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POLYION ION-PAIR LIQUID CHROMATOGRAPHY

I. A NEW METHOD FOR THE REGULATION OF THE RETENTION OF IONIC COMPOUNDS IN LIQUID CHROMATOGRAPHY

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SUMMARY

A type of chemical interaction, which has received little attention in analytical and separation procedures, is introduced for the use in reversed-phase column liquid chromatography of low-molecular-weight ionic organic compounds. It is the association between a polyion and oppositely charged ions of low valency in aqueous solution. The stationary phase is a microparticulate octylsilica material. It was found that addition of a polyanion, dextran sulphate, of very high molecular weight to the mobile aqueous phase causes a drastic decrease of the chromatographic retention of divalent ammonium compounds. The results follow a retention model which assumes that the polyion associates with the sample ions in the mobile phase. Monovalent sample ions are only very little affected by the polyion. This opens new possibilities to regulate the separation selectivity between sample ions of different valency and charge type, *e.g.*, in ion-pair chromatography.

INTRODUCTION

In reversed-phase ion-pair chromatography the retention of an ionic compound has most often been regulated by the type and concentration of a counter-ion or a co-ion added to the mobile phase[1,2]. Both the counter-ion and the sample ion distribute between the mobile and the stationary phases and the distribution ratio for the sample ion will be dependent on the hydrophobicity and concentration of the counter-ion. The co-ion can distribute between the phases together with a counter-ion and thereby compete with the sample ion for adsorption on the stationary phase[2].

The degree of ion-pair formation in the mobile aqueous phase has often been regarded to be too low in order to have any significant effect on the distribution ratio of the sample ion[1]. This is also expected from calculations of the degree of ion-pair

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formation due to electrostatic forces according to *e.g.* the Bjerrum theory[3]. This, and other similar theories, predicts that ion-pair formation between two oppositely charged ions increases with the charge of the two ions and may therefore be much greater *e.g.* for 2:2-valent and 1:4-valent electrolytes than for 1:1-valent ones[3,4].

In the reversed-phase liquid chromatography of ionic compounds only a few methods exist to regulate the retention by changing the binding of the sample ion to the mobile aqueous phase, *e.g.* by some complexation equilibrium occurring in this phase. One might well suspect that the use of counter-ions containing several charges can give such high degrees of ion-pair formation or complexation with sample ions of the opposite charge so that the chromatographic retention of the latter will be drastically influenced, especially if also the sample ion has several charges.

One way to strengthen the ion-pair association might be to use, as counter-ion to the sample, a polyion, *i.e.*, a polymer where the monomers contain one or more ionized groups. If the polyion is present in the mobile aqueous phase it may bind sample ions and thereby decrease their retention. The decreased retention will be obtained if the polyion will not or hardly bind to the stationary phase. If it will, it might cause an increased distribution of the sample to the stationary phase which counteracts the effect obtained in the mobile phase. This may even lead to a total increase of the retention.

It may be foreseen that the molecular weight of the polyion determines whether it will only affect the mobile phase, or both phases. When the molecular weight is so high that the polyion becomes excluded from the pores of the solid stationary phase the effect of binding of the sample to the mobile phase may have a dominating influence on the retention.

It must also be expected that the Donnan effects can influence the distribution of counter-ions between the stagnant zone of mobile phase within the pores and the mobile zone outside the pores when the polyion is excluded. Such effects can be used for separation purposes even in cases where the sample ions are unretained on the stationary solid phase. This is somewhat related to work done in gel-permeation chromatography where an excluded neutral polymer dissolved in the mobile phase has been used to influence the retention of macromolecular samples by "steric exclusion" occurring in the mobile phase[5,6].

The ion association between polyions and their counter-ions in aqueous solutions has been discussed previously. Theories have been developed which regard the polyion as being covered by a uniform surface charge and where the counter-ions can interact with this surface charge[7]. But the counter-ion–polyion interaction has also been described as an ion-pair formation localized at the charged functional groups present in the polyion[7]. The counter-ions bound to the polyion have been further divided in those which are regarded as mobile and those which are regarded as localized at the charged groups[8]. More recent literature[9] defines these as territorially bound and "site bound", respectively.

