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PREPARATION AND PROPERTIES OF STATIONARY PHASES CONTAINING IMMOBILIZED, ELECTRICALLY NEUTRAL NON-MACROCYCLIC IONOPHORES FOR LIQUID–SOLID CHROMATOGRAPHY

P. GROSSMANN and W. SIMON*
Department of Organic Chemistry, ETH-Zentrum, CH-8092 Zurich (Switzerland)
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SUMMARY

Novel stationary phases for liquid–solid chromatography have been prepared by immobilizing electrically neutral, non-macrocyclic ionophores on porous glass, silica and styrene–divinylbenzene copolymers. The chromatographic behaviour of these sorbents is described. They show selectivity for alkali and alkaline earth metal cations and have been used to separate such ions.

INTRODUCTION

Stationary phases which provide new selectivity characteristics are of great interest in high-performance liquid chromatography (HPLC). By covalently binding organic complexing agents to insoluble supports it is hoped that the impressive selectivity behaviour of many of these compounds can be transferred to chromatographic systems.

Various approaches have recently been reported for the immobilization of metal-chelating functional groups on insoluble supports, either for the separation or for the preconcentration of different metal cations. Styrene–divinylbenzene copolymers have often been used as supports for the immobilization of various complexing molecules. Brozio[1] synthesized resins containing pyridine-2,6-dicarboxylic acids. Fritz and co-workers demonstrated the applicability of styrene–divinylbenzene copolymers containing immobilized thioglycolate[2,3], aromatic α-hydroxyoxime[4] and amide groups[5,6] for the separation of several transition metal ions. Insolubilized noncyclic poly(oxyethylene) derivatives were used by Yanagida and co-workers[7] to separate alkali and alkaline earth metal cations, with separation times of 1–4.5 h. Sugii and co-workers described the synthesis and applicability of styrene–divinylbenzene resins containing thiohydantoinyl groups[8], nitrosoresorcinol groups[9], β-diketones[10] and 8-hydroxyquinoline[11]. Blasius and co-workers[12–14] made extensive studies of organic resins containing macrocyclic crowns and cryptands.

A number of sorbents based on organic resins other than styrene–divinylbenzene copolymers have been described by Siggia[15], Bowen[16] and Smid[17,18] and their co-workers. The use of silica as a solid support has also received considerable atten-

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tion. For the preconcentration of trace metals, Leyden and co-workers[19–21] used silica with immobilized diamines. Cram and co-workers[22] reported on the separation of chiral amino ester salts using silica with covalently bound macrocyclic compounds, and Siggia and co-workers[23,24] described the synthesis of silica with pyrrolidone groups for the separation of shale oil and oestrogens.

In this paper we describe the synthesis and chromatographic behaviour of new stationary phases prepared by immobilizing electrically neutral, non-macrocyclic molecules (ionophores) on porous glass, silica and styrene–divinylbenzene copolymers. These ionophores induce substantial cation selectivity in solvent polymeric (liquid) membrane electrodes. Therefore, the new column packings described are expected to show selectivity for these cations (alkali and alkaline earth metal cations).

EXPERIMENTAL

Synthesis of stationary phases

Phase 1. The resin was first chloromethylated by adding 30 ml of freshly distilled chloromethyl methyl ether to 5 g Styragel (macroporous styrene–divinylbenzene copolymer, 20–40 μm spherical particles, 100-Å pores; Waters Assoc. No. 27123) in a flask immersed in a water thermostat. After stirring (overhead stirrer) for 1.5 h at room temperature, the temperature was raised to 66°C and 1.52 g (5.84 mmol) freshly distilled anhydrous stannic chloride in 15 ml chloromethyl methyl ether were added dropwise. Stirring was then continued under reflux for 1 h. The product was cooled, filtered off and washed successively with the following solvents: dioxan–water (3:1); dioxan with 10% concentrated hydrochloric acid; dioxan; dioxan with increasing amounts of methanol; methanol. The chloromethylated Styragel was dried for 4 days at 0.02 Torr and analysed for chlorine: 15.40 wt.-% chlorine (4.34 mmol chlorine per g dry resin).

In the coupling reaction, a solution of 258 mg (0.581 mmol) ionophore II[26] (Fig. 1) in water–ethanol (1:1) was neutralized to pH = 7 by adding aqueous caesium carbonate (0.02 *M*). The solvent was then evaporated and the residue dried over P_2O_5 for several days. A solution of this residue (caesium salt of ionophore II) in 7 ml dimethyl sulphoxide–dimethylformamide (5:7, v/v) was added to 1313 mg chloromethylated Styragel (see above). This slurry was then stirred for 8 h at 50°C and afterwards for 40 h at 80°C. After cooling, the product (stationary phase 1) was filtered off and washed successively with the following solvents: dimethyl sulphoxide; dimethyl sulphoxide–water; dimethylformamide; dimethylformamide–water; dimethylformamide; acetone, methanol. After drying at room temperature and 0.02 Torr, 1731 mg of stationary phase 1 were obtained.

