

## Recueil Reviews

### Transduction of molecular recognition into electronic signals

David N. Reinhoudt

Laboratory of Organic Chemistry and MESA Research Institute, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands  
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#### Introduction

Almost from the beginning<sup>1-3</sup> supramolecular chemistry has been associated with possible applications based on shape-selective interactions between molecules or between ions and molecules. These applications involve the translation of molecular properties to a macroscopic level and will lead to new materials or devices. The real challenge is to find ways to communicate with individual molecules or assemblies of molecules and to exploit events at the molecular level. Molecular electronics<sup>4</sup>, wires<sup>5</sup>, switches<sup>6</sup>, etc., have been discussed and nanostructures already approach this level. One way to transduce molecular properties into macroscopic properties is to incorporate supramolecular structures in (polymeric) materials, to link them to (polymeric) supports or to use spontaneous self-assembly to produce materials which exhibit the molecular properties of the individual components, *e.g.*, transport, sensing, switching, optical non-linearity, conductance, etc. Recently we have observed a development from incorporation in thin polymeric films to self-assembly into monolayers on surfaces of solids<sup>7</sup> and the spontaneous assembly of molecular objects via a process that is programmed by specific interactions between the molecular components that form the supramolecular structure<sup>8</sup>. Most practical applications so far have come from the former approach. This review describes the transduction of molecular-recognition processes directly into electronic signals, a process which connects the worlds of solution chemistry and solid-state electronics.

#### Chemical sensors

A chemical sensor measures, preferentially continuously, the activity of one species. A sensor is composed of two elements, a selector which is responsible for the selective recognition and a transducer which converts the recognition event at the molecular level into a signal at the macroscopic level. The transduction process is linked to a physical parameter that changes during the recognition process, such as an increase in mass or change in optical properties. When the recognition process involves a change of charge or redox properties, electrochemical methods of transduction are attractive, either amperometric or potentiometric. The advantage of a potentiometric transducer over the other transducers is its large dynamic range as the signal is proportional to the logarithm of the chemical concentration. There are two types of potentiometric sensing devices.

In the classical ion-selective electrode the signal is the potential drop developed at the interface of a non-electronic conductive material. Alternatively, the field-effect transistor (FET) is able to measure the conductance as a function of an electrical field perpendicular to the conductivity channel of a semiconductor.

#### Ion-sensitive field-effect transistors (ISFETs)<sup>9</sup>

Ion-sensitive field-effect transistors combine the ion-selective electrode and solid-state (IC) technologies<sup>11</sup>. As transducing elements, they have several attractive properties such as rapid response, small dimensions, low output impedance, and the possibility to integrate other electronic functions<sup>11</sup>. The structure resembles the metal-oxide semiconductor field-effect transistor (MOSFET) (Figure 1). The ISFET (Figure 1) consists of two *n*-type diffusion regions (source and drain) embedded in a *p*-type silicon substrate. The conductance between source and drain in the semiconductor is a function not only of the applied potential but also of the electrical field perpendicular to the gate oxide. The first experiments with ISFETs in 1970 by Bergveld<sup>13</sup> showed that the state of ionization of the SiO<sub>2</sub>-gate surface is correlated with the conductance between source and drain. This observation was the basis for the pH-sensitive ISFET because the state of ionization of the SiOH functions is determined by the pH of the contacting solution. The pH sensitivity of the ISFET can be described by the site-dissociation model<sup>14</sup> which relates the reversible protonation and deprotonation reactions at the oxide surfaces of the ISFET gate oxide with the potential difference between the surface and the bulk solution. The model correctly describes Nernstian (59.8 mV · decade<sup>-1</sup>) or non-Nernstian (< 59.8 mV · decade<sup>-1</sup>) behavior of the different oxides (SiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Ta<sub>2</sub>O<sub>5</sub>), depending on the proton dissociation and association constants and the surface concentration of the oxide groups<sup>15</sup>. This pH sensitivity nicely illustrates the above-mentioned objective, the communication between a chemical world in solution and the world of solid-state electronics. Although several attempts have been reported to replace the surface-pH sensitivity by sensitivity towards other ions by surface modifications, they have been largely unsuccessful. Clechet and Jaffrezic-Renault<sup>16</sup> modified the silica surface by silylation with (3-cyanopropyl)dimethyl (dimethylamino)silane. They reported a sensitivity of 20 mV · decade<sup>-1</sup> towards Ag<sup>+</sup> but also a residual pH sensi-

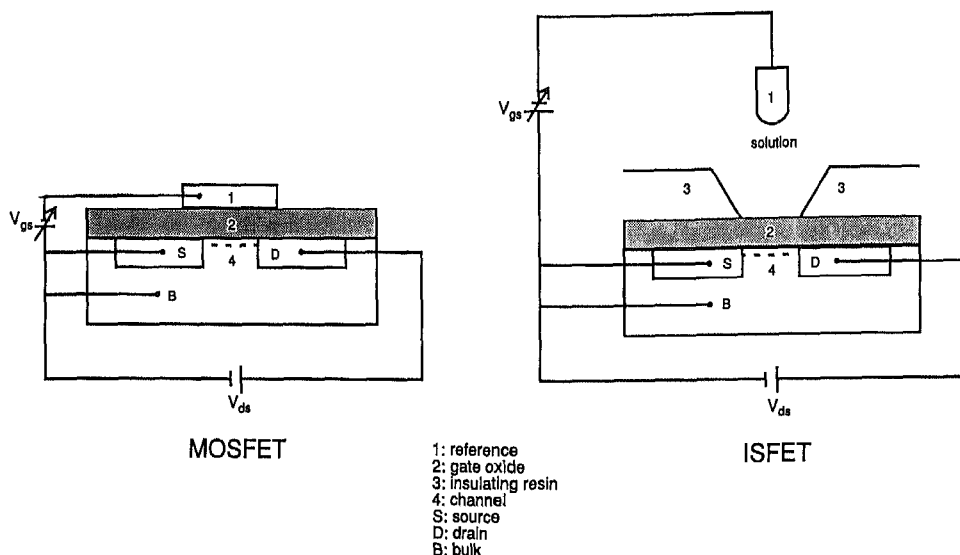


Figure 1. Schematic drawing of the metal-oxide field-effect transistor (MOSFET) and ion-sensitive field-effect transistor (ISFET).

tivity of 15 mV per pH unit. This sub-Nernstian response is due to a low site density which limits both the response and the dynamic range. Using the site dissociation model for pH-sensitive ISFETs, the relation between the number of binding sites per  $\text{cm}^2$  ( $N_s$ ), the association constant ( $K_a$ ), the concentration of ions ( $I^+$ ) and the surface potential ( $\Psi_0$ ) is given by Eqn. 1 (Figure 2)<sup>17</sup>. In this equation  $[I_b^+]$  is the bulk concentration of ions and,  $C_{eq}$  the equivalent double-layer capacitance ( $20 \mu\text{F} \cdot \text{cm}^{-2}$ ).

