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Very Lipophilic $Ca^{2\oplus}$-Selective Ionophore for Chemical Sensors of High Lifetime**

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Abstract: Ionophores based on 3-oxapentane-1,5-diamide derivatives with water/1-*n*-octanol partition coefficients up to 10^{22} were synthesized. They have sufficient lipophilicity to be used in solvent polymeric membranes for optodes, ISFET's, and ion-selective electrodes with a lifetime from 600 to $4 \cdot 10^6$ hours even in permanent contact with whole blood or undiluted blood serum. Selectivities of $Ca^{2\oplus}$ over $Mg^{2\oplus}$, $Na^{\oplus}$ and $K^{\oplus}$ of $10^{4.2}$, 10^6 and 10^8, respectively, were obtained.

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Only very recently it has been realized[1] that chemical sensors such as optodes[2] and ion-selective field effect transistors (ISFET's)[3] based on ionophore-doped solvent polymeric membranes require membrane components of extremely high lipophilicity to achieve an analytically relevant lifetime. To confine ionophores over an adequate period of time to such liquid membrane phases, water/1-*n*-octanol partition coefficients[4] of more than 10^{15} are necessary for an application in typical ISFET's or optodes in contact with whole blood or undiluted blood serum[1,2]. Since no such ion carriers exhibiting relevant selectivities have been known, we designed suitable representatives. Here we report on the synthesis of a lipophilic $Ca^{2\oplus}$-selective ionophore and on the electromotive behaviour of membranes based on it.

Experimental

Reagents:

For all experiments, doubly quartz distilled water and chemicals of *puriss.* or *p.a.* grade were used.

Lipophilicity Determinations:

The lipophilicities of the ionophores were determined by thin layer chromatography (TLC) on reversed phase silica plates according to the procedure described previously[5]. The P_{TLC} values obtained by this method are closely related to water/1-*n*-octanol partition coefficients.

Electromotive Force Measurements:

The membrane preparation and measuring technique are described in detail elsewhere[5]. Membrane

composition: 1 wt% ligand, 64–66 wt% *o*-nitrophenyl octyl ether (*o*-NPOE; puriss. p.a., Fluka AG, Buchs, Switzerland) and 33 wt% poly(vinyl chloride) (PVC high molecular; purum p.a., Fluka). As lipophilic anionic sites, potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB; purum p.a., Fluka) was added in a ratio of 46 mol% relative to the ligand. The membrane thickness was about 0.2 mm.

The electromotive force (E) measurements were taken at 21 ± 1 °C on cells of the type Hg; Hg_2Cl_2, KCl (sat.)/3 M KCl/sample//membrane//10^{-2} M $CaCl_2$, AgCl; Ag. The external half-cell was a free flowing free diffusion liquid-junction calomel reference electrode[6]. The solvent polymeric membranes were mounted in electrode bodies Philips IS-561 (N.V. Philips Gloeilampenfabrieken, Eindhoven, Holland). The measured E values were corrected for changes in the liquid-junction potential using the Henderson formalism[7]. The activity coefficients used are described in detail in Ref.[8].

Selectivity Determinations:

Separate Solution Method (SSM): The selectivity coefficients, log K^{Pot}_{CaM}, were determined in 10^{-1} M unbuffered metal chloride solutions.

Fixed Interference Method (FIM): The selectivity factor, log K^{Pot}_{CaK}, was evaluated from the electrode response function for the primary ion determined in solutions with a fixed concentration of the interfering ion. The solutions were buffered with pH buffers and $Ca^{2\oplus}$ buffers and contained 125 mM $K^{\oplus}$ as background electrolyte. The preparation of the buffer solutions is described in Ref.[9].

Interference by Hydrogen Ions:

About 50 mL of a $CaCl_2$ solution of a given concentration (10^{-3}, 10^{-2}, or 10^{-1} M) was used for the measurements. By stepwise addition of small amounts of NaOH and HCl solutions, the pH was gradually changed to about 12 and 1, respectively. The E values were corrected for changes in the liquid-junction potential and in the activity due to the ionic strength.

