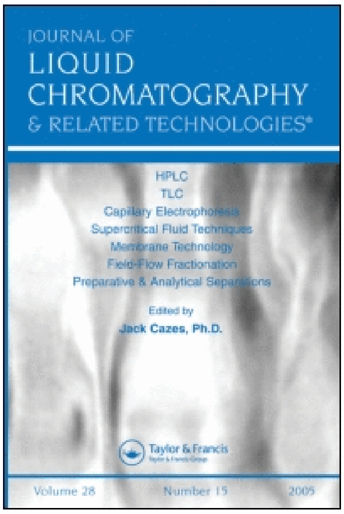

**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Adsorption of Tetralkyl Ammonium Ions on Reversed-Phase HPLC Columns

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**To cite this Article** Rey, M. E. Del and Vera-avila, L. E.(1987) 'Adsorption of Tetralkyl Ammonium Ions on Reversed-Phase HPLC Columns', Journal of Liquid Chromatography & Related Technologies, 10: 13, 2911 — 2929
**To link to this Article: DOI:** 10.1080/01483918708066835
**URL:** http://dx.doi.org/10.1080/01483918708066835

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(13), 2911-2929 (1987)

# ADSORPTION OF TETRALKYL AMMONIUM IONS ON REVERSED-PHASE HPLC COLUMNS

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## ABSTRACT

A systematic study of the influence of several chromatographic parameters upon adsorption of tetralkyl ammonium ions on reversed-phases has been made. Results show that organic solvent content in mobile phase, tetralkyl ammonium hydrophobicity and tetralkyl ammonium concentration in eluent are the most influent variables. A mathematical expression to calculate the adsorbed ion concentration for different combinations of these variables is deduced. Results from this study can be applied in Ion Pair Reversed-Phase Chromatography to calculate column equilibration times, for setting gradient elution separation methods and for the study of retention mechanisms.

## INTRODUCTION

Ion Pair Reversed-Phase Chromatography (RP-IPC) is actually a technique of common use for the separation of ionic compounds. The mobile phase is composed, generally, of water-methanol or water-acetonitrile mixtures with pH and ionic concentration adjusted by addition of buffers and salts. Specially important for the separation of ionic solutes are the ions of opposite charge present in the mobile phase. These ions have received many different names [counterions (1), hetaerons (2), ion-interaction reagents (3) and so on] which shows the uncertainty existing about the retention mechanism operating in these systems. In the present work we will call them counterions.

For the separation of ionic compounds whose molecules have small hydrocarbonate surfaces, the counterions in the mobile phase will have to be compounds with a strong hydrophobicity such as alkyl sulphates or alkyl sulphonates for cationic solutes or tetralkyl ammonium ions for anionic solutes. Several investigators have proved that this type of ions do adsorb by hydrophobic effect on the surface of the stationary phase (4-6).

The process of adsorption of counterions has several implications:

First, it can be deduced that the mobile phase volume required for reaching column equilibrium will change with the mobile phase composition because of changes in the adsorption isotherm of the counterion. Knox et al (7) reported a case where equilibrium was reached after passage of 8 L of mobile phase through the column. Then, when setting a separation method for routine work by RP-IPC with gradient elution, a main requirement is reproducibility but it is also very important that the time for going baçk to initial conditions be as short as possible. Nevertheless during the developpment of the gradient there will be changes in the concentration of adsorbed counterion so that the reproducibility will depend on the kinetics of the desorption process for this species. Besides, if initial and final conditions of gradient correspond to very different concentrations of adsorbed counterion,the time required to return to initial conditions may be fairly long.

Finally, to study the retention mechanism in RP-IPC is necessary a knowledge of the previous state of the stationary phase, that is, the adsorbed counterion concentration before injection of solutes, which would be very different with different experimental conditions.

This discussion shows the importance of the study of adsorption of heavy counterions on reversed-phase columns. Even if many investigators have published adsorption isotherms of some counterions with several mobile phase compositions (4,6-10), a more complete and systematic study of this process is still necessary.

Here we present the results of the study of tetralkyl ammonium ions adsorption on reversed-phase columns. We have studied the main variables that fix the stationary counterion concentration. A related study for anionic counterions has been published elsewhere (10).

## EXPERIMENTAL

### Instrumentation and Chromatographic Procedures

The liquid chromatograph used consisted of a Perkin-Elmer 3B Series Pump System, a Model 7105 Rheodyne injector, a Model R 401 Waters refractive index detector and a Perkin-Elmer Model 023 recorder. Column temperature was fixed at 40° C by means of a Perkin-Elmer Model LC-100 oven. All mobile phases were degassed before use in an ultrasonic bath (Branson Instruments Model D-50).