$$[I_b^+] = \frac{C_{eq} \cdot \Psi_0}{q \cdot K_a} \cdot e^{-q \cdot \Psi_0 / (k \cdot T)} \cdot \frac{1}{N_s - C_{eq} \cdot \Psi_0 / q} \quad (1)$$

### Chemically modified field-effect transistors (CHEMFETs)

The alternative to surface modification is the attachment of a membrane to the gate oxide. This was first reported by Janata et al.<sup>18</sup> in 1978 for ISFETs of which the gate oxide was covered with a thin, plasticized, PVC membrane containing the  $\text{K}^+$ -selective ionophore valinomycin (1). This MEMFET-type sensor exhibited a response selective for variations of the  $\text{K}^+$  concentration even in the presence of a large excess of  $\text{Na}^+$  ions. Their observation has stimulated research in this area because not only sensitivity but also selectivity at the molecular level could be introduced. By systematic variation of the structure of the ionophores a number of cation-selective MEMFET

devices have been constructed. However, because of the physical adhesion of the hydrophobic membrane to the hydrophilic gate oxide, the lifetimes of these devices were short<sup>19</sup>. Moreover, it was found that the measured response was also dependent on pH and  $\text{CO}_2$  because the state of protonation of residual  $\text{SiOH}$  groups at the gate surface also influenced the measured potential<sup>20</sup>. In a typical experiment we have shown that  $\text{CO}_2$  causes a rapid response of such a PVC MEMFET (Figure 3)<sup>21</sup>.

Despite the research contributions of many groups, the unsatisfactory reproducibility of the sensor response and its limited lifetime seriously reduced initial enthusiasm for ISFET-based sensors and, in some cases, led to frustration<sup>22</sup>. This is not surprising given that at least four different parameters influence the response and limit the lifetime:

- (i) The thermodynamically ill-defined potential at the membrane-gate-oxide interface.
- (ii) Physical adhesion of the apolar membrane to the polar gate oxide.
- (iii) The leaching of electro-active components that determine the membrane potential (ionophore and lipophilic anions).
- (iv) The leaching of plasticizer from the membrane.

We have systematically studied the influence of these parameters and have developed a novel sensor architec-

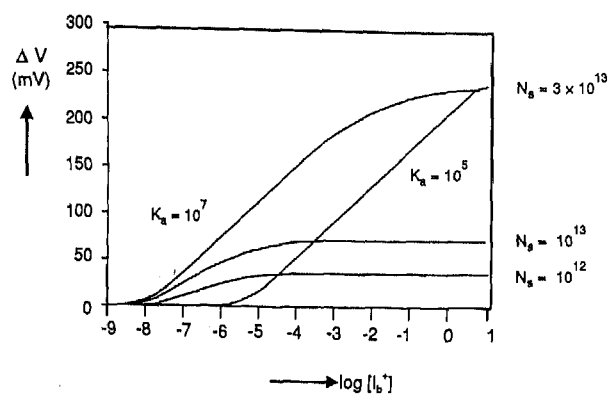


Figure 2. Relation between the relative surface potential ( $\Delta V$ ) and the logarithm of the ion concentration in solution  $[\log(I_b^+)]$  of a surface-modified ISFET, for different association constants ( $K_a$ ) and number of binding sites per  $\text{cm}^2$  ( $N_s$ )<sup>17</sup>.

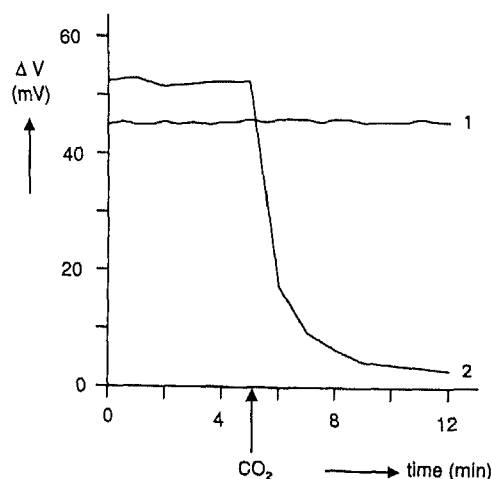


Figure 3. Effect of  $\text{CO}_2$  on the response of a PVC MEMFET with (1) and without (2) a buffered poly(2-hydroxyethyl methacrylate) hydrogel membrane attached to the gate oxide<sup>21</sup>.

ture which allows a thermodynamic description of the membrane potential and leads to long sensor lifetimes. In order to eliminate the ill-defined potential at the membrane-gate-oxide interface we introduced between the gate oxide and the hydrophobic sensing membrane, a hydrophilic poly(2-hydroxyethyl methacrylate) (poly-HEMA) hydrogel membrane in which an aqueous solution of buffer salts is "immobilized". In this structure the silanol groups at the gate oxide are in equilibrium with the buffered solution and *although the surface potential is unknown, it will be fixed as long as the pH in the hydrophilic membrane is constant.* This was confirmed experimentally by the much improved signal-to-noise ratio and the elimination of CO<sub>2</sub> interference<sup>21</sup>.

The physical adhesion in the MEMFETs was replaced by a chemical adhesion of the membrane via the (partial) silylation of the silanol groups of the gate oxide with (3-methacryloxypropyl)trimethoxysilane<sup>21,23</sup>. The methacryl groups react in the photo(co)polymerization with a mixture of monomeric 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate (10/1 w/w) under the formation of a hydrophilic cross-linked membrane layer of 10–20 μm. The additional advantage of this photo(co)polymerization is the compatibility with standard IC technology. A hydrophobic (e.g. PVC) membrane deposited on top of the hydrogel, in which the ionophore is incorporated together with hydrophobic anions<sup>24</sup>, gives sensors that function very well for weeks under laboratory conditions when the ionophores are sufficiently hydrophobic<sup>25</sup>. The overall potential of these sensors can be described as the sum of four contributions (Figure 4).

Since the hydrogel is buffered (pH 4), the equilibrium with the silanol groups keeps the surface potential ( $E_s$ ) constant. The other contributions can be described by the model of Teorell<sup>26</sup>, Meyer and Sievers<sup>27</sup> that we have generalized to describe free or immobilized neutral ligands<sup>28</sup> and ion-pair formation<sup>29</sup>. The boundary potentials  $E_{B1}$  and  $E_{B2}$  are determined by the ratios of the activities of ionic species in solution and the membrane phase according to Nernst's law. The diffusion potential ( $E_D$ ) is a function of the activities of all ionic species at both inner sides of the membrane and their respective mobilities.

In our CHEMFET architecture, the activity of the ions in the polyHEMA layer is kept constant during the measurement and this fixes one boundary potential ( $E_{B1}$ )<sup>28</sup>. Consequently, the overall potential difference is only a function of the diffusion potential and the boundary potential ( $E_{B2}$ ) at the interface between membrane and solution. In the absence of an ion-selective ionophore, these

boundary and diffusion potentials will depend on the concentration of all ionic species and this would be detrimental for a sensor designed to detect (differences in) activities of one species. In order to eliminate all other influences except this crucial parameter, specific molecular recognition properties must be incorporated and this is the field of supramolecular chemistry.

