Transduction of Selective Recognition by Preorganized Ionophores; K^+ selectivity of the Different 1,3-diethoxycalix[4]arene Crown Ether Conformers

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Three different conformers of 1,3-diethoxy-*p*-*tert*-butylcalix[4]arene crown ethers have been used to study the effect of the ionophore preorganization on the potentiometric K^+-selectivity. Selectivities were measured for chemically modified field effect transistors (CHEMFETs) and membrane ion-selective electrodes (ISEs) by two different methods. The ionophores show decreasing K^+/Na^+ selectivities in the order: *partial cone* > 1,3-*alternate* > *cone*. As a function of time the *cone* conformer maintained a constant selectivity whereas a continuous decrease of the selectivity values was observed for the other two conformers. This supports our prediction based on the association constants and our previous ^1H NMR studies on conformational stability.

A system that has the potential to transfer molecular recognition by synthetic receptors into an electric signal is a chemically modified field effect transistor (CHEMFET),[1] an electronic device based on an ion sensitive field effect transistor (ISFET).[2] The ISFET (Fig. 1) consists of two n-type diffusion regions (source and drain) embedded in a p-type silicon substrate (bulk). The conductance in the channel between the source and the drain is a function of the electrical field perpendicular to the gate oxide. When an ion-selective membrane is attached to the gate of the ISFET the developed membrane potential determines the CHEMFET response. We have developed a CHEMFET technology based on a chemically attached poly(2-hydroxyethyl methacrylate) (poly-HEMA) hydrogel interlayer in between a hydrophobic membrane and the gate oxide insulating layer.[3] This solves the problem of the thermodynamically ill-defined membrane–gate oxide interface and suppresses interference from carbon dioxide. A CHEMFET combines the principle of detection by a membrane ion-selective electrode (ISE) with the solid state integrated circuit (IC) technology. Its selectivity is the macroscopic transduction of the molecular recognition processes by receptor molecules present in the hydrophobic membrane.[4]

The potentiometric selectivity coefficient (K_{ij}) is derived from the Nikolsky–Eisenmann equation [$E = E^\circ + (RT/z_iF)\ln(a_i + K_{ij}a_j^{zi/zj})$] where E is the potentiometric response, E° the standard potential, z is the charge of ion i and j and a_i and a_j are the activities of the primary and the interfering ion, respectively).

Our work on selective receptors is based on the concept of preorganization of the ligating atoms. One type of receptor with an increased hydrophobicity are the calix[4]arene crown ethers.[5] Other calix[4]arene derivatives have already proved to be versatile neutral carriers in potassium-selective electrodes by Diamond *et al.*, Kimura *et al.* and our group.[6]

Our previous studies on the 1,3-dialkoxy-*p*-*tert*-butylcalix[4]arene crown ethers in solution[5] showed peak selectivity for K^+ cations based on the two-phase (water–chloroform) picrate extraction method. ^1H NMR studies revealed that the flexible 1,3-dimethoxy-*p*-*tert*-butylcalix[4]arene crown ether (**2**) undergoes a conformational reorganization from *cone* to *partial cone* upon complexation. We have also shown that 1,3-dialkoxy-*p*-*tert*-butylcalix[4]arene crown ethers with alkyl groups larger than methyl have fixed conformations that do not interconvert at temperatures below 100 °C.[7] This means that it is possible to study the effect of the different conformations on the thermodynamic stability of complexes with alkali cations and on the selectivity of complexations. The three different conformers (**1a**–**c**) show a sequence of binding efficiency for alkali-metal cations in solution which decreases from *partial cone* > 1,3-*alternate* > *cone*. The *partial cone* conformer (**1b**), which is ideally preorganized for the complexation of K^+ according to X-ray analysis,[5] exhibits a K^+/Na^+ selectivity of 1.2×10^4.