Membrane Resistance:

Resistance measurements were carried out as described in Ref.[10], the membrane resistance R having a precision of $\Delta(\log R) < 0.2$.

Practical Response Time:

The electrodes were conditioned in 20 mL of 10^{-4} M $CaCl_2$ solution 1–2 min. Then 0.2 mL of 0.1 M $CaCl_2$ solution was added and a magnetic stirrer was turned on for approximately 2 s. E values were taken at 100 ms intervals.

Stability/Drift:

E stabilities and E drift were examined by use of a cell assembly in a thermostated beaker containing a 10^{-3} M $CaCl_2$ solution at 21 ± 1 °C. The total measuring time was 16 h.

Synthesis of the Ionophore N,N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentane-1,5-diamide (ETH 5234):

3-Oxapentanoic acid-*N*,*N*-dicyclohexylamide: A solution of 3-oxapentanedioic acid anhydride (4.0 g, 31 mmol; Aldrich) and *N*,*N*-dicyclohexylamine (5.6 g, 31 mmol; Fluka, puriss. p.a.) in toluene (150 mL; Fluka, puriss. p.a.) was stirred at room temperature for 4 h and then at 100 °C for 2 h. The solvent was evaporated and the residue recrystallized from ethyl acetate/hexane 4:1 to yield the product (3.5 g, 11.8 mmol, 38%).

N,*N*-Dicyclohexyl-*N'*,*N'*-dioctadecyl-3-oxapentane-1,5-diamide: To a solution of 3-oxapentanoic acid-*N*,*N*-dicyclohexylamide (1.19 g, 4 mmol) in CH_2Cl_2 (80 mL; Fluka, puriss. p. a.), triethylamine (0.81 g, 8 mmol; Fluka, puriss. p.a.), bis(2-oxo-3-oxazolidinyl)phosphinic acid chloride (1.02 g, 4 mmol; Fluka, purum), and freshly recrystallized dioctadecylamine (2.09 g, 4 mmol; Fluka) were added at room temperature. After stirring for 16 h, the reaction mixture was washed with 100 mL saturated aqueous $NaHCO_3$ solution, the organic phase filtered and the solvent evaporated. The residue was purified by filtration through silica gel 60 (30 g, Fluka) with hexane/ethyl acetate 3:2 to yield ligand ETH 5234 (2.3 g, 2.87 mmol, 71.8%). – The ^{1}H-NMR, IR and mass spectra correspond to the constitution of the ionophore. Elemental analysis, calculated for $C_{52}H_{100}N_2O_3$ (801.3): C 77.94%, H 12.58%, N 3.50%; found: C 77.75%, H 12.54%, N 3.48%.

Scheme

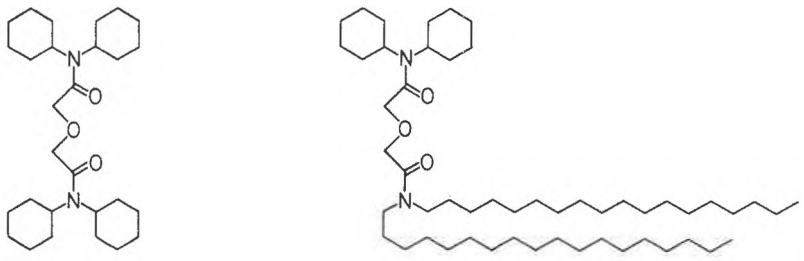

Table 1. Properties of $Ca^{2\oplus}$-selective ionophores.