Phase 2. The chloromethylated resin was aminated as follows. Chloromethyl-

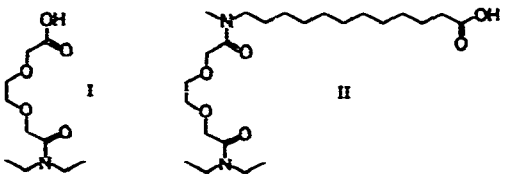

Fig. 1. Structures of ionophores I and II.

ated Styragel, 2.5 g containing 15.4 wt.-% Cl (see above), was swollen in chloroform for 2 h at room temperature and then heated at 60–80°C in dioxan for 3 h. The solvent was then sucked off and a solution of 11.4 g (190 mmol) freshly distilled ethylenediamine in 1.27 g water was added dropwise while stirring and cooling to 0°C. The mixture was then stirred at 0°C for 2 h and afterwards overnight at room temperature. The mixture was then allowed to stand for 6 days at room temperature, after which the beads were filtered off, washed thoroughly with water and dried for 4 days at 0.02 Torr. The aminated resin beads contained 1.19 wt.-% chlorine and 7.99 wt.-% nitrogen (5.71 mmol nitrogen per g resin).

In the coupling reaction, 1419 mg of the aminated Styragel were swollen on a glass frit for 1 h with dimethylformamide, filtered off and washed for 10 min with dimethylformamide–triethylamine (10:1). The resin was then washed three times each with dimethylformamide and methylene chloride, transferred to a flask and 945 mg (4.05 mmol) ionophore I[26], dissolved in 3 ml methylene chloride were added. After stirring for 20 min under a dry atmosphere, a solution containing 836 mg (4.05 mmol) N,N′-dicyclohexylcarbodiimide in 4 ml methylene chloride was added. The reaction mixture was stirred for 2 h at room temperature, for 30 min at 40–50°C and finally for 3 h at room temperature. The product was filtered off, washed with methylene chloride, ethanol, acetic acid, water and methanol and dried under vacuum (12 Torr) for 4 h at 40°C. Yield of phase 2: 1818 mg.

Phase 3. Porous glass was silanized as follows. Three grams of porous glass (Bio-Glass 200, 200–400 mesh irregular particles, 200-Å pores, pore volume 35%; Bio-Rad Labs.) were first cleaned by heating at 80–90°C in 10% nitric acid solution for 1 h. The glass beads were then filtered off, washed thoroughly with water and dried for 4 h at 60–70°C under vacuum (12 Torr). The beads were conditioned to a relative humidity of 15% by placing them over a saturated solution of lithium chloride in a desiccator for 2 days. N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (Pierce Inorganics, No. P. 98008), 24.5 mmol dissolved in 50 ml toluene, was then added. After stirring (overhead stirrer) for 1 h at room temperature, the temperature was slowly raised to 110–120°C and the slurry was refluxed for 16 h. The product was then cooled, filtered over a glass frit and rinsed with toluene, ethanol and acetone. The silanized glass beads were then dried overnight at 100–110°C. Elemental analysis of the product: 5.24 wt.-% carbon; 0.98 wt.-% hydrogen; 1.71 wt.-% nitrogen.

In the coupling reaction, 1137 mg silanized glass beads were dried for 2 days over P_2O_5 at 0.02 Torr and then slurried in 4 g benzene and 4 g chloroform. To this mixture were added 2 g freshly distilled triethyleneamine. The acid chloride of ionophore I[26], 4.21 mmol dissolved in 5 ml benzene–chloroform (4:1, v,v), was then added dropwise under nitrogen. After shaking the mixture for 3 days at room temperature, the product was filtered off, washed with chloroform, ethanol, water and methanol and dried for several hours at 60–70°C. Yield: 1219 mg.

Phase 4. Silica (Lichrospher SI 100, 10-μm spherical particles, 100-Å pores, specific surface area 250 m^2/g; Merck No. 9312) was silanized with N-(2-aminoethyl)-3-aminopropyltrimethoxysilane by the procedure described above. To 1871 mg of this silanized silica, 4.03 mmol of the acid chloride of ionophore I[26] were coupled by the procedure described for stationary phase 3, to yield 2064 mg phase 4.

Characterization of stationary phases

Infrared spectra of the stationary phases were obtained on a Perkin-Elmer spectrophotometer (Model 283) from pellets consisting of 4 mg stationary phase in 300 mg KBr.

Two methods were used for the determination of the ionophore content of the stationary phases. In the first the change in weight of the stationary phase was measured before and after the reaction. In the second method, the changes in the elemental composition of the stationary phases were determined. For further details see ref. 26.