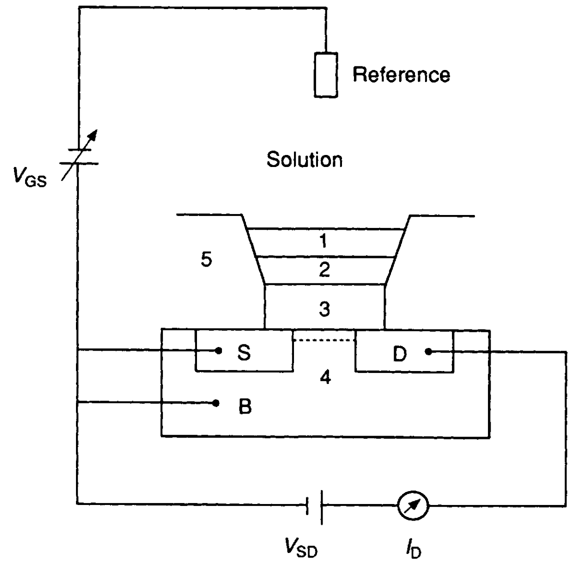

Fig. 1 Scheme of a chemically modified field effect transistor (CHEMFET)

In this paper we describe the evaluation of the K^+-selectivity of **1a**–**c** and **2** for different alkali- and alkaline-earth-metal cations based on CHEMFET and ISE devices.

Results and Discussion

The different conformers of calix[4]arene crown ether derivatives (see Fig. 2) were incorporated into solvent polymeric membranes and their K^+-selectivities were compared based on the different electrode features (CHEMFET and ISE). Typical responses of CHEMFETs towards the K^+ activities of sample solutions are given in Fig. 3. The lower detection limit (at low K^+ activity) is determined by the cation interference (0.10 mol

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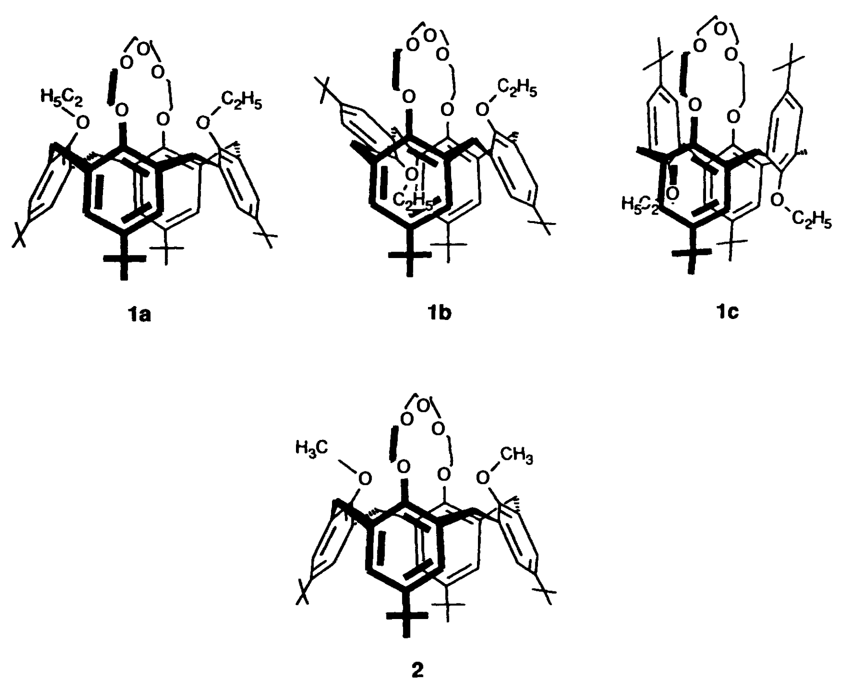

Fig. 2 Structures of the calix[4]arenes crown ether derivatives (ionophores **1a–c**, **2**)

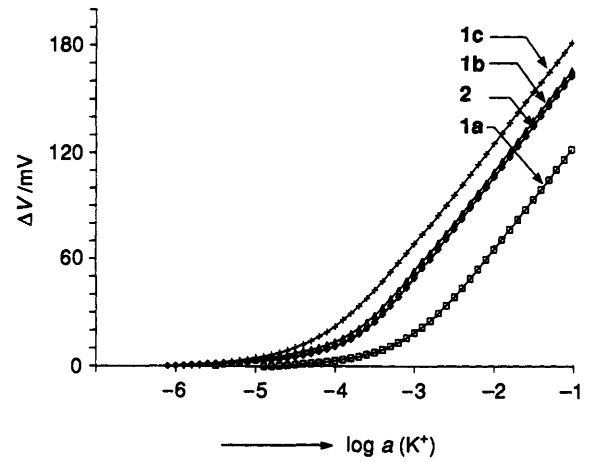

Fig. 3 K^+ responses of CHEMFETs based on PVC membranes containing ionophores **1a–c** and **2** (0.10 mol dm^{-3} sodium chloride)

