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RETENTION MECHANISM FOR REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHY

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SUMMARY

The retention mechanism of reversed-phase "ion-pair" liquid chromatography was investigated. The study demonstrates that for a methanol–water (35:65), pH 3 mobile phase and a μBondapak C_{18} stationary phase: sodium pentane-, hexane-, heptane-, and octanesulfonate, and octylamine hydrochloride ion-interaction reagents are retained by the stationary phase; ion-pair formation does not occur in the mobile phase; the capacity factor of solute ions is increased by mobile phases containing ion-interaction reagents of the *opposite* charge over the range 0 to 20 m*M*; the retention behavior of *neutral* molecules is independent of ion-interaction reagent concentration from 0 to 20 m*M*; solute ions are rapidly eluted by mobile phases containing ion-interaction reagents of the *same* charge, and the capacity factor is relatively independent of the ion-interaction reagent concentration from approximately 2 to 20 m*M*; ion-interaction reagents are locally *desorbed* from the stationary phase by the injection of solute ions of the *same* charge; ion-interaction reagents are locally *adsorbed* by the injection of solute ions of the *opposite* charge.

Because neither the conventional ion-pair formation hypothesis nor a simple ion-exchange hypothesis has been entirely satisfactory in interpreting the data, a broader mechanistic model is presented to account for experimental results.

INTRODUCTION

The application of reversed-phase paired-ion chromatography to the separation of charged solutes has gained wide acceptance as an alternative to ion-exchange. Haney and co-workers[1,2], Waters Associates[3], and Knox and co-workers[4,5] found that the addition of long-chained alkyl ions to the mobile phase gave enhanced separation of oppositely charged solute ions. This technique has been called "soap chromatography"[4], "ion-pair chromatography"[6], "solvent-generated (dynamic) ion-exchange chromatography"[7,8], "hetaeric chromatography"[9], "Paired-Ion Chromatography™"[3], "detergent-based cation-exchange"[7], "solvophobic-ion chromatography"[10], and

"surfactant chromatography"[11]. The variety of nomenclature indicates the uncertainty which exists concerning the retention mechanism in this mode of liquid chromatography. Two hypotheses are presently embraced.

The first view stipulates the formation of an ion pair in the aqueous mobile phase, prior to its adsorption onto the bonded, hydrophobic stationary phase[1,3]. Recently, Horváth *et al.*[9] have used solvophobic theory[12] to defend this concept of ion-pair formation in the mobile phase.

The second view stipulates an ion-exchange mechanism[7,10,13,14]. In this hypothesis, it is the unpaired lipophilic alkyl ions that adsorb onto the bonded phase surface and cause the column to behave as an ion exchanger.

In this paper, we investigate the behavior of neutral and charged solutes in systems containing lipophilic ions added to the mobile phase. Neither of the previous restrictive views (ion pair or ion exchange) has proved to be entirely satisfactory in interpreting our data. Instead, the results suggest a retention mechanism that is best described as one of "ion interaction".

The less restrictive term "ion interaction" is taken to mean any process in which ions interact because of Coulombic and other forces. These forces will be referred to as *electrostatic*, *eluophilic* (having an affinity for the mobile phase), *eluophobic* (having an aversion for the mobile phase), *adsorbophilic* (having an affinity for the stationary phase), and *adsorbophobic*. The adsorbophilic ion that is intentionally added to the mobile phase will be referred to as the "ion-interaction reagent".

EXPERIMENTAL

Chromatographic system

The chromatographic system has been described previously[15] and includes the following components: Model 6000A solvent delivery system (Waters Assoc., Milford, Mass., U.S.A.), Model U6K injector (Waters), 5 cm × 2 mm I.D. Bondapak C_{18}/Porasil pre-column (Waters), 30 cm × 4 mm I.D. μBondapak C_{18} main column (Waters), and a Model R401 differential refractometer (Waters). Column temperature was held at 25.0 ± 0.1 °C by a Model FK constant temperature circulating bath (Haake, Saddle Brook, N.J., U.S.A.). Injection volumes were 2.0, 5.0, or 10.0 μl. Mobile phase flow-rate was maintained at 2.03 ± 0.03 ml min^{-1}. The time equivalent of the void volume (t_0) was determined by injecting 5 μl of methanol and measuring the time from injection to the first deviation from baseline. Water soluble vitamins were separated using a Model 244 chromatographic system (Waters), which included an M6000A solvent delivery system, U6K injector, M440 absorbance detector, and R401 refractometer.

Additional instrumentation

Analog ultraviolet (UV) and refractive index (RI) detector outputs were recorded by a Model 281 dual-pen strip-chart recorder (Soltec, Encino, Calif., U.S.A.). Simultaneously, the signal from one detector was digitized by a Model ADC-12QZ analog-to-digital converter (Analog Devices, Norwood, Mass., U.S.A.) interfaced[16] to a Model 9830A digital computer (Hewlett-Packard, Calculator Products Division, Loveland, Colo., U.S.A.). Chromatograms of less than 15 min were digitized at 0.5-sec intervals; longer chromatograms were digitized at 1.0-sec intervals. Chromato-

grams were drawn from digitized data on a Model 9862A plotter (Hewlett-Packard).

Conductance measurements were made with a Model RC16B2 conductivity meter (Industrial Instruments, Cedar Grove, N.J., U.S.A.) operated at 1000 Hz. A 30-ml conductance cell (Cat. No. S-29865, Sargent-Welch, Skokie, Ill., U.S.A.) with a cell constant of approximately 1 cm^{-1} was modified to have a slightly rounded bottom and used for the conductance titrations.

Mobile phases and samples

Distilled water, concentrated HCl (Mallinckrodt, St. Louis, Mo., U.S.A.), and spectrophotometric grade methanol (Aldrich, Milwaukee, Wisc., U.S.A.) were used for solution preparation. Sodium pentane-, hexane-, heptane-, and octanesulfonate were obtained from Waters. Other compounds used as samples were obtained from a variety of suppliers.