Column chromatography

An Altex pump (model 110A; Altex Scientific, Berkeley, CA, U.S.A.), a pulse dampener (Model 716583; Pye Unicam, Cambridge, Great Britain) and a sample injector, Type 70-10 (Rheodyne, Berkeley, CA, U.S.A.) were combined with a conductivity detector, consisting of a flow-through measuring cell constructed in this laboratory[27] and a conductivity meter PW 9501 (Philips, Eindhoven, The Netherlands). The stainless-steel columns 100–150 × 4.1–4.6 mm I.D.) were filled by the slurry packing technique (ethanol, with a pressure of about 1000 p.s.i. for stationary phases 1 and 2, 2000 p.s.i. for phases 3 and 4. A 1-ml volume of a 0.5 *M* solution of a potassium salt (with a specified anion) was then passed ten times through the column in order to convert the anion-exchange sites of the sorbents (primary and secondary amino groups) into the desired form. The columns were rinsed with pure solvents until the eluent was free of ionic impurities (conductivity detection). Before each injection, the anion-exchange sites of the packings had to be converted by use of the above procedure into the anion contained in the injected solution in order to avoid anion exchange between different anions and hence irreproducible retention volumes of the injected salts.

Sample volumes of 20 μl ($1 \cdot 10^{-3}$ to $5 \cdot 10^{-2}$ *M*) were injected at a mobile phase flow-rate of about 0.5–2 ml/min. Experiments at room temperature were conducted without thermostatting. For elevated temperatures, the columns were jacketed and the temperature was controlled by circulating water through the system from a thermostat. Retention volumes were calculated from the measured flow and retention times; plate numbers, N, were calculated from $N = (t_R/\sigma)^2$ where t_R is the retention time and σ is the peak width at 60.7% of the peak height.

RESULTS AND DISCUSSION

Preparation and characterization of the stationary phases

The stationary phases were prepared by binding one of the two ionophores shown in Fig. 1 to porous glass, silica or macroporous styrene–divinylbenzene copolymer. The expected structures of the prepared stationary phases are shown in Fig. 2. Some of the functional groups used to couple the ionophore to the supporting material will remain unsubstituted and thus necessitate conditioning of the stationary phase prior to use, as described in the Experimental.

The KBr-pellet IR spectra obtained after each step in the synthesis are in agreement with the expected structures. For example, in Fig. 3 the IR spectrum of the starting material (macroporous styrene–divinylbenzene copolymer) is compared with

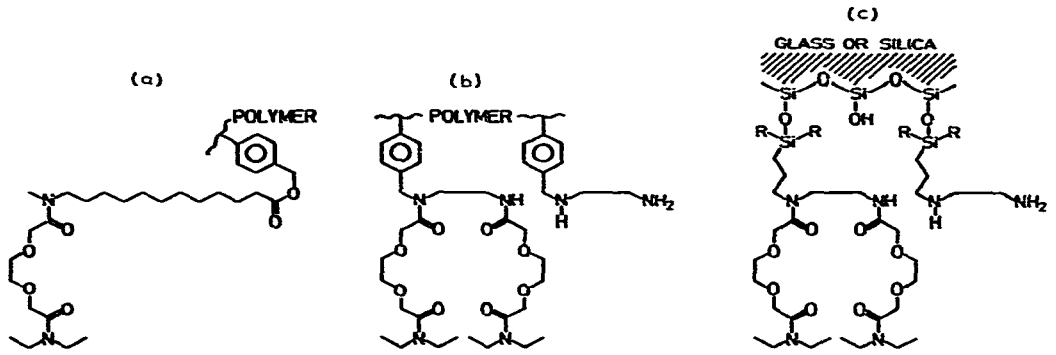

Fig. 2. Expected structures of stationary phases 1 (a), 2 (b), 3 (c) and 4 (c).

that of the final product (stationary phase 1), in which ionophore II is immobilized on the copolymer by an ester bond. This bond leads to characteristic absorption bands at 1730 cm^{-1} (C–0 stretching frequency) and 1260 cm^{-1} (C–O stretching frequency). In addition to the absorption bands of the starting material (Fig. 3A), prominent bands appear in the spectrum of the final product (Fig. 3B) at 1645 cm^{-1} and 1110 cm^{-1}. These are similar to the C–O stretching vibration of the disubstituted amide groups and to the C–O–C stretching vibration of the ether groups found in the IR spectrum of ionophore II.

The properties of the stationary phases, the results of elemental analyses and the calculated loadings with ionophore are summarized in Tables I and II. According to Table II, the prepared stationary phases are loaded with about 0.2–1.0 mmol ionophore per gram of product.

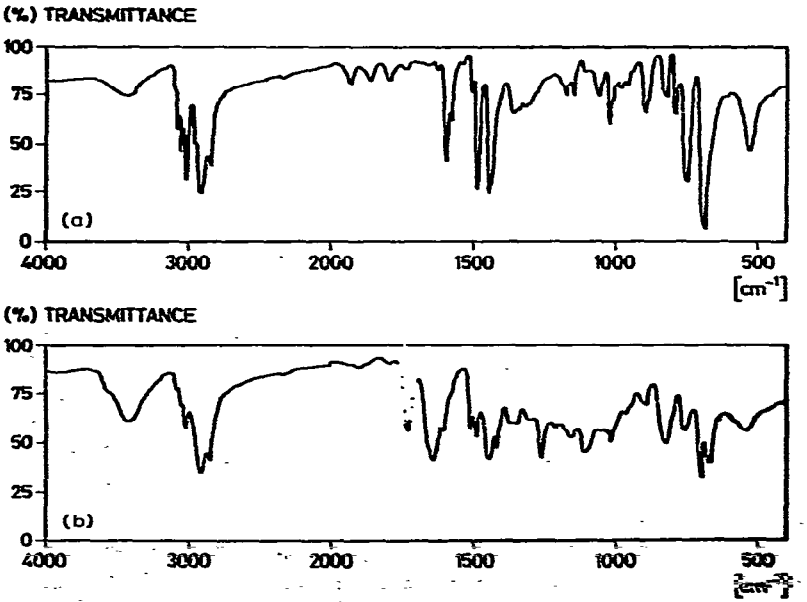

Fig. 3. Infrared spectra (in KBr pellets) of styrene–divinylbenzene copolymer (a) and stationary phase 1

