Preparation of Ca^{2+} Selective Sorbents by Molecular Imprinting using Polymerisable Ionophores

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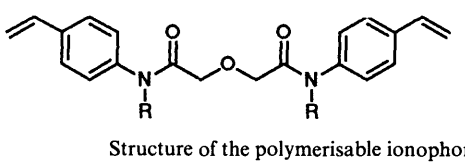
Molecular imprints against calcium and magnesium ions, respectively, were prepared in divinylbenzene-based polymers. A vinylic, Ca^{2+}-selective, neutral ionophore *N,N'*-dimethyl-*N,N'*-bis-(4-vinylphenyl)-3-oxapentanediamide was synthesized and used as the ion-complexing monomer. In order to investigate the ion-complexing abilities of this neutral ionophore, this compound was tested as a neutral carrier in the plasticised PVC membrane of an ion-selective electrode. Electrode response and selectivity coefficients recorded for the ionophore proved to be equivalent to the values recorded for similar parent diglycolic acid amides. In analogy with other 3-oxapentanediamide derivatives, the ionophore is expected to form complexes with calcium and magnesium ions with a molar ratio, ligand to metal ion, of 3:1 and 2:1, respectively. Therefore the metal ion, added to the polymerisation mixture, was expected to act as a template for the ionophore during the polymerisation. The resulting polymers were analysed for their ability to extract calcium ions from methanolic water. The polymers prepared against calcium and magnesium ions were found to bind calcium ions with 6- and 1.7-times lower K_{diss}-values, respectively, when compared with reference polymers prepared in the absence of metal ions. The increased binding strength is attributed to the spatial arrangement of ionophore units in the resulting polymers by the template ions during the polymerisation. In addition, the number of binding sites for calcium ions, determined for the respective polymer preparation, fitted well with theoretical values calculated from the stoichiometry of complexation of the ionophore by calcium and magnesium ions, respectively.

Molecular imprinting of small molecules in highly cross-linked polymers has become accepted as a technique for the creation of specific recognition sites in synthetic polymers.[1] This technique has been applied to studies of the recognition of a substrate by a macromolecule[2,3] and to the preparation of 'specialty' separation media for chromatography.[2] In particular, molecularly imprinted polymers have been successfully used as chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC).[4–6] Furthermore, molecular imprinting may be used as a tool to obtain polymers with enzyme-like properties.[7–9]

3-Oxapentanediamide derivatives have been used widely as electrically neutral carriers in calcium-ion-selective electrodes (ISE).[10] In the case of *N,N,N',N'*-tetracyclohexyl-3-oxapentanediamide (ETH 129) the neutral carrier was shown to form complexes in organic solvents with Ca^{2+} in a molar ratio of ligand to cation of 3:1 and with Mg^{2+} in a molar ratio of ligand to cation of 2:1.[11] When incorporated into solvent polymeric membranes this ligand exhibits a high selectivity for calcium ions over all other alkali and alkaline earth metal ions, in particular over magnesium ions.[12] Extremely sensitive, miniaturised calcium-selective electrodes with sub-nanomolar detection limits have been prepared.[13]