Mobile phases were aspirated through 0.47-μm cellulose acetate filters (HAWP04700, Millipore, Bedford, Mass., U.S.A.) and degassed in an ultrasonic bath (ME4.6, Mettler Electronics, Anaheim, Calif., U.S.A.) before use. Samples were dissolved in a mobile phase containing 10^{-3} *M* HCl and a percent methanol corresponding to the composition of the eluent. (Although the use of halogen acids is not recommended by equipment manufacturers, HCl was used for pH adjustment in this study to avoid confounding the results obtained for the amine hydrochloride samples and for the conductance titrations. After each set of experiments, the system was purged with methanol). The alkylsulfonates and octylamine samples were prepared at a concentration of 100 m*M*; toluene, ethylbenzene, benzenesulfonic acid, chromatropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid), aniline, benzylamine, and phenethylamine samples were 5 m*M*.

RESULTS AND DISCUSSION

Retention of alkylsulfonates

Fig. 1 is a plot of the logarithm of the capacity factor, k', as a function of the number of carbon atoms in the alkyl chain of the sodium salts of pentane-, hexane-, heptane-, and octanesulfonic acids for methanol–water (20:80, 35:65 and 50:50, v/v) eluents. The alkylsulfonates were injected as samples; the eluents did not contain ion-interaction reagents. The data indicate that the four alkylsulfonates are retained by the column in a regular manner. Fig. 2 is a plot of log k' as a function of the percent methanol in the eluent for the same alkylsulfonates; again, the regular retention of these compounds can be seen.

Together, Figs. 1 and 2 suggest that at a given methanol concentration, log k' is a first-order function of the number of carbon atoms, and that for a given number of carbon atoms, log k' is a first-order function of methanol concentration. Both observations are consistent with Martin's equation[17]

$$RT \ln K = a\Delta\mu_{CH_3} + b\Delta\mu_{SO_3^-} + c\Delta\mu_{CH_2} \quad (1)$$

where R is the gas constant, T the temperature in °K, K the partition coefficient, a, b, and c are the numbers of methyl, sulfonate, and methylene groups in a molecule; and $\Delta\mu$ is the differential chemical potential —the free energy required to transport

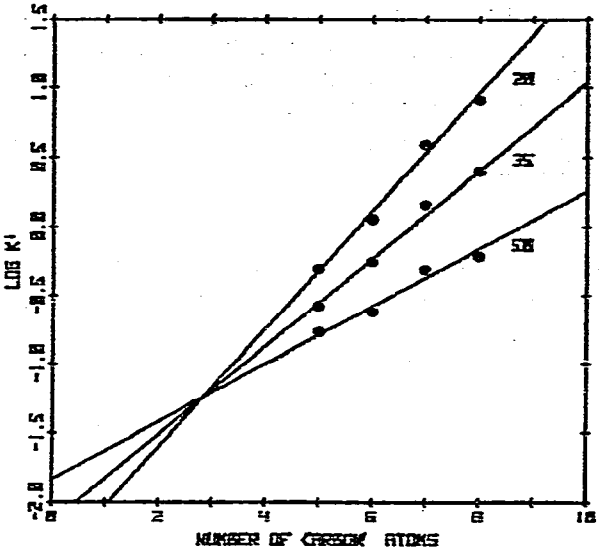


Fig. 1. Dependence of the logarithm of the capacity factor, k', on the number of carbon atoms in the alkyl chain of sodium alkylsulfonates. Numbers beside the straight lines indicate the percent methanol in the eluent. Lines drawn from eqn. 4 (least squares fit using all data points simultaneously).

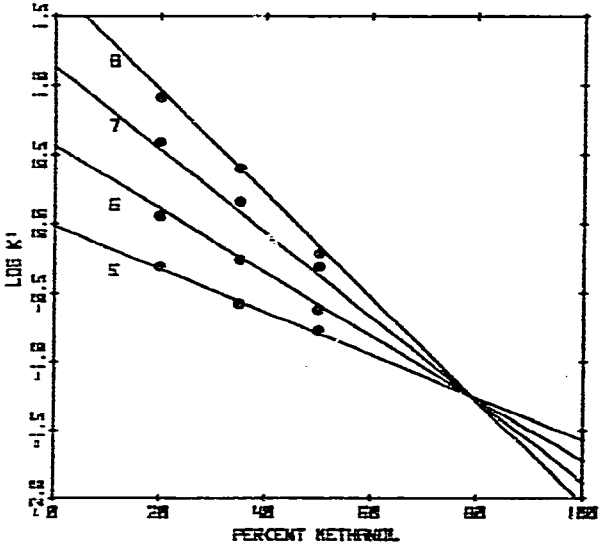


Fig. 2. Dependence of the logarithm of the capacity factor, k', on the percent methanol in the eluent. Numbers beside the straight lines indicate the number of carbon atoms in the alkyl chain of sodium alkylsulfonates. Lines drawn from eqn. 4 (least squares fit using all data points simultaneously).

one mole of a given group (CH_3, SO_3^-, or CH_2) from the stationary phase to the mobile phase. When Martin's equation is applied to homologous straight-chain alkylsulfonates, $a = b = 1$, and eqn. 1 can be written as

$$\log k' = \beta_0' + \beta_1' c \tag{2}$$

where β_0' is an offset term, and β_1' is the slope (a measure of $\Delta\mu_{CH_2}$).

