Enhancement of Retention by Ion-Pair Formation in Liquid Chromatography with Nonpolar Stationary Phases

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In Ion-pair reversed-phase chromatography, the retention of ionized analytes on a nonpolar bonded stationary phase is enhanced by the presence of a "hydrophobic" counterlon (hetaeron) in the mobile phase. Either ion-pair formation in the mobile phase with relatively strong retention of the complex or the conversion of the stationary phase into an ion-exchanger may explain the phenomenon. Analysis of the pertinent equilibria shows that the observed hyperbolic or parabolic dependence of the capacity factors on the hetaeron concentration cannot shed light on the mechanism. The experimental data obtained for the retention of catecholamlnes by using C_4-C_{10} alkyl sulfates and other similar hetaerons in a wide concentration range, however, could be mechanistically interpreted from the chain length dependence of the parameters for the relationship between the capacity factors and hetaeron concentration. Although the results clearly demonstrate that in the system investigated, ion-pair formation governs retention, ion-exchange mechanism can be operative under certain conditions. Changes in retention upon addition of salt to the eluent are treated both theoretically and experimentally. The effect of organic solvents on the behavior of the chromatographlc system is discussed in view of the proposed theory.

According to the popular notion, the selectivity in chromatographic separations is determined by the differences in the equilibrium retribution of eluite molecules, i.e. analytes, between the stationary and mobile phases. Quite frequently, however, secondary equilibria between the eluites and certain species present in the eluent can drastically affect retention *(1).* In a recent paper (2) we analyzed the effect of protonic equilibria in the mobile phase on retention in liquid chromatography with nonpolar stationary phases. The selectivity of the chromatographic system for ionogenic eluites was shown to be greatly influenced by their dissociation constant and the hydrogen ion concentration in the eluent because the binding of protons increases and decreases the retention of weak acids and bases, respectively.

Recent work has demonstrated that retention of charged eluites on nonpolar bonded stationary phases can be augmented by the presence of suitable counterions, which have a substantial hydrophobic moiety, in the mobile phase (3-5). This technique is often referred to as "soap" or "ion-pair reversed-phase" Chromatography. The counterions used in the mobile phase belong in the group of detergents such as alkyl sulfonates and sulfates or tetraalkylammonium compounds, and ion-pair formation between the eluite and counterion is assumed to be responsible for the increase in retention (6).

This approach is particularly interesting because the use of nonpolar bonded stationary phases for liquid chromatographic separations has a wide currency (7). In most cases octadecyl-silica is the stationary phase and hydro-organic mixtures with methanol or acetonitrile as well as neat aqueous or organic solvents are used as eluents. The technique is simple and can be used for the separation of a wide variety of substances. For this reason it is the most popular method in high performance liquid Chromatography. The mechanism of retention is believed to be the same as involved in the so-called hydrophobic effect (8) and we recently adapted the solvophobic theory (9) to treat the interaction of eluites with the hydrocarbonaceous functions of bonded phases in a rigorous thermodynamic fashion (2,10). We have shown that the magnitude of retention is governed by the effect of the solvent on these species and their adducts.

The increasing popularity of using an ion-pair forming agent in the mobile phase to increase the retention of oppositely charged eluites prompted us to investigate the fundamental aspects of this technique. Our treatment, however, is quite general and applicable to a variety of cases where the retention of an eluite on nonpolar bonded phases is enhanced by complex formation with a component, of the eluent. For sake of convenience we propose to call the complexing agent *hetaeron,* a term derived from the Greek work for companion *(εταιρον).* Thus "hetaeric" Chromatography would denote a technique in which a certain concentration of a complexing agent is intentionally maintained in the mobile phase in order to affect the selectivity of the chromatographic system by secondary equilibria. The name should be restricted to situations where the eluite-hetaeron complex is formed in the mobile phase and distributed between the two phases. Of course, secondary equilibria may change the properties of the stationary phase. Indeed, it was recently suggested (11) that in ion-pair reversed-phase Chromatography the stationary phase acts as a dynamically coated ion exchanger because of the adsorption of the detergent ions. By using extensive experimental data and applying the solvophobic theory for the interpretation of the results, we intend to demonstrate in this article that in the situations examined the mechanism of the chromatographic process entails ion-pair formation in the mobile phase and binding of the neutral complex to the stationary phase.

THEORY

Phenomenological Treatment. In order to shed light on the relationship between the capacity factor, which is a convenient measure of retention, and the equilibrium constants, which govern the retention on nonpolar bonded stationary phases in the presence of various concentrations of a complexing agent (hetaeron) in the mobile phase, the process first will be treated phenomenologically.

Figure 1 illustrates the various equilibria which are involved in the chromatographic process. The eluite, E, whose retention is of interest, can interact with the hetaeron, *H,* to form a complex, E*H,* which is bound to the hydrocarbonaceous ligand, L, of the stationary phase to form LE*H*. Alternatively, the hetaeron may bind first to the stationary phase and then form LE*H*. In addition the species E and *H* can individually form the adducts LE and L*H* with the ligands of the stationary phase. It is assumed that binding of the eluite and the heteron

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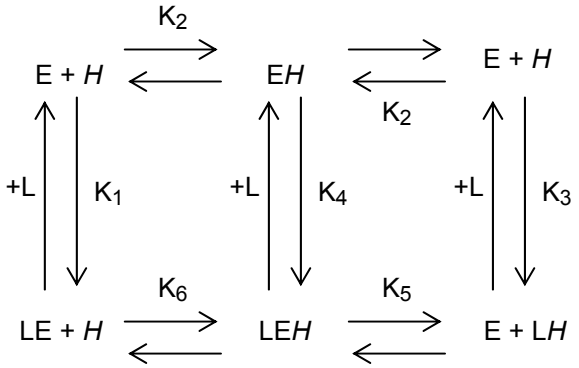

Figure 1. Schematic illustration of the equilibria Involved in the chromatographic process with nonpolar bonded stationary phases and a complexing agent in the mobile phase. The meaning of the symbols is: E, eluite; *H,* complexing agent (hetaeron); L, hydrocarbonaceous ligand bound to the support; K_1 to K_6 are the corresponding equilibrium constants

to the stationary phase ligands takes place independently. The equilibrium constants are expressed by the following set of equations in which the species concentrations in the mobile and stationary phase are denoted by the subscripts m and s, respectively. The concentration of L is defined as the accessible ligand concentration in the stationary phase.

$$K_1=[\mathrm{LE}]_s/[\mathrm{E}]_m[\mathrm{L}]_8 \quad (1)$$

$$K_2=[\mathrm{E}H]_m/[\mathrm{E}]_m[H]_m \quad (2)$$

$$K_3=[\mathrm{L}H]_s/[H]_m[\mathrm{L}]_s \quad (3)$$

$$K_4=[\mathrm{LE}H]_s/[\mathrm{E}H]_m[\mathrm{L}]_s \quad (4)$$

$$K_5=[\mathrm{LE}H]_s/[\mathrm{L}H]_s[\mathrm{E}]_m \quad (5)$$

$$K_6=[\mathrm{LE}H]_s/[\mathrm{LE}]_s[H]_m \quad (6)$$

The capacity factor of the eluite, k, is defined in the usual way as

$$k = ø([\mathrm{LE}H]_s + [\mathrm{LE}]_s)/([\mathrm{E}]_m + [\mathrm{E}H]_m) \quad (7)$$

where $ø$ is the phase ratio, i.e. the ratio of the volume of stationary phase to the volume of the mobile phase in the column.

In chromatographic practice when the column is equilibrated with the eluent the hetaeron concentration in the mobile phase is constant. If $[\mathrm{E}] \ll [H]_m$, only a negligible fraction of the hetaeron is in the form of a complex so that the hetaeron concentration can be considered invariant and we can write

$$[H]_m \equiv [H]_m \quad (8)$$

Since the extent of binding of the eluite by the stationary phase is expected to be small and the total ligand concentration $[\mathrm{L}]_T$ is conserved, we may write that

$$[\mathrm{L}]_T = [\mathrm{L}]_s + [\mathrm{L}H]_s \equiv [\mathrm{L}] \quad (9)$$

There are several ways to evaluate from Equations 1-6 a combination of the equilibrium constants which govern the chromatographic process. If we assume that the eluite is bound by the stationary phase as its complex with the hetaeron, which is formed in the mobile phase, then the combination of Equations 1-4 and 7-9 yields the following expression for the capacity factor

$$k = ø[\mathrm{L}](K_1 + K_2K_4[H])/(1 + K_2[H])(1 + K_3[H]) \quad (10)$$

The assumption that complex formation occurs with the hetaeron already bound to the stationary phase yields, by the combination of Equations 1-3, 5 and 7-9 the following expression for the capacity factor.

$$k = ø[\mathrm{L}](K_1 + K_3K_5[H])/(1 + K_2[H])(1 + K_3[H]) \quad (11)$$

A third possible combination uses Equations 1-3 and 6-9 and yields

$$k = ø[\mathrm{L}](K_2 + K_1K_6[H])/(1 + K_2[H])(1 + K_3[H]) \quad (12)$$

Equation 12 would imply that the eluite first binds to the stationary phase and then forms a complex with the hetaeron. Equations 10-12 all express the dependence of the capacity factor on the hetaeron concentration and have the general form

$$k = (k_0 + B[H])/(1 + K_2[H])(1 + K_3[H]) \quad (13)$$

where k_0 is the capacity factor of the eluite in the absence of hetaeron, K_2 is the association constant for the eluite and the hetaeron, K_3 is the binding constant of the hetaeron to the stationary phase and B is the product of the two equilibrium constants as shown in Equations 10-12. A plot of k vs. $[H]$ according to Equation 13 yields a parabola provided $1/K_3[H] < 1/K_2$.

