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MECHANISTIC STUDY ON ION-PAIR REVERSED-PHASE CHROMATOGRAPHY

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SUMMARY

The mechanism of "ion-pair" chromatography that employs a non-polar stationary phase and an amphiphilic ion in the eluent to augment the retention of oppositely charged sample components is investigated. Examination of literature data indicates that neither of the simple limiting mechanisms, "ion pairing" or "dynamic ion exchange", can account for the observed dependence of the retention factor on the concentration of the amphiphilic ion over a sufficiently wide range of conditions. According to the ion-pairing mechanism the analyte traverses the column in the form of an ion pair whereas the dynamic ion-exchange mechanism implies that the interaction of the analyte with the hydrophobic counter-ion adsorbed on the stationary phase surface is responsible for retention. Results of the present study indicate that a mechanistic model based on the assumption that both phenomena take place concurrently does not give adequate agreement with experimental findings either. Therefore, a so-called dynamic complex exchange model is proposed that assumes a metathetical exchange of the analyte between the amphiphilic ion bound to the stationary phase and the ion pairs formed in the mobile phase. Re-examination of earlier experimental data and the results of computer simulation suggest that this model appropriately reflects the experimentally observed hyperbolic dependence of the retention factor on the concentration of the "ion-pairing" agent as long as the retention-attenuating effect of micelle formation is negligible.

INTRODUCTION

The use of ionic detergents, such as alkylsulfates or quaternary alkylamines, in the eluent for modulation of selectivity in reversed-phase chromatography of samples containing ionized components has found widespread acceptance and the technique is frequently referred to as "ion-pair" chromatography.

The physico-chemical phenomena underlying retention in this type of chromatography have yet to be fully elucidated and neither of the two mechanisms that have received the greatest attention has been supported by unambiguous experimental evidence. According to the ion-pairing mechanism, the charged sample components and detergent form neutral ion pairs in the mobile phase which are retarded more

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than the charged components. On the other hand dynamic ion exchange occurs when the detergent adsorbs to the surface of the non-polar stationary phase, thereby converting it into a dynamically coated ion exchanger. It has been stated, however, that the two mechanisms represent limiting cases and the retention process is not expected to follow any of them over a wide range of chromatographic conditions[1]. Moreover, no unambiguous mechanistic determination is possible from the chromatographic data alone. Comparison of chromatographic parameters and extrachromatographically determined stability constants can be used to discriminate between mechanisms, but the paucity of relevant physico-chemical data in the literature as well as the difficulties involved in precise measurement of the equilibrium constants of chromatographic interest greatly impedes progress in this field. However, notable advances have been made in christening this important branch of chromatography. At the latest count there are a dozen names listed for ion-pair chromatography in the literature that include ion-association[2] and solvophobic-ion chromatography[3]. In view of the mechanistic uncertainties and the quite general use of secondary equilibria with a similar physico-chemical bases in reversed-phase chromatography, we have used the terms hetaeric chromatography for the technique and hetaeron for the complex forming agent in the eluent from the Greek word *ἑταιρον* (companion). In fact knowledge obtained in the study of "ion-pair" chromatography can be readily applied to shed light on the mechanism of chromatographic separations involving complexation[4–7].

In this study we attempt to formulate a mechanism which removes the shortcomings observed with ion-pairing or dynamic ion-exchange mechanisms. The method of analysis used here is to relate the retention factors to the thermodynamic equilibrium constants of the individual steps involved in the retention process, *i.e.*, the study of the dependence of retention factors on the hetaeron concentration in the eluent. The scope of the investigation is restricted to the concentration range of the amphiphilic hetaeron where the effect of micelle formation is negligible.

THEORY

Hyperbolic retention behavior

In many cases the retention factor, k, is related to the hetaeron concentration $[T]$ by

$$k = \frac{A + B[T]}{1 + C[T]} \tag{1}$$

Eqn. 1 represents a rectangular hyperbola and the parameters A, B, and C can be determined experimentally. A and the ratio B/C are the retention factors in the absence of hetaeron and the maximum value of the retention factor obtained at sufficiently high hetaeron concentrations, respectively. Depending on the physical meaning of parameter C, eqn. 1 can describe the dependence of k on $[T]$ for either ion-pairing or dynamic ion exchange. When C is the ion-pair formation constant in the mobile phase retention proceeds only via ion pairing. On the other hand, when C is the equilibrium constant for hetaeron binding to the stationary phase then dynamic ion exchange occurs.