TABLE I

PROPERTIES OF THE STATIONARY PHASES

S/DVB = Styrene–divinylbenzene copolymer.

Phase	*Support material*			*Immobilized ionophore*	*Bond type (ionophore/ support material)*
	Type	*Particle diameter (μm)*	*Pore diameter* (Å)		
1	S/DVB	20–40	100	II	Ester
2	S/DVB	20–40	100	I	Amide
3	Glass	40–80	200	I	Amide
4	Silica	10	100	I	Amide

TABLE II

ELEMENTAL ANALYSIS AND CAPACITY OF THE STATIONARY PHASES

Phase	*Elemental analysis (%)*				*Capacity* obtained by*			
	C	*H*	*N*	*O*	*Weight increase*	*Elemental analysis*		
						C	*N*	*O*
1	73.89	7.13	0.89	3.06	0.592	–	0.318	0.319
2	73.32	7.98	7.43	1.51	1.021	–		0.236
3	8.68	1.48	2.39		0.314	0.316	0.659	–
4	18.61	2.81	4.08		0.436	0.374	0.600	–

* Ionophore (mmol) per gram of dry stationary phase.

Chromatographic behaviour

Selectivity. After conditioning of the columns, the selectivities of the stationary phases were determined by injecting solutions of various salts and measuring their retention volumes. The results (Fig. 4) demonstrate that the stationary phases described lead to a different retention volume for each salt and thus induce cation selectivity. These differences in retention volume are mainly due to variations in the complex formation. From a comparison of Fig. 4C and 4D, the selectivity depends not only on the ionophore–cation interactions, but also on the type of support (resin, glass, silica) used. Except in the case of phase 4, all the stationary phases retain barium and calcium much more strongly than the alkali metals. This is in agreement with the selectivity sequence induced in solvent polymeric membranes by similar ionophores[25]. Ion-selective liquid membrane electrodes and chromatographic systems are basically different, so that utmost care should be taken when correlating experimentally determined selectivity factors[27].

Influence of the mobile phase. The complex formation constant for the ionophore–cation interaction is also influenced by the dielectric constant of the solvent: usually, the lower the dielectric constant the stronger is the interaction[28]. Thus, low selectivities for the present systems are expected when water is used as the mobile

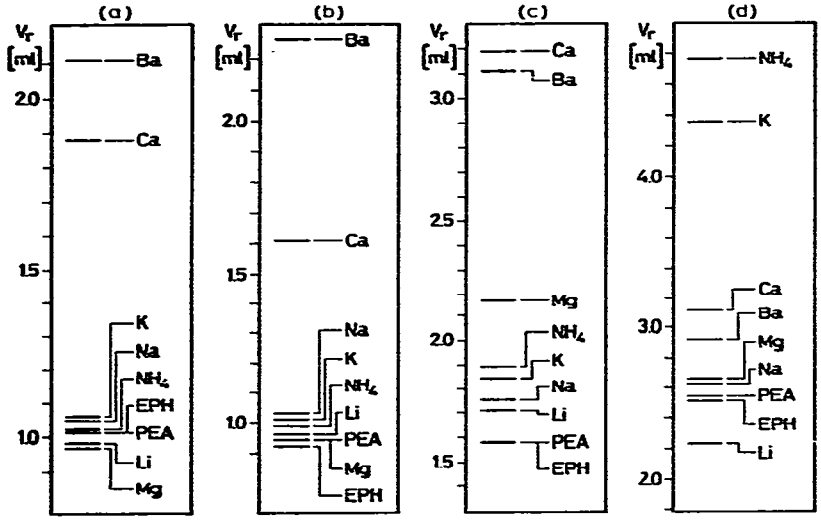

Fig. 4. Selectivities. a, Stationary phase 1. Column: 150 × 4.6 mm. Mobile phase: water–ethanol (2:8 v/v). b, Stationary phase 2. Column: 150 × 4.6 mm. Mobile phase: water–ethanol (3:7 v/v). c, Stationary phase 3. Column: 130 × 4.1 mm. Mobile phase: water–ethanol (1:1 v/v). d, Stationary phase 4. Column: 150 × 4.6 mm. Mobile phase: water. Injection in each case: 20 µl, $2 \cdot 10^{-2}$ *M*. PEA = (±)-α-Phenylethylammonium chloride; EPH = (±)-ephedronium chloride.

phase. If methanol or ethanol is added to the mobile phase to decrease the dielectric constant, the retention volumes should increase. The experimental results for stationary phases 1 and 2 shown in Fig. 5 confirm this.