If either $K_2[H] \ll 1$ or $K_3[H] \ll 1$, Equation 13 can be written as

$$k = (k_0 + B[H])/(1 + \mathrm{P}[H]) \quad (14)$$

where P can be either K_2 or K_3. Equation 14 is the equation of a rectangular hyperbola, for the dependence of the capacity factor on the hetaeron concentration. Both parabolic and hyperbolic dependence of the capacity factor on the hetaeron concentration have been observed in ion-pair reversed phase chromatography (5, *6).*

When the complex is an ion-pair, both the eluite and the hetaeron have to be fully ionized for the above treatment to be valid. Whereas the hetaeron is usually a strong electrolyte in ion-pair chromatography, the eluites are often weak bases or acids and therefore the pH of the eluent can have an influence on the retention. The corresponding protonic equilibria can readily be incorporated into the above model as will be shown by the example of a weakly basic eluite.

The protonation of the neutral eluite, E°, is characterized by Its acid dissociation constant, K_a, related to the equilibrium

$$\mathrm{E}^0 + \mathrm{H}^+ \leftrightarrows \mathrm{EH}^+ \quad (15)$$

Both forms, E^0 and EH^+, can bind to the ligands of the stationary phase according to the following equilibria

$$\mathrm{E}^0 + \mathrm{L} \leftrightarrows \mathrm{LE}^0 \quad (16)$$

and

$$\mathrm{EH}^+ + \mathrm{L} \leftrightarrows \mathrm{LEH}^+ \quad (17)$$

The equilibrium constants corresponding to Equations 16 and 17 are denoted by K_1^0 and K_1, respectively.

In this case, however, only the protonated eluite molecules can form an ion-pair [HE*H*] with the hetaeron-counterion and bind as a complex, [LHE*H*], to the stationary phase. Consequently, mass balance yields the following expression for the capacity factor

$$k = ø\ \frac{[\mathrm{LHE}H]_s + [\mathrm{LE}]_s + [\mathrm{LEH}^+]_s}{[\mathrm{E}]_m + [\mathrm{EH}^+]_m + [\mathrm{HE}H]_m} \quad (18)$$

Following the previous approach and using Equations 1-4 and 15-17 to substitute the equilibrium constants into Equation 18, we obtain for the capacity factor the expression

$$k = \text{ø}[\text{L}] \frac{\frac{K_1^0 K_a}{(K_1 + [\text{H}^+] + K_2 K_4[\text{H}])_s}}{(1 + \frac{K_a}{[\text{H}^+]} + K_2[\text{H}])(1 + K_3[\text{H}])} \quad (19)$$

Equation 19 can also be written in a form similar to that of Equation 13, but in this case the magnitude of the parameters would also be dependent on the acid dissociation constant of the eluite and the hydrogen ion concentration in the mobile phase.

Enhancement Factor. Experimental data show that in ion-pair reversed phase chromatography the dependence of the capacity factor on the detergent concentration often follows hyperbolic behavior (6) such as represented by Equation 14. According to this expression the two limiting values of the capacity factor are k_0 and B/P at zero and at sufficiently high hetaeron concentrations, respectively. The ratio of the two quantities gives the highest possible amplification of the capacity factor due to the presence of the hetaeron. It is termed the enhancement factor, η, and given by

$$\eta = B/k_0 P \quad (20)$$

where k_0 is the capacity factor of an eluite in the absence of hetaeron, P is either the stability constant of the eluite hetaeron complex or the equilibrium constant for the binding of the hetaeron to the stationary phase and the physical meaning of B also depends on the particular mechanism which governs eluite retention in the presence of the hetaeron. We shall see later how η can be used for both the elucidation of mechanism and the practical selection of a hetaeron.

Mechanistic Implications of the Solvophobic Theory. In view of the preceding section, on a closer examination of the process, the retention of the eluite in ion-pair chromatography on nonpolar bonded phases can occur either by *"dynamic ion-exchange",* i.e., ion-pair formation takes place between the eluite and the hetaeron bound to the stationary phase, or by *ion-pair formation in the mobile phase* and binding of the complex to the nonpolar stationary phase. Since a phenomenological approach cannot distinguish between the two cases on the basis of the dependence of the capacity factor on the hetaeron concentration, we shall use the solvophobic theory to estimate the relative magnitude of the equilibrium constants on the basis of the molecular properties of the hetaeron and eluites.

The solvophobic theory was developed to describe the effect of solvent on chemical phenomena (*9*) and has successfully accounted for inter alia, the solubility of small nonelectrolytes in water and other solvents (*12*) and the effect of solvent variation on reaction rates for several different chemical reactions (*13*). It is not restricted to water as the solvent and expresses the energetics of the solvent effect in terms of, at least in principle, measurable properties of the solute and solvent, unlike other theories for the hydrophobic effect.

We have recently adapted this approach to quantitatively treat the effect of eluite and eluent properties on chromatographic retention using polar solvents, especially water, and a nonpolar stationary phase (*2,10*). In our model, we assumed that, the chromatographic process entails a reversible association of a solute, S, with the hydrocarbonaceous ligand, L, of the stationary phase to form a complex SL. The logarithm of the corresponding equilibrium constant for a nonionized solute, K_1^0, was expressed for fixed column and eluent properties at a given temperature by Equation 47 in Ref. (10)

which for our purpose can be written in a simplified form as

$$\ln K_l^0 . a - b + c \in A \quad (21)$$

where *a, b,* and *c* can be regarded as constants dependent upon solvent and column properties; *b* also depends on the eluite properties such as dipole moment, polarizability and molecular volume. $\in$A is the difference between the molecular surface area of the complex, A_{SL}, and those of the eluite, A_S, and the hydrocarbonaceous ligand, A_L, so that

$$\in A = A_{SL} - A_S - A_L \quad (22)$$

For the capacity factor of an ionized solute, the following simplified expression can be derived from Equation 20g of Ref. (2)

$$\ln K_l = a' + b' f(Z) + c' \in A \quad (23)$$

where *a', b',* and c' are again solvent and column dependent parameters. The effect of charge on the solute molecule is represented by the function $f(Z)$ which goes approximately as the absolute value of the product of charges on the ion and its counterion.

The simplified expressions in Equations 21 and 23 allow us to make some qualitative and semiquantitative statements regarding the constants lumped together in the enhancement factor as far as the mechanism of ion-pair reversed-phase chromatography is concerned. It is recalled that the parameters k_0, B, and P in Equation 20 are directly related to the equilibrium constants defined in Equations 1-5.

As k_0 is the capacity factor of the eluite in the absence of hetaeron, in view of Equations 1,10, and 14 we may write that

$$\ln k_0 = \ln(\text{ø}[\text{L}]\, K_1) = a' + b' f(Z) + c \in A \quad (24)$$

Obviously the value of k_0 is independent of any kind of ion-pair formation. On the other hand, the meaning of the constants B and P in Equation 20 is dependent on the actual mechanism of the chromatographic process.

The energy of any electrostatic interaction and hence the logarithm of the corresponding equilibrium constant depends upon the product of the charges on the interacting species. Thus, for the ion-pair formation in the mobile phase the equilibrium constant, K_2, in Equation 2 can be expressed by

$$\ln K_2 = f'(Z_E Z_H) + \text{const.} \quad (25)$$

where Z_E and Z_H are the charges on the eluite and hetaeron, respectively. Similarly, in the case of dynamic ion-exchange, represented by Equation 5, the equilibrium constant, K_5, for the interaction between the eluite and the hetaeron bound to the stationary phase can be expressed by

$$\ln K_5 = f''(Z_E Z_H) + \text{const.} \quad (26)$$

The other equilibrium constants of interest, K_3 and K_4, correspond to the binding of the hetaeron and the complex to the stationary phase ligands, as shown by Equations 3 and 4, respectively. According to the solvophobic theory, both equilibrium constants can be expressed as a function of the decrease in the molecular surface area upon binding of the species to the stationary phase. Thus, by using Equation 23, we can write for the equilibrium constant representing the binding of the charged hetaeron, that

$$\ln K_3 = a' + b' f(Z_H) + c' \in A_3 \quad (27)$$

On the other hand, the equilibrium constant for the binding of the neutral ion-pair can be expressed from Equation 21 as

$$\ln K_4 = a - b + c \in A_4 \quad (28)$$

Table I. Relationship between Hetaeron Properties and the Parameters of Equation 14 as Predicted for the Two Limiting Mechanisms in Ion-Pair Reversed-Phase Chromatography

Paramete	Ion-pair formation occurs in the mobile phase	Ion-pair formation occurs in the stationary phase
k_0	...	...
B	hydrophobic surface area (carbon number)	hydrophobic surface area (carbon number)
P	charge type (P=K2)	hydrophobic surface (carbon number) area + charge type (P=K3)
B/k_0P	hydrophobic surface area (carbon number)	charge type

According to the previous discussion the meanings of $\in A_3$ and $\in A_4$ in Equations 27 and 28 are different and given by

$$\in A_3 = A_{HL} - A_H - A_L \quad (29)$$

and

$$\in A_4 = A_{HEL} - A_L - A_{HE} \quad (30)$$

where A_i is the molecular surface area of the species i denoted by the subscripts.