Recently the association of counter-ions to polyions has been treated according to the so called counter-ion condensation phenomenon which is based on an electrostatic theory where the amount of counter-ions associated is related to critical values which have to be reached for the net charge density on the polyion[8–11]. This model seems to have found acceptance and experimental support but it is clear that other interaction forces may also have to be taken into account[9,12].

The most important characteristic of the counter-ion condensation phenomenon is that the polyion becomes "saturated" by a number of bound counter-ions that is less than the number of charges on the polyion (for monovalent counter-ions) in order to obtain the net charge density. This net charge density is determined by the valence of the counter-ions and the charge density of the polyion[9].

Even if most discussions on the counter-ion–polyion association were for monovalent counter-ions, there is evidence that counter-ions of higher valency will associate to a much higher degree[4,8,9,11,13]. Studies seem to be concentrated on cases where the high valency is located to one functional group[8], such as for divalent metal ions and complexes thereof, and not to several functional groups in the same molecule as may be the case in many organic compounds, *e.g.* divalent ammonium compounds. However, some studies have been performed on the binding of polyamines, *e.g.* spermine, to the polynucleotide DNA[11,14]. Also, studies of counter-ion association are scarce for systems containing a mixture of several different types of counter-ions[13].

Stoichiometric data for the degree of binding are rather scarce, especially for organic counter-ions, but some studies show high binding degrees for di- and trivalent metal ion–phenantroline complexes with polyanions[15].

Experimental studies of counter-ion binding to polyions have received attention partly because of the biological importance of polyelectrolytes, such as the nucleic acids and the sulphated polysaccharides[16], and have been performed by a variety of physico-chemical methods[10]. Chromatography does not seem to have been used very much for the study of counter-ion binding. The method by Hummel and Dreyer[17], which has been used for the study of binding of low-molecular-weight ligands to proteins and which is based on gel-permeation chromatography, has occasionally been used for studies of binding of copper ions to the macromolecular polyelectrolyte DNA[17]. This method, or modifications thereof[18], might be used for the study of binding of organic ions to polyions.

This paper is an investigation of the possibility to use the association between a dissolved polyion and counter-ions in order to obtain new methods for the regulation of the chromatographic retention of low-molecular-weight ionic organic compounds (counter-ions to the polyion). This study may also lead to new possibilities for the chromatographic separation of polyions and metal ions.

EXPERIMENTAL

Equipment

An LDC solvent delivery system 711-47 (Milton-Roy Minipump with pulse dampener; Laboratory Data Control, Riviera Beach, FL, U.S.A.), a Rheodyne syringe loading injector 7120 (Rheodyne, Berkeley, CA, U.S.A.) with a 20-μl loop, and an LDC UV III Monitor measuring the absorbance of the eluate at 254 nm in a 10-μl flow-cell were used. The separation columns were LiChroma tubes (316 stainless steel, Handy and Harman), 100 $\times$ 4.6 mm I.D., equipped with modified Swagelok connectors and Altex 2-μm stainless-steel frits (Altex, Berkeley, CA, U.S.A.).

The eluent reservoir and the separation column were thermostated to 25.0°C by water circulating through glass jackets.

Chemicals

Dextran sulphate sodium salt was obtained from Sigma (St. Louis, MO, U.S.A.) and contained 0.5–1.0% phosphate to give pH 6.5–7.0 aqueous solution. The salt is based on a dextran of molecular weight 500,000 and was used without any purification. Dextran sulphate is stable towards hydrolysis of the glucosidic bonds at neutral pH even at high temperatures but easily autohydrolyses when present as acid, *e.g.* after ion exchange to the hydrogen form. As used in this study, at pH 2, it is believed that hydrolysis is very slow at room temperature and indeed no indications of hydrolysis were observed.

All other chemicals were of analytical or pharmacopoeial grade. The amines (drugs) were obtained as the salts: quinidine bisulphate, quinine chloride, chlorpheniramine maleate, brompheniramine maleate, phenylpropanolamine chloride, ephedrine chloride, and N-ethylephedrine chloride.

LiChrosorb RP-8 was obtained from Merck (Darmstadt, F.R.G.) and had a mean particle diameter of 5 μm. According to the manufacturer it is prepared from a silica of 10 nm mean pore-diameter (LiChrosorb SI-100) which has a specific pore volume of 1.0 ml/g. The exclusion limit is not known but may perhaps be compared with the related material (LiChrospher SI-100) which has an exclusion limit of 50,000–80,000 determined from linear polystyrenes. LiChrosorb RP-8 is prepared by covalently binding octyl chains to the silica surface which leads to a decreased pore diameter and pore volume.