1 R = CH_3
2 R = H

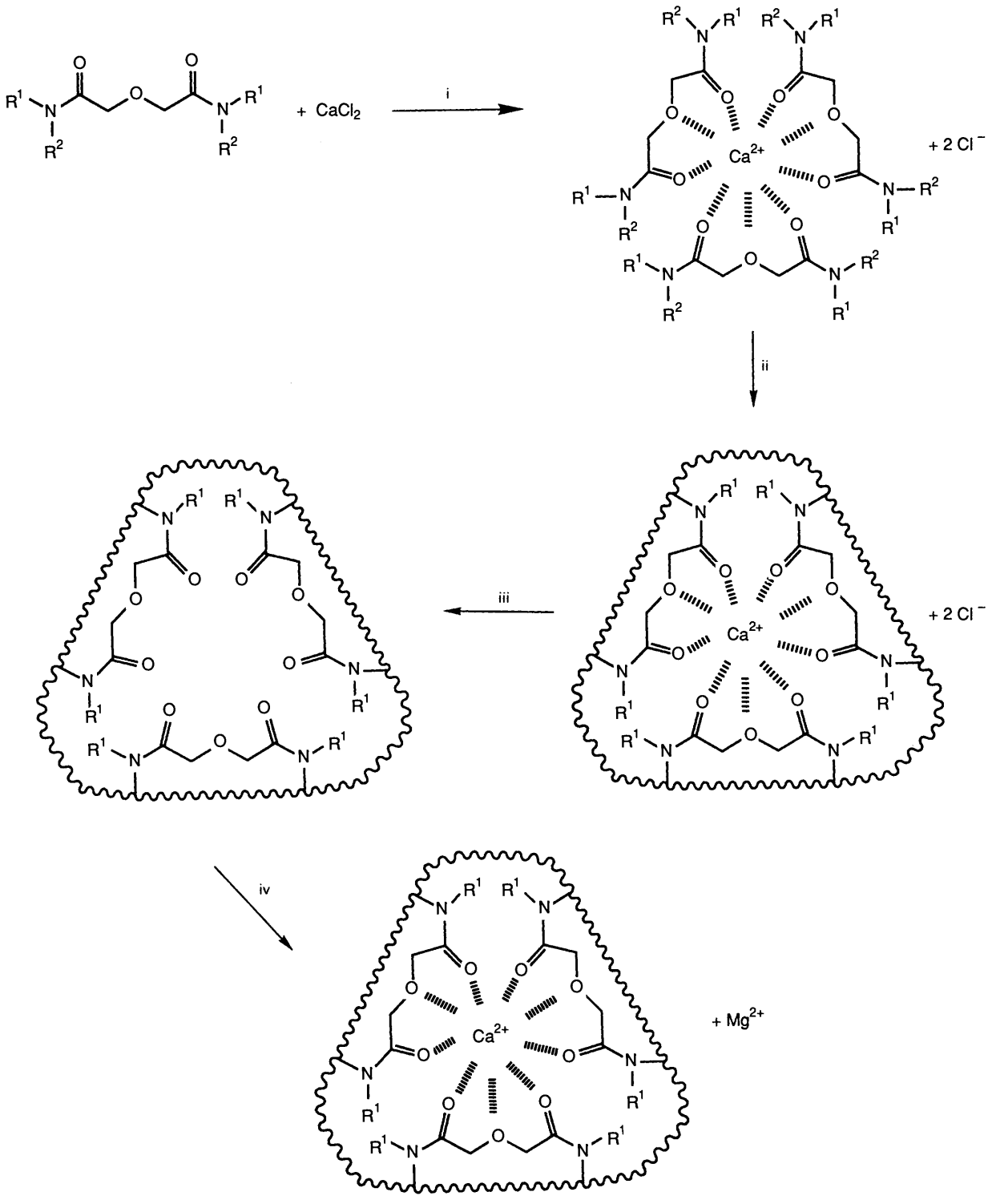
Structure of the polymerisable ionophore.

The present investigations were initiated as a feasibility study with the objective of obtaining specific imprints of metal ions. Such polymers may have potential for applications in (*i*) the specific removal of, for example, toxic heavy metals, (*ii*) the preparation of matrices for the emerging new bioseparation technique of immobilised metal affinity chromatography (IMAC) and (*iii*) the preparation of metal-ion-selective sensors. A polymerisable, calcium-selective, 3-oxapentanediamide derivative, compound **1**, was synthesized. Functional tests of this new monomer were carried out by using compound **1** as a component of ion-selective electrodes. This compound proved to be as selective and efficient in calcium extraction as the parent 3-oxapentanediamide compounds. Polymers with molecular imprints against calcium and magnesium ions were prepared, using compound **1** as ion-complexing monomer. These polymers were shown to bind calcium ions efficiently. By the imprinting approach the ionophore units were spatially arranged within the polymeric network to facilitate the binding of calcium ions (see Scheme 1). The molecularly imprinted polymers bound calcium ions more efficiently than did polymers prepared in the absence of metal ions, in regard to both binding strength and uptake capacity.