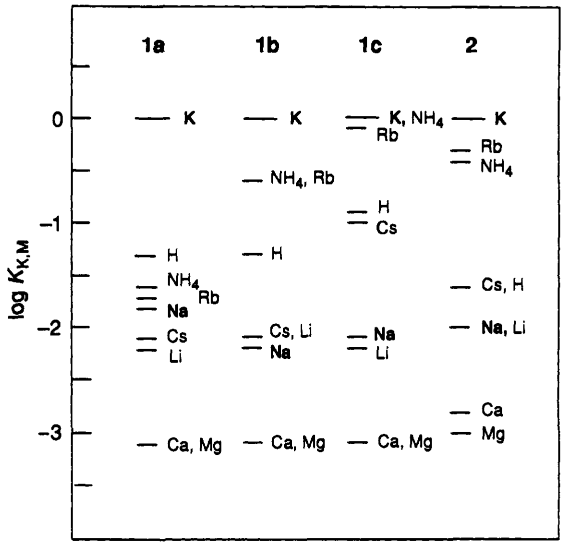

Fig. 4 K^+ selectivity, log $K_{K,M}$, of CHEMFETs based on PVC membrane containing ionophores **1a–c** and **2** (fixed interference method, 0.010 mol dm^{-3} solutions of chlorides)

dm^{-3} Na^+) and varies depending on the conformer used, *e.g.*, −3.1, −4.0, −3.8, and −3.8 for ionophores **1a**, **1b**, **1c** and **2**, respectively. The slopes of the linear part of the dynamic ranges were, in all cases, between 56.8 and 59.0 mV. The selectivity coefficients log $K^{Pot}_{K,M}$ were calculated from the CHEMFETs responses and are given in Fig. 4. The K^+ selectivity *versus* Mg^{2+} and Ca^{2+} ions is high and constant for all conformers of the diethoxy derivatives (**1a–c**). The most significant differences of relative K^+ selectivity of the different conformers were found for Rb^+ and NH_4^+ cations. While the *cone* conformer (**1a**) exhibits significant K^+/NH_4^+ or K^+/Rb^+ selectivities (−1.6 and −1.7, respectively), the 1,3-*alternate* (**1c**) does not show significant selectivities between K^+, NH_4^+ and Rb^+ cations. The ionophores show good K^+/Na^+ selectivities in decreasing order: **1b** > **1c** > **2** > **1a**.

In order to demonstrate the different responses of the K^+ selective ionophores investigated, all measurements were also made with ISEs. Fig. 5 shows electrode responses to the K^+ activity with constant (0.10 mol dm^{-3}) concentration of sodium chloride as the background electrolyte. The responses of the ionophores employed deviate only marginally from CHEMFET measurements. Their K^+ selectivities over other interfering cations are slightly better than that observed with CHEMFETs. For alkaline-earth-metal cations (Ca^{2+}, Mg^{2+}) and small ions (Li^+, H^+) there are pronounced differences in K^+-selectivity between conformers **1a**, **1b** and **1c**.

The selectivity coefficients were evaluated by two different methods. According to the literature,[8] the separate solution method (SSM) provides more information about the *affinity* of an ionophore for a specific ion but the fixed interference method (FIM) is more reliable for the estimate ion of the *selectivity* of an ionophore towards a specific ion in the presence of interfering ions. The potentiometric K^+/Na^+ selectivity values of CHEMFET and ISE obtained by these both methods are given in Table 1. A membrane containing ionophore **1a** exhibits an almost constant K^+/Na^+ selectivity, independent of