In several studies, however, parabolic concentration dependence of retention factors has been obtained[4,8,9], *i.e.*, with increasing hetaeron concentration the retention factor increases to a maximum from which it decreases with further increase in hetaeron concentration. This effect can be treated by modification of the model below to include the possible effect of entrapment of eluite in micelles formed by the detergent. This discussion will be limited to models which conform to the general hyperbolic behavior.

Eqn. 1 can be written in linearized form[11] as

$$\frac{[T]}{(k - \mathrm{A})} = \frac{1}{(\mathrm{B} - \mathrm{AC})} + \frac{\mathrm{C}}{(\mathrm{B} - \mathrm{AC})}[T] \tag{2}$$

so that plots of $[T]/(k - \mathrm{A})$ against $[T]$ yield straight lines with $\mathrm{C}/(\mathrm{B} - \mathrm{AC})$ and $1/(\mathrm{B} - \mathrm{AC})$ as the slope and intercept, respectively, allowing the evaluation of parameter C as the ratio of slope to intercept.

In all cases discussed here, we assume that the uncomplexed analyte binds independently to the stationary phase as

$$\mathrm{E} \overset{K_0}{\rightleftharpoons} \mathrm{E_s} \tag{3}$$

where K_0 is the equilibrium constant and E and $\mathrm{E_s}$ are the eluites in the mobile and stationary phases, respectively. In view of eqn. 3 the retention factor of the eluite in the absence of hetaeron, A, is given by

$$A = \varphi K_0 \tag{4}$$

where φ is the phase ratio.

Dynamic ion exchange

According to the dynamic ion-exchange mechanism the analyte, E, forms a complex $\mathrm{E}T_\mathrm{s}$ with a hetaeron already bound to the surface of the stationary phase, T_s, according to the equilibrium.

$$T_\mathrm{s} + \mathrm{E} \overset{K_1}{\rightleftharpoons} \mathrm{E}T_\mathrm{s} \tag{5}$$

If the hetaeron binds to the stationary phase surface according to Langmuir isotherm, the surface concentration of hetaeron, $[T]_\mathrm{s}$, is given by

$$[T_\mathrm{s}] = \frac{K_2[T]\,[T_\mathrm{s}]^*}{1 + K_2[T]} \tag{6}$$

where $[T]$, $[T_\mathrm{s}]^*$ and K_2 are the concentration of hetaeron in the eluent, the maximum surface concentration of bound hetaeron and the equilibrium constant for binding of hetaeron to the stationary phase surface, respectively. Eqn. 6 assumes that a monolayer will be formed on the surface at sufficiently high hetaeron concentration in the eluent.

The concentration of each species can be found by combining eqns. 5 and 6 and the surface concentration of the complex can be expressed as

$$[\mathrm{E}T_\mathrm{s}] = \frac{K_1K_2[\mathrm{E}]\,[T]\,[T_\mathrm{s}]^*}{1 + K_2[T]} \tag{7}$$

Combination of eqns. 3, 5, 6 and 7 yields the following expression for the retention factor when dynamic ion exchange governs the chromatographic retention

$$k = \varphi \frac{K_0[T_s]^* + K_1K_2[T]\,[T_s]^*}{1 + K_2[T]} \tag{8}$$

Ion pairing

According to this model the analyte and hetaeron first form a complex, ET, in the mobile phase, and then the complex binds reversibly to available sites on the surface as ET_s. The corresponding equilibria are given by

$$T + \mathrm{E} \underset{}{\overset{K_3}{\rightleftharpoons}} \mathrm{E}T \tag{9}$$

and

$$\mathrm{E}T \overset{K_4}{\rightleftharpoons} \mathrm{E}T_s \tag{10}$$

where K_3 and K_4 are the appropriate equilibrium constants. The concentration of available surface sites, *i.e.*, the sites not occupied by hetaeron, is given by the difference between the maximal surface density of binding sites and the actual surface concentration as

$$[T_s]^* - [T_s] = \frac{[T_s]^*}{1 + K_2[T]} \tag{11}$$

where $[T_s]$ is the concentration of the hetaeron bound to the surface. Combining eqns. 9, 10 and 11 we obtain the following expression for the surface concentration of the complex E$[T_s]$, when ion pairing is the dominant mechanism

$$[\mathrm{E}T_s] = \frac{K_3K_4[\mathrm{E}]\,[T]\,[T_s]^*}{1 + K_2[T]} \tag{12}$$

