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FORMATION OF ION PAIRS UNDER CONDITIONS EMPLOYED IN REVERSED-PHASE CHROMATOGRAPHY

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SUMMARY

The existence of ion pairs under conditions employed in “ion-pair” chromatography has been examined by re-analysis of electrical conductance data reported in the literature for octylamine and octylsulfonate in methanol–water (35:65) solutions as well as by titration experiments with systems containing catecholamines and alkylsulfates. The results indicate that the stability constants of ion pairs formed between these species in aqueous solution range from 10 to 100 M^{-1} . As a consequence such ion pairs may play a significant role in the chromatographic process in contradistinction to assertions put forward in the literature.

INTRODUCTION

“Ion-pair” chromatography on hydrocarbonaceous bonded stationary phases has become a widely used branch of high-performance liquid chromatography (HPLC)^{1,2}. The mechanism of the chromatographic process in this technique, in which amphiphilic ions such as alkylsulfonates or tetraalkylquaternary ammonium compounds are added to the eluent for selective retention of oppositely charged sample components, has been subject of controversy^{2–5}.

Several authors have even questioned the existence of ion pairs formed between organic ions and amphiphilic counter-ions in aqueous or hydroorganic eluents commonly used in reversed-phase chromatography^{4,6}. Generally, mechanistic understanding of the chromatographic process has been encumbered by the paucity of pertinent data on the stability constants of ion pairs formed under conditions of chromatographic relevance.

The goal of this communication is to investigate the possible existence of ion pairs under conditions employed in ion-pair chromatography. The analysis of conductivity data reported in the literature⁴ and of the results from titrimetric experiments in our laboratory has yielded stability constants for ion pairs of chromatographic interest that are sufficiently large to be of importance in the retention process.

THEORY

Conductance and ionic composition of solutions

Conductometry. The simplest expression for the conductance, Λ , of a solution is given by

$$\Lambda = \Lambda_0 + \sum_i \lambda_i c_i \quad (1)$$

where Λ_0 is the background conductance of the solvent and λ_i and c_i are the specific conductance and concentrations of ionic species i . The ions may aggregate to form non-conductive species



where A and B are ions and D is neutral. The formation constant of D is given by K . At constant volume mass balances yield

$$\bar{c}_A = c_A + c_D \quad (3a)$$

$$\bar{c}_B = c_B + c_D \quad (3b)$$

where \bar{c}_A and \bar{c}_B are stoichiometric concentrations and c_A , c_B and c_D are actual concentrations of A, B and D, respectively.

The conductance of a solution containing AA' and BB' where A' and B' are the respective counter-ions of A and B, is given in view of eqn. 1 as

$$\begin{aligned} \Lambda &= \lambda_A c_A + \lambda_B c_B + \lambda_{A'} c_{A'} + \lambda_{B'} c_{B'} + \lambda_D c_D + \Lambda_0 \\ &= (\lambda_A + \lambda_{A'}) (\bar{c}_A - c_D) + (\lambda_B + \lambda_{B'}) (\bar{c}_B - c_D) + (\lambda_{A'} + \lambda_{B'}) c_D + \Lambda_0 \end{aligned} \quad (4)$$

The first and second terms on the right-hand side of eqn. 4 can be considered the conductance due to the salts initially comprising the solution reduced by the complex formation and the third term represents the conductance of the new salt formed from the ions A' and B' . Eqns. 2–4 will be used for model calculations in order to simulate the effect of complex formation on conductance under conditions of chromatographic interest.

Evaluation of ion-pair stability constants from titration experiments

Stability constants of ion pairs can be evaluated by using an extension of Bjerrum's method^{6,7} for the determination of metal complexation constants.

The pH of an aqueous buffer solution, pH_0 , containing a complex forming base, B, and its conjugate acid, BH, can be expressed by

$$\text{pH}_0 = \text{p}K_a + \log (\bar{c}_B / \bar{c}_{\text{BH}}) \quad (5)$$

where \bar{c}_B and \bar{c}_{BH} are the appropriate stoichiometric concentrations.