For a given column, the differential chemical potential of each of the various functional groups depends upon the mobile phase composition, either directly as the composition affects the chemical potential of the mobile phase itself, or indirectly if preferential adsorption of one component of the mobile phase affects the chemical potential of the stationary phase. Thus, β_1' in eqn. 2 is, in fact, a function of the percent methanol in the eluent. We may then write

$$\log k' = \beta_0 + \beta_1(m - \beta_2)(c - \beta_3) \tag{3}$$

where β_0 is an offset term for $\log k'$, β_1 is a combined slope with respect to percent methanol and number of methylene groups, m is the percent methanol in the eluent, β_2 is an offset term in percent methanol, and β_3 is an offset term in the number of methylene groups. (The parameters β_0, β_2 and β_3 supply degrees of freedom to prevent the model from being forced to pass through the point $\log k' = m = c = 0$.)

Eqn. 3 was fit to the data of Fig. 1 and 2 by a non-linear least squares multivariate regression technique similar to that described by O'Neill[18]. The fitted equation is

$$\log k' = -1.2389 - 0.0072956\,(m - 78.741)\,(c - 1.8603) \tag{4}$$

The straight lines in Figs. 1 and 2 were drawn from this fitted model.

The intersection of the straight lines at $c = 1.86$ (total number of carbon atoms = 2.86) in Fig. 1 suggests that the capacity factor for the three-carbon alkylsulfonate should be approximately independent of percent methanol in a methanol–water mobile phase. This can be explained if

$$\partial\Delta\mu_{CH_3}/\partial m + 2 \times \partial\Delta\mu_{CH_2}/\partial m \approx -\partial\Delta\mu_{SO_3^-}/\partial m \tag{5}$$

That is, variations in the mobile phase composition produce changes in the differential chemical potential of the eluophilic sulfonate group that are compensated by the sum of the changes in the differential chemical potentials of the three eluophobic carbon groups. It is reasonable to expect the sign of $\partial\Delta\mu_{SO_3^-}/\partial m$ to be opposite that of $\partial\Delta\mu_{CH_2}/\partial m$ and $\partial\Delta\mu_{CH_3}/\partial m$ for reversed-phase systems. Eqn. 5 would explain a similar convergence point for the retention of fatty acids on porous polymers in the work of Uchida and Tanimura[19].

The intersection of the straight lines at $m = 78.7$ in Fig. 2 suggests that at a mobile phase composition of approximately 79% methanol, k' for the alkylsulfonates is independent of the number of methylenes in the alkyl chain. The methylene group would be equally attracted (and/or repelled) by both the mobile and stationary phases; that is, $\Delta\mu_{CH_2} = 0$. This "isopotential" mobile phase composition is a function

of the chemical potential of the stationary phase, and its value might serve as a means of evaluating the degree of non-polarity of stationary phases, similar to Scott and Kucera's "wettability"[20].

Conductance titrations

Conductance measurements are a classical means of determining ion-pair formation in solvents[21–24]. However, while many workers have assumed that ion-pair formation occurs in the mobile phase of reversed-phase systems[1–3,9], conductance measurements have not yet been used to aid in the elucidation of chromatographic "ion-pairing" mechanisms. If an ion pair were formed between oppositely charged eluophobic ions in solution, a conductometric titration of one ion with the other would show a distinctive break at the equivalence point[24].

Fig. 3 presents the results of an investigation designed to detect ion-pair formation between octanesulfonate ion and octylammonium ion in pH 3.0 methanol–water (35:65). The lower three curves were obtained for the titrations of sodium octanesulfonate (curve A), octylamine hydrochloride (curve B), and sodium chloride (curve C) against solvent blanks. The upper two curves were obtained for the titrations of sodium octanesulfonate with octylamine hydrochloride (curve D), and octylamine hydrochloride with sodium octanesolfonate (curve E).

If octanesulfonate ions and octylammonium ions form ion pairs, then titration of one with the other would produce a stoichiometric amount of ion pairs, and

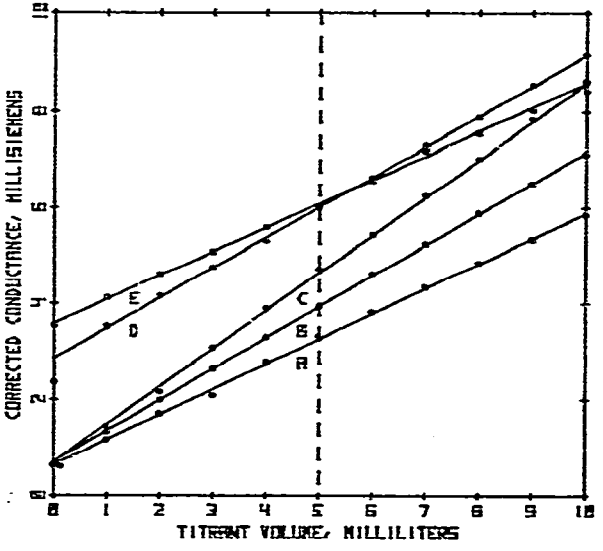


Fig. 3. Conductance titration results. All solutions prepared in methanol–water (35:65) solvent containing 10^{-3} *M* HCl. Curve A: 10 ml solvent titrated with 40 m*M* sodium octanesulfonate. Curve B: 10 ml solvent titrated with 40 m*M* octylamine hydrochloride. Curve C: 10 ml solvent titrated with 40 m*M* sodium chloride. Curve B: 10 ml 20 m*M* sodium octanesulfonate titrated with 40 m*M* octylamine hydrochloride. Curve E: 10 ml 20 m*M* octylamine hydrochloride titrated with 40 m*M* sodium octanesulfonate. Equivalence point for curves D and E occurs at 5 ml titrant volume. Conductances corrected for dilution by the factor $(V + V_0)/V_0$, where V_0 is the initial volume of the solution being titrated and V is the volume of added titrant.

equivalent amounts of sodium ions and chloride ions at the equivalence point. The ion pairs would not contribute to the conductance of the solution, and the conductance at the equivalence point would be the same as the conductance corresponding to the 5-ml point in curve C (NaCl blank) in Fig. 3. This behavior is not observed. Instead, in curves D and E (titrations of sodium octanesulfonate and octylammonium chloride with each other) the conductance is accounted for by the sum of the conductances of the individual ions involved in the titration. Clearly, ion-pair formation does not occur.