Experimental

Divinylbenzene (DVB, technical grade), poly(vinyl chloride) (high molecular PVC), bis(2-ethylhexyl) sebacate (DOS) and potassium tetrakis(4-chlorophenyl)borate (KT*p*ClPB) were all obtained from Fluka (Buchs, Switzerland). Styrene, 2,2'-azoisobutyronitrile (AIBN), diglycolic acid, potassium *tert*-butoxide and dicyclohexyl-18-crown-6 were from Janssen (Beersee, Belgium). Thionyl dichloride and iodomethane were from Merck-Schuchardt (Darmstadt, Germany), and *p*-aminostyrene was from Polysciences (Warrington, PA, USA). All solvents were of highest available grade and doubly quartz-distilled water was used.

Syntheses.—N,N'-*Bis*(4-*vinylphenyl*)-3-*oxapentanediamide* **2**. To a stirred solution of diglycolic acid (10 g, 74.6 mmol) in dry toluene (100 cm^3) containing a catalytic amount of *N,N*-



Scheme 1 *Reagents and conditions:* i, $CHCl_3$; ii, DVB, styrene, AIBN, N_2, 60 °C, 36 h; iii, extraction; iv, Ca^{2+} and Mg^{2+} in methanolic water

dimethylformamide was slowly added thionyl dichloride (27.5 cm^3, 373 mmol). The mixture was stirred at 40 °C for 9 h and then at room temperature for an additional 15 h. The solvent was evaporated off under reduced pressure, the crude acid chloride was dissolved in dry toluene (40 cm^3), and the solution was added dropwise to a solution of *p*-aminostyrene (41.5 g, 336 mmol) in pyridine–toluene [1:1 (v/v); 80 cm^3]. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated off. The residue was dissolved in ethyl acetate, washed three times with 0.5 mol dm^{-3} HCl, twice with 0.1 mol dm^{-3} $NaHCO_3$, and then with water. The organic layer was finally dried over Na_2SO_4 and evaporated under reduced pressure. Crystallisation from chloroform–heptane [5:1 (v/v)] gave pure compound **2** (15.0 g, 59.9%) [Found: C, 70.8; H, 5.85; N, 7.9. $C_{20}H_{20}N_2O_3$ (336.39) requires C, 71.41; H, 5.99; N, 8.33%]; δ_H(300 MHz; $CDCl_3$) 4.26 (4 H, s, CH_2), 5.23 (2 H, d, *J* 10.98, C*H*H=CH), 5.71 (2 H, d, *J* 17.58, CH*H*=CH), 6.69 (2 H, dd, *J* 17.58 and 10.98, CHH=C*H*), 7.40 (4 H, d ArH), 7.56 (4 H, d ArH) and 8.30 (2 H, br s, NH).

N,N′-*Dimethyl*-N,N′-*bis*(4-*vinylphenyl*)-3-*oxapentanediamide* **1**. To a solution of compound **2** (3.36 g, 10 mmol), potassium *tert*-butoxide (8.98 g, 80 mmol) and dicyclohexyl-18-crown-6 (200 mg, 0.5 mmol) in dry tetrahydrofuran (THF) (80 cm^3) at 0 °C was added iodomethane (11.36 g, 80 mmol). The

Table 1 Polymer preparations and analysis. All polymers were prepared using divinylbenzene (DVB, 80 mol%) as cross-linking monomer, chloroform as solvent, and AIBN (1 mol% per vinyl group) as the initiator at 60 °C for 36 h under nitrogen. The amount of AIBN present is not accounted for in the mol% figures given.

Polymer[a]	Print ion (mol %)	1 (mol %)	Styrene (mol %)	Amount of 1 found (μmol/g polymer)[b]	Amount of 1 found (μmol/g polymer)[c]
A (Ca^{2+})	5	15		856	868
B (Mg^{2+})	5	15		856	707
C		15	5	856	752
D			20		

[a] Print ion in parentheses. [b] Calculated from elemental analysis. [c] Calculated from FT-IR spectroscopy data: the carbonyl stretching band between 1750–1615 cm^{-1}, reduced by subtraction of the spectrum recorded for polymer preparation D, was used for the calculations. The molar extinction coefficient was calculated from the spectrum recorded for pure compound **1**.

reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate, and the solution was washed successively with 0.5 mol dm^{-3} HCl, 0.5 mol dm^{-3} $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated under reduced pressure. Crystallisation from THF–heptane [1:1 (v/v)] yielded compound **1** (1.88 g, 51.3%), m.p. 115–117 °C [Found: C, 72.25; H, 6.75; N, 7.6. $C_{22}H_{24}N_2O_3$ (364.44) requires C, 72.51; H, 6.64; N, 7.69%] δ_H(300 MHz; $CDCl_3$) 3.22 (6 H, s, NCH_3), 4.00 (4 H, s, CH_2), 5.32 (2 H, d, *J* 10.99, C*H*H=CH), 5.76 (2 H, d, *J* 17.58 CH*H*=CH), 6.71 (2 H, dd, *J* 17.58 and 10.99, CHH=C*H*), 7.12 (4 H, d, ArH) and 7.42 (4 H, d, ArH); δ_C(300 MHz; $CDCl_3$) 37.2 (NCH_3), 68.9 (CH_2), 115.2 (=CH_2), 127.2 (Ar), 127.6 (Ar) 135.6 (-C*H*=), 137.5 (Ar), 141.6 (Ar) and 169.0 (C=O); *m/z* 364 (M^+, 11%), 232 (63), 204 (100), 175 (74), 174 (75), 146 (34), 132 (46) and 118 (49).

Characterisation of Compound **1** *as an Ionophore in ISEs.*—The lipophilicity of compound **1** was determined by TLC on reversed-phase silica plates according to a procedure described previously.[14] The PTLC values obtained by this method are closely related to water/octan-1-ol partition coefficients.

The electrode calibration curve and selectivity coefficients induced by compound **1** were measured as described.[14] The plasticised PVC membrane was composed of poly(vinyl chloride) (32.1 wt%)–bis(2-ethylhexyl) sebacate (65.3 wt%)–**1** (1.85 wt%)–potassium tetrakis(*p*-chlorophenyl)borate (0.7 wt%) and was approximately 0.2 mm in thickness.

The electromotive force (EMF) measurements were taken at 21 ± 1 °C on cells of the type Hg; Hg_2Cl_2, KCl (sat.)/3 mol dm^{-3} KCl/sample//membrane//10^{-2} mol dm^{-3} $CaCl_2$, AgCl; Ag. The external half-cell was a free flowing, free-diffusion, liquid-junction calomel reference electrode. The solvent polymeric membrane was mounted in electrode bodies Philips IS-561 (N. V. Philips Gloeilampenfabrieken, Eindhoven, Holland). The measured EMF values were corrected for changes in the liquid-junction potential using the Henderson formula.[15] The activity coefficients used are literature values.[16]

The selectivity coefficients, log K_{CaM}, were determined in 10^{-1} mol dm^{-3} unbuffered metal chloride solutions by the separate-solution method (SSM) according to the IUPAC recommendations.[17]

Polymer Preparation.—The compositions of the polymerisation mixtures are shown in Table 1. To a solution of compound **1** (728 mg) in chloroform (3 cm^3) was added anhydrous $CaCl_2$ (74 mg) or $MgCl_2$ (95 mg) and the print ions were dissolved by stirring at room temperature overnight (16 h). Cross-linking monomer (DVB) (1388 mg), initiator (AIBN) (32 mg), and the appropriate amount of styrene (0, 70, or 280 mg, see Table 1) were added. The polymerisation mixtures were degassed by sonication *in vacuo* and were subsequently purged with nitrogen for 5 min. The tubes were then sealed with Parafilm and placed in a water-bath at 60 °C for 36 h. The bulk polymers were ground in a mechanical mortar (Retsch, Haan, Germany) and wet sieved {water–ethanol [1:1 (v/v)]} through a 25 μm sieve (Retsch, Haan, Germany). The fines were removed from the preparation by settling twice in methanol–water–acetic acid [18:1:1 (v/v/v)] and twice in pure methanol. The metal ions were extracted from the polymer by continuous washing with methanol–water–acetic acid [18:1:1 (v/v/v); 300 cm^3] and methanol–water [1:1 (v/v); 300 cm^3] for 24 h in each solvent mixture. After this treatment the eluent was determined to be calcium-free by the *o*-cresolphthalein complexone colour test (Sigma, St. Louis, MO). All washing eluents were combined and the total amount of calcium washed out of the polymer was found to be 84 mg (112% of the amount added to the polymerisation mixture).