If the conjugate acid forms a complex with species T according to



the stability constant of the complex is given by

$$K = c_{TBH}/c_T c_{BH} \quad (7)$$

where c_T , c_{BH} and c_{TBH} are the actual concentrations in the solution. In this case the concentration of the conjugate acid, which can act as a buffer, is reduced as seen from the mass balances

$$c_{BH} = \bar{c}_{BH} - c_{TBH} \quad (8a)$$

and

$$c_T = \bar{c}_T - c_{TBH} \quad (8b)$$

where \bar{c}_T is the stoichiometric concentration of the complexing agent. In this treatment we assume that the hydrolysis of the base is negligible. The pH of the solution with the complexing agent added is given as

$$\text{pH} = \text{p}K_a + \log \{ \bar{c}_B / (\bar{c}_{BH} - c_{TBH}) \} \quad (9)$$

and the pH change, ΔpH , which occurs upon complexation with the conjugate acid of the buffer is given by

$$\Delta\text{pH} = \text{pH} - \text{pH}_0 = \log \{ \bar{c}_{BH} / (\bar{c}_{BH} - c_{TBH}) \} \quad (10)$$

Eqn. 10 can be rewritten in the form

$$10^{-\Delta\text{pH}} = 1 - c_{TBH}/\bar{c}_{BH} \quad (11)$$

An expression for the concentration of complex c_{TBH} is obtained by combination of eqns. 8a and b as

$$c_{TBH} = \frac{(\bar{c}_{BH} + \bar{c}_T + 1/K) - \sqrt{(\bar{c}_{BH} + \bar{c}_T + 1/K)^2 - 4\bar{c}_{BH}\bar{c}_T}}{2} \quad (12)$$

The stability constant K can be estimated from the dependence of the pH shift on the stoichiometric concentration of the complexing agent by use of eqns. 11 and 12. The initial stoichiometric concentration of the conjugate acid is determined from the initial pH value, the total concentration of the buffer, *i.e.*, $\bar{c}_B + \bar{c}_{BH}$, and the $\text{p}K_a$ of the buffer according to eqn. 5. Alternatively the initial solution may be prepared by mixing the base (or the acid) and its salt, in which case the initial concentrations are known. A suitable iterative technique must be used in the calculations; *e.g.* least-squares parameter estimation of non-linear equations according to Marquardt⁸. By substituting \bar{c}_B for \bar{c}_{BH} analogous formulations can be derived for the formation of complexes with the base.

EXPERIMENTAL

Analysis of conductance data

Experimental results reported by Bidlingmeyer *et al.*⁴ were used in conjunction with eqns. 2-4 to simulate the effect of the ion pair on the conductance. The following values were used: octylammonium chloride, $\lambda = 176$; sodium octylsulfate,

$\lambda = 137$ and NaCl, $\lambda = 217$; all molar conductivities are expressed in $\text{mS } M^{-1}$. The conductance of water, λ_0 , is 0.33 mS. For the calculation of conductances we assumed formation constants ranging in magnitude from 10 to $10^6 M^{-1}$.

Job's plots⁹ were calculated for the system octylammonium chloride and sodium octylsulfonate by using these data and by assuming that the sum of the stoichiometric concentrations of the two species was fixed at 40 mM. Conductances were calculated with stability constants ranging from 10 to $10^6 M^{-1}$ and an increase in the concentration of octylammonium chloride from 0 to 40 mM was assumed to take place in 4 mM increments. The calculations were done on a PDP 11/10 mini-computer (Digital Equipment Corp., Maynard, MA, U.S.A.).

Titrimetric experiments

Materials. Dopamine, epinephrine and octopamine hydrochlorides were purchased from Aldrich (Milwaukee, WI, U.S.A.) or Sigma (St. Louis, MO, U.S.A.). Butyl-, hexyl-, octyl- and decylsulfates were supplied by Eastman Organic Chemicals (Rochester, NY, U.S.A.) and Brand-Nu Laboratories (Meriden, CT, U.S.A.).

Procedure. Catecholamine hydrochlorides were dissolved in distilled water to obtain 10 mM solutions that were first back titrated with 1 M NaOH solution to near their $\text{p}K_a$ values by using a thermostatted stirred vessel and a Model 26 pH-meter (Radiometer, Copenhagen, Denmark). Before and during the procedure the solution was purged with nitrogen. Known amounts of solid sodium alkylsulfates were added to increase the concentration by 10 mM. The pH was recorded at each step when the alkylsulfate had been completely dissolved. The procedure was repeated until the concentration of alkylsulfate reached 100 mM.