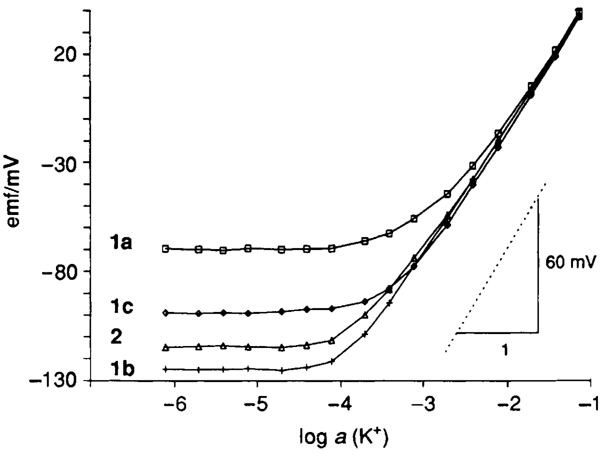

Fig. 5 K^+ responses of ISE based on PVC membrane containing ionophores **1a–c** and **2** (0.10 mol dm^{-3} sodium chloride).

Table 1 Comparison of K^+/Na^+ selectivity of calix[4]arene crown ethers for different kinds of electrode and methods of selectivity determination ($C_{Na^+} = 10^{-1}$ mol dm^{-3})

Ionophore	CHEMFET-FIM	ISE-FIM	ISE-SSM
1a	−2.1	−2.0	−1.9
1b	−3.0	−3.6	−3.7
1c	−2.8	−3.3	−3.3
2	−2.8	−2.8	−3.3

Table 2 Comparison of selectivity coefficients, log $K_{K,Na}$, of ionophores **1a–c** and **2** to the difference of binding free energies $\Delta(\Delta G)$ of their complexes with alkali-metal picrates in $CDCl_3$ ($C_{Na^+} = 10^{-1}$ mol dm^{-3})

Ionophore	$\Delta(\Delta G)$(K/Na)/kcal mol^{-1}	log $K_{K,Na}$ of CHEMFETs
1a	2.7	−2.1
1b	5.5	−3.0
1c	4.0	−2.8
2	4.7	−2.8

the type of electrode and the method of determination. The membrane containing the most selective ionophore **1b** shows the highest selectivity in the case of ISE and the SSM method. This supports our reported data[5] which indicate higher thermodynamic stabilities of the complexes of the *partial cone* conformation (ionophore **1b**). Table 2 relates K^+/Na^+ selectivity values with the differences in binding free energy $\Delta(\Delta G)$ of complexation (K^+ *versus* Na^+).[5] Similar relationships were observed for the other ions investigated.

In order to study the selectivity with respect to time, the ISEs were stored in aqueous solution of KCl for three months. Fig. 6 illustrates changes with time of K^+/Na^+ selectivity values for all of the tested ionophores. While ionophores **1a** and **2** maintained constant values (even after three months of continuous exposure to 10^{-2} mol dm^{-3} KCl solution), a continuous decrease in selectivity was observed for ionophores **1b** and **1c**. Because their lipophilicities are almost equal, the observed time-dependent differences in selectivity suggests a slow isomerization of **1b** and **1c** to the thermodynamically more stable *cone* conformer **1a**.[7]

Experimental

Chemicals.—High molecular weight poly(vinyl chloride) (PVC), bis(2-ethylhexyl) sebacate (DOS) and potassium tetrakis(4-chlorophenyl)borate (KTpClPB) were obtained from Fluka. Tetrahydrofuran (THF) was freshly distilled from a benzophenone–sodium mixture before use. All the inorganic salts were of analytical reagent grade from Merck-Schuchardt, except for potassium and sodium chlorides (*Suprapur*). All solutions were made with doubly distilled and deionized water. Compounds **1a–c** and **2** were prepared according to literature methods.[5]

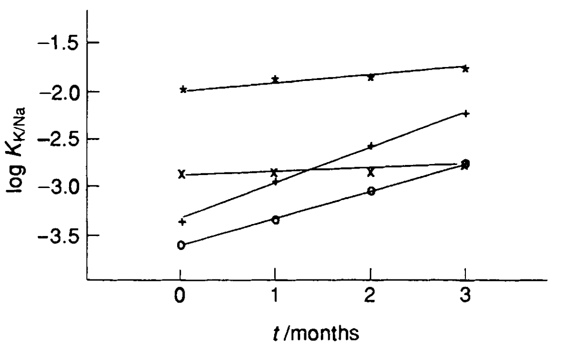

Fig. 6 Time-dependent K^+/Na^+ selectivity of ionophores **1a–c** and **2** (ISE, fixed interference method, 0.10 mol dm^{-3} NaCl, continuous conditioning of ISE in 0.010 mol dm^{-3} KCl): ionophores:*, **1a**; ○, **1b**; +, **1c**; ×, **2**