We can express the enhancement factor, n, by two different combinations of the equilibrium constants. In the first case, assuming dynamic ion exchange we obtain from Equations 11, 14, 20, 24, and 26 that

$$\ln \eta = \ln (K_5/K_1) = \text{const.} + f''(Z_E Z_H) - f(Z_E) - c(A_{LEH} - A_{EL} - A_{LH}) \quad (31)$$

In the second case, when ion-pair formation in the mobile phase dominates, the enhancement factor can be expressed by using a similar combination of the pertinent equations as

$$\ln \eta = \ln (K_4/K_1) = \text{const.} - f(Z_E) + c(A_{HEL} - A_{HE} + A_E - A_{LE}) \quad (32)$$

From the results presented earlier (2) we know that

$$A_{EL} - A_E - A_L \propto A_E \quad (33)$$

Since this relationship is expected to hold for all species under investigation we can write for the last term in Equation 32 that

$$A_{HEL} - A_{HE} + A_E - A_{LE} \propto (A_{HE} - A_E) \quad (34)$$

In other words the last term in Equation 32 depends upon the difference in the surface area of the complex and eluite. If the complex is formed to maximize the electrostatic effect, this difference is very nearly the surface area of the hetaeron, A_H, alone. Hence, if the retention proceeds primarily by the formation of ion-pairs in the mobile phase, the enhancement factor will depend upon the surface area of the hetaeron as

$$\log \eta \propto A_H \quad (35)$$

Consequently, in the case of normal alkyl sulfates or sulfonates $\log \eta$ is expected to be proportional to the carbon number of the hetaeron.

On the other hand, no such a dependence is expected in the "ion-exchange" mechanism. Indeed, the relevant hetaeron property, as seen from Equation 30, is a function of its charge, $f(Z_H Z_E)$, only. In view of these relationships, the analysis of experimental data obtained with hetaerons containing the same ionic groups but different alkyl chain lengths can shed light on the actual mechanism of the process. The dependence of the enhancement factor on the properties of the hetaeron and eluite is shown in Tables I and II, which summarize the conclusions of this approach.

Table II. Relationship between Eluite Properties and the Parameters of Equation 14 as Predicted for the Two Limiting Mechanisms with Ion-Pair Formation

Parameter	Ion-pair formation occurs in mobile phase	Ion-pair formation occurs in stationary phase
k_0	charge and hydrophobia surface area	charge and hydrophobic surface area
B	charge and hydrophobia surface area	charge and hydrophobic surface area
P	charge (P = Kt2)	...
B/k_0P	charge	charge and hydrophobic surface area

EXPERIMENTAL

A Model 601 (Perkin-Elmer, Norwalk, Conn.) high pressure liquid chromatograph was used with a Model 7010 sampling valve (Rheodyne, Berkeley, Calif.), a Model FS 770 (Schoeffel, Weetwood, N.J.) variable wavelength detector at 254 nm, and a Perkin-ElmerModelR-56 recorder were used, Partisil 1025 ODS (Whatman, Clifton, N.J.) columns packed with 10-μm octadecyl-silica containing about 5% (w/w) carbon were used in the study of hetaeron behavior. The chromatogram in Figure 2 was obtained with a LiChrosorb RP-18 column (Rainin, Boston, Mass.) packed with 5-μm octadecyl-silica. All columns were 250 mm long and had 6.4 mm o.d. and 4.6 mm i.d. Most experiments were carried out by isocratic elution using neat aqueous 5 x 10^{-2} M phosphate buffer, pH 2.5, and the hetaerons were also dissolved in this buffer. In most cases the flow rate and the column temperature were 2 mL/min and 40 °C, respectively. Some experiments were carried out with hetaerons dissolved in mixtures of methanol and the above mentioned phosphate buffer as the eluent.

Catecholamine derivatives were obtained from Aldrich (Milwaukee, Wis.) or Schwartz/Mann (Orangeburg, N.Y.) and reagent grade H_3P0_4 and KH_2PO_4 were supplied by Fisher (Pittsburgh, Pa.). The alkyl sulfates and hexylsulfonate used were Eastman products (Rochester, N.Y.), whereas the alkyl phosphates were gifts from Hooker Chemicals Corp. (Buffalo, N.Y.). The perfluorated carboxylic acids were purchased from Aldrich. Methanol was "distilled in glass" from Burdick and Jackson (Muskegon, Mich.).

Retention times were measured from the distance between the injection point and the peak maxima on the chromatogram. The mobile phase hold-up times were measured as described previously (2) and the capacity factors have been calculated in the usual way (14).

The analysis of the data was performed on a PDP 11/10 minicomputer equipped with a RX01 floppy disc, a VT55 display unit, and a Decwriter. The computer program used for parameter estimation by the least squares method was written in BASIC language.

The symbols used in this study for the sample components are as follows: DOPA, 3,4-dihydroxyphenylethylamine (dopamine); EP, l-(3,4-dihydroxyphenyl)-2-(methylamino)ethanol (epinephrine, adrenaline); OP, l-(4-hydroxyphenyl)-2-aminoethane (octopamine); NE, 2-amino-l-(3,4-dihydroxyphenyl)ethanol (norepinephrine, noradrenaline); DOS, 3,4-dihydroxyphenylserine.

RESULTS AND DISCUSSION

A typical chromatogram in Figure 2 illustrates the separation of certain catecholamines on octadecyl-silica in the absence and in the presence of n-octylsulfate in the neat aqueous phosphate buffer used as the eluent. As the chemical nature and concentration of the alkyl sulfate have great

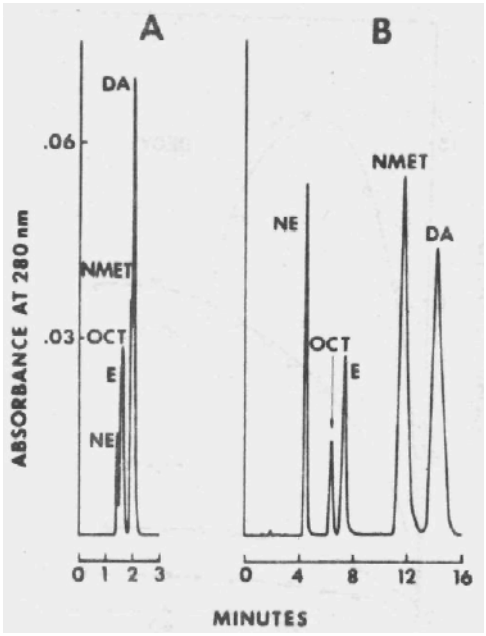

Figure 2. Chromatograms illustrating the effect of ion-pair formation with *n*-octylsulfate in the eluent on the separation of catecholamines by reversed-phase chromatography. Column, 5 μm LiChrosorb RP18; flow rate, 2,0 mL/min; temp., 70 °C; inlet pressure, 2200 psi. Eluents: A, 5 X 10^{-2} M phosphate in water, pH 2.2; B, 5 X 10^{-2} M phosphate and 3 X 10^{-3} M octylsulfate in water, pH 2.2

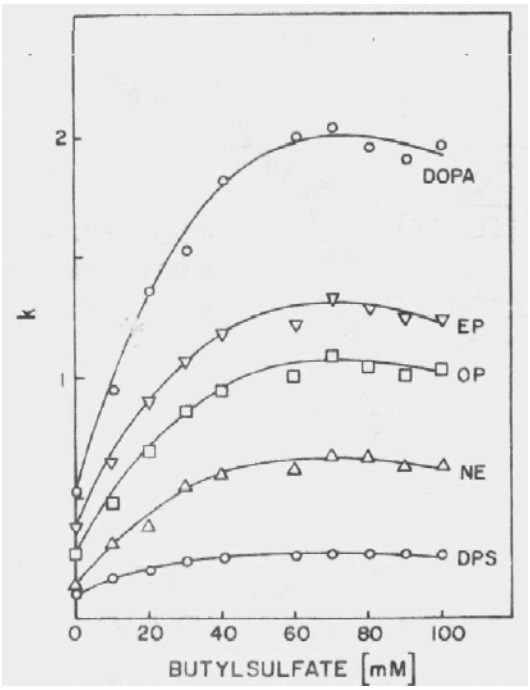

Figure 3. Dependence of the capacity factor of protonated catechol amine derivatives on the concentration of n-butyisulfate in the eluent, Column, 10 μm Partisil ODS; flow rate, 2.0 mL/min; temp., 40°C; inlet pressure. 400 psi; eiuent, 5 X 10^{2} M phosphate in water. pH 2.55. containing various concentrations of the hetaeron