Eqns. 11 and 12 were used to estimate the stability constants of the catecholamine-alkylsulfate complexes by using a non-linear least-squares program written in BASIC language and a PDP 11/10 computer (Digital Equipment Corp.).

RESULTS AND DISCUSSION

In an earlier work from our laboratory¹⁰ on reversed-phase chromatography of catecholamines with alkylsulfates of different chain length in the eluent, two limiting models were proposed: (a) ion-pair formation in the mobile phase and retention of the ion pairs by the stationary phase, and (b) adsorption of alkylsulfate to the stationary phase that is converted into a dynamically coated ion exchanger. As the pertinent equilibrium constants were not available from extrachromatographic measurements the dependence of binding on the alkyl chain length of the amphiphilic anions was used to conclude that *under the conditions investigated* the chromatographic results were consistent with the ion-pairing but not with the dynamic ion-exchange model.

A number of workers^{4,5,11-18} have disagreed with this proposition by claiming that ion pairs are weak in water and therefore cannot contribute materially to retention. Disbelief in the chromatographic significance of ion-pair formation is not entirely groundless as the pertinent stability constants for simple salts such as LiNO_3 and tetraisoamylammonium nitrate¹⁹⁻²¹ and moderately complex salts such as tetraethylammonium picrate²² have been found to be rather low, of the order of $1 M^{-1}$ or less. The situation, however, is different if the components of the complex are

comprised of ions having bulky non-polar moiety. Diamond²³ reported that the dependence of the osmotic coefficients of tetraalkylammonium chlorides and iodides on concentration in aqueous solution at the freezing point is very different from the values predicted on the basis of the Debye-Hückel equation. In a similar fashion, the osmotic coefficients of fatty acids at 25°C were also anomalous. The effects were attributed to ion pairing promoted by hydrophobic effects.

Since that time evidence of strong ion pairing in water has become abundant. For instance, solubility product of 1:1 ion pairs formed from benzyltriphenylphosphonium and dodecylsulfate ions²⁴ are in the range $2 \cdot 10^{-8}$ to 10^{-7} *M* (ref. 25). Upon decrease of the chain length of the alkyl sulfate from 14 to 7 the corresponding solubility product increases from $6 \cdot 10^{-9}$ to $5 \cdot 10^{-6}$ *M*. Also 2:1 complexes show comparable stability and the solubility constants are of the order of 10^{-13} *M* (ref. 26). Tomlinson and Davis²⁷ found that 2:1 complexes of sodium cromoglycate and alkylphenyldimethylammonium chlorides have solubility constants ranging from $3 \cdot 10^{-8}$ to $4 \cdot 10^{-7}$ *M* as the alkyl chain increases from decyl to octadecyl.

Of more relevance to the present issue are stability constants of ion pairs formed in water. Benzyltriphenylphosphonium ion pairs with dodecyl sulfate have a stability constant of 156 *M*⁻¹ at 25°C (ref. 28). Alkyltrimethylpyridinium-alkylsulfate ion pairs have stability constants between $4 \cdot 10^{-4}$ and $2 \cdot 10^{-6}$ *M*⁻¹ when the alkyl groups range between hexyl and hexadecyl, respectively²⁵. It has been asserted that complex formation is insignificant when the total number of carbon atoms in the imminent ion pair is less than twenty²⁹. This rule is not absolute in view of the observation that the formation constants for ion pairs of tetramethylammonium and tetraethylammonium with dodecylsulfate are 12.5 and 18.5 *M*⁻¹, respectively, which are not markedly different from the constant for corresponding tetrapropylammonium ion pair³⁰. High ion-pair formation constants are apparent in ion pairs formed from naphthalene-, anthraquinone-1- and azobenzene-4-sulfonate with butyl-, hexyl-, octyl- and decyltrimethylammonium ion³¹.

Ion-pair formation is favored due to the decreasing dielectric constant of the medium when the concentration of the organic component in hydroorganic mixtures is increased^{22,32,33}. As a result, for instance, the formation constant of a relatively small ion pair such as tetrabutylammonium nitrate increases by nearly 15 orders of magnitude in water-dioxane mixtures from pure water to pure dioxane. On the other hand hydrophobic (solvophobic) interactions, responsible for the relatively large stability constant in water, decrease with increasing organic solvent concentration. Consequently, the ion-pair stability constant might not increase monotonically with increasing organic composition but go through a minimum. Nevertheless, there are claims made in the literature³³ that at low ethanol concentrations the hydrophobic contribution to the formation constant of trimethylammonium-alkylcarboxylate ion pairs increases with the concentration of ethanol.