Influence of anions on the retention of the cations. The retention of a salt depends on both the cation and the anion because electroneutrality requires the binding of a cation together with an anion. The influence of the type of anion for stationary phases 2 and 3 is shown in Table III: it is seen that the retention volume of a given cation increases with increasing polarizability and lipophilicity of the anion[14].

Column efficiency. In addition to the selectivity of the stationary phase, the

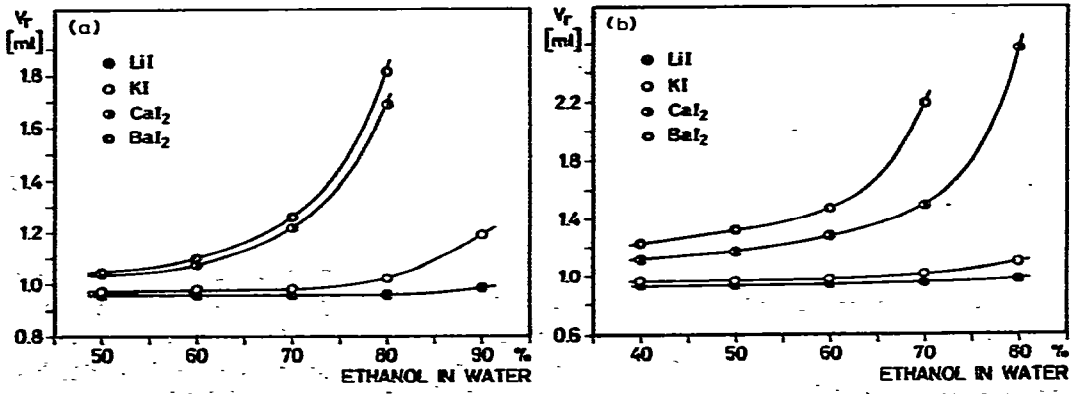

Fig. 5. Influence of the mobile phase on retention volumes on stationary phases 1 (a) and 2 (b). Column: 150 × 4.6 mm. Mobile phase flow-rate: 1.1–1.3 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

TABLE III

INFLUENCE OF ANIONS ON THE RETENTION OF CATIONS ON STATIONARY PHASES 2 AND 3

Anion	*Retention volumes on stationary phase 2* (ml)*			*Retention volumes on stationary phase 3** (ml)*	
	K^+	Ca^{2+}	Ba^{2+}	K^+	Ca^{2+}
Acetate	0.96	1.00	1.05	1.57	2.15
Chloride	0.98	1.14	1.35	1.61	2.26
Bromide	0.98	1.24		1.69	2.55
Nitrate	1.00	1.27	1.55	1.81	2.81
Iodide	1.04	1.73	2.54	1.87	3.23
Thiocyanate	1.12	2.68	4.49	1.93	3.55
Perchlorate	1.08	3.80	7.35	1.96	4.12

* Column: 150 × 4.6 mm. Mobile phase: water–ethanol (3:7 v/v); flow-rate 1.2 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

** Column: 130 × 4.1 mm. Mobile phase: water–ethanol (1:1 v/v); flow-rate 1.35 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

efficiency of the packed column is also important. The stationary phases described yield columns for which the plate number markedly decreases with increasing retention volume; a typical example is provided by phase 2 (see Fig. 6). This reduction in plate number cancels the advantage gained through increased selectivity. Methods of reducing this effect by variation of the major parameters (mobile phase flow, injected sample amount, temperature, additives to the mobile phase) were investigated.

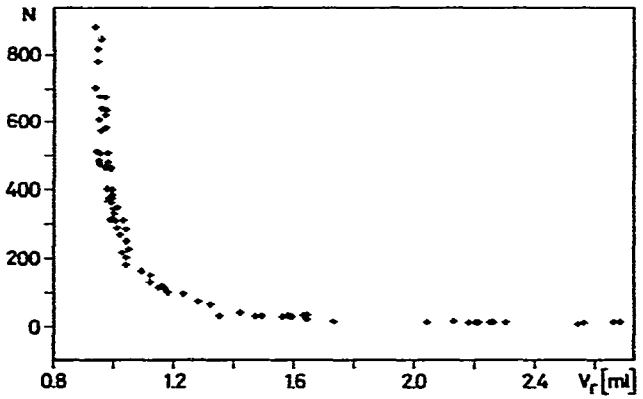

Fig. 6. Column efficiency for stationary phase 2. Column: 150 × 4.6 mm. Mobile phase flow-rate: 1–1.2 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

Influence of the mobile phase flow-rate. Using stationary phase 4 (Fig. 7), the influence of the flow speed on the retention volume and the plate number was studied. For lower velocities the retention volume slightly increases and the plate number decreases somewhat. This means that in the flow-rate range chosen (0.1–3 ml/min) plate number fall-off is always present with increasing retention and nothing is gained from flow reduction.