The retention factor under these conditions is obtained by combining eqns. 3, 9, 10 and 12 so that

$$k = \varphi \frac{K_0[T_s]^* + K_3K_4[T]\,[T_s]^*}{(1 + K_2[T])\,(1 + K_3[T])} \tag{13}$$

Mixed dynamic ion-exchange–ion-pairing mechanism

As the limiting mechanisms dynamic ion exchange and ion pairing do not always reflect the physical reality we shall combine the features of the two in order to formulate a "mixed" mechanism for expressing the dependence of retention factor on the hetaeron concentration.

When both ion pairing and dynamic ion exchange can occur, the retention factor can be expressed by combining eqns. 8 and 13 so that

$$k = \frac{K_0[T_s]^* + K_1K_2[T]\,[T_s]^* + K_3K_4[T]\,[T_s]^*}{(1 + K_4[T])\,(1 + K_2[T])} \tag{14}$$

In the limiting cases of K_2 or K_3 going to zero, retention factors are given by eqn. 8 or 13, respectively.

The experimentally observed dependence of the retention factor on the hetaeron concentration has not always followed the predictions of eqn. 14. Therefore, another mechanism was developed as described below in order to supplement the "mixed" mechanism.

Dynamic complex exchange

When ion pairs formed in the mobile phase bind to the stationary phase surface covered by bound hetaeron and a subsequent metathetical process takes place, the mechanism is termed dynamic complex exchange. The description of dynamic complex exchange requires the following equilibria.

The ion pair formed in the mobile phase may bind to the surface in the process

$$ET + T_s \overset{K_5}{\rightleftharpoons} TET_s \tag{15}$$

which is followed by decomposition of the ternary complex

$$TET_s \overset{K_6}{\rightleftharpoons} TE_s + T_s \tag{16}$$

The concentrations of the complexes bound to the surface are given by

$$[TET_s] = K_5[ET]\,[T_s] = \frac{K_1K_2K_5[E]\,[T]^2\,[T_s]^*}{1 + K_2[T]} \tag{17}$$

and

$$[ET_s] = K_6\,[TET_s]/[T_s] \tag{18}$$

or

$$[ET_s] = K_3K_5K_6[T]\,[E] \tag{19}$$

The total concentration of the eluite bound to the surface, $[E_s]_t$, by this mechanism is given by

$$[E_s]_t = [TET_s] + [ET_s] = \frac{K_3K_5K_6[T]\,[E]\,(1 + K_2[T]) + K_2K_3K_5[E]\,[T]^2\,[T_s]^*}{(1 + K_2[T])} \tag{20}$$

so that the retention factor obtained is

$$k = \varphi\,\frac{K_0[T_s]^* + K_3K_5K_6[T]\,(1 + K_2[T]) + K_2K_3K_5[T]^2\,[T_s]^*}{(1 + K_2[T])\,(1 + K_3[T])} \tag{21}$$

Eqn. 21 illustrates that the relatively simple dynamic complex-exchange model, which does not involve the effect of micelles, gives rise to a rather complicated expression. Nevertheless, as will be shown later, in many cases the dependence of the retention factor on the hetaeron concentration given by eqn. 21 reduces to the hyperbolic behavior of eqn. 1 most commonly observed in practice and the apparent parameters thus obtained have meaningful physical interpretations.

Model testing by computer simulation

Retention factors were calculated as a function of hetaeron concentration by using eqns. 14 and 21 after introducing appropriate dimensionless parameters and a dimensionless hetaeron concentration to simulate predictions of the "mixed" and dynamic complex-exchange models, respectively. The dimensionless hetaeron concentration was defined as $K_2[T]$ and the parameters used were $K_0[T_s]^*$, $K_1[T_s]^*$, $K_4[T_s]^*$ and K_3/K_2 for mixed ion exchange and $K_0[T_s]^*$, K_5K_6, K_3/K_2 and $[T_s]^*/K_6$ for dynamic complex exchange and their values covered the full range of practical conditions.