Although amphiphilic ions like those used in ion-pair reversed-phase chromatography can form ion pairs, direct evidence for their existence under conditions used in chromatography has not been presented to date. On the contrary, the conductance of the solutions containing sodium octanesulfonate and octylamine hydrochloride has been taken as evidence against ion-pair formation because the conductance, corrected for dilution, of solutions containing either octanesulfonate or octylamine upon titration with the octylamine and octanesulfonate, respectively,

appeared to increase linearly with titrant volume⁴. This would be expected if ion pairs are not formed.

We repeated that analysis by computer simulation of the conductance expected if ion-pair formation occurred for several values of the stability constants for ion-pair formation. The conductance of the salts used were determined from the data in Fig. 3 of Bidlingmeyer *et al.*⁴. The results along with corresponding curve from Bidlingmeyer *et al.* are shown in Fig. 1. Points of the calculated data show that as long as the stability constant is less than $100 M^{-1}$ apparently straight lines are obtained and only when the constant exceeds $1000 M^{-1}$ does curvature become evident. Consequently an apparently linear plot of experimental data should not be taken as evidence for the absence of ion pairs.

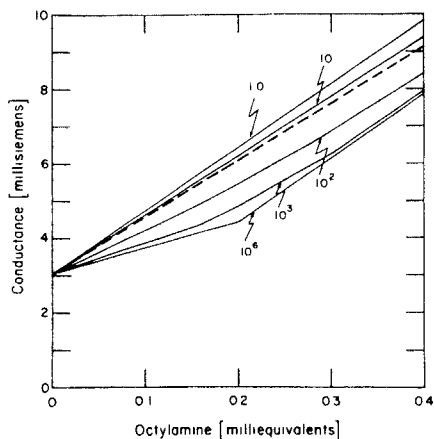


Fig. 1. Results obtained by simulating conductometric titration of octylsulfonate by octylamine in aqueous solution that has been reported by Bidlingmeyer *et al.*⁴. Calculated conductance is plotted against octylamine concentration with the ion-pair formation constant as the parameter. The dashed curve shows the experimental results given in the literature⁴.

Inspection of Fig. 1 strongly suggests that the stability constant of the ion pair octylamine–octanesulfonate should be less than $100 M^{-1}$ and greater than $10 M^{-1}$. Upon assuming that retention occurs via ion-pair formation our estimate for the stability constant from data given on the dependence of octylamine retention factors on octylsulfonate concentration (Fig. 4 of ref. 4) is $500 M^{-1}$ which is much higher than the value suggested by Fig. 1. Therefore, an apparently linear conductance *versus* concentration plot may not exclude ion-pair formation as a chromatographically significant phenomenon.

An alternative technique to the use of simple plots of conductance *versus* concentration of potential pairing ion to discover the existence of ion pairs is the use of Job's plot⁹ of conductance data. Job developed the method of continuous variation to detect the formation of weak complexes in solution. In the method, a series of solutions are prepared which contain mixtures of the compounds that are expected to form complexes. In all solutions the sum of the concentrations of the putative complex formers is constant. An extensive property such as optical absorption or electrical conductivity of one or both complex formers is measured. In Job's

plot, the value of that extensive property is plotted *versus* the mole fraction of one of the complex formers. If no interaction occurs, the data will form straight line connecting the points corresponding to the values for the solutions containing the pure components. However, if complex is formed, the plots will not be straight. In the usual case, the composition at which the deviation from the straight line is maximal corresponds to the stoichiometry of the complex.

The value of Job's plots in discovering complexation is shown in Fig. 2 which is a simulation of the octylsulfonate–octylamine system for various values of ion-pair formation constants in which the parameters used in the previous simulation also were used. The sum of the concentration of octylamine and octylsulfonate were set at 40 mM in order to correspond to the conditions used in Fig. 1. In contrast to Fig. 1, the deviation from linear behavior is striking when the stability constant is as small as $10 M^{-1}$ and the figure clearly demonstrates that the use of the method of continuous variations and a Job's plot can signal the incidence of interactions which may be weak and therefore not readily discerned in other graphical data presentations.