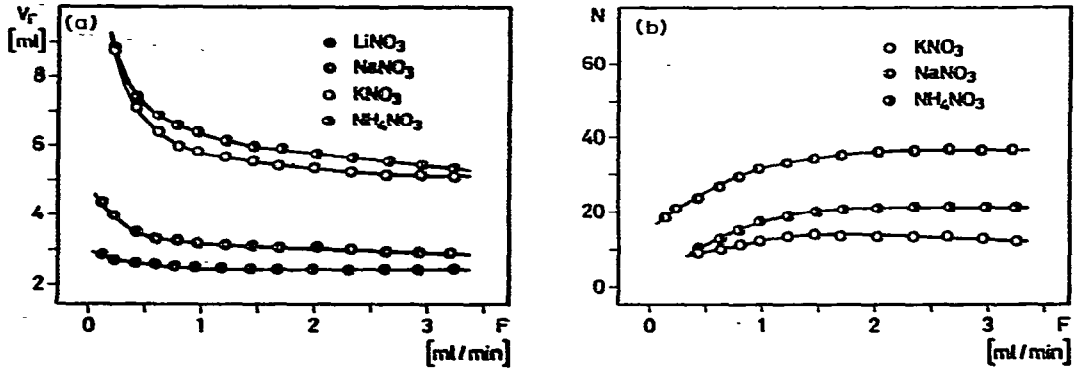

Fig. 7. Influence of mobile phase flow-rate on retention volumes and column efficiency for stationary phase 4. Column: 150 × 4.6 mm. Mobile phase: water doubly distilled. Injection: 20 μl, $2 \cdot 10^{-2}$ *M*.

Influence of the sample amount. The loading of a chromatographic system through injection of too large samples results in broad, asymmetric elution profiles because of operation in the non-linear portion of the distribution isotherm. The effect of the amount of sample injected was studied for phase 1 (see Fig. 8). The retention volume decreases with decreasing sample concentration. This is a strong indication of non-linearity of the adsorption isotherm. At the same time the plate number increases. However, even for very low sample concentrations, the plate number decreases with increasing retention.

Influence of the temperature. Increasing the temperature of a liquid chromatographic system generally leads to faster elution and an increase in plate number (a lowering of the viscosity of the mobile phase and an increase in the mobility of the sample molecules lead to faster mass transport). For the stationary phases described, heating should accelerate the kinetics of complexation and hence improve the plate number. This is illustrated for stationary phase 2 in Fig. 9. The change in plate number is decidedly less than expected; for barium and calcium the plate numbers are less than 50 at 60°C, as compared to 350 (20°C) and 450 (60°C) for potassium. From Table IV it is seen that, for stationary phase 3, an increase in temperature from 20 to

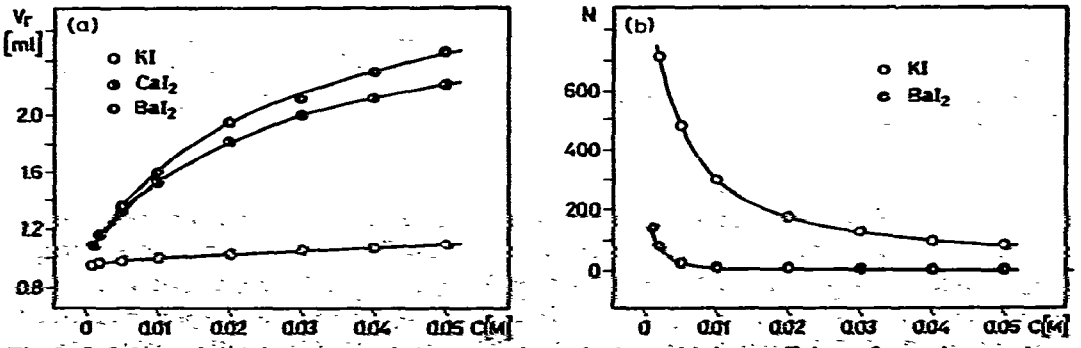

Fig. 8. Influence of sample concentration on retention volumes and column efficiency for stationary phase 1. Column: 150 × 4.6 mm. Mobile phase: water–ethanol (2:8 v/v); flow-rate 1.2 ml/min. Injection: 20 μl.

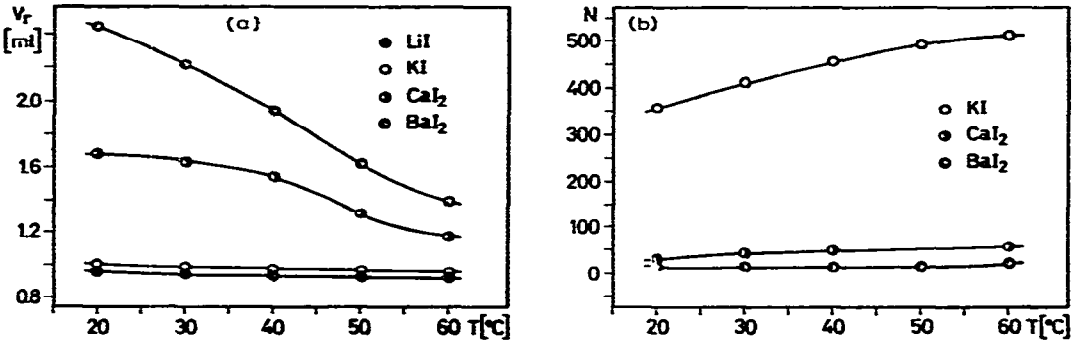

Fig. 9. Influence of temperature on retention volumes and column efficiency for stationary phase 2. Column: 150 × 4.6 mm. Mobile phase: water–ethanol (3:7 v/v); flow-rate 0.9-1.0 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