The retention factor data calculated with different sets of parameters at different hetaeron concentrations were analyzed according to the linearized expression given in eqn. 2 and parameter C was evaluated from the slope and intercept. For each plot the correlation coefficient was calculated in order to measure the linearity of data when plotted according to eqn. 2. The hetaeron concentration range under investigation bracketed the hetaeron concentration at which half-maximal retention was obtained by about two orders of magnitude. Retention factors ranged from 1 to 101. In the algorithm for generating data for mixed ion exchange the group K_3/K_2 was changed. On the other hand dynamic complex-exchange data were generated by varying the ratio $[T_s]^*/K_6$ from 10^{-2} to 10^2 in $\sqrt{10}$ increments. For each value of that group the ratio K_3/K_2 was allowed to vary from 10^{-3} to 10^3 in steps of $\sqrt{10}$.

The program for the above calculations was written in BASIC language and a PDP 11/10 minicomputer (Digital Equipment Company) was used.

RESULTS AND DISCUSSION

This study and an earlier one from our laboratory[1] are focussed on the relationship between chromatographic retention and hetaeron concentration in the mobile phase. An attractive feature of this method of analysis is that, when the equilibrium constants are known, the dependence of retention on hetaeron concentration can be predicted quantitatively and compared to experimental data. More important is that the equilibrium constants can be evaluated from extrachromatographic measurements. Their value often can be estimated from the structure and properties of the species involved. In order to gain mechanistic insight the prediction can be tested against values obtained from chromatographic experiments. In the present study of the mechanism of "ion-pair" chromatography the main emphasis is placed on the elucidation of the physico-chemical meaning of parameter C in eqn. 1.

No single retention mechanism described in the literature has been found to account satisfactorily for all observations made in "ion-pair" chromatography. As it was pointed out earlier[1] the actual mechanism is expected to depend on the conditions and neither the limiting dynamic ion-exchange nor the ion-pairing mechanism is likely to operate over the entire range of hetaeron concentration and solvent composition.

A mechanistic determination requires the evaluation of some of the equilibrium constants by extrachromatographic means in order to relate the parameters of eqn. 1 to the appropriate equilibria, *i.e.*, assign to them physical meaning. As such data were not available, attempts were made[1] to employ extrathermodynamic free energy

relationships to infer the physical meaning of thermodynamic constants. Retention data of catecholamines obtained with octadecylsilica and alkylsulfates of various chain length in the eluent could be interpreted by either ion pairing or dynamic ion exchange. However, no linear dependence of the logarithm of parameter C in eqn. 1 on the carbon number of the alkyl sulfates was observed. Since in the case of dynamic ion exchange C is the binding constant of the hetaeron to the stationary phase its logarithm should be linearly dependent on the carbon number of the alkyl moiety. Therefore, the analysis of the data gave sufficient support to the proposition that dynamic ion exchange was not the dominant mechanism under the experimental conditions investigates. For sake of simplicity it was assumed that the retention proceeds via the other limiting mechanism since the data presented were consistent with ion pairing. Findings of several other investigations[3,12] have also found ion pairing to be the dominant mechanism in "ion-pair" chromatography. In contradistinction, many investigators argued that detergents bind so "strongly" to the hydrocarbonaceous surface of the stationary phase that dynamic ion exchange ought to be the mechanism of retention[2,13–15]. The implications of this observation in favor of dynamic ion exchange, however, are greatly attenuated by the low surface coverage, of the order of a few percent, which can be calculated from experimental data representing "strong" binding of hetaerons under conditions usually employed in ion-pair reversed-phase chromatography[3,8,14,16–19].

Another argument for the important role of the bound hetaeron and for dynamic ion exchange has been put forward on the basis of the observation that with increasing concentration of cationic hetaeron, retention of anionic and cationic sample components increases and decreases, respectively[2,16]. Whereas anionic hetaerons are expected to promote the elution of anionic eluites and to enhance the retention of cationic eluites, the quantitative data given in the literature are not wholly consistent with the model since the hetaeron concentrations at which the effects are half-maximal are different for anionic and cationic eluites. If the observed phenomena were due to the presence of bound hetaeron in both cases, the two effects would have identical dependence on the hetaeron concentration in the mobile phase.

It has also been claimed on the basis of conductance measurements, that there are no ion pairs in "ion-pair" chromatography[2,13]. In fact, the majority of investigators have interpreted their data by evoking dynamic ion exchange; yet, no study has been carried out to relate the observed retention behavior, *i.e.*, the dependence of the retention factor on the hetaeron concentration, to the intrinsic thermodynamic parameters of the chromatographic system.