Elemental analysis:* Polymer A (Found: C, 79.95; H, 7.5; N, 2.6. Calc.: C. 85.03; H, 7.78; N, 2.63%); Polymer B (Found: C, 80.2; H, 7.4; N, 2.6. Calc.: C, 85.03; H, 7.78; N, 2.63%); Polymer C (Found: C, 82.3; H, 7.65; N, 2.6. Calc.: 85.26; H, 7.77; N, 2.55%); Polymer D (Found: C, 87.6; H, 8.05; N, ≤0.2. Calc.: C. 91.72; H, 8.28; N, 0.66%).

Equilibration Experiments.—Polymer particles (50 mg) were incubated with solutions (1 cm^3) of $CaCl_2$ or $MgCl_2$ (0.5–250 mmol dm^{-3}) prepared in methanol–water [1:1 (v/v)]. The particles were suspended by sonication and the samples were placed on a rocking table at room temperature for 36 h. The polymer particles were then removed by centrifugation (20 min, 14 000 rpm, Eppendorf centrifuge) and the supernatant was analysed by atomic absorption spectroscopy (Varian AA 1275). The amount of cation initially added, reduced by subtraction of the amount of cation found in the supernatant, constituted the amount of cation adsorbed on the polymer. No cation could be detected in the supernatant after incubation of polymer particles with pure solvent. The dissociation constant (*K*) and the number of binding sites (*n*) of the polymer were calculated using Scatchard-plot analysis[18] assuming identical and independent binding sites.[19]

Results and Discussion

The type of ionophore used in the present study has previously been shown to be very selective for calcium ions.[12,20] Compound **2** was prepared by a method similar to one described earlier for the preparation of diglycolic acid amides.[20] Compound **2** was then *N*-methylated by reaction with potassium t-butoxide and iodomethane[21] to form the *N,N*-disubstituted amide **1**.

In analogy with the parent diglycolic amides,[12,20] ionophore **1** forms complexes with calcium and magnesium ions in organic solvents. This is shown by the observation that solid, anhydrous $MgCl_2$ and $CaCl_2$ readily dissolve in a solution of compound **1** in chloroform. On the other hand, addition of $MgCl_2$ and $CaCl_2$ to a solution of compound **2** resulted in precipitates of compound **2** and the respective metal ion. This observation is in agreement with results described previously for other *N*-monosubstituted diglycolamides.[22] Ionophore **1** was used throughout all subsequent studies.

* The calculated values were calculated by assuming complete incorporation of all components of the polymerisation mixture except the template ions.