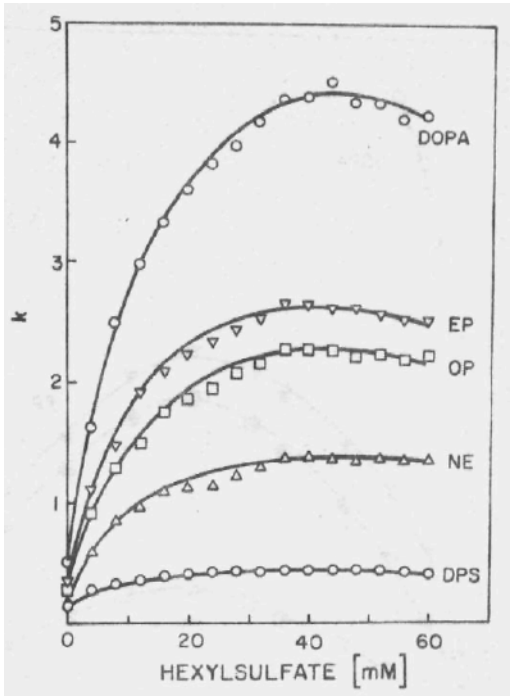

Figure 4. Dependence of the capacity factor of charged catecholamine derivatives on the concentration of n-hexylsulfate in the neat aqueous mobile phase. Conditions are given in Figure 3

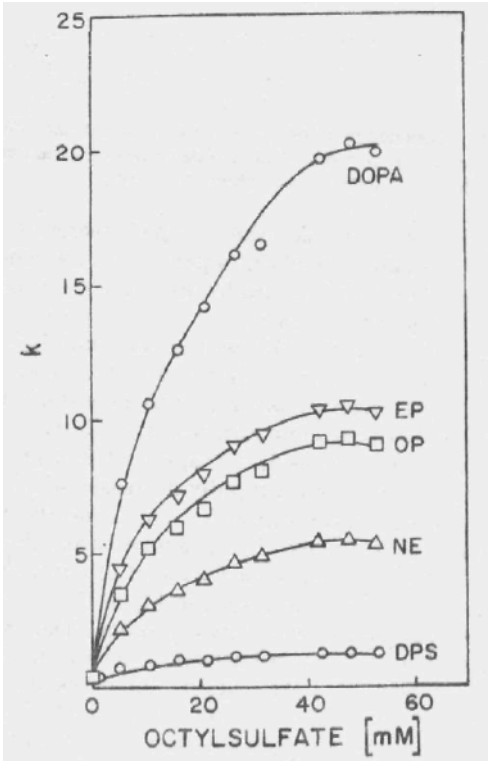

Figure 5. Dependence of the capacity factor of charged catechoiamtne derivatives on the concentration of o-octyisulfale In the neat aqueous mobile phase. Conditions are given in Figure 3

influence on the retention of the eluites, experiments were carried out in a wide range of conditions in order to shed light on the chromatographic process in view of the preceding theoretical anaysis. In addition to alkylsulfates of different chain lengths, hexylsulfonate as well as butyl- and amyl-phosphates were also employed.

Equation 13 predicts that the observed capacity factor will initially increase with increasing hetaeron concentration followed by a monotonic decrease at high hetaeron concentrations. However, if over the experimentally accessible range of hetaeron concentrations, either the binding of hetaeron to the stationary phase, $K_3[H]$, or the extent of ion-pair formation in the mobile phase, $K_2[H]_t$ *is* negligible, the capacity factor can be expressed by Equation 14. In this case the capacity factor first rises and eventually becomes practically independent of the hetaeron concentration. Therefore, if Equation 14 holds over the experimental range of hetaeron concentration, a plot of k vs. $[H]$ yields a rectangular hyperbola. The capacity factor of four catecholamines and the amino acid, 3,4-dihydroxyphenylserine, as a function of the concentration of various n-alkyl sulfatee in the eluent is shown

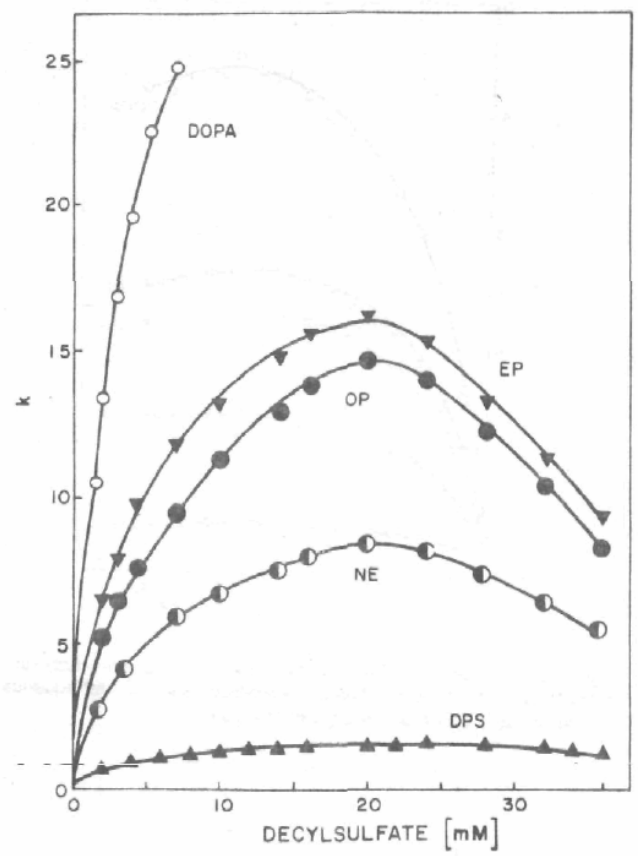

Figure 6. Dependence of the capacity factor of charged catecholamine derivatives on the concentration of *n*-decylsulfate In the neat aqueous mobile phase. Conditions are given in Figure 3

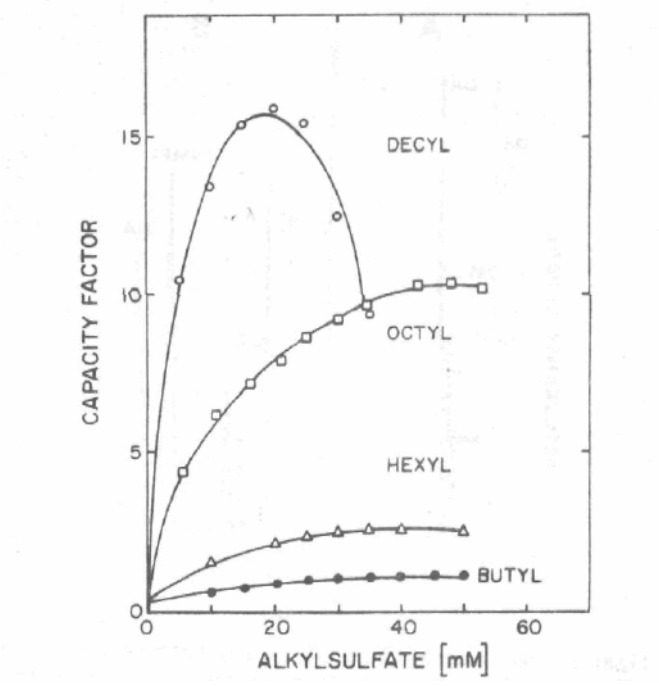

Figure 7. Plots of the capacity factor of adrenaline vs. the hetaeron concentration for various n-alkylsulfates. Conditions are given In Figure 3

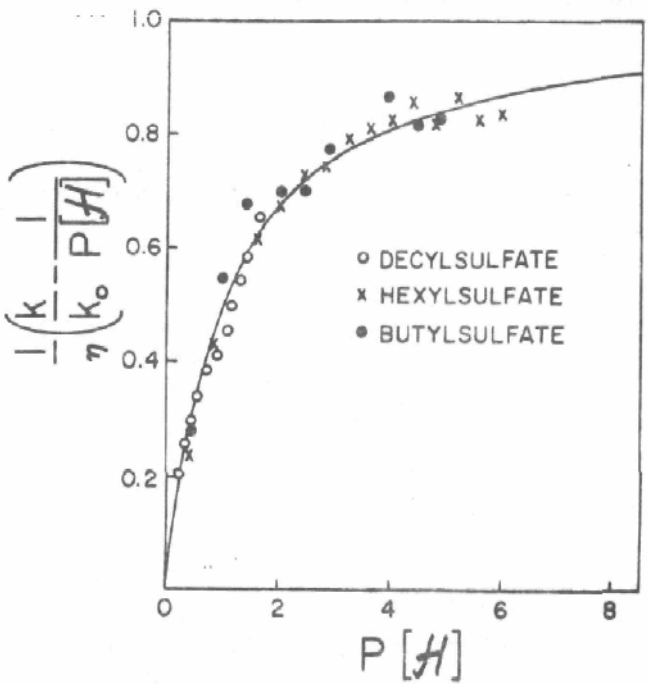

Figure 8. Normalized plot of the capacity factor data obtained for dopamine using three different n-alkyl sulfates as the hetaerons. The theoretical curve calculated from Equation 41 is given by the solid line. The data were obtained under conditions described in Figure 3

in Figures 3-6. The alkyl chain of the hetaerons ranges from butyl to decyl groups and the upper limit of the concentration range was usually determined by the solubility of the hetaeron in the neat aqueous eluent.