Recently, we showed[20] in contrary to earlier claims[2,13] that ion pairs indeed form between alkyl sulfates and catecholamines under conditions used in chromatography. However, the stability constants are somewhat lower than those that had been calculated from chromatographic analysis of the catecholamine–alkyl sulfate system on the assumption that the retention mechanism is ion-pair formation[1]. A comparison of the results obtained by the two methods is given in Table I. The mean values of the stability constants of a given alkyl sulfate are much smaller when determined titrimetrically than when determined from the concentration dependence of retention. Whereas the data do argue that ion pairs form in solution, stability constants determined by the two methods are inconsistent, implying that retention does not occur by the simple ion-pair mechanism. On the other hand the

TABLE I

COMPARISON OF THE MEAN VALUES OF ION-PAIR FORMATION CONSTANTS OBTAINED IN AQUEOUS SOLUTION FOR THREE CATECHOLAMINES AND THE ALKYL SULFATES INDICATED BY TITRIMETRIC AND CHROMATOGRAPHIC METHOD

The amines were dopamine, epinephrine and octopamine. The stability constants are given in M^{-1}.

Method	*Ref.*	*Alkyl sulfate*			
		Butyl	*Hexyl*	*Octyl*	*Decyl*
Titration	20	9.4 ± 3	8.0 ± 1.5	18.7 ± 1.0	20.5 ± 2.1
Chromatography	1	46 ± 3	107 ± 8	68 ± 10	125 ± 40

chromatographic data did not support dynamic ion exchange as the retention mechanism although the binding of amphiphiles to non-polar stationary phases used in reversed-phase chromatography is known. Nevertheless, the results make a strong case for the proposition that ion-pair formation indeed plays a role in "ion-pair" chromatography.

As neither of the simple mechanisms, ion pairing or dynamic ion exchange, accounts fully for the experimental observations, the two mechanisms introduced under Theory will be discussed here. In the case of the mixed ion-exchange mechanism, both ion-pair formation in the eluents and dynamic ion exchange are assumed to contribute to retention and the dependence of the retention of the hetaeron concentration is given by eqn. 14. According to the other mechanism, dynamic complex exchange, the surface of the stationary phase may be coated with adsorbed hetaeron but ion exchange in the conventional sense plays a negligible role in determining the magnitude of retention. On the other hand ion pairs formed between the sample component and hetaeron in the mobile phase do migrate to the stationary phase surface where a metathesis takes place and the analyte is released to form an ion pair with a hetaeron bound to the surface.

In order to assess which of the two mechanisms predicts retention behavior consistent with all experimental findings, the relationships between the stability constant, C, calculated by fitting data generated by the computer according to the "mixed" and dynamic complex-exchange mechanisms to eqn. 2, and the ion-pair stability constant used in the computer simulation was examined. The values of C, which may be considered an apparent stability constant, were obtained from linear regressions of linearized form of the computer-generated data as described above.

For the "mixed" mechanism eqn. 14 was used to determine the apparent stability constant upon varying the ratio of K_3/K_2 in the range from 10^{-3} to 10^3. Fig. 1, in which the ratio of the observed stability constant to the ion-pair stability constant is plotted *versus* the logarithm of K_3/K_2 shows that in the case of the "mixed" mechanism, the apparent stability constant exceeds the actual value of the ion-pair stability constant and, if the adsorption constant equals or exceeds the ion-pair formation constant, the apparent stability constant will be exceedingly large.

However, it was found that when the ratio K_3/K_2 is less than 10, poor linearity is obtained ($r < 0.9$) due to the parabolic nature of eqn. 14. As a result, only when the ion-pairing constant is greater than the adsorption constant yields

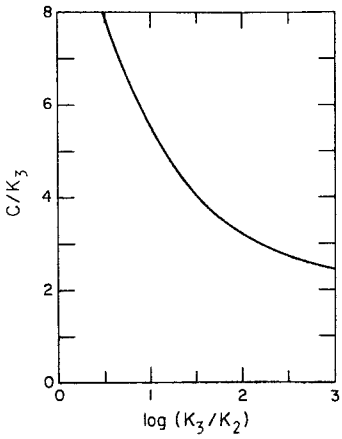

Fig. 1. Graphical illustration of the results obtained by computer simulation of the dependence of retention factor on the hetaeron concentration according to the "mixed" mechanism, see eqn. 14, for the case of hyperbolic retention behavior given by eqn. 1. The ratio of parameter C, the calculated "ion-pair formation constant" to the true ion-pair stability constant, K_3, is plotted against the logarithm of the ratio of K_3 to K_2, the equilibrium constant for hetaeron binding to the stationary phase surface.

eqn. 14 acceptable straight lines according to the form of eqn. 2. But this condition does not seem physically realistic and the mechanism must therefore be tentatively discarded.