Chromatographic procedures

The separation columns were packed by a high-pressure slurry technique. Sample volumes injected were in the range 2–20 μl. The concentration of solutes in the samples were 0.001 *M* or lower depending on their molar absorptivity. Phosphate buffers were prepared by mixing 258.5 ml 1 *M* phosphoric acid with 90.0 ml 1 *M* sodium hydroxide and diluting to 1 l to give an ionic strength of 0.1 and pH 2.

Eluents were prepared by first mixing volumes of buffer and methanol in the ratios given and then dissolving dextran sulphate. The eluent flow-rate was usually 1.0 ml/min. At introduction of a new eluent the columns were equilibrated with *ca.* 50 ml of eluent or until the retention times were constant. After that, the eluent was recycled in a volume of 100–200 ml.

Capacity ratios, k', were measured from the retention volume, V_R, at the peak maximum by $k' = (V_R - V_m)/V_m$, where V_m is the retention volume, after compensation for the dead volume in the detector inlet tubing, of the unretained low-molecular-weight solute nitrate when the eluent contained no dextran sulphate or the retention volume of a perturbation peak obtained at injection of the samples and which gave the same value as for nitrate. The porosity (V_m divided by the volume of the empty separation column) of the columns was 0.63.

RESULTS AND DISCUSSION

A polyion salt, sodium dextran sulphate, prepared from dextran of molecular weight 500,000, was used as additive in the eluent of reversed-phase systems consisting of an alkylsilica as the solid phase and a mixture of methanol and aqueous buffer as the eluent. The polysaccharide dextran contains linear chains of α(1,6)-

linked D-glucopyranosyl residues with occasional branches of $\alpha(1,3)$-linkages. The (1,6)-linkages are due to 90–95% of the glucosidic linkages of the polymer.

Due to its high molecular weight the polyion will probably be excluded to a large extent from the pores of the solid phase, which is based on a silica of 10-nm average pore diameter. Thus the polyion will be present in the moving zone of the mobile phase but not in the stagnant zone within the pores.

The influence of dextran sulphate on the retention of mono- and divalent cationic compounds and monovalent anionic compounds was studied. Table I gives a list of the structures of the compounds studied. They were difunctional and monofunctional amines and one sulphonic acid. The two pK_a values of the difunctional amines are in the ranges 4–5 and 8–10, respectively. The results are given in Fig. 1. They were obtained at pH 2.0 in the mobile phase where the difunctional amine (quinidine) is present mainly as divalent cation, the monovalent amines are present as monovalent cations and the sulphonic acid is present as monovalent anion. At increasing concentration of the polyion the capacity ratio for the divalent amine decreases drastically whereas only minor changes occur for the other samples.

The exact composition of the eluent was not known because the sulphatation

TABLE I

STRUCTURES OF SUBSTANCES STUDIED

Formula	*Name*	R_1	R_2
CH_3O, N, HO–CH, N, $CH=CH_2$	Quinidine	–	–
	Quinine (diastereomer of quinidine)	–	–
R_1, $CH–CH_2–CH_2–N(CH_3)_2$, N	Chlorpheniramine	Cl	–
	Brompheniramine	Br	–
$CH(OH)–CH(CH_3)–N(R_1)(R_2)$	Phenylpropanolamine	H	H
	Ephedrine	H	CH_3
	N-ethylephedrine	C_2H_5	CH_3
CH_3–C_6H_4–SO_3H	Toluene-4-sulphonic acid	–	–