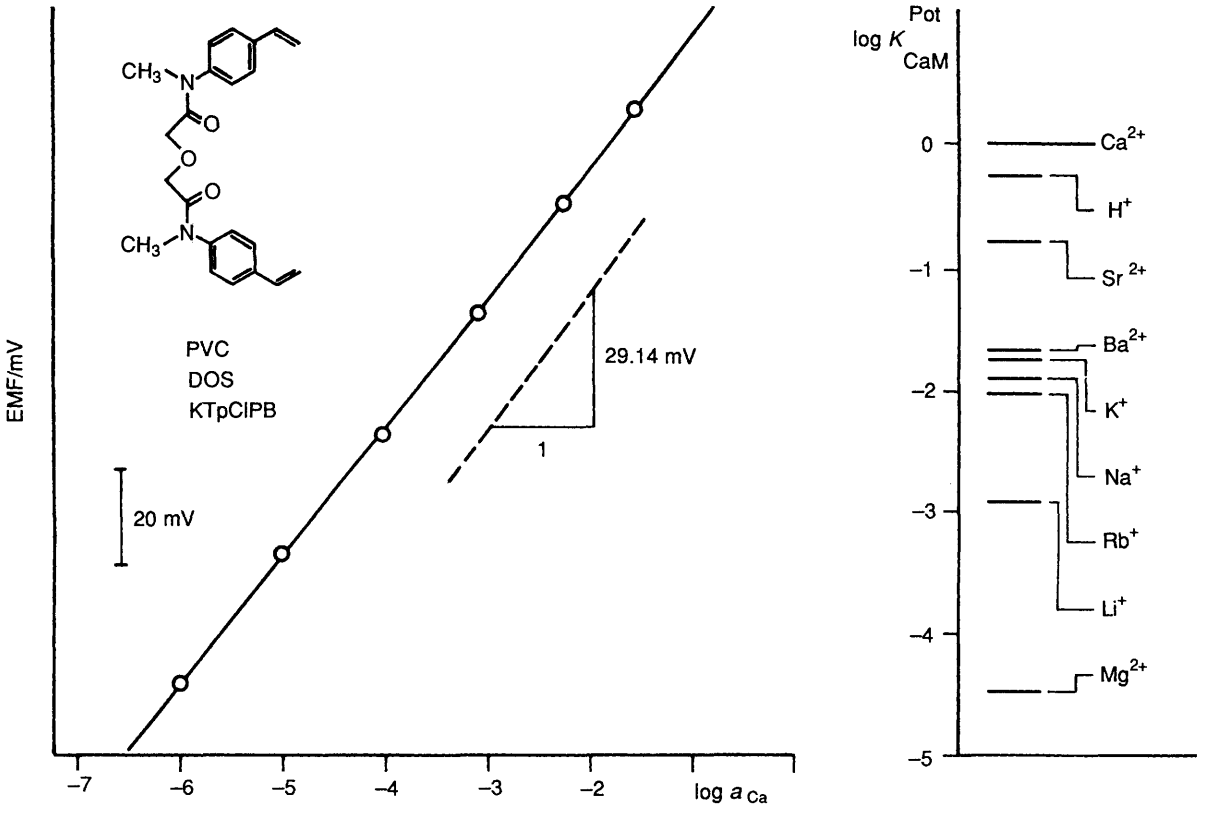


Fig. 1 Electrode calibration curve (left) and graphical representation of the selectivity coefficients, log K_{CaM}, (right) for the solvent polymeric membranes based on compound **1** as carrier, K_{CaM} indicates the preference of the membrane for M relative to Ca^{2+}. Therefore, small values of K_{CaM} represent a high selectivity for Ca^{2+} over M. The log K_{CaM}-values were determined in unbuffered metal chloride solutions using the separate-solution method (SSM).

In order to investigate further the ion-complexing abilities of compound **1**, this ionophore was tested as a carrier in an ion-selective electrode.[10] Ionophore **1** was incorporated into plasticised PVC membranes, which were then mounted in electrode bodies for EMF measurements.[14] The low lipophilicity of compound **1** (log P_{TLC} 2.74 ± 0.26) was sufficient to guarantee a stable potentiometric signal within the measurement time of 15 min. The measured EMF values were corrected for changes in the liquid-junction potential by applying the widely used Henderson formula.[15] This equation gives correct values for measurements performed in pure metal chloride solutions of known activity, which is the case in this study. The calibration curve for calcium ions was linear with an almost Nernstian response of 27.16 mV/dec ± 0.15 mV per decade at 20.6 °C (theoretical slope s_{th} 29.14 mV per decade) over a concentration range of 10^{-6} to 10^{-1} mol dm^{-3} $CaCl_2$ (Fig. 1). Furthermore, compound **1** was selective for calcium ions over all other alkaline and alkaline earth metal ions (Fig. 1). The membrane in question showed the highest rejection for Mg^{2+} with a selectivity coefficient of log K_{CaMg} −4.5. This value means that a Ca^{2+} solution with an activity of $10^{-4.5}$ mol dm^{-3} Ca^{2+} induces the same potential as a Mg^{2+} solution with an activity of 1 mol dm^{-3} Mg^{2+} in a potentiometric device such as an ion-selective electrode. The high selectivity observed for calcium ions is in agreement with previous findings for the parent 3-oxapentanediamide derivatives.[12,20]

Molecular imprints against calcium and magnesium ions were prepared by using compound **1** as the ion-complexing monomer. The polymer preparations are shown in Table 1. Incorporation of compound **1** into the polymers was confirmed both by elemental analysis (see Experimental section) and by Fourier-transform IR (FT-IR) spectroscopy (spectra not shown). The IR spectra for polymer preparations A, B and C as well as for compound **1** showed the bands characteristic of amide carbonyl stretching at 1670 cm^{-1} and for ether stretching at 1115 cm^{-1}. In polymer preparation D, a reference polymer prepared without the use of compound **1**, both signals were missing. Calculations based on elemental analysis and FT-IR spectroscopy showed that compound **1** was incorporated into the resulting polymers to a high extent (Table 1). Furthermore, the addition of metal ions in some of the polymerisation mixtures did not change to any significant extent the amount of compound **1** incorporated (Table 1). The template metal ions added during polymerisation were quantitatively removed from the resulting polymers by extraction (see Experimental section).