Firstly, for a cation-sensitive CHEMFET the recognition process should reject all other positive ions from the membrane, even when the natural partition ( $K_p$ ) would allow transfer from the aqueous to the membrane phase. Since the extraction coefficient ( $K_{ex}$ ) is the product of the partition coefficient ( $K_p$ ) of the ion and the association constant ( $K_a$ ) of the ionophore-ion complex, this can be achieved by designing receptor molecules with a large preference for one cation.

Secondly, the association equilibrium of the complex determines the free cation concentration. According to Nernst's law the membrane potential is given by  $E_B = [R \cdot T / (z_i \cdot F)] \cdot \ln(a_i / \bar{a}_i)$ . The maximum response that can be obtained as a function of  $\log(a_i)$  is 59.5 mV · decade<sup>-1</sup> for monovalent cations at room temperature, when the activity of ion  $i$  in the membrane ( $\bar{a}_i$ ) is constant. In general, the activity of ion  $i$  in aqueous solution ( $a_i$ ) will influence the concentration in the membrane ( $\bar{a}_i$ ) because they are related by the partition coefficient  $K_p$ . In order to keep the concentration in the membrane ( $\bar{a}_i$ ) constant, the ionophore has a second role, *viz.* to "buffer" the membrane with respect to  $\bar{a}_i$ . This is achieved by a fixed ratio of complexed and uncomplexed ionophore in the membrane. For monovalent cations, this ratio can be fixed when the ratio of concentrations of ionophore and lipophilic anions is 2. Because of electroneutrality under these conditions in the bulk of the membrane, the ratio of complexed to free ionophore will be close to 1 and consequently  $\bar{a}_i \approx K_a^{-1}$ . When a change of ion activity in solution would result in a proportional change of activity in the membrane phase, the complexation equilibrium will counteract this as long as the "buffer capacity" of the free ionophore is sufficient<sup>29</sup>.

Thirdly, the role of the ionophore is to eliminate the diffusion potential ( $E_D$ ) which is a function of the concentration of charged species and their mobility. A high absolute association constant  $K_a$  will establish a low free-ion concentration at both membrane interfaces, almost independently of the ion concentration in the aqueous phases, and consequently will reduce  $E_D$ .

A quantitative description of  $E_M$  would require the absolute values of activities,  $K_p$ ,  $K_a$ , and mobilities of all ionic species in the membrane, but these are not known. How-

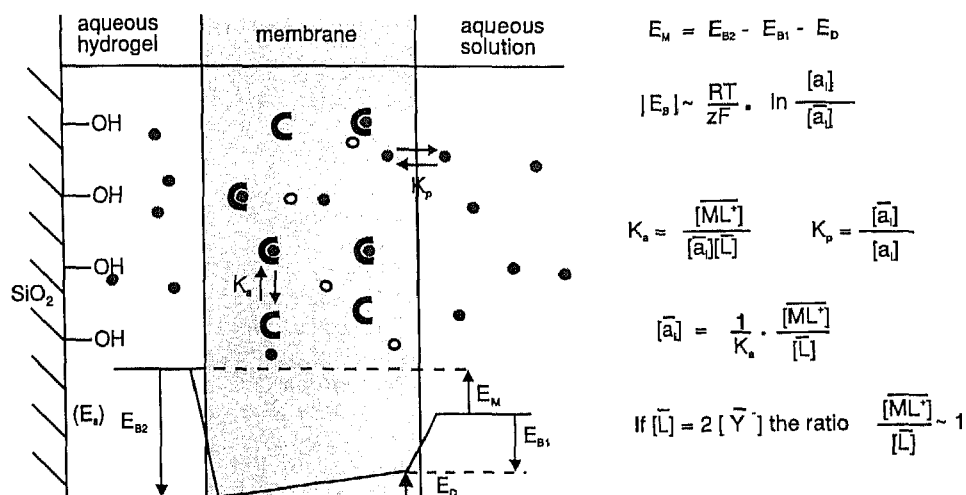


Figure 4. Schematic drawing of a CHEMFET depicting the effect of an ionophore on the membrane potential.

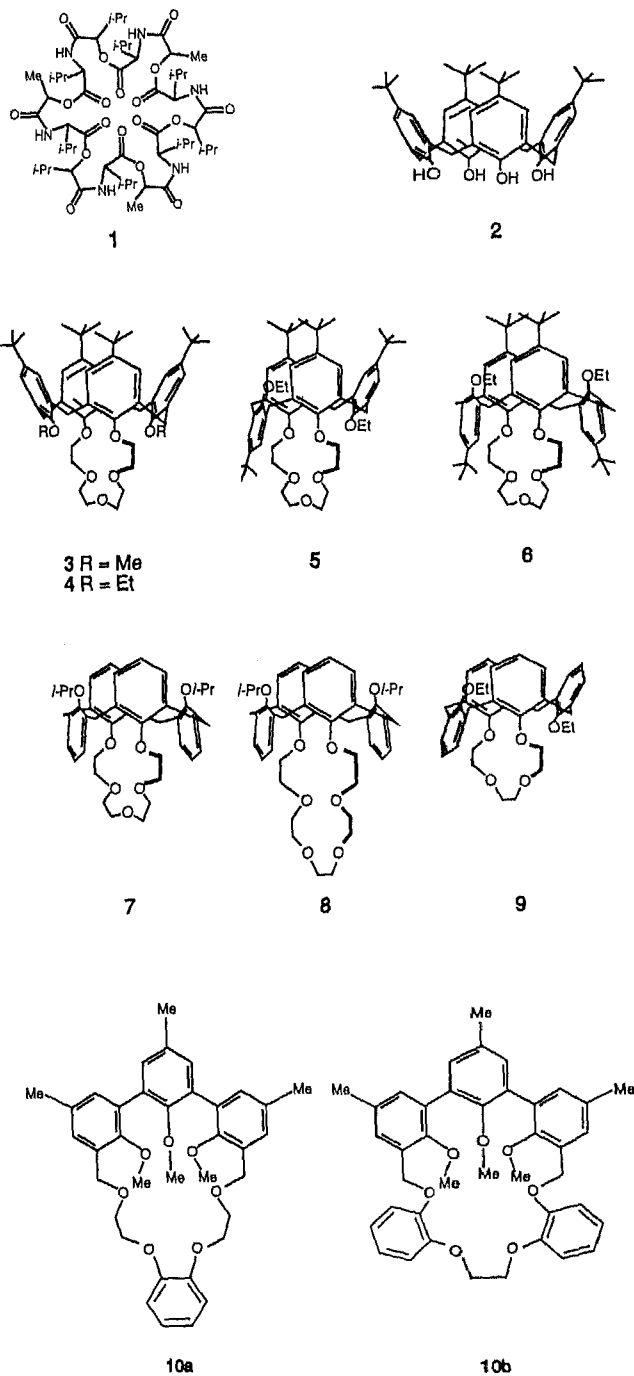
ever, computer simulations provide a good understanding of the effect of the different parameters on the membrane potential<sup>29b</sup>. Experimental results of CHEMFETs with different ionophores (e.g. for Na<sup>+</sup>, K<sup>+</sup>, Cs<sup>+</sup>, Ca<sup>2+</sup>, Ag<sup>+</sup>, ...) incorporated in plasticized PVC membranes exhibit Nernstian responses over 3–4 decades of activities in the presence of other cations.