Inspection of the data shows that with butyl-, hexyl-, and decylsulfates the capacity factor in most cases rises with increasing hetaeron concentration to a constant value from which it does not decline significantly. On the other hand when decylsulfate is used, the capacity factor increases to a maximum from which it rapidly decreases with further increase in the hetaeron concentration. Thus, the qualitative predictions are supported by the data as is also illustrated by the dependence of the capacity factor of adrenaline on the concentration of the hetaerons in Figure 7.

In order to test the validity of Equations 13 and 14, the data shown in Figures 3-6 were analyzed by a least-square fit. The data obtained using butyl-, hexyl-, and octylsulfates did not converge by using Equation 13 but they did fit Equation 14. On the other hand, the data obtained using decylsulfate could be well fitted to Equation 13, whereas the fit to Equation 14, was very poor.

The quality of the fit can be illustrated by a generalized plot of the data. Equation 14 is divided by k_{0t} the expression can be rearranged to obtain

$$(k/k_0\eta) - [1/\eta(1 + P[H])] = P[H]/(1 + P[H]) \quad (36)$$

The RHS of Equation 36 represents a normalized capacity factor corrected for the effect of unconjugated eluite binding. When it is plotted against the normalized hetaeron concentration, $P[H]$, a rectangular hyperbola should be obtained. Such a plot of data obtained with butylsulfate, hexylsulfate, and decylsulfate as hetaerons and DOPA as the eluite is shown in Figure 8. In the case of other acidic hetaerons such as hexylsulfonate, butyl- and amylphosphates in neat aqueous eluents, the relationship between the capacity factors measured with these eluites and the hetaeron concentration was found to conform well to Equation 13.

We noted in the theoretical section that the dependence of the capacity factor on the hetaeron concentration alone does not shed light on the actual mechanism of the process. However, as one changes from one hetaeron to another, both representing the same type of compounds, predictions of the effect on the capacity factor can be made by recourse to solvophobic theory as shown in Tables I and II. If ion-pair formation occurs in the mobile phase, the enhancement factor will depend strongly on the hydrophobic area of the hetaeron

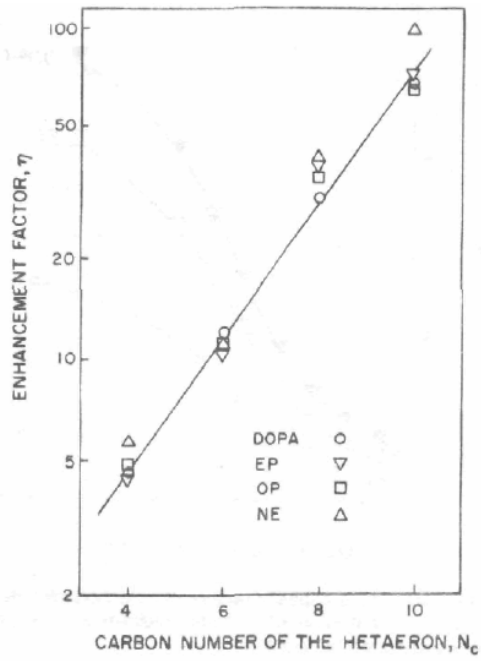

Figure 9. The dependence of the enhancement factor of catecholamine derivatives on the carbon number of n-alkyl sulfates as the hetaerons. The straight line obtained by least squares analysts fits the expression, log η = 0.225 (±0.0317) N_c, where η is the enhancement factor and N_c is the carbon number. The intercept is zero within experimental error

and the charge of eluite but it will be independent of the size of the eluite. On the other hand, if ion-pair formation occurs on the stationary phase, i.e., in the case of the ion-exchange mechanism, the enhancement factor will depend on the charge of the hetaeron and the charge and size of the eluite.

The predictions based on the solvophobic treatment of chromatographic retention support the concept that in our experiments soap chromatography proceeds through the formation of ion-pairs in the mobile phase followed by adsorption onto the nonpolar stationary phase. The logarithm of the enhancement factor evaluated with the least-squares parameters of Equation 14 is linear in the carbon number of the hetaeron and largely independent of the size of the eluite as illustrated in Figure 9. This is predicted for the ion-pairing mechanism according to Equation 35, whereas no dependence on the hetaeron size but a dependence on the molecular area of the eluite is expected for the ion-exchange mechanism. The constant P of Equation 14 should be equal to K_2 and independent of hetaeron size in the ion-pair mechanism but will depend upon the charge of both eluite and hetaeron. It will also be dependent upon the charge type since the distance of closest approach of the two ions is implicitly included in the function $f(Z_E Z_H)$ in Equation 25 and 26.

The mean values of the pertinent parameters as obtained by least squares analysis are shown in Table III. The standard deviation of the values is also indicated. The capacity factor of the eluite in the absence of hetaeron, k_0, is not shown because it is not a property of the hetaeron. It is seen from the data in Table III that the enhancement factors are fairly independent of the eluite, but they strongly depend and, in fact, increase exponentially with the carbon number of the hetaeron in agreement with the data shown in Figure 9. The only exception to this rule is 3,4-dihydroxyphenylserine which was not entirely in the cationic form at the eluent pH employed. However, we can estimate the enhancement factor of the cationic form by using Equation 19. Assuming a value of 2.8 for K_1^0/K_1 on the basis of data obtained with similar eluites in a previous work (2), we obtain corrected enhancement factors for the amino acid which fall into the range of n shown in Table III for the fully protonated amines.

given hetaeron at the saturation level may be too great or

Table III. List of the Parameters of Equation 13 and the Enhancement Factors us Evaluated from Experimental Data Obtained at Various Hetaeron Concentrations in the Mobile Phase on Octadecyl- Silica Column. The Eluitesa Were the Same as Those in Figures 3-7

Hetaeron	$K_2[M]^{-1}$, Ion-pair formation constant	$K_3[M]^{-1}$ Hetaeron binding constant	n = B/k0K2, Enhancement factor
Alkyl sulfates			
Butyl	46 ± 3	· · ·	4.9 ± 0.6
Hexyl	107 ± 8	· · ·	11.1 ± 0.6
Octyl	68 ± 10	· · ·	36.6 ± 3.8
Decyl	125 ± 40	12	104 ± 30
Hexylsulfonate	68 ± 3	· · ·	12.1 ± 1.3
Alkyl phosphates			
Butyl	270 ± 77	· · ·	6± 1.4
Amyl	341 ± 72	· · ·	16.6 ± 3.7

On the other hand, in the ion-exchange mechanism, the constant P will depend upon the charge of the hetaeron and the size of the hetaeron. Even though the experimentally determined value of P ranges widely, it is apparent from an inspection of the data for the alkyl sulfates in Table III that the value of P does not depend upon hetaeron size in a strong fashion, if at all. If we consider the data obtained with hetaerons other than alkyl sulfates, this conclusion is reinforced. The value of the enhancement factor for hexylsulfonic acid is virtually identical to that for hexylsulfate in accord with their identical carbon numbers.

The results of the preceding analysis can be sustained without an explicit dependence on the solvophobic theory. In interactions between ionized species, one expects the magnitude of the effect to be determined by the charges on the species. On the other hand, retention by the hydrocarbonaceous stationary phase should depend upon the "hydrophobicity" of the species, i.e. on the carbon number or some related parameter. As a consequence, we expect the parameter P of Equation 14 to depend upon the charge and not the size of the hetaeron if ion-pair formation occurs in the mobile phase, whereas in the dynamic ion-exchange a strong size-dependence would be expected. The enhancement factor for ion-pairing depends upon the difference between the carbon number of the hetaeron-eluite complex and the carbon number of the eluite, since the ratio K_4/K_1 depends upon two binding constants. On the other hand the enhancement factor for ion-exchange would depend upon the ratio K_5/K_1 which corresponds to the process of ion-pairing in the stationary phase and eluite binding. As a consequence, the only hetaeron property affecting this ratio would be the charge.