The dynamic complex-exchange mechanism was examined in a similar fashion by use of eqn. 21 and evaluation of the ratio of the apparent stability constant obtained from linear plots to the ion-pair formation constant used in the calculation. With dynamic complex-exchange data the plots usually showed good linearity and correlation coefficients exceeding 0.96 and frequently 0.99 were obtained. The results are plotted in Fig. 2 with the ratio $[T_s]^*/K_6$ as the parameter that measures the proportion of retention by simple adsorption to that by complex exchange. Its value was allowed to vary over the range 0.01 to 100.

Analysis of the dynamic complex-exchange model yielded linear plots over nearly the entire range of conditions examined. Furthermore in the physically realistic domain where $K_3/K_2 \leqslant 1$, the calculated stability constant is almost always greater than the stability constant used in the calculation. As expected, the ratio increases as the relative significance of exchange increases, *i.e.*, as $[T_s]^*/K_6$ decreases. It appears that when the role of exchange is significant, *i.e.*, $[T_s]^*/K_6 \leqslant 0.1$, the apparent stability constant is always greater by a factor of about three than the actual ion-pair formation constant. Even when complex exchange does not dominate retention, the ratio C/K_3 is greater than unity and frequently approaches three whenever K_3/K_2 is less than one, *i.e.*, under physically realistic conditions.

Recently the ion-pair stability constants of chromatographic interest for alkyl sulfates and catecholamines were evaluated potentiometrically[20] and were found to be different from those obtained from chromatographic experiments[1]. It is interesting therefore to attempt to explain the discrepancy in view of the present mechanism. The *t*-statistic was calculated for comparison of the chromatographically obtained

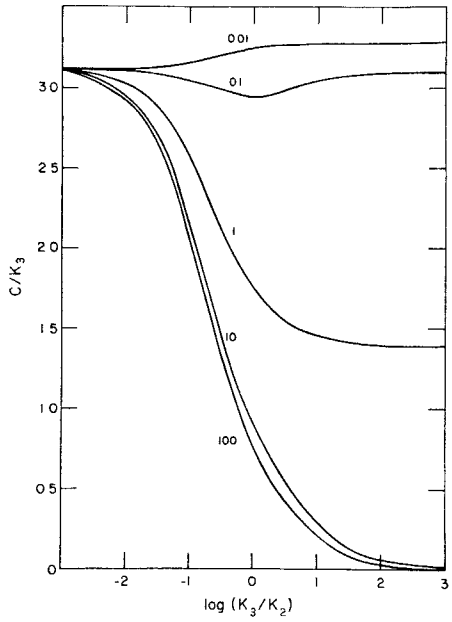

Fig. 2. Graphical illustration of the results obtained by computer simulation of the dependence of retention factor on the hetaeron concentration according to the dynamic complex-exchange mechanism, see eqn. 21, for the case of hyperbolic retention behavior given by eqn. 1. The ratio of parameter C, the calculated "ion-pair formation constant" to the true ion-pair stability constant, K_3, is plotted against the logarithm of the ratio of K_3 to the equilibrium constant for hetaeron binding to the stationary phase surface K_2. Each curve was calculated with the stated value of the parameter $[T_s]^*/K_0$, the magnitude of which increases with the significance of dynamic complex exchange in the retention process.

stability constants to the titrimetrically determined constants which were multiplied by the factor three in view of the discussion above. Only for the stability constants for ion pairs with hexylsulfate did the chromatographic values differ from those expected on the basis of the potentiometric results and the simulation at the $P = 0.05$ level of significance. Thus not only the disturbing inconsistency between the two sets of data could be reconciled, but the results of the calculations also lend support to the proposition that retention in ion-pair chromatography proceeds via dynamic complex exchange.