The above model is based on the assumption that exchange processes at the interfaces are fast (and limited to very small interfacial regions) on the time-scale of the measurement. Our experiments showed response times of  $\geq 250$  ms<sup>30</sup>. Impedance spectroscopy with K<sup>+</sup>-selective CHEMFETs (valinomycin, 1) demonstrated the rejection of Na<sup>+</sup> ions from the membrane<sup>31</sup>, even when the Na<sup>+</sup>/K<sup>+</sup> ratio was 10<sup>4</sup>.

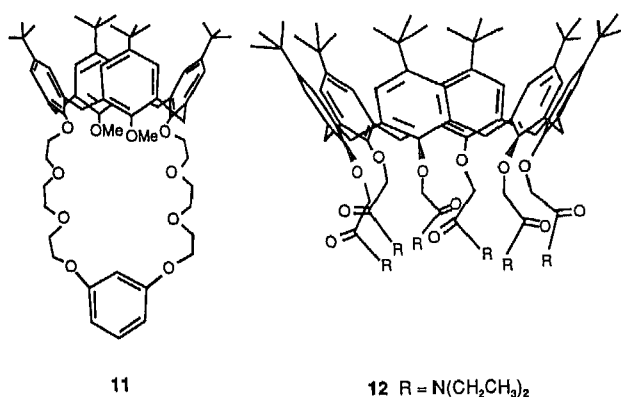
### Ionophores

Until recently, most experiments with cation-sensitive IS-FETs were carried out with plasticized PVC membranes that contained natural ionophores, non-cyclic synthetic ligands or simple crown ethers. We have developed several classes of ionophores specifically for these sensors, based on calix[4]arenes<sup>32</sup> and terphenyls<sup>33</sup>. These are not only more selective but also much more lipophilic. This increases the lifetime of the sensor as it reduces the partitioning of ionophore to the contacting aqueous phase. In particular the calix[4]arene building block 2 provides the 3-dimensional skeleton that is needed to fix different donor ligands in the spacial positions that are optimal for binding one ion selectively. Ungaro et al.<sup>34</sup> showed that for a "hard" cation (like Na<sup>+</sup>) ester, ketone, or amide carbonyl groups, assembled at the "lower" rim of the calix[4]arene, can introduce high selectivity for Na<sup>+</sup> vs. K<sup>+</sup>. In plasticized PVC membranes such calix[4]arenes show<sup>25a,35</sup> Na<sup>+</sup>/K<sup>+</sup> selectivities [ $\log(K_{Na,K}^{pot})$ ] of  $-2.5$  to  $-2.7$ .

For K<sup>+</sup>-selective sensors, we have developed calix[4]arene crown ethers with five oxygen atoms in the bridge and the two remaining phenolic oxygen atoms alkylated with C<sub>1</sub>–C<sub>3</sub> alkyl chains<sup>36</sup>. The conformationally flexible 1,3-dimethoxy calix[4]arene crown-5 3 exhibits, in two-phase extraction experiments, a peak selectivity for K<sup>+</sup> comparable to the other alkali cations; this selectivity was confirmed in CHEMFET response measurements. NMR studies of K<sup>+</sup> complexes of 3 in CDCl<sub>3</sub> solution revealed that upon complexation the 1,3-dimethoxy calix[4]arene crown-5 skeleton undergoes a conformational change from "cone" to "partial cone". The latter structure was confirmed by single-crystal X-ray analysis in the solid state. Since such a "partial-cone" conformation is thermodynamically unfavorable for the ligand, we have synthesized the 1,3-diethoxy calix[4]arene crown-5. We could separate the three different conformations because, at room temperature, the more bulky ethoxy groups block the conformational interconversion. Separate experiments with the cone 4, partial cone 5 and 1,3-alternate conformer of 6 showed the partial-cone conformer 5 to have the highest K<sup>+</sup>/Na<sup>+</sup> selectivity and the 1,3-alternate conformer 6 to have a surprisingly high selectivity<sup>25c</sup>. Recently Ungaro et al.<sup>37</sup> synthesized the 1,3-diisopropoxy calix[4]arene crown-5 7 in the 1,3-alternate conformation, but without the *tert*-butyl groups in the *para* positions of the calix[4]arene. This favors the access of ions to the cavity of this ionophore and enhances the K<sup>+</sup>/Na<sup>+</sup> selectivity to a record value of  $\log(K_{K,Na}^{pot}) = -4.2$ , higher than is found for the natural ionophore valinomycin (1) under comparable conditions. When the ring size of these 1,3-dialkoxy calix[4]arene crown ethers is increased by one CH<sub>2</sub>CH<sub>2</sub>O unit the selectivity is changed in favour of Cs<sup>+</sup>. Ungaro et al.<sup>38</sup> have shown that this high selectivity is at least partly due



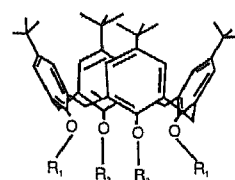
to the participation of the two aromatic rings in 8. The presence of *p*-*tert*-butyl groups renders complexation of the larger Cs<sup>+</sup> ion almost impossible. Reduction of the ring size by a CH<sub>2</sub>CH<sub>2</sub>O unit leads to calix[4]arene crown-4 ethers 9 and Shinkai et al.<sup>39</sup> have reported unprecedented Na<sup>+</sup>/K<sup>+</sup> selectivities of  $\log(K_{Na,K}^{pot}) \leq -5$ . Despite the high thermodynamic stabilities of the complexes, the rates of complexation and decomplexation remain sufficiently high to give fast responses on the time-scale of the CHEMFET measurements. A further shielding of the ion-binding cavity, e.g. by bridging the calix[4]arene with a rigid terphenyl rather than with a flexible polyethylene glycol, gives complexes that are kinetically stable<sup>40</sup>. On the other hand the hemispherands 10, that are formed by reaction of terphenyls and polyethylene glycols, give acceptable K<sup>+</sup>/Na<sup>+</sup> selectivities<sup>41</sup>. Increasing the ring size of the calix[4]arene crown ethers or the size of the calixarene to calix[6]arene gives the ionophores 11 and 12, respectively, that are selective for guanidinium ions<sup>42</sup>. The substitution of the hard-oxygen donor ligands for the softer thioether (13), and thioamides



(14 and 15) introduces<sup>43</sup> selectivities for the softer metal cations like Ag<sup>+</sup>, Pb<sup>2+</sup>, and Cd<sup>2+</sup>. Typical selectivities in CHEMFET measurements vary from -2.7 to 5.2 with sensitivities as high as 10<sup>-6</sup> M (for Pb<sup>2+</sup>)<sup>43</sup>.