The Enhancement Factor. As shown above, the enhancement factor may be justifiably regarded as a property peculiar to the hetaeron and largely independent of eluite properties. This conclusion is supported by both experimental results and theoretical considerations. As a consequence, it may be used as a guide to hetaeron selection or to extract information about the eluite which is otherwise experimentally inaccessible. In the following development, it is assumed that chromatographic experiments are carried out in the range of hetaeron concentration where the capacity factor is at the maximum or in the plateau region of the saturation curves where the effect of the hetaeron on the retention is the greatest. The advantage of working at saturation concentration is that the capacity factors are least sensitive to small errors in hetaeron concentration. On the other hand an error in the hetaeron concentration can generate a large error in the capacity factor at low hetaeron concentrations where the curve is almost linear. The capacity factor obtained with a

too small or the eluite-hetaeron complex may elute together

with another species whose retention is unaffected by the hetaeron under such conditions. The retention of the complex may be affected by choosing another hetaeron with a shorter or longer aliphatic sidechain, respectively, rather than by changing the hetaeron concentration.

By use of the enhancement factor, determined in an appropriate solvent system, one can readily calculate the effect of the alkyl chain length of the hetaeron upon the capacity factor of the eluite complex. In the example of the alkyl sulfates examined here in water, the effect of two additional carbons is to increase the capacity factor by a factor of 2.5. Thus, increasing or decreasing the carbon number of the alkyl chain can shift the capacity factor into the usually favored range of 1-10.

The analysis of the enhancement factor also allows the effect of simultaneously using two hetaerons of the same kind but having different chain lengths to be explicated. If the mobile phase contains two such hetaerons of equal concentration, the capacity factor is the arithmetic mean of the capacity factors which are obtained when the two are used alone at the same total molar concentration. On the other hand from the data shown in Figure 9 it follows that the capacity factor of a hetaeron, which has the arithmetic mean alkyl chain length of the two hetaerons, is given by the geometric mean of their capacity factors. If the chain lengths of the two hetaerons are not too different, the arithmetic and geometric mean capacity factors are about the same. For example, a 1:1 mixture of hexyl- and octylsulfates would have an en-hancement factor of 24, whereas heptylsulfate at the same total hetaeron concentration would show an enhancement of 20. Therefore, a safe rule of thumb is that at the same molar concentration 8 mixture of hetaerons has the same capacity factor as that of a hetaeron which has their mean chain length.

In many cases it is difficult to obtain precise values for the capacity factors of eluites poorly retarded in the absence of a hetaeron. However, it may be desirable to determine the value of these capacity factors in physicochemical mea-surements. If the enhancement factor is available from ex-periments, the capacity factor in the absence of hetaeron can be estimated as the quotient of the observed capacity factor to the enhancement factor. Alternatively, an equivalent calculation is

$$k_0 \,.\, kk_{0,\mathrm{R}}/k_{\mathrm{R}} \qquad (37)$$

where k_0 is the capacity factor in the absence of hetaeron, $k_{0,\mathrm{R}}$ is the capacity factor of a reference eluite obtained in the absence of hetaeron, and k and k_R are the observed capacity factors of the test and reference eluite in the presence of hetaeron.

Depletion of Hetaeron. In one case we have observed a sigmoidal dependence of the capacity factors on the hetaeron concentration as shown in Figure 10. Under the conditions of the experiment, the concentration of dodecysulfate, which is sparingly soluble in aqueous salt solutions, was below the concentration range used in this study for the other alkyl-sulfates having relatively short hydrocarbon chains.

The observed behavior can readily be explained by a break-down of the theoretical model at sufficiently low hetaeron concentrations. When the concentrations of the hetaeron and eluite are commensurable, a significant fraction of the hetaeron can be complexed so that the total hetaeron concentration in the mobile phase is not conserved, i.e., the assumption stated in Equation 8 is not fulfilled. The non-linearity arising from a depletion of the dodecylsulfate ions by the eluites at very low hetaeron concentrations can be responsible for the observed sigmoidal behavior.

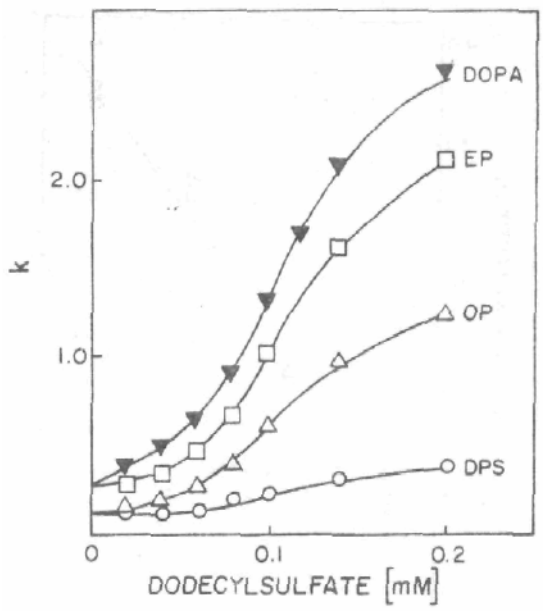

Figure 10. Plots of the capacity factors of catecholamine derivatives against the concentration of *n*-dodecylsulfate as the hetaeron. Conditions are stated in Figure 3

Salt Effects. If salt is added to the eluent, the model shown in Figure 1 can be extended by the equilibria related to the salt-eluite complex. In general, added salts would tend to reduce the capacity factor because they would compete with the hetaeron in forming ion-pairs with the eluite. Equation 10 can be modified in order to take into account salt competition in the calculation of the capacity factor as follows:

$$k = \phi[\mathrm{L}](K_1 + K_2K_4[H] + K_7K_8[\mathrm{S}])/(1 + K_7[\mathrm{S}] + K_2[H]) \qquad (38)$$

where K_7 is the equilibrium constant for the formation of salt-eluite complexes, K_8 is the binding constant of the complex to the stationary phase, and [S] is the salt concentration. The values of K_7 and K_8 are unknown. We can estimate, on the basis of the change in capacity factor of acids upon ionization, that K_8/K_1 . 3.5 (2). Since in the case of the hydrophobic hetaerons investigated the capacity factor is much greater than this value, according to Equation 38, the capacity factor is expected to fall precipitously with addition of salts until it descends to a value which is unaffected by the addition of more salts. Data obtained with several eluites and the use of decylsufate as the hetaeron are shown in Figure 11, where the capacity factor is plotted as a function of $(NH_4)_2SO_4$ concentration. As expected from Equation 38 and the above discussion, the capacity factors rapidly decrease with the salt concentration. However, the capacity factor at high salt concentrations does not reach a constant value but increases somewhat, The increase of retention on nonpolar stationary phases with increasing salt concentration has already been observed and explained in the rubric of the solvophobic theory by the increase in the surface tension of the mobile phase (2, *10).* In a rigorous treatment, each term of Equation 38 should be treated for ionic strength effects by an expression which has been used previously, Equation 20 in Ref. (2) and can be written as

$$\ln K = \ln K^0 + \alpha[BI^{1/3} + CI) + \beta I \qquad (39)$$

where *B, C, α,* and *β* are constants, *I* is the ionic strength, and *K* and K^0 are the equilibrium constants in the presence and absence of salt, respectively. However, at high salt and hetaeron concentrations we can assume that practically only

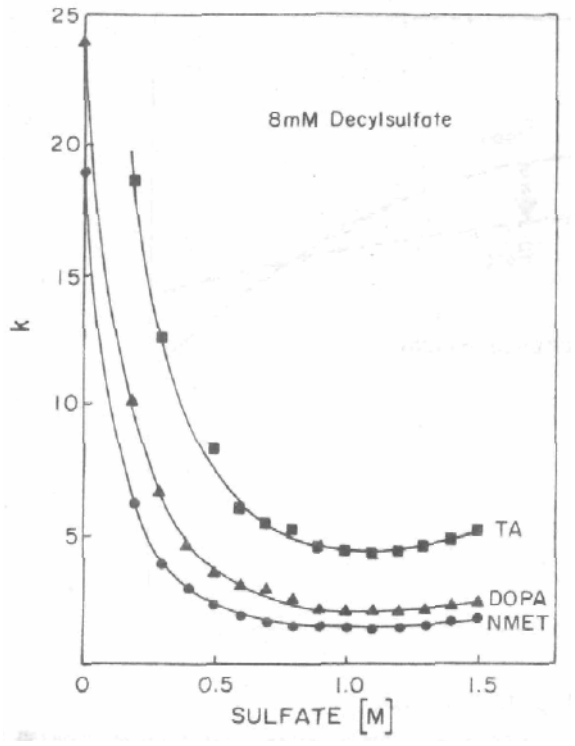

Figure 11. Effect of salt on the retention in ion-pair reversed phase chromatography with 8 X 10^{-3} M *n*-decylsulfate in the neat aqueous eluent. The chrorriatbgraphtc conditions are the same as those described in Figure 3 except that in addition various concentrations of Na_2S0_4 were used in the eluent

neutral complexes bind and, as a consequence, the expression can be rewritten as

$$\ln K = \ln K^0 + \beta' I \tag{40}$$

where

$$\beta' = \beta + \alpha C \tag{41}$$

Since the value of β' is practically constant for different eluites (2) Equation 38 can be rewritten as

$$k = \text{ø}[\text{L}][(1 + K_2K_4[H] + K_7K_8[\text{S}])/(1 + K_7[\text{S}] + K_2[H])] \exp(\beta' I) \tag{42}$$

The inspection of Figure 11 shows that the data conform to this expression.