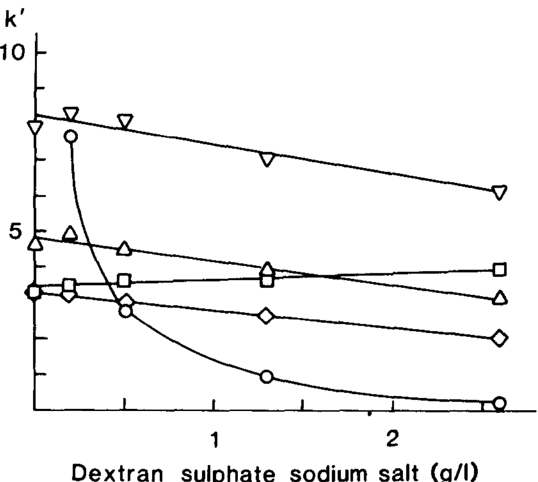

Fig. 1. Retention of cationic and anionic solutes when the concentration of dextran sulphate is changed in the eluent. Samples: ○ = quinidine; ▽ = N-ethylephedrine; △ = ephedrine; ◇ = phenylpropanolamine; □ = toluene-4-sulphonic acid. Eluent: dextran sulphate in phosphate buffer (pH 2.2, ionic strength 0.01)–methanol (9:1). Stationary phase: LiChrosorb RP-8, 5 μm. The curves only connect the data points in order to illustrate the tendency.

degree of the dextran was not known. It may, however, be assumed to be close to two sulphate groups per glucoside unit (*cf.* refs. 19 and 20) and 1.61 g of dextran sulphate will then correspond to 0.01 moles of sodium. This means that the sodium concentration in the eluent may have varied from 0.009 *M* to 0.025 *M* and the ionic strength similarly (including the polyion in the calculation). This can explain the slight increase of the retention of toluene-4-sulphonate which would be increasingly retained as the sodium ion-pair when the sodium concentration increases. Another explanation is that the excluded polyanion favours the distribution of small anions to the stagnant zone of the mobile phase relative to the moving zone, where the polyanion is present, and that this will increase the retention volume of small anions. This is the Donnan effect and it is probable that it will occur in this case since the buffer electrolyte concentration in the mobile phase is not in excess of the polyelectrolyte concentration.

The cationic samples would be retained only as ion pairs with dihydrogen phosphate as counter-ion if it is assumed that the large size of the polyion prevents it from entering the pores of the stationary phase and act there as a counter-ion to retained cations. The retention of cations is therefore expected to be unchanged unless their state in the mobile phase is influenced by the added polyelectrolyte.

The weak decrease of the retention of the monovalent amines seems too large to be caused by a decrease of their activity coefficients due to the change in ionic strength. Furthermore, it is doubtful whether the polyelectrolyte should be included in a calculation of the ionic strength. The increased concentration of sodium might decrease the retention of the ammonium ion pairs by competitive adsorption of sodium phosphate but this seems rather unlikely and therefore the retention decrease is most likely caused by binding to the dextran sulphate polyion in the mobile phase.

The marked difference of the effect of the polyion on monovalent amines *versus* the divalent amine can be ascribed to a difference in the nature of binding of mono- and multivalent ions to the polyion[9].

Retention model for a divalent sample ion

The pronounced retention decrease for quinidine can only be explained by its binding to dextran sulphate in the mobile phase. A plot of the reciprocal capacity ratio *versus* the concentration of dextran sulphate gave a linear relation (Fig. 2), except for the highest concentration where the capacity ratio is uncertain. The linearity indicates that the effect of dextran sulphate can be expressed by a simple complexation to the sample in the mobile phase, where the complexed amount is proportional to the concentration of dextran sulphate. This is expressed in the following tentative retention equation for quinidine

$$k' = \frac{W_s}{V_m} \cdot \frac{\left(1 + \frac{V_i}{V_o}\right) K_0 \, K_{H_2QZ_2} \, [Z^-]_m^2}{1 + \frac{V_i}{V_o} + K_{H_2QU_P} \, n_U \, [P]_o} \tag{1}$$

where k' is the capacity ratio, $[P]_o$ the concentration of polyion in the eluent, W_s the mass of the solid stationary phase, V_m the volume of mobile phase in the column:

$$V_m = V_i + V_o \tag{2}$$

where V_i is the volume of mobile phase inside the particles (in the pores) and V_o is the volume outside the particles. K_0 is the total concentration of adsorbing sites on the solid phase where each site can adsorb the species H_2QZ_2 (*cf.* ref. 1). Z^- represents the dihydrogen phosphate ion and H_2Q^{2+} the sample ion.

