explained by our theory. This effect can possibly be ascribed to a decreased ion mobility, as a result of the presence of the membrane network. This would mean that R_m of an uncharged membrane is always higher than the resistance of a comparable compartment filled with solution.

In order to estimate the absolute value of R_m, curve b of Fig. 4 was extrapolated to $d = d_m = 50$ μm. A value of approximately 425 kΩ was found. This is the resistance of a 50 μm thick solution compartment in front of the bare ISFET. Using ΔR_{tot} of ISFET no. 1 from Table 1, we find: $R_m = 425 - 227 = 198$ kΩ. Thus, it can be concluded that the Donnan effect caused a change in resistance of about 53%. Using eqn. (6) we find $R_m = d_m/(FA_m\bar{u}_- C_P)$, under the approximation that $C_P = \bar{c}_-$, whereas for a comparable compartment filled with solution the resistance becomes $R_s = d_m/(2FA_m u_s c_s)$, assuming that $c_+ = c_- = c_s$ and $u_+ = u_- = u_s$. Together with the result that the Donnan effect caused a change in resistance of about 50% this leads to: $C_P \approx 4c_s u_s/\bar{u}_-$. Up to now, we have assumed $\bar{u}_-$ to be equal to u_s, leading to a fixed charge density $C_P \approx 4c_s$. However, C_P is probably higher, because it is known that the counterion mobility in a charged membrane is often lower than in a solution [7].

Conclusions

Charged proteins deposited on an ISFET can, in principle, be detected by means of a simple a.c. impedance measurement. Qualitatively, the measurements are in good agreement with our theoretical description. However, more experimental results are necessary to explain the exact relation between the membrane resistance and the amount of fixed membrane charge. The measurement method has proven to be adequate and might also be suitable for other types of sensors, e.g. immunosensors and enzyme sensors.

Acknowledgement

This project is financially supported by the Dutch Ministry of Economic Affairs.

References

1 J. F. Schenck, *U.S. Patent No. 4 238 757* (1980) (filed 1976).
2 R. B. M. Schasfoort, P. Bergveld, R. P. H. Kooyman and J. Greve, Possibilities and limitations of direct detection of protein charges by means of an immunological field-effect transistor, *Anal. Chim. Acta, 238* (1990) 323–329.
3 J. Kruise, J. A. Voorthuyzen, P. Bergveld, F. J. B. Kremer, D. Starmans, E. R. J. Sudhölter, J. Feijen and D. N. Reinhoudt, Protein covered ISFETs, theory and measurements, *Proc. 3rd Int. Meet. Chem. Sensors, Cleveland, OH, USA, Sept. 24–26, 1990*, pp. 264–267.
4 F. G. Donnan, The theory of membrane equilibrium, *Chem. Rev., 1* (1924) 73–90.
5 R. B. M. Schasfoort, G. J. Streekstra, P. Bergveld, R. P. H. Kooyman and J. Greve, Influence of an immunological precipitate on d.c. and a.c. behaviour of an ISFET, *Sensors and Actuators, 18* (2) (1989) 119–129.
6 P. Bergveld and A. Sibbald, in G. Svehla (ed.), *Comprehensive Analytical Chemistry*, Vol. XXIII, Elsevier, Amsterdam, 1988.
7 Y. Toyoshima, M. Yuasa, Y. Kobatake and H. Fujita, Studies of membrane phenomena. Part 3: Electrical resistance of membrane, *Trans. Faraday Soc., 63* (1967) 2803–2813.