The addition of inorganic acids such as H_2SO_4 and H_3PO_4 to the eluent has been observed to have an effect similar to that of added salt in agreement with the findings of others (5). The decrease in the capacity factors can be readily explained by the competition between the organic and inorganic anions to form ion-pairs with the eluites. At sufficiently high concentrations, however, a strong inorganic acid may also reduce the degree of dissociation of the hetaeron acid, and thereby further diminish retention. Of course, if such a hetaeron would be used for the separation of basic eluites at an eluent pH where they are partially or fully undissociated, the addition of an acid first would result in a reduction of pH and a concommitant protonation of the eluitss. Under such conditions the observed capacity factors would increase as expressed by Equation 19 before a decrease occurs upon further increasing the acid concentration.

Nature of the Hetaeron. Among the anionic hetaerons investigated, the use of alkyl sulfates and sulfonates is the most convenient according to our experience. As shown previously, commercially available compounds can provide a sufficiently broad scale of enhancement factors to meet the requirements in chromatographic practice.

The alkyl phosphates used were mixtures of the corresponding mono- and dialkyl derivatives. These substances have a very low solubility in water but they can be used in hydro-organic eluents. Nevertheless we have not observed in our chromatographic system any specific effect which would warrant the use of such hetaerons over that of alkyl sulfates or sulfonates.

A number of experiments were carried out with perfluorooctanoic and decanoic acids. The enhancement factors of these hetaerons, which are almost fully dissociated above pH 2, were estimated to be commensurable to those obtained with alkyl sulfates having the same chain length. An accurate evaluation of the properties of the perfluorated hetaerons, however, was not possible because of an instability of the chromatographic system which gave rise to poor reproducibility. We cannot, close out the possibility that the mechanism of retention with such bulky and highly hydrophobic hetaerons would involve dynamic ion exchange rather than ion pairing in the mobile phase. It should be emphasized, therefore, that the conclusions from the experimental data obtained with alkyl sulfates and similar compounds, together with the predictions therefrom, may not be applicable to ion-pairing agents of greatly different chemical makeup. On the other hand, alkyl sulfonates and sulfates enjoy great popularity in ion-pair reversed phase chromatography so that the results can be of wide interest.

Hydro-organic Eluents. The effect of an organic solvent in eluents containing the hetaerons investigated can be estimated in a general fashion. The predictions are based on our knowledge of the behavior of the individual terme in Equation 11 upon the dielectric properties of the medium. The effect of the organic solvent is qualitatively illustrated in Figure 12, where the solid curve represents the dependence of the capacity factor on the hetaeron concentration in neat aqueous eluents. When an organic solvent is added to the eluent, the relationship is modified as shown by the dashed curve. At low hetaeron concentrations, the increase in capacity factor with increasing hetaeron concentration is expected to be greater in the hydro-organic mixture than in the neat aqueous eluent. According to Equation 11, the rise of k in this domain is determined by the stability constant for ion-pair formation, K_2, whose magnitude increases with decreasing dielectric constant of the medium. Hence, the addition of organic components, and the attendent depression of the dielectric constant of the eluent, causes the left side of the curve to shift to the left.

The magnitude of the change as a function of the organic solvent concentration in the eluent is illustrated in Figure 13 for methanol and acetonitrile. The dependence of K_2 on the medium dielectric has been estimated using the Born approximation for the energy of an ion in solution which is given by

$$K_2 = A \exp(-2Z_1Z_2e^2/\alpha\varepsilon kT) \tag{43}$$

where Z_1 and Z_2 are the charges of the ions, e is the charge of the electron, α is the radius, ε is the dielectric constant of the medium, and A is a preexponential. The results illustrated in Figure 13 show the maximum change in K_2 one would expect due to solvent variation on the basis of Equation 43. With acetonitrile of methanol as solvent, the ion pair formation constant, K_2, may be enhanced by three orders of magnitude with respect to the value in neat aqueous medium, K_2^0. Similar results can be expected with other solvents of chromatographic interest.

On the other hand the value of the capacity factor at the maximum or plateau region of the curve is expected to decrease as shown in Figure 13. According to Equation 11, the saturation value of k is determined by the association constant

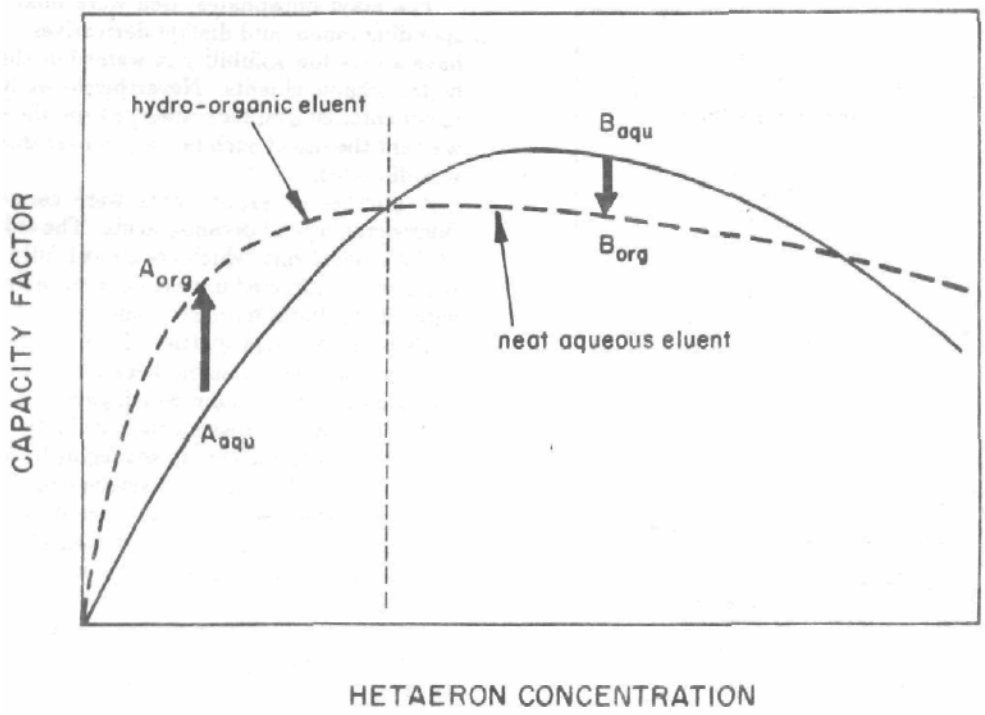

Figure 12. Schematic illustration of the effect obtained when instead of neat aqueous eluent a hydro-organic mixture is used in ion-pair reversed-phase chromatography

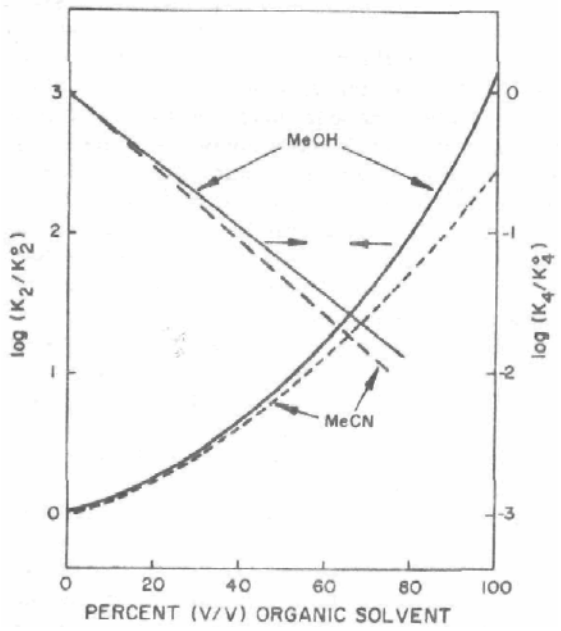

Figure 13. Variation of the normalized stability constant, K_2/K_2^0, of the ion-pair and that of the normalized binding constant K_4/K_4^0, of the complex with the organic solvent content of the eluent in ion-pair reversed-phase chromatography. The equilibrium constants K_2^0 and K_4^0 are obtained with neat aqueous eluents under otherwise identical conditions. The dielectric constants for water-methanol and water-acetonitrile mixtures were taken from Åkerlof (*15*) and Douhéret and Morénas (16), respectively. The dependence of the normaized binding constant on the solvent composition is taken from various sources (*10, 17*, *18*)

for the hetaeron-eluite complex and the ligand of the stationary phase, K_4. The magnitude of this term is primarily determined, in the hermeneutics of the solvophobic theory, by the surface tension of the mobile phase and the eluite surface area. As the addition of an organic solvent reduces the surface tension of the eluent, this term is also reduced in magnitude. In fact, properties other than the surface tension are also involved but the chief effects come from the surface tension and a closely related solvent property as discussed in previous work (*10*), The constant K_3 in Equation 11 is the association constant of the hetaeron and the hydrocarbonaceous ligand. Therefore, it is affected by the organic solvent in a similar way, i.e., K_3 also decreases with added organic component. The effect of organic solvent concentration on K_3 or K_4 can be estimated on the basis of prior results (*10, 17,18*) and is shown for K_4 in Figure 13, It is seen that with methanol or acetonitrile as cosolvents with water, the binding constant of the hetaeron to the stationary phase, K_4 (or the maximum capacity factor) may be reduced as much as two or three orders of magnitude with respect to the corresponding value, K_4^0, in neat aqueous eluent.