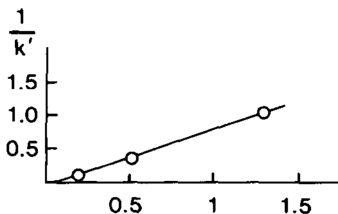

Fig. 2. Test of retention model according to the reciprocal of eqn. 1. Sample: Quinidine. Conditions as in Fig. 1.

Eqn. 1 can be derived from the following expressions for k'

$$k' = \frac{n_s}{n_m} = \frac{n_s}{n_i + n_o} = \frac{[H_2QZ_2A]_s \, W_s}{C_{Q,i} \, V_i + C_{Q,o} \, V_o} \tag{3}$$

where n_s and n_m are the amounts of solute in the stationary and mobile phase. The amount of solute in the mobile phase is divided in that within the pores (i) and that outside the pores (o), because the solute, when complexed to the polyion, is supposed not to enter the pores. All low-molecular-weight compounds are assumed to have the same concentration within and outside the pores (Donnan effects are neglected). $[H_2QZ_2A]_s$ is the concentration (mol/g) of sample species H_2QZ_2 adsorbed to adsorption sites A on the stationary phase and C_Q is the total concentration of the sample in the mobile phase. At the pH used here C_Q is equal to the total concentration of the divalent form of quinidine:

$$C_{Q,i} = [H_2Q^{2+}]_i = [H_2Q^{2+}]_o \tag{4}$$

$$C_{Q,o} = [H_2Q^{2+}]_o + [H_2QU_P]_o \tag{5}$$

U_P is a binding unit on the polyion which will be discussed further on.

The following equilibria, with the corresponding equilibrium constant given in parentheses, form the basis for eqn. 1:

$$H_2Q_i^{2+} + 2Z_i^- + A_s \rightleftharpoons H_2QZ_2A_s;\ (K_{H_2QZ_2})$$

representing the distribution of the sample to the stationary phase and

$$H_2Q_o^{2+} + U_{P,o} \rightleftharpoons H_2QU_{P,o};\ (K_{H_2QU_P})$$

representing the binding of the sample ion to the polyion.

It is thus assumed that quinidine is retained only as the divalent ammonium ion together with two phosphate ions. There is some evidence, however, that it is also retained in monovalent form even if the divalent form dominates in the mobile phase at the actual pH as the two pK_a values are 4.2 and 8.8. This is based on the observation that an increase of pH from 2 to 3, in absence of the polyion, caused a drastic increase of the retention of quinidine when the phosphate concentration and the ionic strength were kept constant, thus indicating a favoured retention of the monovalent sample ion. The only alternative explanation would be that this retention increase was caused by the decreased concentration of phosphoric acid in the mobile phase which can lead to a decreased competitive adsorption of phosphoric acid on the stationary phase.

The evaluation of the effect of the polyion in the mobile phase, according to the proposed model, can be performed even if the retained species of the sample are unknown.

Only if the pH and phosphate concentration are changed eqn. 1 has to be modified to take into account the change of sample protolysis in the mobile phase and a possible change of the ratio of monovalent to divalent species retained. A further prerequisite for the use of eqn. 1 is that the polyion does not distribute to the stationary phase or indirectly influences its composition so that the sample retention is affected.

U_P is a unit of a polyion which is assumed to bind the sample ion. If there are

n_U such units on one polyion molecule the concentration of units, $[U_P]_o$, will be given by

$$[U_P]_o = n_U [P]_o \tag{6}$$

Eqn. 1 is a simplification in that it is valid for a situation when the adsorption and binding isotherms are linear, which is the case when symmetrical chromatographic peaks are obtained. Only at higher sample concentrations, when the concentration of free adsorption sites and binding units may decrease, the equation takes another form (*cf.* refs. 1 and 2). Such overloading of the stationary phase would give rise to tailing peaks whereas overloading of the polyion would result in fronting peaks. A further simplification is that the fact that the polyion probably has a rather broad molecular weight distribution has not been taken into account.