The columns of Lichroma tubing (15 cm x 4.6 mm i.d.) were homepacked with a 5μ C 18 irregular shaped reversed-phase packing Supelcosil LC-7 (250 $m^2/g$ , supplier data). Elementary analysis of packing showed a carbon content of 13.6% which corresponds to a 0.63 mmol/g concentration of C 18 chains chemically bounded to silica. The weight of packing in the columns was 1.24 g ± 2% (from a 5 column analysis). These columns were used in almost all the experiments except for the study of the effect of different salts on counterion adsorption where the packing was irregular shaped 5μ Rsil C 18 HL (Alltech) with a carbon content of 16 % (supplier data). The weight of packing in the column was, in this case, 1.45 g. For the sulfas' separation we used a prepacked 10μ Lichrosorb RP 8 column (25cm x 4.6mm i.d. from Chromapon Inc.).

The void volume of the chromatographic system was measured for every composition of mobile phase (without counterion present) by injection of a $NaNO_3$ solution. This volume varied significatively with changes in acetonitrile content in the mobile phase (Table 1). Changes in other mobile phase variables (salt content or salt type) did not modify appreciably the void volume.

The adsorbed tetralkyl ammonium ion concentration was evaluated by means of the mobile phase volume passed through the column until there was a detection of the migration front of the counterion by refractive index (5). It was possible to detect migration fronts with counterion concentrations in mobile phase as low as $10^{-4}$ M.

For each experiment, before passing the mobile phase with counterion, the column was pre-equilibrated with a solvent of the same composition but without counterion. After each experiment, the column was washed with 40 mL of pure methanol in order to eliminate all counterion from the stationary phase.

The tetralkyl ammonium concentration in the stationary phase was calculated from each experiment with the following relation:

$$[C^+]_{st} = \frac{(V_f - V_o)}{W_{st}} \cdot [C^+]_m \qquad (1)$$

TABLE 1

Variation of Void Volume with Acetonitrile Content in Mobile Phase

Conditions: column (15cm x 4.6mm i.d.) packed with 5μ Supelcosil LC-7; mobile phase acetonitrile-water (pH=7), containing 0.25 M ammonium acetate; temperature 40°C.

| % acetonitrile (v/v) | void volume (mL) |
|---|---|
| 40 | 1.98 |
| 35 | 2.04 |
| 30 | 2.14 |
| 25 | 2.16 |
| 20 | 2.22 |
| 10 | 2.35 |

where $[C^+]_{st}$, is the counterion concentration in the stationary phase (mmol/g), $[C^+]_m$ is the counterion concentration in the mobile phase (mmol/mL), $V_f$ is the mobile phase volume passed through the column until elution of the migration front (mL), $V_o$ is the void volume of the chromatographic system (mL) and $W_{st}$ is the mean packing weight in the column (g).

## Standard and Reagents

Analytical grade acetonitrile and methanol were from Merck. Water was bidistilled and degassed thoroughly by vacuum. The bromide salts of tetraethyl ammonium (TEA), tetrapropyl ammonium (TPA), tetrabutyl ammonium (TBA) and tetrahexyl ammonium (THA) were purchased from Eastman Chemicals. Salts added to the mobile phase, sodium sulphate (Baker), mono and disodium phosphates (Merck), citric acid (Merck) and crotonic acid (Eastman Chemicals) were analytical grade. The sodium hydroxide used was from Reactivos Químicos Monterrey. The sulfa drugs were obtained from Laboratorio Central de la Dirección General de Aduanas.

Mobile phases were prepared in volumetric flasks taking aliquots from concentrated solutions of tetralkyl ammonium ions and of inorganic salts and adding the required volume of water and acetonitrile. The solvents to pre-equilibrate the column were prepared in a similar way but without the counterion. All mobile phases were degassed in an ultrasonic bath before use.

The concentrated solution of tetralkyl ammonium ions were prepared dissolving the calculated amount of the bromide salt in water to give a concentration of $10^{-2}$ M. For THA, which is sparingly soluble in water, the "concentrated" solution was $10^{-3}$ M. Concentrated solutions of the salts were prepared dissolving the calculated amount of the salt (sodium sulphate or ammonium acetate), the mixtures of salts (mono and disodium phosphates) or the acid (crotonic or citric acid) in water and adjusting the pH to 7 with a NaOH solution. For the ammonium acetate solution where it was necessary to know the concentration with better precision, a titration was made using the formaldehide method (11).

## RESULTS

### Adsorption Isotherms of Tetralkyl Ammonium Ions

Figure 1 shows the adsorption isotherms of TBA and TPA ions at 40°C using a water rich mobile phase with a constant ammonium acetate concentration to fix pH and ionic strength. The two ions follow a Freundlich type adsorption isotherm described by the mathematical relation:

$$[C^+]_{st} = A\ [C^+]_m^B \qquad \text{or} \qquad \log[C^+]_{st} = A' + B\ \log[C^+]_m \qquad (2)$$

where A, B and A' are constants.