Conductance measurements also demonstrated that ion-pair formation does not occur in the octylamine hydrochloride titrations of sodium pentanesulfonate, sodium hexanesulfonate, or sodium heptanesulfonate.

Additional conductance titrations were carried out looking for evidence of ion-pair formation in methanol–water solvents ranging from 0 to 100% methanol. Sodium octanesulfonate was used as a titrant for the drugs procaine, dopamine, methapyrilene, and ephedrine, some of which are reported to have been separated by "ion-pair" chromatography[9,25–27]. No evidence of ion-pair formation was found in any of these conductance studies.

Because we find no measurable evidence of ion-pair formation in the mobile phase, we will not use the term "ion-pair reagent," but will instead use the term "ion-interaction reagent" to refer to the adsorbophilic ion that is intentionally added to the mobile phase.

Effects of ion-interaction reagents on capacity factors

Fig. 4 illustrates the effect of the concentration of negatively charged sodium octanesulfonate on the k' values of three positively charged homologs, aniline

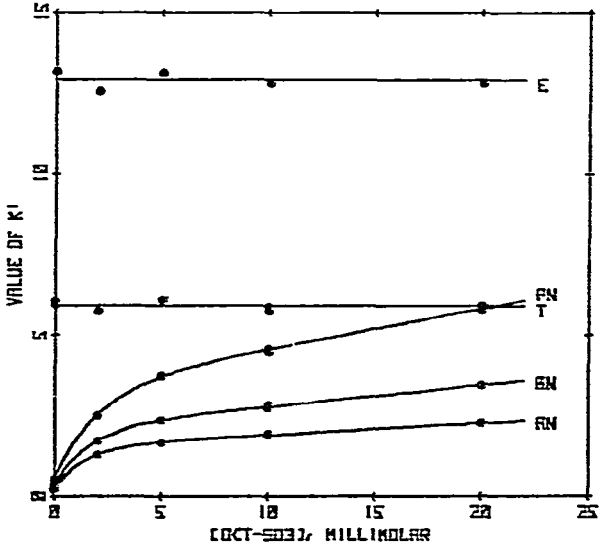


Fig. 4. Effect of sodium octanesulfonate concentration on the capacity factors, k', of neutral and positively charged solutes. Mobile phase: methanol–water (32.5:67.5) pH 3 containing the indicated amount of ion-interaction reagent. E = Ethylbenzene; T = toluene; AN = aniline; BN = benzylamine; PN = phenethylamine.

(AN), benzylamine(BN), and phenethylamine (PN). As expected[1,4], the k' value of each amine increases with increasing octanesulfonate concentration. At each concentration, aniline is eluted first, followed in sequence by the longer chain members of the series, benzylamine and phenethylamine. Because the charge on each amine is the same (+1), the differences in retention behavior must be caused not only by electrostatic attraction, but also by other forces acting within the system (*i.e.*, eluophobic, eluophilic, adsorbophobic, and adsorbophilic).

While the effect of ion-interaction reagents on the k' values of oppositely charged ionic samples is well documented in the literature[11], the effect of ion-interaction reagents on the k' values of uncharged samples and similarly charged ionic samples has received less attention[28,29]. Fig. 4 shows that the retention behavior of the neutral homologs, toluene (T) and ethylbenzene (E), is not appreciably affected by variations in the concentration of octanesulfonate. Apparently, the adsorption of octanesulfonate on the stationary phase is transparent to these neutral species. As expected, the longer chain-length homolog, ethylbenzene, elutes last.

Fig. 5 shows the effect of octanesulfonate concentration on the k' values of several negatively charged species. In each case, k' decreases rapidly between 0 and 2 m*M* octanesulfonate, and then remains relatively constant with further increases in octanesulfonate concentration. This type of behavior has been observed previously in paper chromatography when surface active ions were added to the mobile phase[28]. At each concentration of octanesulfonate, the four alkylsulfonate homologs (S5, S6, S7, and S8) are eluted in order of increasing chain length. Again, because the charge on each alkylsulfonate is the same (−1), the differences in retention behavior must be caused not only by electrostatic repulsion, but also by other forces acting within the system.

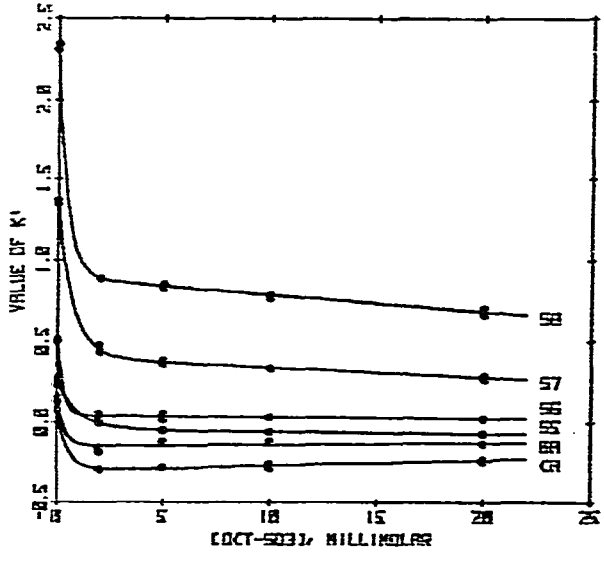


Fig. 5. Effect of sodium octanesulfonate concentration on the capacity factors, k', of negatively charged solutes. Conditions the same as in Fig. 4. CA = Chromatropic acid; BA = benzenesulfonic acid; S5 = sodium pentanesulfonate; S6 = sodium hexanesulfonate; S7 = sodium heptanesulfonate; S8 = sodium octanesulfonate.