All polymer preparations were analysed for their ability to extract calcium and magnesium ions from methanolic water solutions. Polymers A, B and C bound calcium ions to a variable extent. The binding data were analysed by Scatchard-plot analysis,[18] assuming identical and independent binding sites.[19] The dissociation constant (K) and the number of binding sites (n) of the polymer were calculated for each polymer preparation (Table 2). The binding strength was found to be 6 times and 1.7 times higher for polymers A and B, prepared against calcium and magnesium respectively, than for polymer C, prepared in the absence of metal ions (Table 2). Furthermore, the Scatchard plots were almost linear over the concentration range investigated, which indicates uniform binding sites. Binding of magnesium ions could not be detected for any of these polymer preparations under the conditions used. Over the entire concentration range investigated the same amount of Mg^{2+} was found in the supernatant, within experimental error (±4.3%), as was initially added to each sample (see Experimental section). The extreme selectivity observed for calcium ions is in agreement with the selectivities found for

Table 2 Binding data for calcium ions recorded for polymers A–C (Table 1)

Polymer	K_{Diss}[a] mmol dm^{-3}	Number of binding sites (μmol g^{-1} polymer)[a]	Theoretical number of binding sites: FT-IR analysis (μmol g^{-1} polymer)	Theoretical number of binding sites: Elemental analysis (μmol g^{-1} polymer)
A	2.95	228	290[b]	285[b]
B	10.56	334	354[c]	428[c]
C	17.60	212	752[d]	856[d]

[a] Calculated from Scatchard-plot analysis. [b] Assuming a 1:3 complex prior to polymerisation. [c] Assuming a 1:2 complex prior to polymerisation. [d] Assuming random distribution of compound **1**.

compound **1** as a ionophore in an ion-selective electrode (see above). Neither of the metal ions was bound to polymer D, prepared without use of compound **1**. This observation shows that the adsorption of metal ions is a specific event due to the presence of residues of compound **1** in the polymer. We conclude that the selectivity for calcium ions inherent in the 3-oxapentanediamide structure is preserved during the incorporation of this unit into the highly cross-linked polymeric network (see Scheme 1).

From the data presented in this present study it is evident that by imprinting of metal ions the ionophore units are placed in a favourable position to bind calcium ions (Scheme 1). Previously, it had been shown that 3 and 2 molecules of the 3-oxapentanediamide derivative bind to calcium and magnesium, respectively, in the crystalline state[11] and in solvent polymeric membranes.[12] Supposing the same ratio in solution, the ionophore units will become incorporated during the polymerisation into sites containing, respectively, 3 and 2 units of ionophore in the polymers. The number of sites calculated from the binding data for each polymer preparation is very close to the theoretical values of 287 and 391 for polymers A and B, respectively, (the mean value of FT-IR measurement and elemental analysis, Table 2) taking into consideration the observed incorporation of compound **1** into the polymers (Table 1). The specificity of recognition of the polymers was retained after storage, in dry form, at room temperature for at least six months.

In conclusion, we have prepared highly cross-linked polymers containing a metal-complexing structure, with selectivity for calcium ions, by copolymerisation of compound **1** with divinylbenzene. The binding strength and uptake capacity were increased by performing the polymerisation in the presence of metal ions. We attribute the significant selectivity of our calcium-binding model system to the fact that ligand preorganisation to the metal ion, followed by polymerisation, results in recognition sites in which the co-ordinating oxygen atoms of the ionophoric residues are comfortably oriented (Scheme 1).

The results presented here further underline the great potential of molecular imprinting. Molecularly imprinted polymers have already been successfully used for resolutions of many racemic compounds, especially of amino acid derivatives,[2,4–6] and as selective sorbents in a flow-through column electrode.[23] In this report the preparation of molecular imprints in synthetic polymers against metal ions is demonstrated. Such polymers may find applications in metal-ion-selective sensors,[10] and in fact this aspect initiated this study. Investigations in this direction are currently being pursued. Another application may be the development of enzyme-like catalysts,[7–9] since a metal ion often is a constituent of the active site of enzymes.

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