When the tetralkyl ammonium concentration in the stationary phase raised over a value of, approximately, 0.12 mmol/g the relation 2 was no longer obeyed and a rapid and irreversible degrading of the column was observed (i.e. a dramatic lost of efficiency followed by pic dedoubling). This limiting value of overcharge of the stationary phase with counterion corresponds to about one TBA molecule for each five hydrocarbonate chains.

It was not possible to obtain the isotherms for TEA,because of a very weak adsorption, or for THA,which has a low solubility in mobile phases and consequently a very strong adsorption on the non polar phase that rapidly overpasses the stability limit mentionned before.

### Acetonitrile Content in Mobile Phase

TBA adsorption was measured for several acetonitrile contents in the mobile phase, all other variables being fixed. Results for two different sets of eperiments are showed in

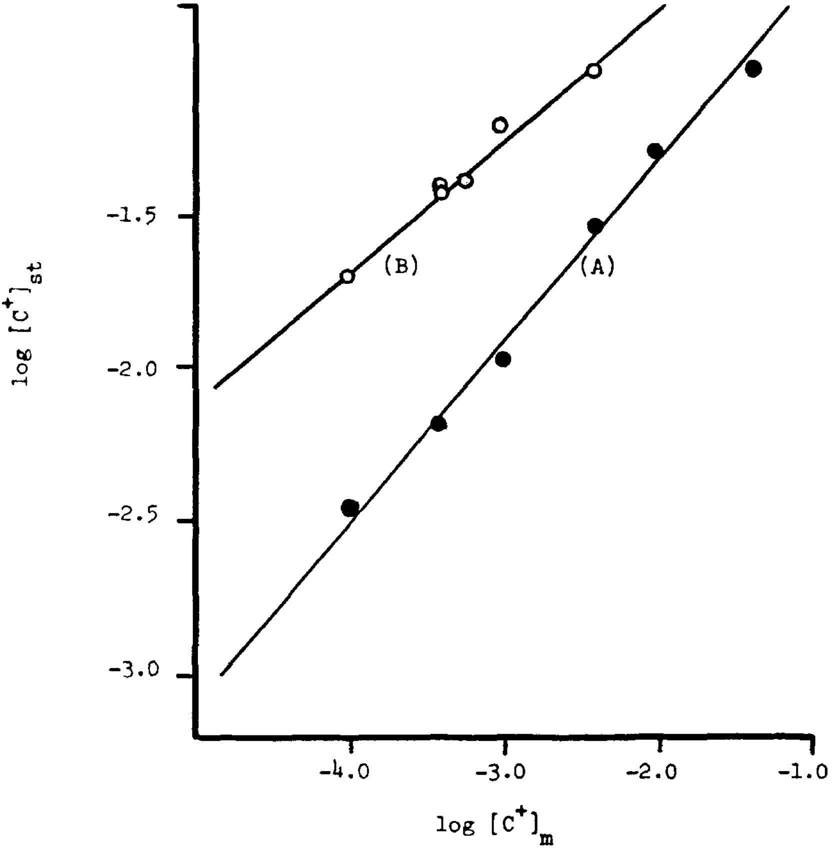

FIGURE 1. Adsorption Isotherms of Tetralkyl Ammonium Ions

Conditions: column (15cm x 4.6mm i.d.) packed with 5μ Supelcosil LC-7; mobile phase acetonitrile-water (pH=7) 10:90 v/v, containing (A) TPA and 0.31 M ammonium acetate, (B) TBA and 0.25 M ammonium acetate; Temperature 40°C.

Regression Equations:

(A) $\log[C^+]_{st} = -0.184 + 0.577 \log[C^+]_m$ (r=0.998)

(B) $\log[C^+]_{st} = 0.082 + 0.443 \log[C^+]_m$ (r=0.986)

Figure 2. In both of them the counterion behaviour is well expressed by the relation:

$$\log[C^{+}]_{st} = X - Y\,\Phi_{org} \qquad (3)$$

where, X and Y are constants for a given set of experiments and $\Phi_{org}$ is the acetonitrile volume fraction in the mobile phase.

## Carbon Atoms in Counterion Molecule

Because of the mentionned problems with THA and TEA, it was only possible to make the two following sets of experiments:
In the first set TPA, TBA and THA adsorption was measured using a rich acetonitrile mobile phase and a low counterion concentration.
In the second set we measured TEA, TPA and TBA adsorption in a water rich mobile phase and a low counterion concentration so as to make it possible to observe a counterion migration front at an elution volume well differentiated from system void volume.