Fig. 6 shows the effect of the concentration of positively charged octylamine hydrochloride on the k' values of neutral and negatively charged species. Ethylbenzene again elutes after toluene; neither is affected by the octylamine hydrochloride concentration.

At each concentration of octylamine hydrochloride, the alkylsulfonate samples are eluted in order of increasing chain length (heptanesulfonate and octanesulfonate were so strongly retained —$k' > 15$ for octylamine hydrochloride concentrations > 5 mM— that it was not practical to obtain a complete set of data for them). Even though the charge of the ion-interaction reagent in Fig. 6 is the opposite of that in Fig. 5, the *order* of retention of the negatively charged alkylsulfonate samples is the same in both figures. Thus, the retention order of the alkylsulfonates must be caused by forces other than purely electrostatic interactions.

Fig. 7 shows the effect of the concentration of octylamine hydrochloride on the capacity factors of positively charged species. In each case, the capacity factor decreases sharply between 0 and 2 mM octylamine hydrochloride and then remains relatively constant with further increases in concentration. The behavior of octylamine hydrochloride as a sample in an octylamine hydrochloride eluent (Fig. 7) is very similar to the behavior of sodium octanesulfonate as a sample in a sodium octanesulfonate eluent (Fig. 5).

The difference in retention behavior of chromotropic acid disodium salt (CA) and benzenesulfonic acid sodium salt (BA) in Figs. 5 and 6 might be explained on the basis of charge alone. The anionic benzenesulfonate is singly charged (—1), whereas the anion of chromotropic acid (a disulfonic acid) is doubly charged (—2). When the ion-interaction reagent is negatively charged (Fig. 5), electrostatic forces exert a greater repulsion on the dianion of chromatorpic acid than they do on benzenesulfonate, and chromotropic acid elutes first. When the ion-interaction reagent is positively charged (Fig. 6), the dianion of chromotropic acid is more strongly retained and benzenesulfonate elutes first. However, in this case (Fig. 6) it should also be noted that the doubly charged anion of chromotropic acid (—2) is retained *less* than the singly charged hexane-, heptane-, and octanesulfonate (—1) ions. This is contrary to what one would expect if the retention mechanism were exclusively ion-exchange, and again suggests the importance of forces other than electrostatic forces acting to control retention.

Negative capacity factors

Many of the capacity factors in Figs. 5 and 7 are less than zero in the presence of ion-interaction reagents. Negative capacity factors imply a decrease in the apparent void volume of the column. Adsorption of ion-interaction reagent onto the stationary phase probably does increase the volume of the stationary phase slightly and, thus, decrease the void volume of the column; but t_0, measured by injection of a methanol sample, remained experimentally the same for all concentrations of the ion-interaction reagent. Thus, negative k' values cannot be rationalized by a decrease in the solvent void volume of the column.

The stationary phase, which is made by bonding a C_{18} moiety to porous silica, retains its porous character after the binding process. When ion-interaction reagent is adsorbed, the pores become electrically charged and tend to exclude similarly-charged solute molecules, making a portion of the solvent void volume in-

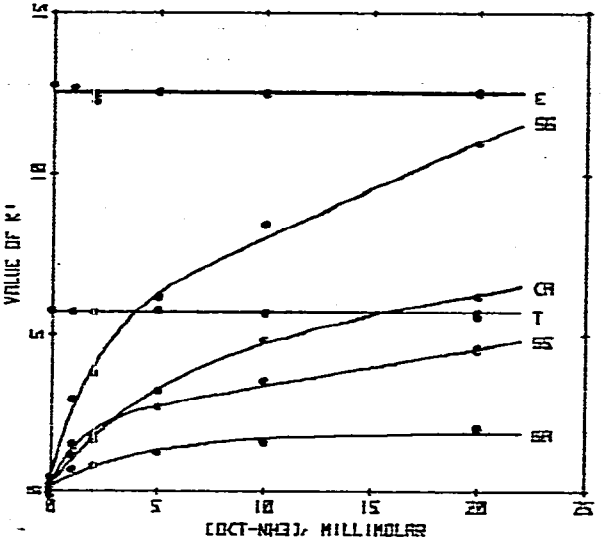


Fig. 6. Effect of octylamine hydrochloride concentration on the capacity factors, k', of neutral and negatively charged solutes. Mobile phase: methanol–water (35:65) pH 3 containing the indicated amount of ion-interaction reagent. Abbreviations as in Figs. 4 and 5.

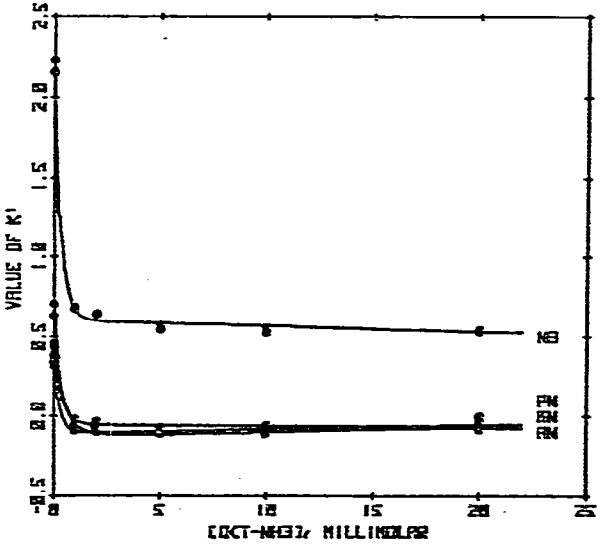


Fig. 7. Effect of octylamine hydrochloride concentration on the capacity factors, k', of positively charged solutes. Conditions the same as in Fig. 6. N8 = Octylamine hydrochloride; other abbreviations as in Fig. 4.

accessible to these solutes. This "charge-exclusion" phenomenon causes the rejected molecules to be eluted more rapidly than otherwise, and leads to negative capacity factors for solutes having little affinity for the stationary phase. Solute molecules with a greater overall affinity for the stationary phase will still be excluded from the

smaller pores, but will elute with positive k' values because of their stronger adsorption on the accessible surface of the stationary phase. Such a charge exclusion mechanism has been proposed before in gel permeation chromatography[30,31].