Many authors proclaimed the dominance of dynamic ion exchange on the basis of strong binding of hetaerons to non-polar stationary phases[2,13–15] and/or referred to the dependence of retention on hetaeron concentration. The latter argument is not entirely convincing as both the ion-pairing and dynamic ion-exchange mechanisms exhibit the same formal dependence of retention on the hetaeron concentration, *cf.* eqns. 8 and 13. The present work shows that this concentration dependence holds for the dynamic complex-exchange mechanism as well. On the other hand, the mechanism proposed here admits, and even requires, strong binding of the detergent to the stationary phase.

Among others, the dynamic complex-exchange mechanism also resolves the

puzzling observation[2] that the hetaeron concentration at which the retention of a substance that forms complexes with the hetaeron is half-maximal is often much larger, apparently even orders of magnitude, than the concentration at which the retention of a substance having the same charge sign as the hetaeron is half-maximal.

According to the dynamic complex-exchange model the retention factor rises with increasing hetaeron concentration to a constant plateau, the value of which is given by $K_5 (1 + [T_s]^* K_6)$. Plots of k *versus* $[T]$ allow the evaluation of a "formation" constant defined as the reciprocal of the hetaeron concentration at which the retention factor is the mean of the limiting values obtained in the absence of hetaeron and at saturation, *i.e.*, when the modulus $\eta = 0.5$ (ref. 11). If K_2, the binding constant of hetaeron to the stationary phase, is much greater than K_3, eqn. 21 yields a "formation" constant of the ion pair that corresponds to the above-defined parameter and will be very nearly the same as the actual ion-pair formation constant. However, when repulsion of a similarly charged substance by the hetaeron bound to the stationary phase surface increases, the concentration of hetaeron at which the repulsion or decrease in retention is half-maximal should correspond to the bulk hetaeron concentration at which half of the stationary phase surface is coated. In the usual chromatographic practice with bulky amphiphiles as hetaerons, this concentration will be much lower than the reciprocal ion-pair formation constant.

The approach employed previously[1] for determination of mechanism by considering the dependence of the parameters of eqn. 1 on molecular properties may be extended to examine dynamic complex exchange as follows. The parameter A is just the retention of eluite in absence of hetaeron so it is independent of hetaeron properties. The parameters B and C are approximately given as $K_2K_3K_5K_6$ and K_2K_3, respectively. K_2 is the stability constant for hetaeron binding to the stationary phase and it is expected to increase with increase of the hydrophobicity of the eluite and decrease with increased charge. K_3 is the ion-pair formation constant and will depend upon the charges of both eluite and hetaeron and only weakly on their hydrophobic surface area. Therefore, the group, K_2K_3, *i.e.*, parameter C, depends on the hydrophobic area and charge of the hetaeron and on the charge of the eluite. Since K_5 is the stability constant for the complex of the ion pair with the bound hetaeron, it is expected to depend on the hydrophobic area of the hetaeron and the eluite and not on the charge of either, whereas K_6 will be nearly independent of the charge of both hetaeron and eluite. As a consequence, the parameter B, which is given approximately as $K_2K_3K_6K_7$ will depend on the hydrophobic surface area and charge of the hetaeron and on the hydrophobic surface area and charge of the eluite. The maximum value of the modulus, η_c, is defined as[11]

$$\eta_c = B/AC \tag{22}$$

It is the ratio of the maximum retention possible in the presence of hetaeron to the retention in the absence of hetaeron and therefore is a measure of the retentive power of a given hetaeron. In the present analysis of the dynamic complex-exchange model it is given by K_5K_6. Therefore, η_c is expected to depend on the hydrophobic area of the hetaeron and to be independent of the eluite charge and hydrophobic surface area. These considerations, along with the results for the ion-pairing and dynamic ion-exchange mechanisms[1], are summarized in Tables II and III.

It is clear from Tables II and III that the dynamic complex-exchange and ion-

TABLE II

HETAERON PROPERTIES DETERMINING THE PARAMETER VALUES OF EQN. 1 AND THE MODULUS, η, IN "ION-PAIR" CHROMATOGRAPHY FOR ION PAIRING (I), DYNAMIC ION EXCHANGE (II) AND DYNAMIC COMPLEX EXCHANGE (III)

Parameter	*Mechanism*		
	I	*II*	*III*
A	—	—	—
B	Hydrophobic surface area	Hydrophobic surface area	Hydrophobic surface area
C	Charge type ($C = K_3$)	Hydrophobic surface area and charge type ($C = K_2$)	Charge type ($C \approx BK_3$)
$\eta_c = B/AC$	Hydrophobic surface area	Charge type	Hydrophobic surface area