Results from these studies can be seen in Figure 3.
Counterion adsorption can be expressed by the relation:

$$\log\,[C^{+}]_{st} = C + D\,N_C \qquad (4)$$

where, C and D are constants for a given set of experiments and $N_C$ is the number of carbon atoms in the counterion molecule.

## Salt Concentration in Mobile Phase

Figure 4 shows the changes produced in counterion adsorption with varying ammonium acetate concentration in the mobile phase. In the three sets of experiments the behaviour of the counterion can be adjusted by the linear realtion:

$$\log\,[C^{+}]_{st} = P + R\,\log[\mathrm{salt}]_m \qquad (5)$$

where, P and R are constants for a given set and $[\mathrm{salt}]_m$ is the molar salt concentration in the mobile phase.

The concentration salt range studied corresponds to buffer concentrations commonly used in RP-IPC to fix pH and ionic

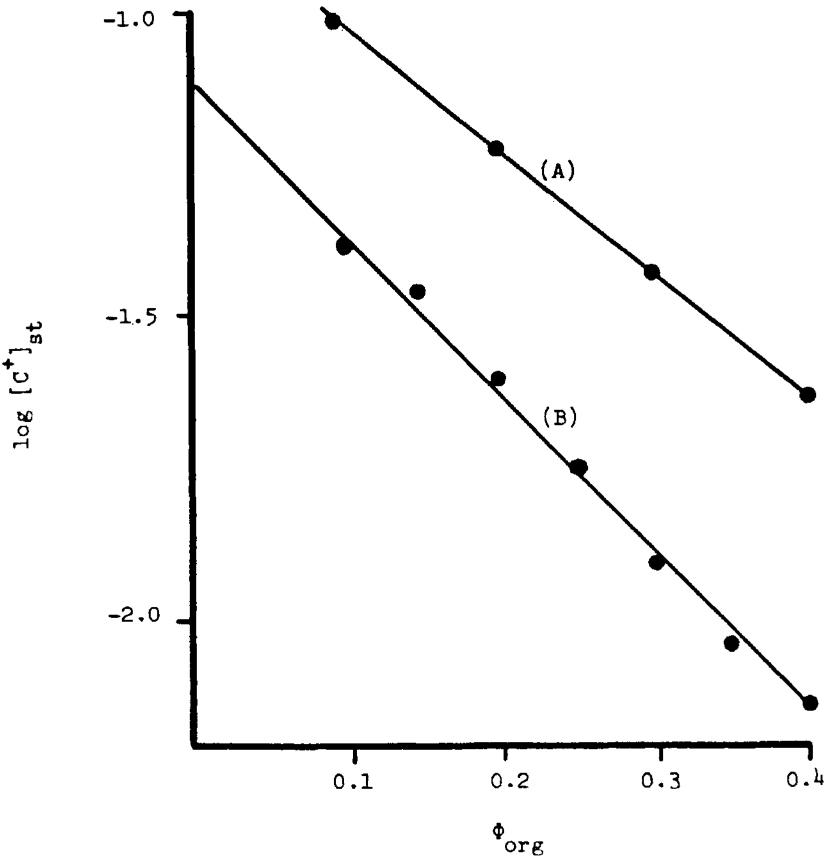

FIGURE 2. Adsorption as a Function of the Acetonitrile Content in the Mobile Phase.

Conditions: column (15cm x 4.6mm i.d.) packed with 5μ Supelcosil LC-7; mobile phase acetonitrile-water (pH=7) containing, (A) 0.004 M TBA (B) 0.0006 M TBA; and 0.25 M ammonium acetate; temperature 40°C.

Regression Equations:

(A) $\log [C^+]_{st} = -0.812 - 2.05\ \Phi_{org}$ (r=-0.999)

(B) $\log [C^+]_{st} = -1.10 - 2.63\ \Phi_{org}$ (r=-0.997)