The existence of peaks occurring before the conventional void volume has been observed by others. Knox and Jurand[5] have reported that when "soap chromatography" is applied to urine analysis, "the bulk of the endogenous compounds . . . elute before the solvent peak . . . These are presumably high-molecular-weight substances that are excluded from the reversed-phase silica gel used as column packing." Karch *et al.*[32] present a chromatogram showing a peak labelled "unknown" eluting before the inert ^2H_2O peak. In each of these cases, an electrostatic exclusion mechanism could be responsible for the appearance of these early peaks.

Chromatographic behavior of alkylsulfonates in the presence of sodium octanesulfonate

Figs. 8 and 9 show chromatograms of RI response for injected samples of heptane- and octanesulfonate, respectively, as a function of increasing sodium octanesulfonate concentration in the eluent. (The chromatograms correspond to points on the upper two curves in Fig. 5.) The dashed line in each figure indicates the time equivalent of the void volume, t_0.

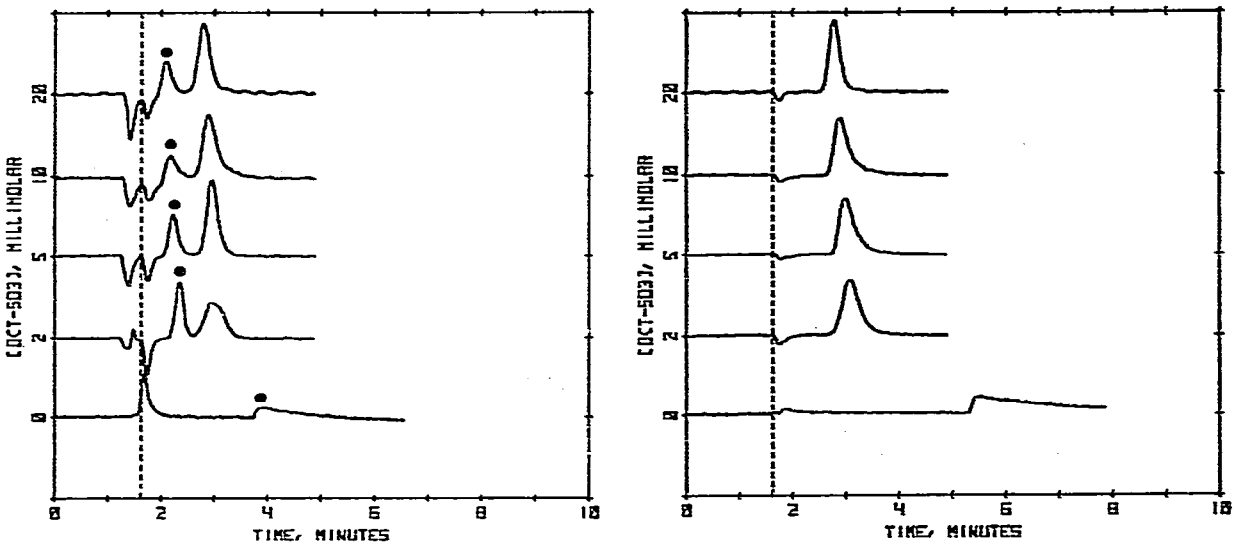


Fig. 8. Chromatograms showing the elution of sodium heptanesulfonate as a function of sodium octanesulfonate concentration in the eluent. RI detector. Mobile phase: methanol–water (32.5:67.5) pH 3 containing the indicated amount of ion-interaction reagent. Dashed line indicates t_0.

Fig. 9. Chromatograms showing the elution of sodium octanesulfonate as a function of sodium octanesulfonate concentration in the eluent. Conditions as in Fig. 8.

The lowest chromatogram in each of Figs. 8 and 9 is the response obtained when a 5 μl sample of 0.10 M sodium alkylsulfonate is eluted with a mobile phase containing no ion-interaction reagent. In each of these two lowest chromatograms the peak eluted first has the same retention time and direction of deflection as a methanol sample injected under the same conditions; thus, the first peak is probably

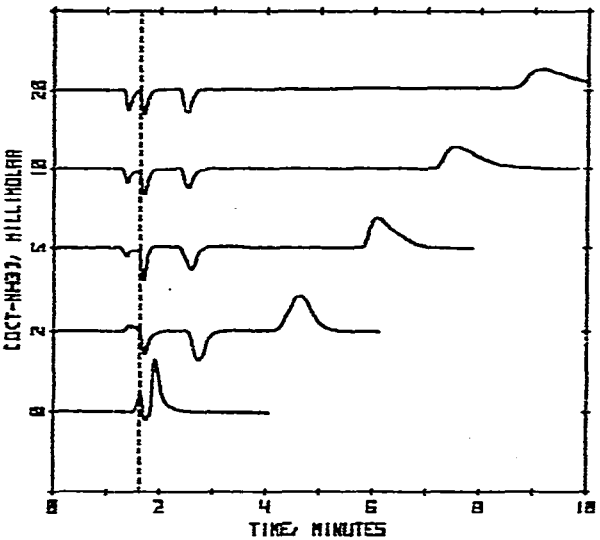
solvent methanol that has been preferentially adsorbed by the stationary phase and is now displaced by the more strongly adsorbed alkylsulfonate. The second peak (last to eluate) is the alkylsulfonate. Similar solvent displacement peaks have been reported previously[33].