### CHEMFET measurements

The CHEMFETs were measured in a constant-drain-current mode ( $I_d = 100 \mu\text{A}$ ) with a constant-drain-source potential ( $V_{ds} = 0.5 \text{ V}$ ) using a source-drain follower type of ISFET amplifier. The membrane potential developed was compensated for by an equal and opposite potential ( $V_{gs}$ ) via the reference electrode (SCE) (Figure 1). The selectivities are expressed as the potentiometric selectivity coefficient  $K_{ij}^{\text{pot}}$  derived from the Nikolsky-Eisenmann equation [ $E = E^0 + [R \cdot T / (z_i F)] \cdot \ln(a_i + K_{ij}^{\text{pot}} \cdot a_j^{z_i/z_j})$ ]. In all cases, the SiO<sub>2</sub> gate oxide of the ISFET was chemically modified by silylation with (3-methacryloxypropyl)trimethoxysilane and subsequently with the *in situ* photopolymerized poly(2-hydroxyethyl methacrylate). In the plasticized PVC, the ionophores are dissolved together with 0.5 molar equivalents of a hydrophobic anion (e.g. a tetraphenylborate). The latter eliminates the partitioning of soft anions and, as a consequence, the undesired anion response<sup>44</sup>. Typical response curves that are obtained by titration of the primary cation solution into a concentrated solution of interfering (or secondary)



- 13 R<sub>1</sub> = CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>, R<sub>2</sub> = CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>  
 14 R<sub>1</sub> = R<sub>2</sub> = CH<sub>2</sub>C(S)N(CH<sub>3</sub>)<sub>2</sub>  
 15 R<sub>1</sub> = R<sub>2</sub> = CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>C(S)N(CH<sub>3</sub>)<sub>2</sub>

ions and the potentiometric selectivities are given for the K<sup>+</sup>-selective ionophores 4–6 (Figure 5), for the Ag<sup>+</sup>-selective ionophore 13 (Figure 6), and for the Pb<sup>2+</sup>-selective ionophore, 14 (Figure 7). Simulations with the mathematical model that describes the membrane processes predict the observed Nernstian response (or deviations thereof) under a wide range of experimental conditions<sup>28,29,45</sup>. The lifetime of these sensors (less than four weeks) is limited by the leaching of the electroactive components, ionophore and lipophilic anions, and of the plasticizer to the aqueous phase, even with these very lipophilic calixarene-based ionophores.

### Durable CHEMFET sensors

Despite the improved lifetime of the sensor when the ionophores are hydrophobic it is obvious that the plasticized PVC in which the electroactive components are dissolved will not give the lifetime needed for practically applicable sensors. Firstly, the organic low-molecular-weight plasticizer will ultimately leach to the contacting aqueous phase. Secondly, both the ionophore and the hydrophobic anion will leach from the membrane because the ultra-thin membranes attached to the gate oxide of the field effect transistor have very small volumes. There have been few reports of alternative membrane materials in the literature. We found that polysiloxanes prepared *in situ* by condensation or hydrosilylation give materials that meet the required low glass-transition temperature ( $T_g$ )<sup>31,41c</sup>. In order to tune the electrical properties of such

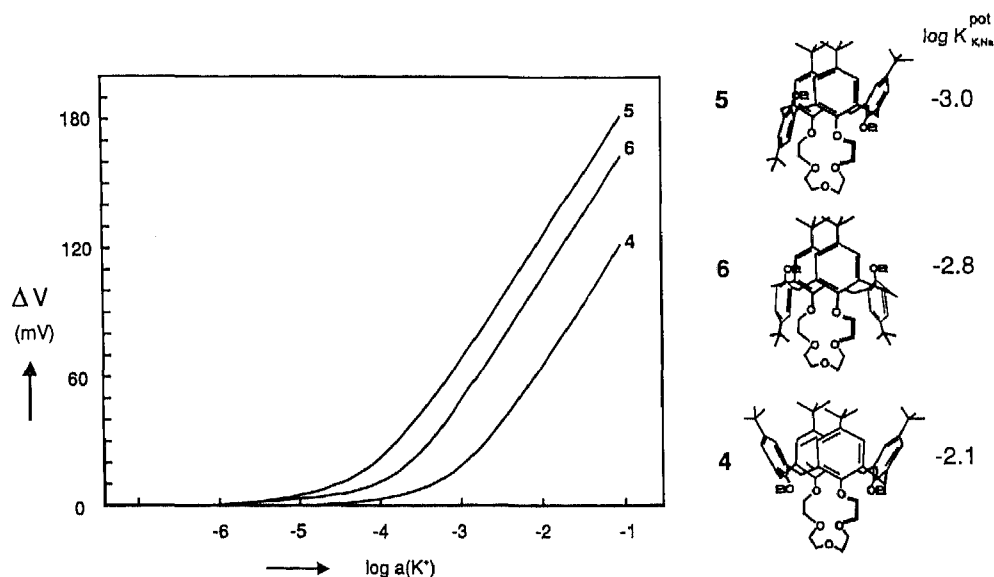


Figure 5. Response of CHEMFETs with the three different 1,3-diethoxy calix[4]arene crown ethers 4–6.

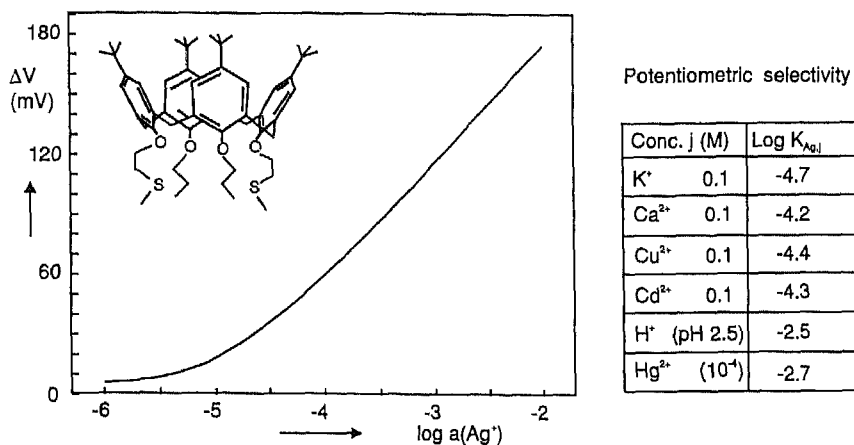


Figure 6. Response of an  $Ag^+$ -selective CHEMFET-containing ionophore 13.

membranes, copolymers of octamethylcyclotetrasiloxane, and the cyclic tetramers of (3-cyanopropyl)methylsiloxane and (3-methacryloxypropyl)methylsiloxane, were synthesized (Scheme 1)<sup>46,47</sup>.