TABLE IV

RETENTION-DEPENDENT PLATE NUMBER FALL-OFF AT 20°C AND 50°C FOR STATIONARY PHASE 3

Column: 90 × 5 mm. Mobile phase: water–ethanol (1:1 v/v); flow-rate 1.5 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

20°C		*50°C*	
V_r *(ml)*	*N*	V_r *(ml)*	*N*
1.77	168	1.73	239
2.09	74	2.12	127
2.70	48	2.59	78
3.20	37	2.89	71

50°C somewhat increases the plate numbers, but the retention-dependent plate number fall-off is still observed.

Additives to the mobile phase. In analogy to the behaviour often observed for reversed-phase systems, an attempt was made to increase the plate numbers through addition of a buffer (phosphate at pH 7) or of salts (sodium nitrate or benzoate, tetramethylammonium nitrate). Fig. 10a and 10b depict the results for stationary phase 4 with $LiNO_3$ and KNO_3: increasing ionic strength leads to a somewhat faster elution of the sample. However, Fig. 10c shows that the plate number fall-off for this phase is not improved by addition of ionic species. This behaviour is typical of all of the investigated systems.

Chromatographic separations

Since the solvent largely determines the degree of interaction between cations and immobilized ionophores, the cation retention on the stationary phases described may be fixed through choice of the mobile phase. Therefore, by appropriate selection of mobile phases, the stationary phases described may be used for rapid separations of alkali and alkaline earth metal cations. Studies conducted with phase 2 using mobile phases of various ethanol to water ratios show clearly that the separation

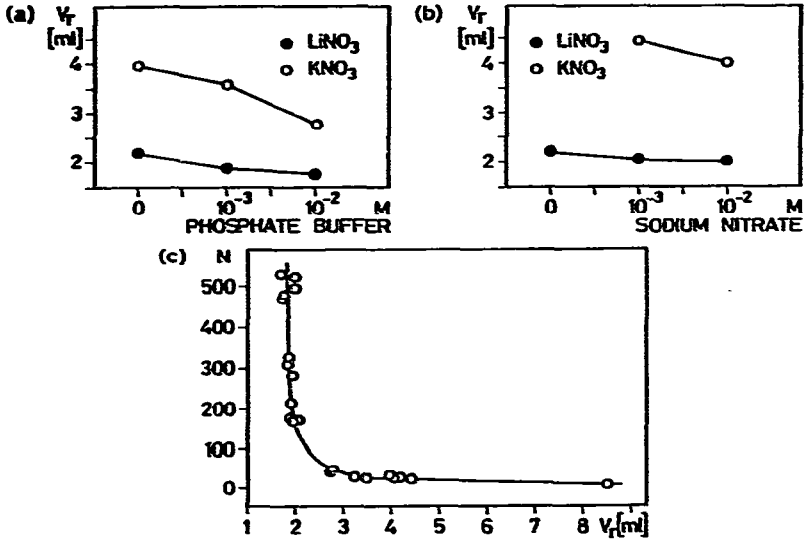

Fig. 10. Influence of additives to the mobile phase for stationary phase 4. a, Mobile phase with sodium phosphate buffer (pH = 7); b, mobile phase with sodium nitrate; c, column efficiency, resulting from experiments with additives to the mobile phase. Column: 150 × 4.6 mm. Mobile phase: water with additives; flow-rate 2 ml/min. Injection: 20 µl, $2 \cdot 10^{-2}$ *M*.

factor between, *e.g.*, magnesium and calcium, increases with increasing ethanol content, *i.e.*, with decreasing mobile phase polarity (Fig. 11). In spite of the increasing separation factor, the resolution between magnesium and calcium is not improved to the desired degree because the plate number of calcium decreases with increasing retention of calcium. For this reason, baseline separation of magnesium and calcium is not possible by isocratic elution. However, as illustrated in Fig. 12, baseline separation on stationary phase 2 between, *e.g.*, lithium and barium can be achieved by using a solvent gradient elution procedure.