Membranes.—The membranes were prepared using a mixture of 1 wt% ionophore, 50 mol% KTpClPB (with respect to the ionophore), 64–66 wt% DOS and 33 wt% PVC in 1 cm^3 of THF.

CHEMFETs.—The ISFETs were fabricated as described previously.[9] The CHEMFETs used in this study contain an intermediate hydrogel layer of poly(2-hydroxyethyl methacrylate) (polyHEMA) between the gate oxide and the sensing membrane. The polyHEMA layer was anchored chemically to the gate oxide[3,10] and the CHEMFETs were encapsulated with epoxy resin (Hysol). Subsequently the hydrogel layer was conditioned by immersion in buffered (pH = 4) 0.1 mol dm^{-3} aqueous potassium chloride solution for 3–6 h prior to solvent casting. The membranes were made by solvent-casting of a liquid mixture in THF on mounted CHEMFETs and the THF was allowed to evaporate overnight. Before the measurements were recorded the membranes were conditioned in 1.00×10^{-2} mol dm^{-3} aqueous potassium chloride solution for one night.

Ion-selective Electrodes.—The solution of membrane in THF was cast into 24 mm i.d. glass rings resting on a glass plate. After overnight solvent evaporation, the resulting membrane was peeled from the glass mould and discs, 7 mm in diameter, were cut out. The membrane discs were mounted in an IS 561 Philips electrode body. The electrode was left overnight in 1.00×10^{-2} mol dm^{-3} KCl.

Procedure.—A saturated calomel electrode (SCE) was used as a reference with a double junction containing 1.0 mol dm^{-3} LiOAc. All experiments were performed at temperature 20 ± 1 °C. When not in use, the CHEMFETs and ISE were stored in 1.00×10^{-2} mol dm^{-3} KCl at room temperature.

The CHEMFETs were measured in a constant drain-current mode (I_d = 100 μA), with a constant drain-source potential (V_{ds} = 0.5 V). This was achieved using a source-drain follower-type ISFET amplifier. The developed membrane potential was compensated by an equal and opposite potential (ΔV_{gs}) *via* the reference electrode. Four CHEMFETs were monitored simultaneously and the data were collected and analysed using an Apple IIe microcomputer. All experiments were performed in a dark and earthed metal box to eliminate interference by static electricity and photosensitivity of the CHEMFETs.

The emf of the ISE was recorded on a custom-made 16-channel electrode monitor (resolution, 200 μV) equipped with one field-effect transistor-operational amplifier per channel (input impedance, 10^{12} Ω per 2pF). The data acquisition was performed with an IBM compatible computer in combination with our own software. Further details are given in reference 11. The emf of the electrodes in each solution was measured over 10 min at 0.5 min intervals. The average of the last five measurements was used for further calculations.

The potentiometric selectivity coefficients, $K^{\text{Pot}}_{\text{K,M}}$, were determined by the fixed interference method (FIM) and by the separate solution method (SSM).[12] The response curves of the CHEMFETs and ISE were measured by the addition, with stirring, of aliquots of concentration 1.00×10^{-2} mol dm^{-3} and 1.00 mol dm^{-3} KCl at a constant pH of 4. The constant background concentrations of all interfering ions were 1.00×10^{-2} or 1.00×10^{-1} mol dm^{-3} at constant pH = 4. In the case of sodium chloride as the background electrolyte concentrations of 1.00×10^{-3} and 1.00 mol dm^{-3} were used. All concentrations were converted into activities using the two-parameter Debye–Hückel equation.[13] The recommended National Bureau of Standards functions can be approximated by this Debye–Hückel model if the ionic strength does not exceed 1 mol dm^{-3}. The activity coefficients used were estimated based on parameters in references 13 and 14.

Acknowledgements

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