It can be shown that after inverting eqn. 1 the reciprocal k' can be plotted *versus* $[P]_o$ to yield a straight line (Fig. 2) and the ratio between the slope and intercept of that line will give

$$\frac{\text{slope}}{\text{intercept}} = \frac{K_{H_2QU_P} \cdot n_U}{1 + \dfrac{V_i}{V_o}} \tag{7}$$

This can be used *e.g.* to calculate the ratio of bound to free sample ion in the mobile phase outside the pores by the equation

$$\frac{[H_2QU_P]_o}{[H_2Q^{2+}]_o} = K_{H_2QU_P} \cdot n_U \cdot [P]_o \tag{8}$$

This will require a determination of V_i and V_o which can be done by measurement of V_m using a low-molecular-weight unretained compound and by measurement of V_o using a totally excluded compound. The value of V_i/V_o can be estimated to range between 0.6 and 1 depending on the specific pore volume of the stationary phase and the packing density of the support particles.

It must be emphasized that even if the experimental data fit well to this model it does not show that it is the divalent sample ion which has been bound by the polyion. This can only be concluded from a study at different pH values of the mobile phase.

The chromatographic measurements do not permit the determination of the value n_U. It is included in the model for formal reasons to indicate that several molecules of the solute probably interact with one polyion molecule. Furthermore, it is doubtful if the binding units on the polyion can have a well-defined character.

In the plot of Fig. 2 the molarity scale for the polyion was not used because the exact composition of the polyion was not known. The binding ratio (eqn. 8) could not be determined due to the low intercept and to the capacity ratio being so high in the absence of the polyion that it could not be measured. Anyway, Fig. 1 indicates a binding ratio well above 10 for the higher concentrations of dextran sulphate.

Similar experiments on another column, obtained at somewhat higher meth-

anol concentrations of the eluent, also gave a linear plot as illustrated in Fig. 3. Here, a measurable retention of quinidine was obtained at zero concentration of the polyion. This may depend on the higher methanol concentration but also on the fact that this column had been treated with dimethyloctylammonium as additive to the eluent (*cf.* ref. 2) in previous experiments and that this agent had not been completely washed off the column. Calculation of the binding ratio from eqns. 7 and 8 gave a value of 9 at the highest concentration of polyion using the value 0.6 for V_i/V_o.

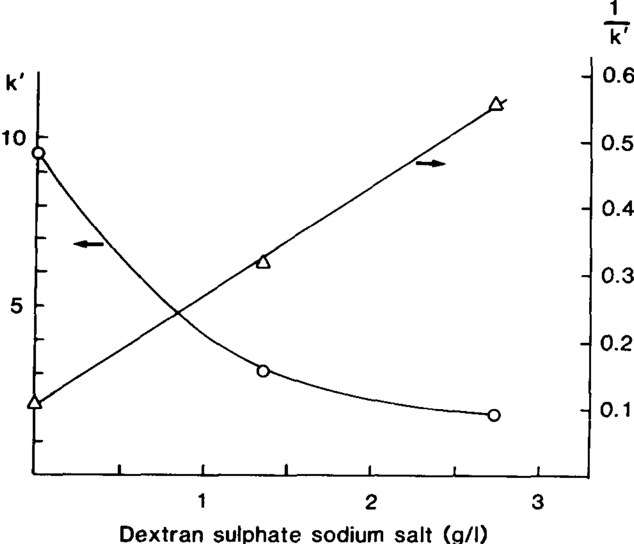

Fig. 3. Retention and test of retention model when the concentration of dextran sulphate is changed in the eluent. Sample: quinidine. ○ = Capacity ratio; △ = reciprocal capacity ratio. Eluent: dextran sulphate in phosphate buffer (pH 2.2, ionic strength 0.01)–methanol (84:16). Stationary phase: LiChrosorb RP-8, 5 μm (previously used with other eluents).

Fig. 1 seems to indicate higher binding ratios than obtained for the results of Fig. 3. This may indicate that dextran sulphate could have had an influence on the properties of the stationary phase in the case of Fig. 1 or that the difference in methanol concentration influences the association to the polyion.

Fig. 4 shows further results on the retention of singly and doubly charged cations and singly charged anions. The conditions are similar to those in Fig. 1 with the exception that the buffer concentration was 10 times higher. This makes the sodium concentration vary from 0.09 up to maximally 0.10 *M* whereas the phosphoric acid and dihydrogen phosphate concentrations are constant. The retention of the monovalent anions and cations is constant and this may depend on the absence of Donnan effects at the high buffer electrolyte concentration used and on the higher and constant sodium ion concentration as compared to the data of Fig. 1. The retention of all divalently charged cations decreases when the concentration of dextran sulphate increases.