When ion-interaction reagent is present in the eluent, the chromatograms of heptanesulfonate (Fig. 8) are markedly different from those obtained in the absence of the ion-interaction reagent. In the chromatograms corresponding to 2, 5, 10 and 20 m*M* octanesulfonate, the last peak to be eluted is the ion-interaction reagent (octanesulfonate). (This last peak elutes at the same time as an octanesulfonate sample injected under the same conditions—see the upper four chromatograms in Fig. 9). The injected heptanesulfonate appears as a positive refractive index peak (marked with a dot) that moves toward shorter retention time with increasing concentration of ion-interaction reagent in the eluent.

These results imply that there is a dynamic equilibrium occurring on the surface such that if another negatively charged sample (*e.g.*, hexanesulfonate) is injected, the locally high concentration of sample will compete with octanesulfonate in the equilibrium with the surface. As a result, the negatively charged sample displaces some of the octanesulfonate which is then eluted from the column.

Chromatographic behavior of alkylsulfonates in the presence of octylamine hydrochloride

Fig. 10 shows chromatograms of RI response for injected samples of pentanesulfonate as a function of increasing octylamine hydrochloride concentration in the eluent. (The data for the pentanesulfonate curve in Fig. 6 were obtained from these chromatograms.) Fig. 11 shows chromatograms of RI response for injected samples of



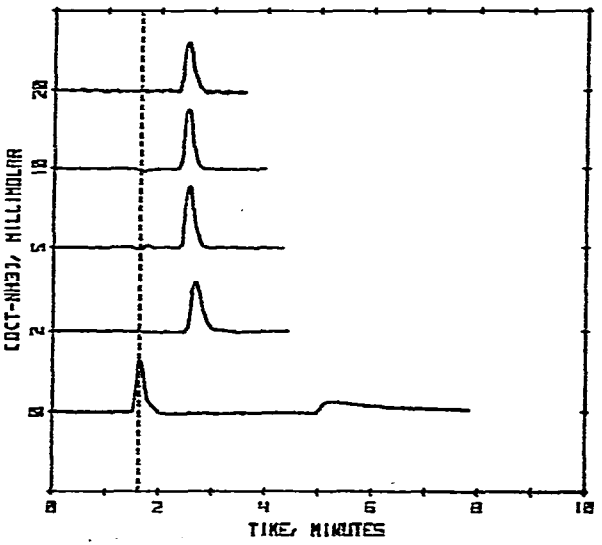


Fig. 10. Chromatograms showing the elution of sodium pentanesulfonate as a function of octylamine hydrochloride concentration in the eluent. RI detector. Mobile phase: methanol–water (35:65) pH 3 containing the indicated amount of ion-interaction reagent. Dashed line indicates t_0.

Fig. 11. Chromatograms showing the elution of octylamine hydrochloride as a function of octylamine hydrochloride concentration in the eluent. Conditions as in Fig. 10.

octylamine hydrochloride as a function of increasing octylamine hydrochloride concentration in the eluent. (The chromatograms correspond to points on the upper curve in Fig. 7.) In each chromatogram, the peak with the longest retention time corresponds to the eluted solute. Fig. 10 shows the usual "ion-pairing" effect of increased solute retention with increasing concentration of ion-interaction reagent.

The most significant feature of Fig. 10 is that when ion-interaction reagent is present in the eluent, a *negative* refractive index peak occurs at the same retention time as the corresponding *positive* octylamine hydrochloride peak in Fig. 11. This phenomenon has also been observed for the separation of positively charged water soluble vitamins (niacinamide, pyridoxine, and thiamine) using octanesulfonate as the ion-interaction reagent.

These observations suggest that the local concentration of ion-interaction reagent is decreased in the eluent when an oppositely charged sample is injected. This behavior would not be expected if dynamic ion-exchange were occurring: there would not be a depletion of the lipophilic ion in the mobile phase —the solute ion would simply be exchanging with other counter ions at the existing surface ion-exchange sites.

A RETENTION MECHANISM

For the conditions used in this investigation:

(a) Sodium pentane-, hexane-, heptane-, and octanesulfonate, and octylamine hydrochloride ion-interaction reagents are adsorbed by the stationary phase (Figs. 1 and 2).

(b) Conductance titrations show that ion-pair formation does not occur in the mobile phase (Fig. 3).

(c) The capacity factor of solute ions is increased by mobile phases containing ion-interaction reagents of the *opposite* charge over the range 0 to 20 m*M* (Figs. 4 and 6).

(d) The retention behavior of *neutral* molecules is independent of ion-interaction reagent concentration from 0 to 20 m*M* (Figs. 4 and 6).

(e) Solute ions are rapidly eluted by mobile phases containing ion-interaction reagents of the *same* charge; the capacity factor is relatively independent of the ion-interaction reagent concentration from approximately 2 m*M* to 20 m*M* (Figs. 5 and 7).

(f) Ion-interaction reagents are locally *desorbed* from the stationary phase by the injection of adsorbophilic solute ions of the *same* charge (Figs. 8 and 9).

(g) Ion-interaction reagents are locally *adsorbed* on the stationary phase by the injection of adsorbophilic solute ions of the *opposite* charge (Figs. 10 and 11).

These observations lead us to propose a retention mechanism for reversed-phase ion-interaction liquid chromatography that is broader in scope than either a dynamic ion-exchange mechanism[7,10,13,14] or an ion-pair mechanism[1,4]. To discuss the model, a negatively charged ion-interaction reagent (sodium octanesulfonate) will be used; however, the proposed mechanism is general and applies equally well to ion-interaction reagents that are positively charged (*e.g.*, octylamine hydrochloride).