The (3-cyanopropyl)methylsiloxanes enhance the dielectric constants of the membrane and the 3-methacryloxypropyl groups allow the covalent binding to the hydrophilic polyHEMA layer. When  $K^+$ - or  $Na^+$ -selective ionophores were added to these membranes together with 0.5 molar equivalent of a hydrophobic tetraphenylborate, excellent Nernstian responses were obtained over more than three decades of salt activity<sup>46,47</sup>.

The final development is the synthesis of ionophores and tetraphenylborates that can be covalently attached to the polysiloxane membrane. This was achieved by modifying the  $K^+$ -selective hemispherand (16), the  $Na^+$ -selective calix[4]arene (17) and the tetraphenylborate (18) with a methacrylate function. The photocopolymerization of the polysiloxane, ionophore and tetraphenylborate mixture in the presence of a photoinitiator<sup>45,48</sup> on the top of the polyHEMA layer gave membranes that exhibited the required, almost Nernstian, response.

The architecture of these CHEMFETs is depicted in Figure 8. All chemical components are chemically connected starting with the silylation of the silanol groups at the gate oxide which introduces methacryl functionalities. Photopolymerization with 2-hydroxyethyl methacrylate connects the hydrophilic (polyHEMA) layer which is subsequently buffered with the appropriate aqueous buffer that contains the ion that is measured. In a third step, the mixture of polysiloxane (that has the required  $T_g$  and

dielectric constant because of the 3-cyanopropyl groups), methacrylated ionophore, and tetraphenylborate, is photopolymerized on top of the polyHEMA layer which still contains some residual methacrylate functions. In the polysiloxane these electroactive components should now be linked as depicted in Figure 9.

Because such a network cannot be fully characterized, the covalent attachment can only be inferred from indirect measurements of long-term stability in comparison with a similar membrane in which the electroactive components are not covalently attached. Such experiments have been carried out with the  $K^+$ -selective hemispherand 16. Initially, these CHEMFETs all exhibited a Nernstian response ( $54\text{--}56\text{ mV} \cdot \text{decade}^{-1}$ ) towards  $K^+$  with selectivity coefficients [ $\log(K_{i,j})$ ] of  $-3.0$  for  $Na^+$ ,  $-4.0$  for  $Mg^{2+}$  and  $-3.5$  for  $Ca^{2+}$ . As shown in Figure 10, the long-term response of the CHEMFETs, without covalently linked ionophore and anionic sites, deteriorated after four weeks when they were exposed to a continuous flow of water in a flow-cell system<sup>49</sup>. However, the CHEMFETs with covalently bound hemispherand exhibited an almost constant response during a period of 20 weeks.

Subsequently, three sets of CHEMFETs with different compositions have been tested under conditions of permanent contact with water<sup>48</sup>. The first set contained valinomycin (1) and covalently attached tetraphenylborate 18, the second covalently attached hemispherand 16 and potassium tetraphenylborate, and the third set both covalently attached hemispherand 16 and covalently attached tetraphenylborate 18. Table 1 shows that over a period of 230 days all sensors of the first set gradually failed to

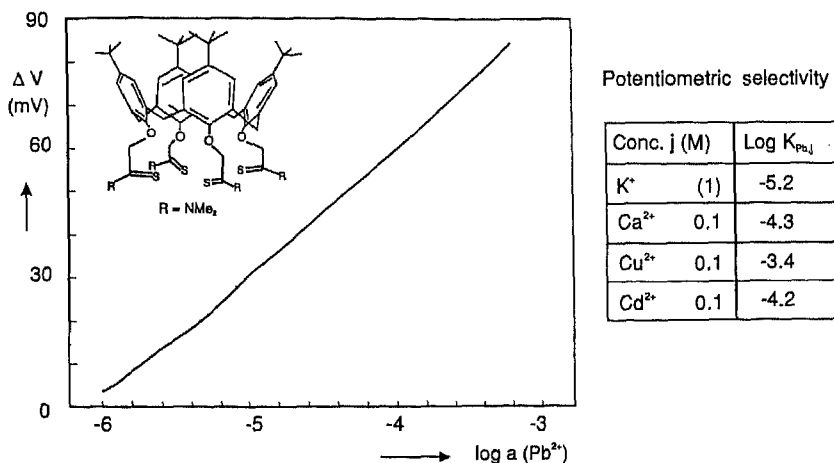
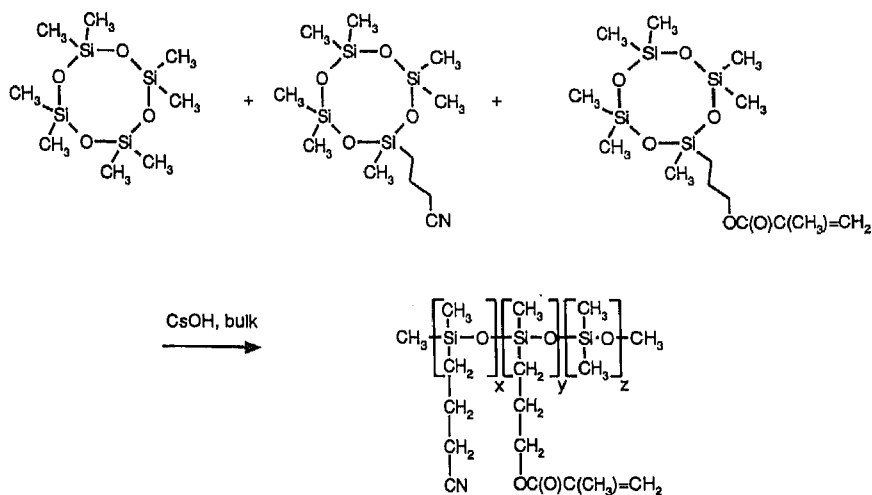


Figure 7. Response of a  $Pb^{2+}$ -selective CHEMFET-containing ionophore 14.



Scheme 1. Synthesis of photopolymerizable polysiloxanes.

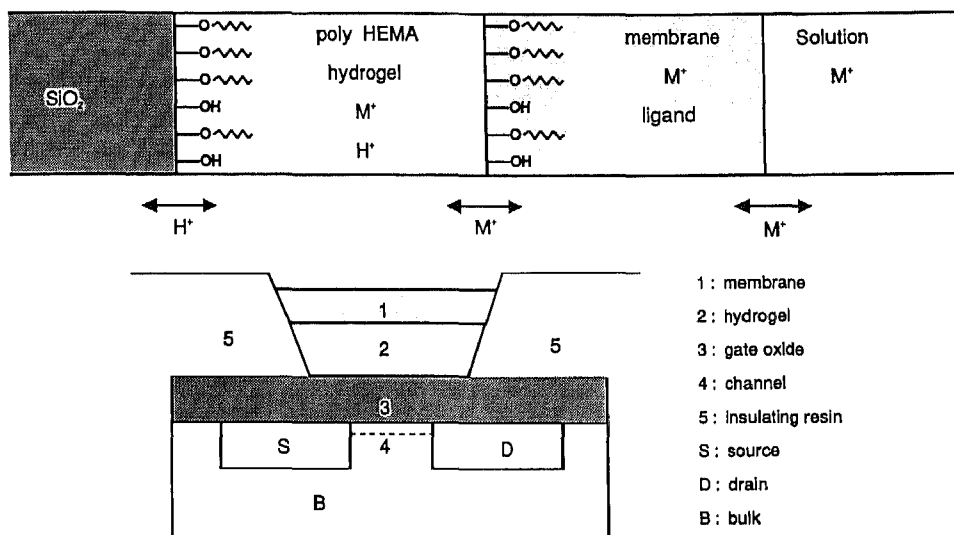


Figure 8. Cross-section of a CHEMFET.