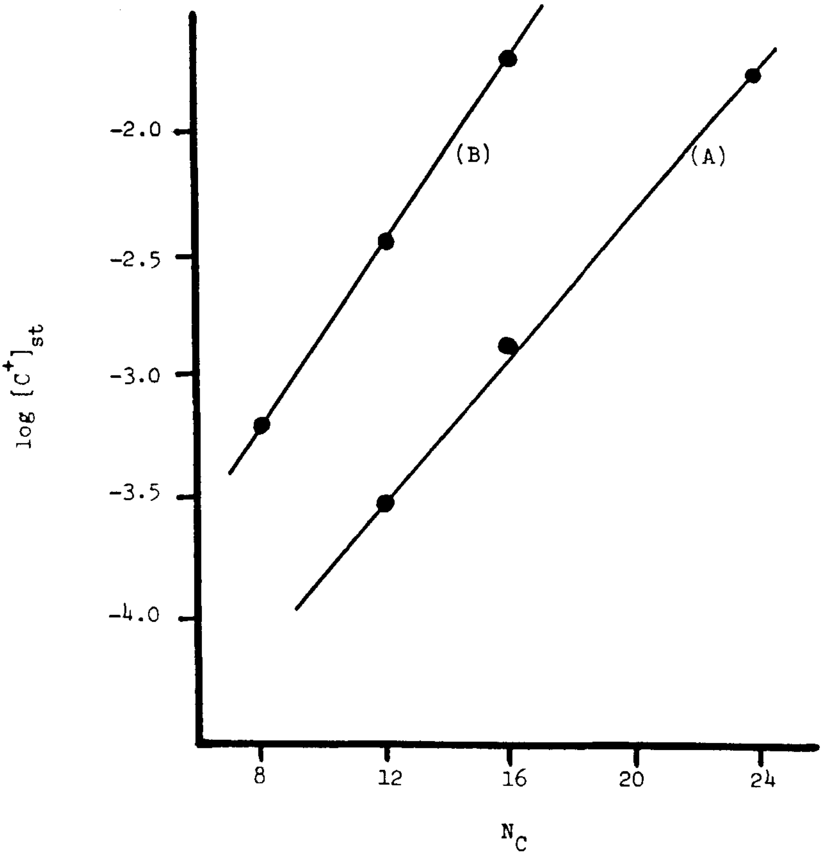

FIGURE 3. Adsorption as a Function of the Number of Carbon Atoms in the Tetralkyl Ammonium Ion Molecule.

Conditions: column (15cm x 4.6mm i.d.) packed with 5μ Supelcosil LC-7; mobile phase acetonitrile-water (pH=7), (A) 40:60 v/v, (B) 10:90 v/v; containing 0.25 M ammonium acetate and 0.0001 M Tetralkyl ammonium ion; Temperature 40°C

Regression Equations:

(A) $\log[C^+]_{st} = -5.21 + 0.144\ N_C$ (r=0.999)

(B) $\log[C^+]_{st} = -4.71 + 0.189\ N_C$ (r=0.999)

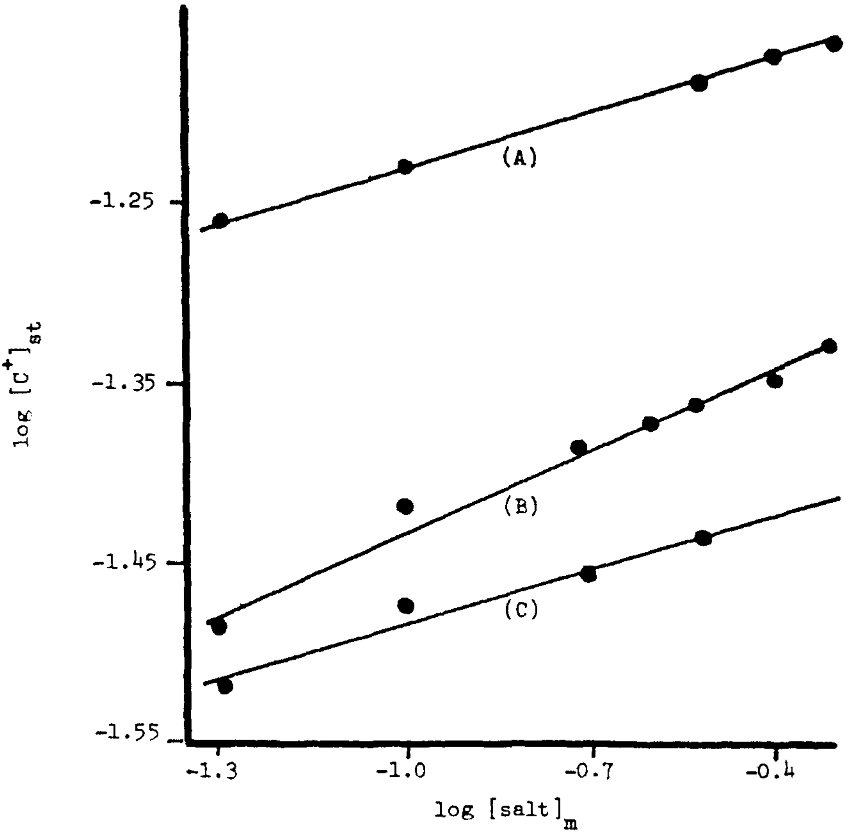

FIGURE 4. Adsorption as a Function of the Salt Concentration in the Mobile Phase.