In the proposed model (see Fig. 12), the ion-interaction reagents ions (octanesulfonate; white squares with 8-carbon tails) are adsorbed and create a negatively

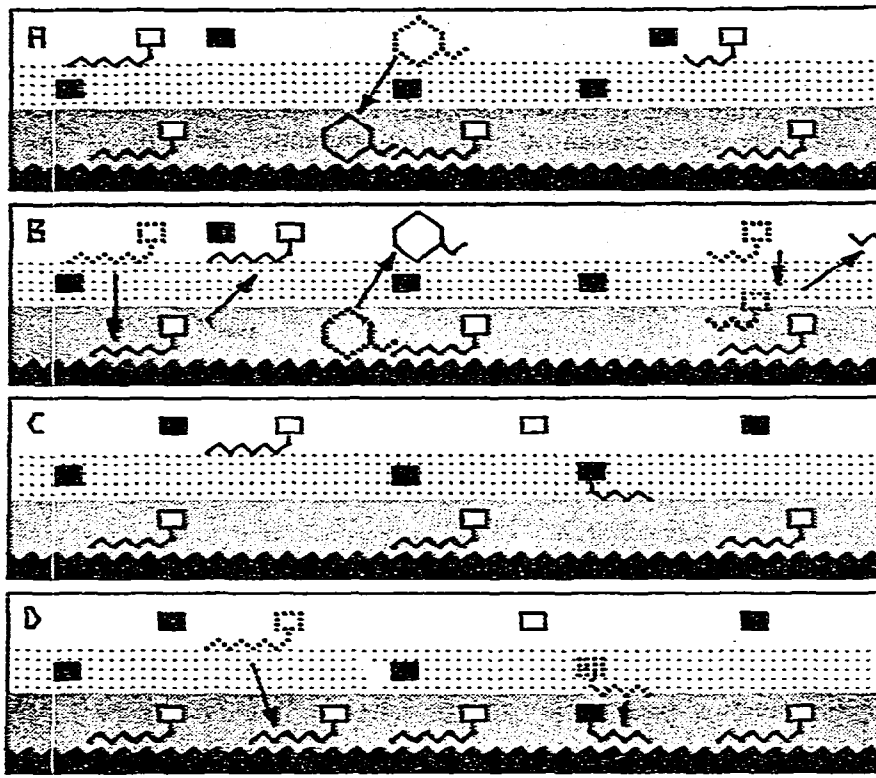


Fig. 12 Retention mechanism for reversed-phase ion-pair liquid chromatography. Black region at bottom of each panel represents C_{18} bonded stationary phase; intermediate shading indicates primary ion layer; light shading shows secondary ion layer; unshaded region represents bulk eluent. See text for further discussion.

charged primary ion layer on the reversed-phase surface. Positively charged ions (*e.g.*, sodium ions; black squares without tails) form a secondary ion layer[34]. The bulk eluent also contains ion-interaction reagent ions and oppositely charged ions

Negatively charged solute ions will experience an electrostatic repulsion by the primary ion layer (see the right sides of Figs. 12a and b) which will tend to keep the solute ions away from the stationary phase (and out of the smaller pores) and will cause them to be eluted rapidly by the mobile phase (see Figs. 5, 7–9 and 11). However, the ion-interaction reagent distribution equilibrium is dynamic so that ion-interaction reagent is constantly *desorbing*, and an equivalent amount of ion-interaction reagent is *adsorbing* at the same rate (see the left sides of Figs. 12a and b). If the ion-interaction reagent concentration in the eluent is increased, the amount of adsorbed ion-interaction reagent will increase; if the eluent concentration is decreased, the amount adsorbed will decrease[4]. Because the steady state is dynamic, negatively charged adsorbophilic (or eluophobic) solute ions in the sample can compete with (and thereby effect a net displacement of) some of the ion-interaction reagent ions; this gives rise to the displacement peaks seen in Fig. 8.

Uncharged molecules do not experience electrostatic repulsion (or attraction);

their transfer across the primary and secondary ion layers is relatively unaffected by the presence of the ionic ion-interaction reagent (see the centers of Figs. 12a and b). Thus, the retention of neutral molecules is not affected by the concentration of ion-interaction reagents (see Figs. 4 and 6).

The secondary ion layer is also in dynamic equilibrium and positively charged ions other than sodium (*e.g.*, hexylammonium ion) can compete for a position in this layer (see Fig. 12c). If an ion with an adsorbophilic (or eluophobic) functional group gets into the secondary ion layer, it will tend to be pulled (or pushed) from the secondary ion layer to the mobile phase–stationary phase interface, where its positively charged ionic functional group will be found in the primary ion layer of the mobile phase and its alkyl chain will be found "on" the stationary phase (see the right side of Fig. 12d). The addition of a positive charge to the negatively charged primary ion layer has the net effect of neutralizing a negative charge from this layer. To restore electrostatic equilibrium, another negative ion can be adsorbed and contribute to the charge of the primary ion layer (see the left side of Fig. 12d). *The net result is that a pair of ions (not necessarily an ion pair) has been adsorbed onto the stationary phase.* This causes a decrease in the concentration of ion-interaction reagent in the eluent, and results in a negative peak in the chromatogram (see Fig. 10).

Once adsorbed onto the stationary phase, the positively charged solute ion will tend to be held rather strongly because of electrostatic attraction in the otherwise negatively charged primary ion layer. As the concentration of ion-interaction reagent in the mobile phase is increased, the amount of negative charge in the primary ion layer will also increase and hold the positive solute ion more often, thus increasing its retention time (Figs. 4 and 6). For a given concentration of ion-interaction reagent, and a given ionic group on the species being eluted, the more adsorbophilic (or eluophobic) the tail, the greater will be the retention time (Figs. 4–6).

CONCLUSION

The retention mechanism presented here for reversed-phase ion-interaction liquid chromatography does not require ion-pair formation in either phase and is not based upon classical ion-exchange. The mechanism assumes dynamic equilibrium, affected not only by electrostatic forces, but also by forces that are eluophilic, eluophobic, adsorbophilic, and adsorbophobic.

We believe this mechanism will be useful for developing future separation techniques.

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