	ETH 129	ETH 5234
Lipophilicity log P_{TLC}	7.5 ± 0.4	22.6 ± 3.7
Electric resistance of membranes [Ω]	$2.4 \cdot 10^5$	$5.2 \cdot 10^5$
Response time $t_{90\%}$ of membranes [s] (10^{-4} to 10^{-3} M $CaCl_2$)	1.1	1.2
Drift [μV/h]	≤ 60	≤ 20
Sensor detection limit log $a_{Ca2\oplus}$ at 0.125 M KCl ion background (see also Fig. 2)	−10.1	−9.7
Slope of electrode response [mV] ($10^{-8.5}$ to 10^{-1} M $Ca^{2\oplus}$); theoretical: 29.1 mV	29.0 ± 1.9	29.7 ± 1.7

Results and Discussion

Although the new ionophore ETH 5234 (see Scheme) is about 10^{15} times more lipophilic than the isolog ETH 129, it induces electromotive properties in membranes which are very similar (see Table 1). The almost identical and very high selectivities (see Fig. 1) are the major reason for the low detection limit, which is at about 10^{-10} M $Ca^{2\oplus}$ even at an ion background of 0.125 M $K^{\oplus}$ (Fig. 2, see also Ref.[11]). The optimal pH working range of the $Ca^{2\oplus}$ electrodes with ETH 5234 was evaluated by the fixed primary ion method (Fig. 3). The data, which have been corrected for changes in the liquid-junction potential

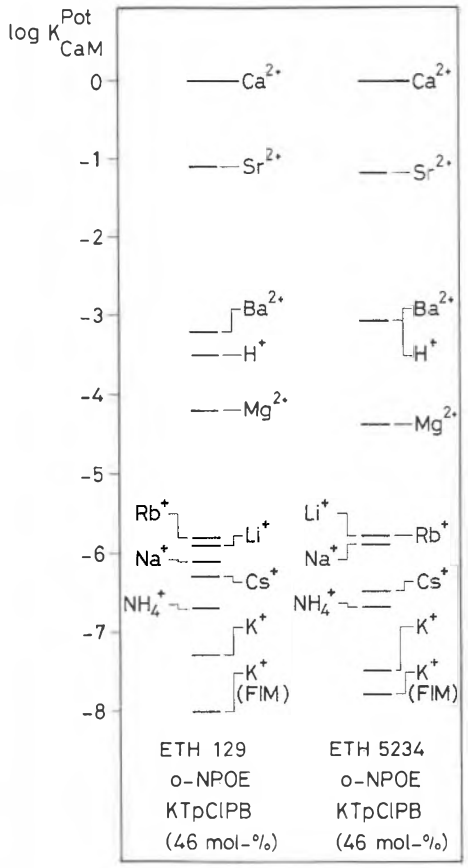

Fig. 1. Selectivity factors, log K^{Pot}_{CaM}, for solvent polymeric membranes with the carriers ETH 129 or ETH 5234, o-nitrophenyl octyl ether (o-NPOE) as membrane solvent, and incorporated lipophilic anionic sites [potassium tetrakis(p-chlorophenyl)borate (KTpClPB), mol-% given].

and the activity coefficients, clearly show that even in millimolar $Ca^{2\oplus}$ solutions no relevant $H^{\oplus}$ interference occurs at pH values of about 2.

The slowdown of the ion transfer between the sample solution and the membrane phase, which is often observed when ionophores with a given constitution of coordinating ligand atoms are replaced by more lipophilic isologues, is fortunately not observed here[12]. Because of the design features of the ionophore ETH 5234 with the lateral lipophilic units on only one amide nitrogen atom, such a behaviour could be expected[13,14]. The ion carrier is therefore suitable for realizing optodes and ISFET's of very long lifetime.

On the basis of such variables as the lipophilicity, the thickness of the membrane, and hydrodynamic conditions, theoretical models and calculations can be used to quantify the loss of membrane components into the sample[2,15]. The lifetime of sensors based on carrier ETH 5234 in permanent contact with whole blood or undiluted blood serum was estimated by such calculations. The resulting lifetime values for typical optodes, ISFET's and ion-selective electrodes are 600, $1.7 \cdot 10^5$ and $4 \cdot 10^6$ hours, respectively.