It is obvious from Fig. 4 that the effect of the polyion is selective for the

divalent cations *versus* the monovalent ions but non-selective within the group of divalent cations. This indicates that the nature of the interaction with the polyion is of electrostatic origin. A plot of the reciprocal k' for the divalent cations *versus* the polyion concentration gave rather scattered data points which was expected already from an inspection of Fig. 4. The reason for this may be that the retention was not quite stabilized between each run when the measurements were made. Nevertheless, reasonably linear relationships were obtained for three of the compounds and slope/intercept values according to eqn. 7 were approximately 60% of those obtained in Fig. 3, which may have to do with the differences in buffer electrolyte and methanol concentration in the eluent. More detailed studies on samples differing more widely in structure and on mobile phases of different compositions are necessary to elucidate the nature of this chromatographic system.

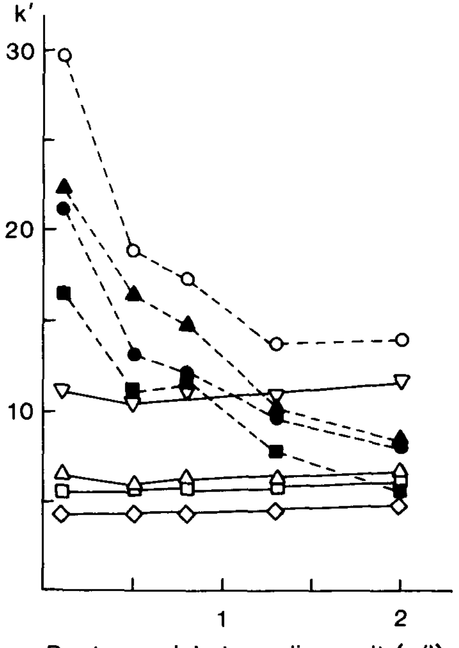

Fig. 4. Retention of cationic and anionic solutes when the concentration of dextran sulphate is changed in the eluent. ---, Divalent cationic solutes; ——, monovalent cationic and anionic solutes. Samples: ○ = quinine; ▲ = brompheniramine; ● = quinidine; ■ = chlorpheniramine; ▽ = N-ethylephedrine; △ = ephedrine; □ = toluene-4-sulphonic acid; ◇ = phenylpropanolamine. Eluent: dextran sulphate in phosphate buffer (pH 1.8, ionic strength 0.10)- methanol (9:1). Stationary phase: LiChrosorb RP-8, 5 μm.

Determination of the binding of small ions to polyions by other methods can serve to confirm the retention model presented here. In fact, a few studies were done on the equilibrium dialysis of quinidine with the polyion present on one side of the membrane using similar conditions as used in the chromatographic studies and a binding ratio of similar magnitude as that presented above was obtained. This supports the retention model. Equilibrium dialysis can also be used to study the binding

of the sample under conditions where the binding isotherm is non-linear, *i.e.* when the binding ratio changes with the sample concentration and fronting chromatographic peaks would have been obtained.

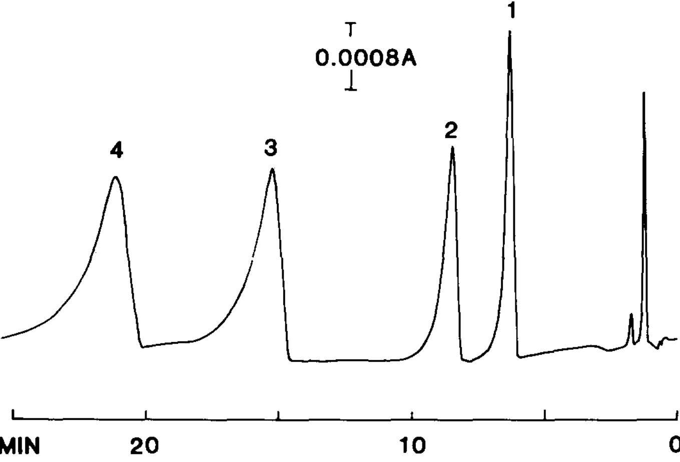

Fig. 5. Chromatogram of divalent and monovalent amines with dextran sulphate in the eluent. Peaks: 1 = phenylpropanolamine; 2 = ephedrine; 3 = quinidine; 4 = quinine. Conditions as in Fig. 4 with dextran sulphate 0.80 g/l. Flow-rate: 1.0 ml/min.