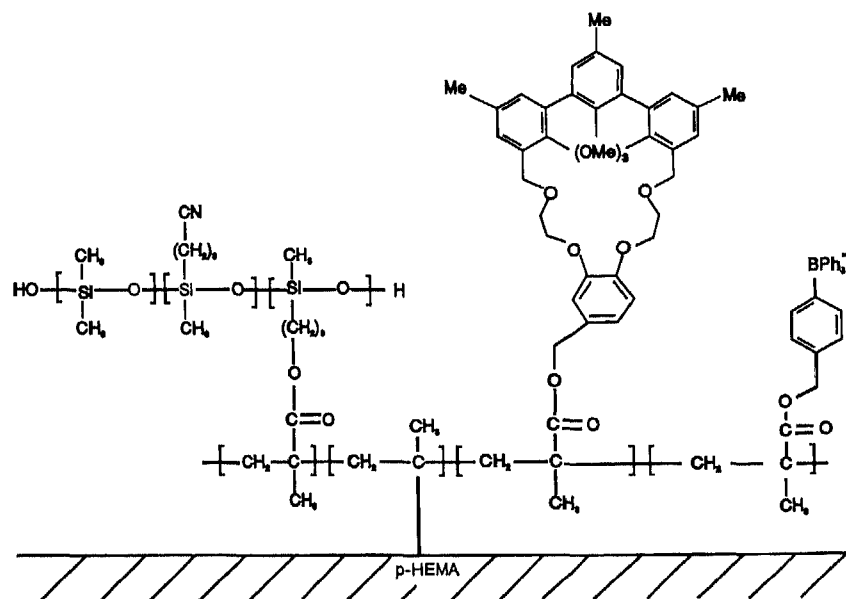


Figure 9. Schematic composition of a polysiloxane membrane with covalently linked hemispherand 16 and tetraphenylborate 18.

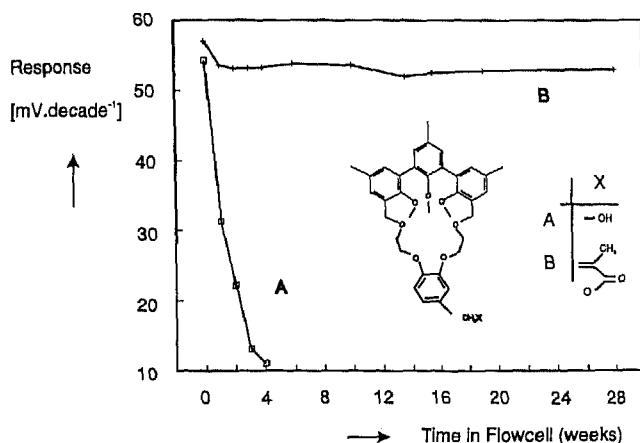
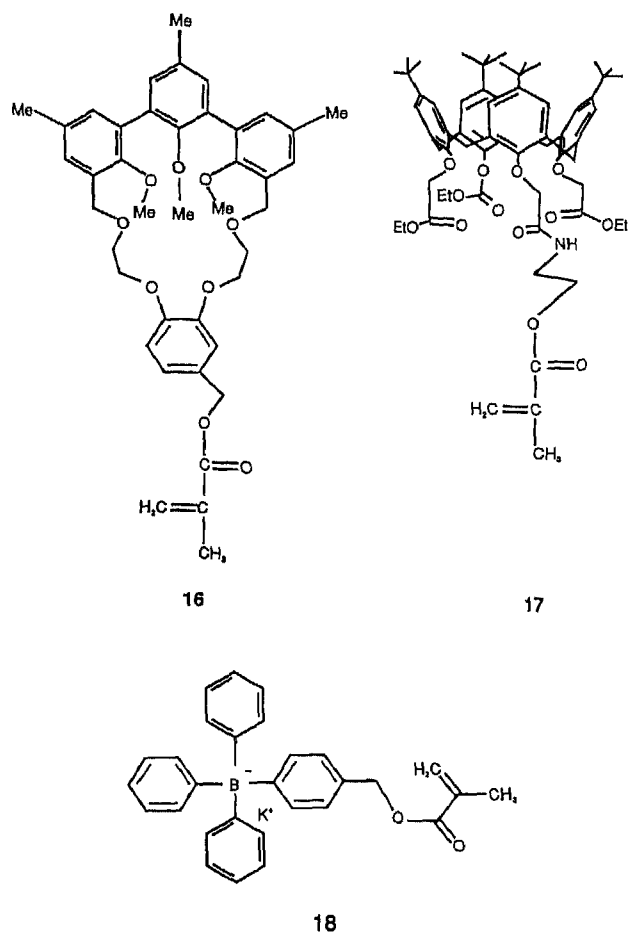


Figure 10. Long-term response of  $K^+$ -selective CHEMFETs with free (A) and covalently linked ionophore (B).

Table I. Long-term performance of CHEMFETs with PS(10)CN polysiloxane membranes.

Ionophore	1	16	16
Borate	18	KITFPB	18
Nr. of sensors tested	22	16	22
Nr. of working sensors			
40 days <sup>a</sup>	2	8	20
60 days <sup>a</sup>	1	6	16
75 days <sup>a</sup>	1	6	16
115 days <sup>a</sup>	1	5	15 <sup>c</sup>
160 days <sup>b</sup>	—	5 <sup>c</sup>	13
230 days <sup>b</sup>	—	4	13

<sup>a</sup> Sensors were continuously washed in tap water (flow 25 ml/min).

<sup>b</sup> Sensors were continuously washed in tap water (flow 500 ml/min).

<sup>c</sup> One sensor showed leakage current.

detect  $K^+$  activities. The sensors with the covalently linked ionophore exhibited, on average, a longer lifetime and with both covalently linked electroactive components showed good responses for 13 out of 22 CHEMFETs. The optimal composition of the polysiloxane membranes was found for 10 mol% 3-cyanopropyl groups [PS(10)CN]<sup>50</sup>, 0.5% methacrylated hemispherand **16**, and 25 mol% methacrylated borate **18**. The  $K^+/Na^+$  sensitivity remained constant ( $52.1 \pm 0.1 \text{ mV} \cdot \text{decade}^{-1}$ ) and the response time as determined by step titrations (100 mM  $K^+$ , 10 mM  $K^+$ , and 1 mM  $K^+$  in the presence of 0.1 M NaCl at pH 3–5) remained fast (Figure 11).