Conditions: column (15cm x 4.6mm i.d.) packed with 5μ, (A) and (C) Supelcosil LC-7 (B) Rsil C 18 HL; mobile phase acetonitrile-water (pH=7), (A) 10:90 v/v, (B) and (C) 20:80 v/v; containing 0.002 M TBA and ammonium acetate; Temperature 40°C.

Regression Equations:

(A) $\log[C^+]_{st} = -1.136 + 0.097 \log[\text{salt}]_m$ (r=0.998)

(B) $\log[C^+]_{st} = -1.282 + 0.149 \log[\text{salt}]_m$ (r=0.990)

(C) $\log[C^+]_{st} = -1.383 + 0.099 \log[\text{salt}]_m$ (r=0.986) strength of the mobile phase. It can be observed that in this variation range the effect of salt concentration changes on counterion adsorption is very small en relation to others variables' effect studied before.

## Salt Type

For this part we considered that the effect of salts in the mobile phase on the tetralkyl ammonium adsorption is given by the anion of the salt. In fact, adsorbed counterions attract toward the stationary phase opposite charge ions forming a double layer, charged surface-ionic cloud, electrically neutral.

In all the experiments we used a 0.04 M concentration of the chosen anion in combination with a 0.01 M concentration of ammonium acetate. The latter was used in order to fix pH in the mobile phase. Experimental results are shown in Table 2.

Results show that TBA adsorption increases with anion type in the following order:

$$CH_3CO_2^- < SO_4^{2-} < [H_2PO_4^-/HPO_4^{2-}] < \text{crotonate}^- < [\text{citrate}^{2-}/\text{citrate}^{3-}]$$

which means that counterion adsorption rises with charge and hydrophobicity of opposite charge ions in the mobile phase, but this effect is rather weak.

TABLE 2

TBA Adsorption with Different Salts in the Mobile Phase.

Conditions: column (15cm x4.6mm i.d.)packed with 5µ Rsil C 18 HL; mobile phase acetonitrile-water (pH=7) 20:80 v/v, containing 0.002 M TBA, 0.01 M ammonium acetate and 0.04 M studied anion salt; temperature 40°C.

| Studied anion | acetate | sulphate | phosphate | crotonate | citrate |
|---|---|---|---|---|---|
| adsorbed TBA mmol/g | 0.0327 | 0.0364 | 0.0391 | 0.0424 | 0.0435 |

## DISCUSSION

It has been observed that, in the usual ranges of variation of the studied parameters, the most important effects on the adsorption of tetralkyl ammonium ions on reversed-phases are given by variations in the acetonitrile content and counterion concentration of the mobile phase as well as by changes in the counterion carbon number.

Acetonitrile content and counterion carbon number effects are typical of reversed-phases and thus they are related to the hydrophobic effect responsible of solute retention in this type of phases. Several investigators have observed linear relations between log k' and the carbon number in solute molecules for homologue series (12-13) in reversed-phases. A linear relation between the organic solvent volume fraction in the mobile phase and log k' has also been observed (13-15).

Freundlich adsorption isotherms have been published before for linear chain anions as alkyl sulphates and alkyl sulphonates (9-10) and for linear chain cations as ionic alkyl amines and cetrimide (4,6). Our results show that this type of isotherm is also observed with globular structure ions such as tetralkyl ammonium, therefore it seems that all heavy ions follow a Freundlich adsorption isotherm on reversed-phases, independently of their structure.

Our results can be compared with those obtained by J.T. Davies (16) in his study of the adsorption of ionic surfactants at the oil-water interface. He observed Freundlich type isotherms and proposed that the electrical potential existing between the charged interface and the associated ionic cloud was responsible for this behaviour. With this base Davies developped the following relation:

$$\log n = A + BN_C + C \log[S]_{water} + D \log[salt]_{water} \qquad (6)$$

where, A, B, C and D are constants, $[S]_{water}$ is the molar ionic surfactant concentration in water, $[salt]_{water}$ is the total molar monovalent electrolyte concentration in water and n is the number of adsorbed surfactant molecules per surface unit.

The strong similarity between Davies equation (6) and our own results (equations 2, 4 and 5) has brought us to propose that for the study of the RP-IPC retention mechanism, the formation of an electrical double layer at the stationary phase-eluent interface has to be considered.

The weak effect observed for salt concentration variations on counterion adsorption has an interesting practical application. It has been observed in RP-IPC that solute retention disminishes proportionally to mobile phase salt concentration (5, 6,9) so, elution gradients based in a gradual increase of salt concentration in the mobile phase would be very reproducible and would require a minimal equilibration time to return to initial conditions because the stationary phase is scarcely modified during gradient developpment. An experimental example of this application is shown (Table 3) in the separation of some sulfa drugs by RP-IPC, with TBA as counterion and with an ammonium acetate concentration gradient. The separation was repeated several times and only a 5 mL volume (2 times the void volume of the system) of initial solvent was passed through the column in between succesive injections. The retention time variation coefficient for each solute was less than 2% which, considering the abrupt changes of salt concentration in the mobile phase during gradient, is fairly good.