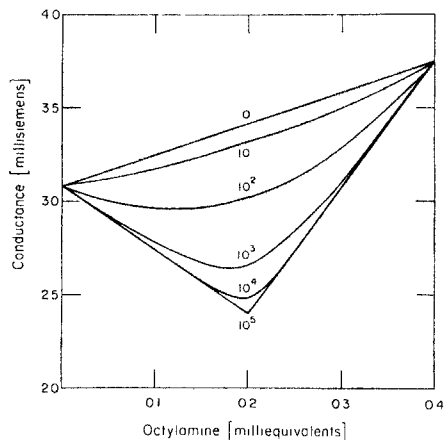


Fig. 2. Job's plot⁹ of simulated conductance data for the aqueous octylamine–octylsulfonate system described by Bidlingmeyer *et al.*⁴. The parameter values used in the calculation were those reported by these authors. It is assumed that the sum of octylamine and octylsulfonate concentration was fixed at 40 mM.

In a previous work from our laboratory¹⁰ formation constants of ion pairs between various catecholamines and alkylsulfates were evaluated from the dependence of catecholamine retention on alkylsulfate concentration in reversed-phase chromatography under the assumption that retention occurs via ion pairing in the particular system investigated. We attempted an independent confirmation of those results by determining stability constants directly. After preliminary experiments, the use of conductance measurements was rejected on the basis of the insensitivity of the method, as seen above. We used the pH change due to addition of detergent to catecholamine solutions to determine stability constants as outlined under theory. This method is derived from the method of Bjerrum⁶ for the determination of metal

complexation constants. The experiments were done at 40°C and under the same conditions used previously¹⁰. The results are given in Table I. The mean value and the standard deviations were calculated for the stability constants obtained with each alkylsulfate and also with each catecholamine. The small standard deviations with mean stability constant value of the alkylsulfates imply that the stability constant is nearly independent of the catecholamine of the ion pair. On the other hand, the standard deviation of the stability constant for a catecholamine is large which implies that the magnitude of the stability constant does depend on the chain length of the alkylsulfate. This can be readily seen by examining the individual constants or their mean values that increase with carbon number of the alkyl chain as shown in Table I. These data do show the existence of ion pairs, yet the stability constants are about three times smaller than those reported earlier on the basis of chromatographic experiments¹⁰. This discrepancy cannot be due to the difference between the column pressure in the earlier determination and that at which the titrations were done. If the discrepancy had been due to pressure, a very conservative estimate shows the volume change in ion-pair formation to be at least $-400 \text{ cm}^3 \text{ mole}^{-1}$.

TABLE I

FORMATION CONSTANTS OF CATECHOLAMINE ION PAIRS WITH ALKYL SULFATES IN AQUEOUS SOLUTION AS DETERMINED BY THE TITRATION METHOD DESCRIBED IN THE EXPERIMENTAL SECTION

The formation constants are expressed in M^{-1} .

| <i>Amine</i> | <i>Alkylsulfates</i> | | | | <i>Mean ± S.D.</i> |
|--------------|----------------------|---------------|----------------|----------------|--------------------|
| | <i>Butyl</i> | <i>Hexyl</i> | <i>Octyl</i> | <i>Decyl</i> | |
| Dopamine | 7.0 | 8.9 | 18.9 | 18.2 | 13.2 ± 6.2 |
| Epinephrine | 12.8 | 6.3 | 19.6 | 22.2 | 12.7 ± 9.6 |
| Octopamine | 8.4 | 8.8 | 17.5 | 21.2 | 14.0 ± 6.4 |
| Mean ± S.D. | 9.4 ± 3 | 8.0 ± 1.5 | 18.7 ± 1.0 | 20.5 ± 2.1 | |

Since these results conflict with those obtained previously¹⁰ it appears that neither the simple ion pairing mechanism, nor the dynamic ion-exchange mechanism adequately account for the phenomena occurring in ion-pair chromatography³⁴.

CONCLUSIONS

The results presented here strongly suggest that ion-pair formation indeed takes place under conditions used in ion-pair chromatography contrary to numerous disclaimers in the literature that were made without access to accurate data on the pertinent stability constants. Nevertheless, abundant experimental evidence suggests that neither the simple ion pairing nor the dynamic ion-exchange model describes adequately the mechanism of the chromatographic process over the full range of practical operating conditions. Therefore a more complicated mechanistic model must be developed in order to account for all experimental observations accumulated in the study of this highly important chromatographic technique.

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