Similar results were obtained with  $Na^+$ -selective CHEMFETs that were made by photopolymerization of the methacrylated calix[4]arene **17**, methacrylated borate **18** and the same polysiloxane PS(10)CN<sup>45</sup>. Over a period of 90 days, the  $Na^+$  selectivities remained unchanged with a response of  $55 \pm 1 \text{ mV} \cdot \text{decade}^{-1}$ . These results clearly demonstrate the advantage of the all-covalently linked architecture for the chemical stability.

The electrical stability which would be required for a constant absolute signal was not investigated. Since the ISFET device itself shows a drift in time as a consequence of inherent solid-state properties, this cannot be solved by the membrane design. Our results clearly show that the membrane architecture gives a constant value in the time-scale of the measurement. It is possible that by transport of water (or salt) the composition of the hydrophilic polyHEMA membrane will slowly change the inner boundary potentials<sup>22</sup>, but this process is irrelevant for the measurement. A suggestion in the literature<sup>22</sup> that osmotic pressure will detach the membrane from the gate oxide is contradicted by the results of our long-term experiments. The chemical attachment of all interfaces and the cross-linking of the polyHEMA layer provides sufficient mechanical strength to compensate for this.

Recently, using regular calibration and a correction algorithm for drift and temperature, we have shown that a constant output signal of the sensor ( $\pm 0.1 \text{ pK}$  units) can be obtained<sup>12</sup>. Because of their fast response times, these CHEMFETs are ideal sensors in flow-injection or flow-through cells<sup>43,49,51,52,53</sup>. In a device with two identical CHEMFETs and a (pseudo)-reference electrode (Figure 12a) a differential measurement eliminates both the problem of a reference electrode and drift. One CHEMFET is used as the reference because it is continuously in contact with a constant solution of *e.g.* 0.1 M NaCl. The second  $K^+$ -selective CHEMFET measures the activity of (different) activities of  $K^+$ ; concentrations of  $10^{-5}$ – $10^{-1}$  M  $K^+$  in 0.1 M NaCl are measured (see Figure 12b). This device has also been used to measure  $K^+$  activities in human

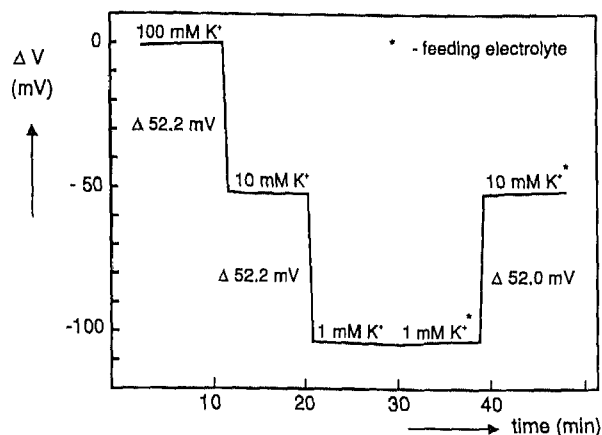


Figure 11. Response of a  $K^+$ -selective CHEMFET to step titrations with 100 mM, 10 mM, and 1 mM of KCl in 0.1 M NaCl at pH 3–5.



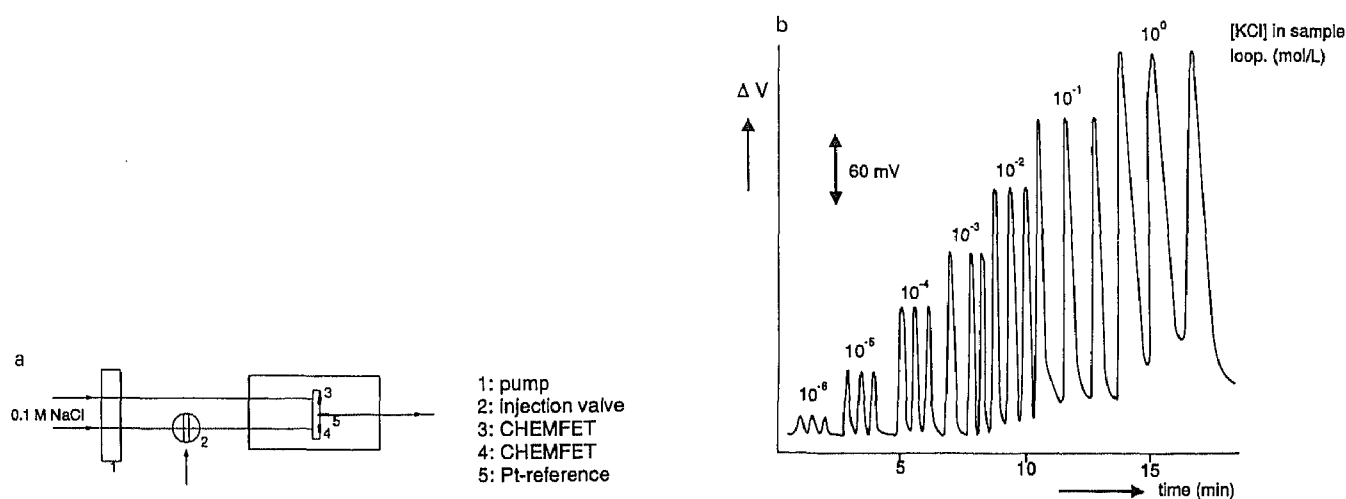


Figure 12. Flow-injection device with two  $K^+$ -selective CHEMFETs and a Pt (pseudo)-reference electrode (a) and responses to different concentrations of KCl ( $10^{-6}$ – $1$  M) in 0.1 M NaCl (results of three consecutive injections at each concentration).

serum and urine samples and the results agree with values obtained by flame photometry<sup>52</sup>.

## Conclusions

Molecular recognition is the basis for chemical sensors but the transduction of the recognition event at the molecular level into a measurable, reliable signal is not a trivial process. In the case of chemically modified field-effect transistors, it requires basic understanding of the process of signal generation and the appropriate material design. For practical applications the basic problems are selectivity and durability. The first has been achieved by design of a series of calix[4]arene-based receptors for  $Na^+$ ,  $K^+$ ,  $Cs^+$ ,  $Ag^+$ ,  $Cd^{2+}$ , and  $Pb^{2+}$ . The second has been achieved by covalent anchoring of the combined membrane system to the gate oxide of the field-effect transistor and by copolymerization of the electroactive components both the ionophore and the lipophilic anions is tuned to buffer the hydrophobic membrane with respect to the ion that is measured. The required electrical properties of the hydrophobic membrane can be achieved by incorporation of polar cyano groups into the polysiloxane. The next challenge will be the design of CHEMFETs that can detect anions. This will first require the synthesis of selective neutral anion receptors. The first results in this field have recently been obtained in our group<sup>54</sup>.

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