TABLE III

ELUITE PROPERTIES DETERMINING THE PARAMETER VALUES OF EQN. 1 AND THE MODULUS, η, IN "ION-PAIR" CHROMATOGRAPHY FOR ION PAIRING (I), DYNAMIC ION EXCHANGE (II) AND DYNAMIC COMPLEX EXCHANGE (III)

Parameter	*Mechanism*		
	I	*II*	*III*
A	Charge and hydrophobic surface area	Charge and hydrophobic surface area	Charge and hydrophobic surface area
B	Hydrophobic surface area and charge	Hydrophobic surface area and charge	Hydrophobic surface area and charge
C	Charge type ($C = K_3$)	—	Charge type ($C \approx 3\ K_3$)
$\eta_c = B/AC$	Charge	Charge type and hydrophobic surface area	Charge

pairing mechanisms are very similar insofar as the qualitative relationships between hetaeron properties and the parameters of eqn. 1 are concerned. The predictions of the magnitude of C are different, however, since C should be greater than K_3 in most cases. The dependence of η_c for dynamic ion exchange on hetaeron properties is different from those in ion pairing and dynamic complex exchange.

The differences in the dependencies of C and η_c on eluite properties are more striking between the three mechanisms. The parameter C will depend upon eluite charge when ion pairing or dynamic complex exchange occurs but not when dynamic ion exchange is the mechanism of retention. The dependence of η_c on molecular properties is different for the three mechanisms since it depends on eluite charge and hydrophobic area in dynamic ion exchange, on charge alone in ion pairing and neither in dynamic complex exchange. In any case these tables serve only as crude guides to relate the parameters to the molecular properties of the species involved. In this regard, the statement that a parameter is independent of a property, *e.g.*, charge,

should be taken to mean than the dependence of that parameter on that property is weak compared to the dependence of another parameter on that property.

Examination of most, if not all, literature data shows consistency with the predictions of the dynamic complex-exchange model, that the retention decreases when, at constant mobile phase hetaeron concentration, the concentration of salt or organic solvent in the eluent is increased. As mentioned above it can also be easily reconciled with the main objection to the ion-pairing model, *i.e.*, that significant hetaeron binding to the surface occurs. Indeed, the complex-exchange model requires the hetaeron binding to occur to a significant extent over the hetaeron concentration range of interest. Yet it predicts that dependence of retention on hetaeron concentration reflects the magnitude of ion-pair formation as both phenomena play an essential role in bringing about retention. It is also interesting to note that according to the dynamic complex-exchange model the three parameters of eqn. 21 show the same dependence on the chemical properties of analyte and hetaeron as that in the ion-pairing mechanism. Thus, the arguments used earlier for ion pairing in neat aqueous mobile phase[1] can be employed with equal force to argue not only against the dynamic ion-exchange but also for the present dynamic complex-exchange model.

The chief defect of the proposed mechanism is its failure to predict a decrease in retention at high hetaeron concentrations. This can be remedied by evoking micelle formation at relatively high detergent concentrations and substituting the factor $(1 + K_3[T] + K_7[T]^n)$ for the factor $(1 + K_3[T])$ in the denominator of eqn. 21. Thus the term $K_7[T]^n$, where n is greater than 1, can be included to account for partitioning of eluite into hetaeron micelles in the mobile phase that do not bind to the stationary phase. Ample data[8–10] suggest that detergent concentration in the eluent may exceed the critical micelle concentration under conditions employed in "ion-pair" chromatography when the alkyl chain is long and the limiting value of the retention factor is reached.

Of course, sound scientific standards demand that the demonstration of a mechanism is complemented by the demonstration that alternatives do not occur. This unified mechanism may be misleading insofar as one could draw the conclusion that a single mechanism prevails for all hetaerons and solvent compositions. That assumption, however, is very likely not true, and therefore, investigations of the mechanism under markedly different conditions may reveal the existence of a simpler mechanism which, of course, will be a limiting case of the comprehensive model described above.

The mechanism suggested here can be regarded as proven only in the sense that all ion-pair chromatography retention data in the literature are consistent with it, according to our knowledge. It does have the attractive feature that it appears to account for all experimental data in one self-consistent mechanism.

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