Applications and conclusions

The main effect of a high-molecular-weight polyion present in the mobile phase under conditions described in this paper will be a reduction of the retention of small sample ions of the opposite charge type. The polyion thus can be used in connection with phase systems that are able to retain solutes which are ionized in the mobile phase, *e.g.* reversed-phase ion-pair chromatographic systems. The results presented show that divalent sample ions of opposite charge to the polyion are affected drastically whereas monovalent cations and anions are not. This gives new possibilities to regulate retention and selectivity of differently charged ions and may be of particular value when a sample contains solutes of the same charge type but different valency which in ordinary ion-pair chromatographic systems often gives rise to much higher retention of the higher charged ions[21,22]. This often demands for a gradient elution. With the aid of the polyion the retention of a divalent ion can be regulated rather independently of the retention of a monovalent ion and even the retention order can be changed. The use of a polyion gradient may be valuable in some cases and with totally excluded polyions the re-equilibration procedure can be expected to be fast.

Fig. 5 shows a separation of two monovalent and two divalent amines using the conditions described for Fig. 4. Many of the cationic solutes showed slightly tailing peaks and future studies will show if tail-reducing additives, like alkylammonium ions[2], can be used in the eluent without complications for the effect of the polyion.

Under the conditions studied no indications were observed for any "saturation" of the polyion. Further studies, *e.g.* with polyions of different charge density,

may show whether this effect, which is expected from the theory of ion condensation, can give rise to difficulties such as fronting peaks.

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REFERENCES

1 A. Tilly-Melin, Y. Askemark, K.-G. Wahlund and G. Schill, *Anal. Chem.*, 51 (1979) 976.
2 A. Sokolowski and K.-G. Wahlund, *J. Chromatogr.*, 189 (1980) 299.
3 J. O'M. Bockris and A. K. N. Reddy, *Modern Electrochemistry*, Vol. 1, Plenum Press, New York, 1970, p. 251.
4 J. E. Gordon, *The Organic Chemistry of Electrolyte Solutions*, Wiley, New York, 1975, p. 388.
5 K. Hellsing, *J. Chromatogr.*, 36 (1968) 170.
6 J. C. Giddings and K. Dahlgren, *Separ. Sci.*, 5 (1970) 717.
7 S. A. Rice and M. Nagasawa, *Polyelectrolyte Solutions*, Academic Press, New York, 1961, p. 427.
8 F. Oosawa, *Polyelectrolytes*, Dekker, New York, 1971, p. 71.
9 G. S. Manning, *Acc. Chem. Res.*, 12 (1979) 443.
10 G. S. Manning, *Annu. Rev. Phys. Chem.*, 23 (1972) 117.
11 G. S. Manning, *Quart. Rev. Biophys.*, 11 (1978) 181.
12 H. Wennerström, B. Lindman, G. Lindblom and G. J. T. Tiddy, *J. Chem. Soc., Farad. Trans. I*, 75 (1979) 663.
13 Y. M. Joshi and J. C. T. Kwak, *Biophys. Chem.*, 13 (1981) 65.
14 W. H. Braunlin, T. J. Strick and M. T. Record, Jr., *Biopolymers*, 21 (1982) 1301.
15 A. Jensen, F. Basolo, H. M. Neumann, *J. Amer. Chem. Soc.*, 81 (1959) 509.
16 W. D. Comper and T. C. Laurent, *Physiol. Rev.*, 58 (1978) 255.
17 G. C. Wood and P. F. Cooper, *Chromatogr. Rev.*, 12 (1970) 88.
18 B. Sebille, N. Thuaud and J. P. Tillement, *J. Chromatogr.*, 180 (1979) 103.
19 V. Hlady, *J. Colloid Interface Sci.*, 98 (1984) 373.
20 *Catalogue*, Pharmacia Fine Chemicals, Uppsala, 1983.
21 W. Jost, K. Unger and G. Schill, *Anal. Biochem.*, 119 (1982) 214.
22 A. Sokolowski, N. Balğobin, S. Josephson, J. B. Chattopadhyaya and G. Schill, *Chem. Scr.*, 18 (1981) 189.