Another interesting parameter to adjust separations by RP-IPC is the salt type in the mobile phase and specifically the ion of opposite charge to counterion. Results in Table 2 show that,when changing mobile phases with different dissolved salts, equilibration time will be small because counterion adsorption remains almost constant. Nevertheless, it has been observed before (5, 9) that changes in salt type may produce appreciable variations in solute retention and selectivity in RP-IPC.

TABLE 3

Retention Time Reproducibility in a RP-IPC Separation with Salt Concentration Gradient (1)

Conditions: column (25cm x 4.6mm i.d.) packed with 10µ Lichrosorb RP 8; Solvent "A" acetonitrile-water(pH=7) 20:80 v/v containing 0.005 M TBA and 0.05 M ammonium acetate , Solvent "B" acetonitrile-water(pH=7) 20:80 v/v containing 0.005 M TBA and 0.5 M ammonium acetate; Flowrate 1 mL/min; Temperature 30°C.

| SOLUTES | retention time (min) in run # : | | | | | $\overline{t_r}$ (2) | σx10$^2$ (3) | %V.C. (4) |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | |
| Sulfa merazine | 7.0 | 7.1 | 7.0 | 7.1 | 6.9 | 7.0 | 8.4 | 1.2 |
| Sulfa methazine | 8.8 | 9.0 | 8.8 | 9.0 | 8.7 | 8.9 | 13 | 1.5 |
| Sulfa methoxazol | 9.7 | 10.0 | 10.0 | 10.0 | 9.7 | 9.9 | 16 | 1.7 |
| Sulfa benzamide | 13.9 | 14.3 | 14.3 | 14.3 | 13.8 | 14.1 | 24.9 | 1.76 |
| Phtalil sulfa thiazol | 19.6 | 20.3 | 20.3 | 20.3 | 19.9 | 20.1 | 31.9 | 1.59 |

GRADIENT:

| | | | | | |
|---|---|---|---|---|---|
| Time (min): | 0 | 10 | 13 | 15 | 30 |
| % B : | 0 | 10 | 30 | 100 | 100 |

(1) At the end of a run a 5 mL volume of Solvent "A" was passed through the column before the next injection.
(2) Mean retention time (min)
(3) Standard deviation in time units
(4) % Variation Coefficient = $(\sigma/\overline{t_r}) \times 100$

## General Model for Counterion Adsorption

Considering only the three parameters with major influence on tetralkyl ammonium adsorption and using only 8 data from experiments corresponding to limiting points in the variation ranges studied (Table 4), we have developped equation (7). This relation permits the calculation of the stationary counterion concentration and so the equilibrium time (equation 8) required, for all posible combinations of parameter values inside these variation ranges.

$$\log [C^+]_{st} = -0.95 + 0.59\Phi_{org} + 0.26N_C + 0.82\log[C^+]_m + [1.08\Phi_{org} - 0.03N_C] \log[C^+]_m \qquad (7)$$

$$t_{eq} = t_o + \frac{[C^+]_{st}}{[C^+]_m} \cdot \frac{W_{st}}{F} \qquad (8)$$

where, $t_{eq}$ is the time required to reach column equilibrium when using a mobile phase containing a tetralkyl ammonium ion (min), $t_o$ is the time of elution of a non retained compound (min) and F is the mobile phase flowrate.

The mathematical model (equation 7) was tested with the results from the 26 experiments made throughout this work, some of them outside the variation range used to calculate the model. The following are the statistical results deduced from this test:

- correlation coefficient (r) = 0.978
- dependent variable ($[\log C^+]_{st}$) variance = $3.02 \times 10^{-3}$
- dependent variable medium value = -1.793
- dependent variable variation coefficient = 3.06 %

The statistical results show that the mathematical model represents correctly the behaviour of the system inside the following ranges:

$10^{-4}\ M < [C^+]_m < 4\times10^{-3} M$ ; $8 < N_C < 16$ ; $0.1 < \Phi_{org} < 0.4$

TABLE 4

Selected Experiments for the Establishment of the General Adsorption Model*

Conditions: stationary phase 5μ Supelcosil LC-7 C 18; mobile phase acetonitrile-water(pH=7) containing 0.25M (1) or 0.31 M (2) ammonium acetate and a variable concentration of tetralkyl ammonium ion; Temperature 40°C.

| PARAMETER | experiment # | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $1^{(2)}$ | $2^{(2)}$ | $3^{(1)}$ | $4^{(1)}$ | $5^{(1)}$ | $6^{(1)}$ | $7^{(1)}$ | $8^{(1)}$ |
| $\Phi_{org}$ | 0.10 | 0.10 | 0.10 | 0.10 | 0.30 | 0.40 | 0.40 | 0.40 |
| $N_C$ | 12 | 12 | 16 | 16 | 12 | 12 | 16 | 16 |
| $\log[C^+]_m$ | -2.4 | -4 | -2.4 | -4 | -2.4 | -4 | -2.4 | -4 |

* The mathematical model and the statistical results were obtained working with reduced parameters in order to have all parameter values at the same level and to form an optimal matrix.