The result of these effects is that the capacity factor vs. hetaeron concentration curve will be broadened and flattened. This analysis implies that quite contradictory observations can be made with regard to the effect of the added organic solvent on retention in this type of chromatography. If, with neat aqueous eluents, one is operating in the quasilinear portion of the curve, marked A_{aqu}, the effect of the organic component will be to enhance the retention because a larger fraction of the eluite will be complexed by the hetaeron and the capacity factor will change from A_{aqu} to A_{org}. On the other hand, in the plateau region an addition of organic component will cause a decrease in the capacity factor for the reasons already adduced. This is illustrated schematically by the shift of point B_{aqu} to B_{org} in Figure 12. At higher hetaeron concentration levels, it is even possible to enhance the retention by increasing the organic solvent concentration. It should be noted that this analysis assumes that the retention is achieved primarily by the ion-pair mechanism. If the ion-exchange mechanism predominates, a different set of predictions will hold. In general, the curve will become narrower and the maximum may become greater. The effect depends upon the relative magnitudes of the two association constants.

Possibility of Dynamic Ion-Exchange. Whereas the analysis of our experimental data clearly shows that chromatographic retention is enhanced by ion-pair formation in the mobile phase and subsequent binding of the complex to the nonpolar stationary phase, dynamic ion-exchange can be the underlying mechanism under some other conditions as has been pointed out earlier.

With alkyl sulfates, the mechanism may be expected to change when the size of the hetaeron increases which results in an increase in its binding constant, K_3. An arbitrary but reasonable definition of the point where the mechanism changes from ion-pairing in the mobile phase to ion-exchange or vice versa is where K_3 is equal to the ion-pair formation constant, K_2. Given that the enhancement factor increases by a factor of 2.5 per ethylene group, a change in mechanism in neat aqueous solutions is expected to occur when the number of carbon atoms in the aliphatic chain is 14 or greater because with such very hydrophobic hetaerons the value of K_2 approaches that of K_3. However, such a situation is difficult to observe experimentally because of the low solubility of long-chain alkyl sulfates. In practice such long-chain species would be used with hydro-organic eluents and under such conditions the various terms are expected to change differently with respect to the neat aqueous solvent. If one considers 50% methanol/water as a "typical" eluent and uses the same criterion for the change in the mechanism, i.e., the ion-pair formation constant equals the hetaeron binding constant, one finds upon recourse to Figure 13 and the data in Table I that the mechanism will be essentially the same as in a neat aqueous eluent unless the carbon number of the hetaeron exceeds 22. This calculation is necessarily crude but it does suggest that in most normally encountered situations with alkyl sulfates or sulfonates the dominant mechanism of "soap" chromatography will be via ion-pairing in the mobile phase.

However, it must be pointed out that a rapid change of mechanism is to be expected with increased molecular size with certain other hetaerons, e.g., with tetraalkylammonium ions of formula R_4N^+. As the number of methylene groups in the alkyl chains increases, the ion-pair formation as measured by K_2 will decrease because the distance between charge centers increases, cf. Equation 43. On the other hand the hetaeron binding constant, K_3, will increase with the chain length since the hydrophobic surface area of the hetaeron increases. As a consequence, the change of mechanism from ion-pairing in the mobile phase to dynamic ion-exchange will be more rapid with increasing size of the hetaeron when the charged group is in the center of the molecule rather than in the ω-position as in the case of alkyl sulfates and sulfonates. A rough calculation, similar to those given in the preceding analyses, indicates that in neat aqueous eluents the onset of dynamic ion-exchange occurs when the hetaeron is changed from tetrapropylammonium to tetrabutylammonium salts.

Since the introduction of ion-pair chromatography for the separation of organic compounds by Horvath and Lipsky in 1966 (*19*), column chromatography with ion-pairing agents present in the liquid stationary phase has been widely used and investigated from the mechanistic point of view (*20-26*). The results obtained in this type of partition chromatography have usually been interpreted by using an extraction model. The predicted linear dependence of the capacity factor on the concentration of the complexing agent, however, was not strictly observed and secondary equilibria have been invoked in order to explain the results.

In ion-pair reversed-phase chromatography, the complexing agent is present in the mobile phase; consequently, the physico-chemical phenomena involved in the separation process are significantly different from those which govern chromatographic retention when a "liquid ion-exchanger" is used as the stationary phase. Therefore, the two techniques have to be clearly distinguished. With suitable bonded phases, ion-pair reversed-phase chromatography offers numerous advantages as far as the versatility of the technique is concerned (27). It can greatly expand the scope of reversed-phase chromatography, which has already become the most popular type of high performance liquid chromatography (27).

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LITERATURE CITED

(1) B. L. Karger, L. R. Snyder, and C. Horvath, "An Introduction to Separation Science". Wiley. New York. N.Y.. 1973, pp 28-30.
(2) C. Horvath. W. Melander. and 1. Molnar, *Anal. Chem.* 48, 142 (1977).
(3) D. P. Wittmer, N. 0. Nuessle. and W. G. Haney, Jr., *Anal Chem.,* 47. 1422(1975).
(4) J. H. Knox and G. R. Laird, *J. Chromatogr.,* 122. 17 (1976).
(5) J. H. Knox and J. Jurand, *J. Chromatogr.,* 125, 89 (1976).
(6) J. H. Knox and J. Jurand, *J. Chromatogr.,* 103, 311 (1975).
(7) C. Horvath, and W. Melander, *J. Chromatogr. Scl.,* 15, 393 (1977).
(8) C. Tanford, "The Hydrophobic Effect". Wiley-Interscience, New York, N.Y., 1973.
(9) O. Sinanoglu, "Molecular Associations in Biology", D. Pullmann, Ed., Academic Press, New York, N.Y., 1968, pp 427-445.
(10) C. Horvath. W. Melander. and I. Molnar, *J Chromatogr.,* 125, 129 (1976).
(11) P T. Kissinger, *Anal* Chem., 49. 883 (1977).
(12) O. Sinanoğlu and S. Abdulnur, *Fed. Proc.,* 24 (2). 12 (196S).
(13) T. Halicioğlu and O. Sinanoğlu, *Ann. N. Y. Acad. Sci..* 158, 308 (1969).
(14) C. Horvath, *Methods Biochem. Anal.,* 21, 79 (1973).
(15) G. Akerlpf, *J. Am. Chem. Soc.,* 54, 4125 (1832).
(16) G. Douhéret and M. Morénas, *C.R. Acad. Habd. Seances Acad. Sci., S6r. C,* 284,729(1967).
(17) S. H. Abbott, J. R. Berg, P. Achener. and R. L. Stevenson, *J. Chromatogr.,* 126. 421 (1973).
(18) B. L. Karger, J. R. Gant, A. Hartkopf, and P. H. Welner, *J. Chromatogr.,* 128, 65 (1976).
(19) C. Horvath and S R. Lipsky. *Nature (London).* 211. 748 (1966).
(20) S. Eksborg, P. 0. Lagerström, R. Modin, and G. Schill, *J. Chromatogr.,* 83, 99 (1973).
(21) S. Eksborg and G. Schill. *Anal. Chem.,* 45, 2092 (1973).
(22) J. C. Kraak and J. F. K. Huber, *J. Chromatogr.,* 102, 333 (1974).
(23) B. L. Karger, S. C. Su, S. Marchese, and B. A. Persson, *J. Chromatogr. Sci.,* 12. 678(1974).
(24) B. A. Persson and B. L. Karger, J. *Chomatogr. Sci.,* 12, 521 (1974).
(25) B. Fransson. K. G. Wahlund, I. M. Johansson, and a Schill, *J. Chromatogr.,* 125,327(1976).
(26) S. C. Su, A. V. Hartkopf, and B. L. Karger, *J. Chromatogr.,* 119, 523 (1976).
(27) R. Gloor and E. Johnson, *J. Chromatogr. Sci.,* 15, 413 (1977).

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