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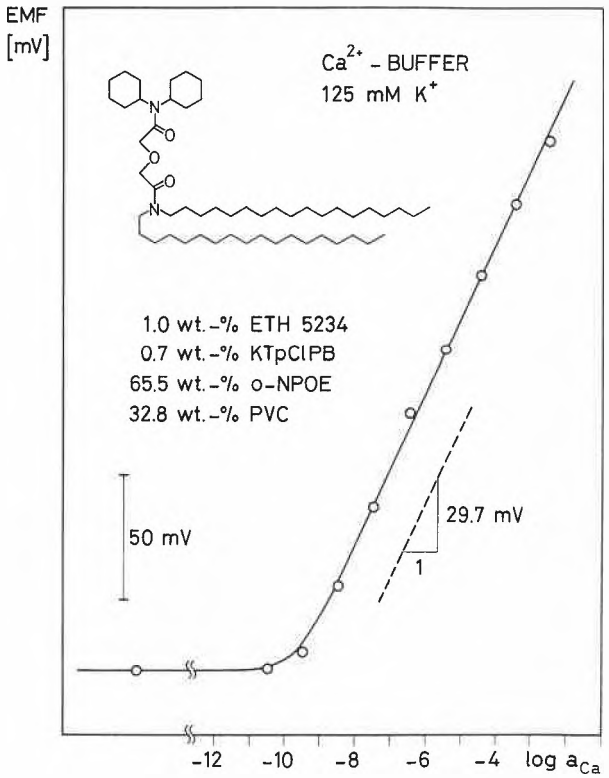

Fig. 2. Electrode response of membrane electrode cell assemblies based on the carrier ETH 5234 in $Ca^{2\oplus}$-buffered solutions containing 125 mM $K^{\oplus}$. The detection limit is at −9.7 log a units.

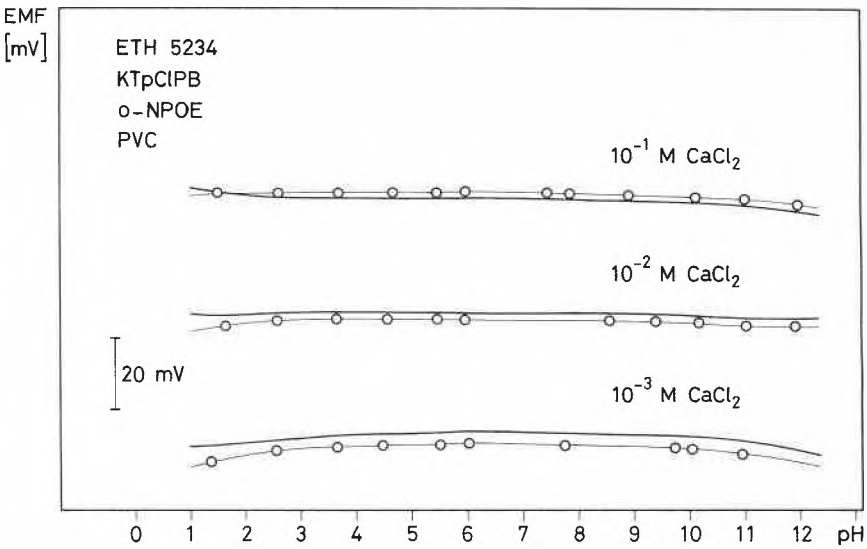

Fig. 3. Dependence of the electromotive force of the $Ca^{2\oplus}$ electrode cell assembly based on the carrier ETH 5234 on the pH of the sample solution for different $CaCl_2$ concentrations: points, experimental values (uncorrected); thick line, E response after corrections for changes in liquid-junction potentials and $Ca^{2\oplus}$ activity coefficients.

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