So it is possible to establish similar models for different conditions (stationary phase carbon content, temperature or type of counterion) with only 8 experimental measures of counterion migration front, selected adequately. It seems important to remark that in a previous work (10) where we studied alkyl - sulphonate and alkyl sulphate adsorption, on a different reversed-phase (Lichrosorb RP-8), we deduced a mathematical model of the same form.

## ACKNOWLEDGMENTS

Authors gratefully acknowledge "Consejo Nacional de Ciencia y Tecnología de México" for the financial support of this work.

## REFERENCES

(1) Westerlund, D. and Theodorsen, A., Reverse-Phase Ion-Pair Chromatography of Naphtalene-Acetic Acid Derivatives with Water and an Organic Modifier as the Mobile Phase, J. Chromatogr., 144, 27, 1977.

(2) Horvath, C., Melander, W., Molnar, I. and Molnar, P., Enhancement of Retention by Ion-Pair Formation in Liquid Chromatography with Nonpolar Stationary Phases, Anal. Chem., 49, 2295, 1977.

(3) Bidlingmeyer, B.A., Deming, S.N., Price, W.P., Sachok, B.Jr. and Petrusek, M., Retention Mechanism for Reversed-Phase Ion-Pair Liquid Chromatography, J. Chromatogr., 186, 419, 1979.

(4) Knox, J.H. and Laird, G.R., Soap-Chromatography - A New High-Performance Liquid Chromatographic Technique for Separation of Ionizable Materials. Dyestuff Intermediates, J. Chromatogr., 122, 17, 1976.

(5) Terweij-Groen, C.P., Heemstra, S. and Kraak, J.C., Distribution Mechanism of Ionizable Substances in Dynamic Anion-Exchange Systems Using Cationic Surfactants in High-Performance Liquid Chromatography, J. Chroamtogr., 161, 69, 1978.

(6) Van de Venne, J.L.M., Hendrikx, J.L.H.M. and Deelder, R.S., Retention Behaviour of Carboxylic Acids in Reversed-Phase Column Liquid Chromatography, J. Chromatogr., 167, 1, 1978.

(7) Knox, J.H. and Jurand, J., Determination of Paracetamol and its Metabolites in Urine by High-Performance Liquid Chromatography Using Ion-Pair Systems, J. Chromatogr., 149, 297, 1978.

(8) Deelder, R.S., Linssen, H.A.J., Konijnendijk, A.P. and Van de Venne, J.L.M., Retention Mechanism in Reversed-Phase Ion-Pair Chromatography of Amines and Amino Acids on Bonded Phases, J. Chromatogr., 185, 241, 1979.

(9) Knox, J.H. and Hartwick, R.A., Mechanism of Ion-Pair Liquid Chromatography of Amines, Neutrals, Zwitterions and Acids Using Anionic Hetaerons, J. Chromatogr., 204, 3, 1981.

(10) Vera-Avila, L.E., Caude, M. and Rosset, R., Chromatographie de Paires d'Ions. 1.- Fixation du Contreion et Mecanismes de Retention, Analusis, 10, 36, 1982.

(11) Charlot, G., Chimie Analytique Quantitative II, Masson, 6th edition, Paris, 1974, p. 342.

(12) Berendsen, G.E. and De Galan, L., Role of the Chain Lenght of Chemically Bonded Phases and the Retention Mechanism in Reversed-Phase Liquid Chromatography, J. Chromatogr., 196, 21, 1980.

(13) Karger, B.L., Gant, J.R., Hartkopf, A. and Weiner, P.H., Hydrophobic Effects in Reversed-Phase Liquid Chromatography, J. Chromatogr., 128, 65, 1976.

(14) Horvath, C., Melander, W. and Molnar, I., Solvophobic Interactions in Liquid Chromatography with Nonpolar Stationary Phases, J. Chromatogr., 125, 129, 1976.

(15) Majors, R.E., Bonded Stationary Phases in Chromatography, Grushka, E. (editor), Ann Arbor Sci. Publ., Mich., 1974, Ch.8.

(16) Davies, J.T., Adsorption of Long-Chain Ions. I., Proc. Roy. Soc., A 245, 417, 1958.