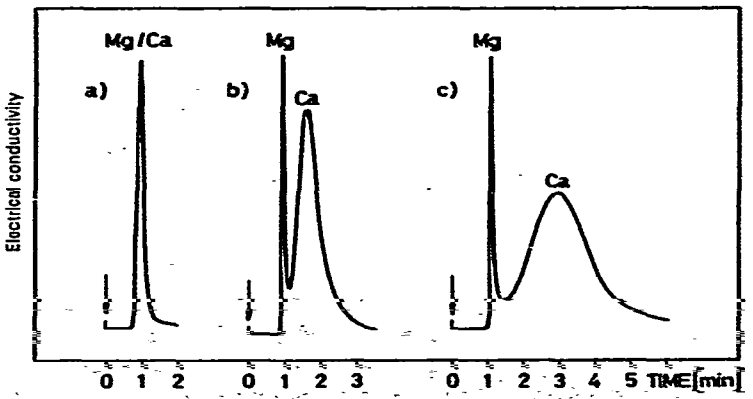

Fig. 11. Separation of magnesium and calcium iodide on stationary phase 2. Column: 150 × 4.6 mm. Mobile phase: water–ethanol [6:4 v/v (a), 3:7 v/v (b) and 2:8 v/v (c)]; flow-rate: 1.2 ml/min (a) and 1 ml/min (b and c). Injection: 20 µl of a mixture of magnesium and calcium; for a and c, $1.8 \cdot 10^{-3}$ *M*, MgI_2, $1.8 \cdot 10^{-2}$ *M* CaI_2; for b, $2.8 \cdot 10^{-3}$ *M* MgI_2, $1.7 \cdot 10^{-2}$ *M* CaI_2.

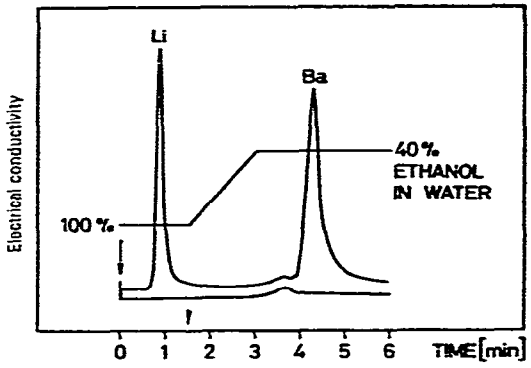

Fig. 12. Separation of lithium and barium on stationary phase 2. Column: 150 × 4.6 mm. Injection: 20 μl; $1.1 \cdot 10^{-2}$ *M* LiI, $9.2 \cdot 10^{-3}$ *M* BaI_2. Mobile phase: gradient, 100% ethanol for 1.5 min, then linear gradient to 40% (v/v) ethanol in water in 1.5 min; flow-rate 1.2 ml/min.

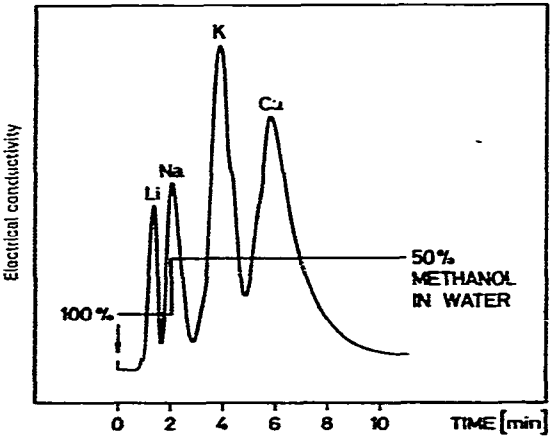

Fig. 13. Separation of lithium, sodium, potassium and calcium iodide on stationary phase 3. Column: 130 × 4.1 mm. Mobile phase: step gradient, methanol for 2 min, then methanol–water (1:1 v/v); flow-rate 1.4 ml/min. Injection: 20 μl; $4.1 \cdot 10^{-3}$ LiI, $4.6 \cdot 10^{-3}$ *M* NaI, $1.3 \cdot 10^{-2}$ *M* KI, $9.2 \cdot 10^{-3}$ *M* CaI_2.

Through judicious choice of the solvent gradient, rapid separations of cations having markedly different elution characteristics are possible with the stationary phases described. For example, Fig. 13 illustrates the quite rapid separation of lithium, sodium, potassium and calcium on stationary phase 3 using gradient elution.

CONCLUSIONS

The results demonstrate that the non-cyclic neutral carriers indeed induce a substantial cation selectivity when immobilized on porous glass, silica and styrene–divinylbenzene copolymer. The possibility of using these new stationary phases for separations of alkali and alkaline earth metal cations was investigated and it is shown that, through careful choice of the mobile phase, they are adequate for this purpose. However, in spite of the high selectivity, this use of these stationary phases was only partially successful, owing to the low efficiency of the packed columns, which resulted

in a substantial broadening and overlapping of bands. It should be noted that similar systems (complexing agents, immobilized on solid supports) show the same behaviour with respect to efficiency[7,14,29,30]. This low efficiency is probably due to two underlying mechanisms.

The first is of a thermodynamic nature. The ionophores used in this work are able to form 1:1 and 1:2 complexes with cations[31,32]. In liquid–liquid chromatography this may lead to non-linear distribution isotherms[27]. Immobilizing these ionophores may therefore provide sorbents with a non-linear adsorption isotherm. The second mechanism is of a kinetic nature. The complexation and decomplexation reaction between the immobilized ionophores and the cations could be so slow that equilibrium is not attained in chromatographic systems. Calculations of elution profiles based on a simple mathematical model indeed show that there should be not only elution profile broadening but also tailing if the sample's distribution equilibrium between the stationary and liquid phase is not attained[26].

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