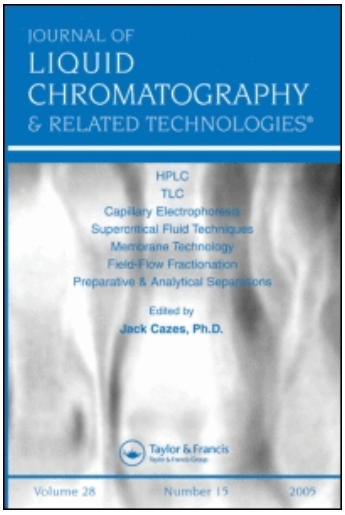

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ADSORPTION OF A HYDROPHOBIC CHELATING AGENT AND ITS CHELATE-METAL COMPLEX ON RP-COLUMNS

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ABSTRACT

The reversed-phase adsorption of the hydrophobic chelating agent 4-dodecyldiethylenetriamine, in the presence of zinc ions, was measured at different compositions of the mobile phase. The effect of the following parameters was analyzed: proportion of metallic ion to chelating agent, concentration of chelating agent, volumic fraction of organic modifier, pH and salt concentration. Results show that, depending on the composition of the mobile phase, the chelating agent can exist in two different forms in both phases: "free" and/or forming a chelate-metal complex. The adsorption of the latter is stronger than that of the free triamine. This phenomenon, which has not been reported before, plays undoubtedly an important role on retention in Reversed-Phase Ligand Exchange Chromatography and should be considered in the study of the retention mechanism.

INTRODUCTION

In the late 70's, knowledge and experience acquired in the study of RP-Ion Pair Chromatography (IPC), led Karger and collaborators to propose the use of metal chelate additives dissolved in the mobile phase, in conjunction with a RP column, to produce a dynamically generated ligand exchanger (1).

Besides possessing high resolution capacity and special selectivity, characteristic of Ligand Exchange Chromatography (LEC), this new technique overcame some limitations of other LEC systems such as poor efficiency and band asymmetry usually found in LEC on resins, or reduced stability and poor column reproducibility found in the chelating bonded phases or in systems based on the deposition of metals on silica.

The chromatographic system in RP-LEC is similar to the system employed in RP-IPC. In both techniques the mobile phase contains a hydrophobic compound (the pairing ion or the chelating agent) which, during the equilibration process, is adsorbed on the column modifing its properties. The main difference between the two systems arises from the species associated with the adsorbed hydrophobic compound and the nature of this association . In RP-IPC, the pairing ion is electrically associated with an exchangeable ion of opposite charge and retention proceeds, at least in part (2-4), by an ion exchange equilibrium. In RP-LEC, the chelating agent is coordinately bond to a metallic ion, with some sites in the coordination sphere of the metal being occupied by exchangeable ligands such as solvent molecules or species coming from salts or buffers dissolved in the mobile phase. In this case, retention probably proceeds by a ligand exchange equilibrium with the metallic ion remaining associated with the stationary chelating agent. Some authors propose that in LEC process, solutes may be retained not only by complexation in the inner coordination sphere of the metal but also by outersphere complexation (5,6).

From this discussion, it seems evident that any study of retention in these dynamic techniques should comprise a prior study of adsorption of the hydrophobic species on the column. In fact, the amount of adsorbed compound determines the system exchange capacity which, in turn, is directly related to solute retention. In addition, the time required to reach equilibrium between the mobile and stationary phases also depends on this.

In the case of RP-IPC, there are many reports on the adsorption of different kinds of pairing ions at different mobile phase compositions (7-9). We have proposed simple algebraic expressions relating the pairing ion concentration in the stationary phase to the eluent composition (10,11). On the other hand, there has not been any corresponding study of the influence of mobile phase composition on the adsorption of chelating agents or chelate-metal complexes for RP-LEC.

In this paper we present results on the effect of different chromatographic parameters on the adsorption of a hydrophobic chelating agent, in the presence of a metallic ion, on a reversed-phase column. We examined the same species used by Karger et al in their works (1,5): i.e. the chelating agent 4-dodecyldiethylene-triamine in the presence of zinc (II) ion.

EXPERIMENTAL

Instrumentation and Chromatographic Procedures.

The LC system used consisted of a Perkin-Elmer 3B Series Pump System, a Rheodyne 7105 valve injector, a R-401 Waters refractive index detector and a Linear 1200 Alltech recorder. The column was thermostated at 40°C by means of a LC-100 Perkin-Elmer oven. All the mobile phases were degassed in an ultrasonic bath from Branson Instruments prior to use.

Acid-base titrations were performed using a 2G8N pHmeter from Tacussel, equipped with a combined glass-calomel electrode.

The chromatographic column consisted of Lichroma tubing (15 cm x 4.6 mm i.d.) which was home-packed with 5 μ irregular shaped reversed phase, Rsil C-18 HL (550 m^2g^{-1} and 16% carbon load) from Alltech.

Void volumes were measured for every mobile phase composition (without chelating agent or transition metal present) by injection of a $NaNO_3$ solution.

The amount of adsorbed chelating agent was determined by frontal analysis. For each experiment, the column was first pre-equilibrated with a solvent containing the appropriate organic modifier, transition metal salt and inorganic salt concentrations, and the refractometer reference cell was filled with this solvent. Then, a mobile phase of the same composition, but also containing the chelating agent, was passed through the column until the migration front of triamine was detected at the refractometer measuring cell.

The quantity of chelating agent in the stationary phase was calculated with the following equation:

$$Q_{t(st)} = (V_f - V_o)\ [Q_t]_{(m)} \qquad (1)$$

where, $Q_{t(st)}$ represents the quantity of adsorbed triamine (mmol), $[Q_t]_{(m)}$ is its concentration in the eluent (mmol/mL), V_f is the mobile phase volume required for its elution and measured at the inflexion point of the front (mL), and V_o is the void volume of the chromatographic system.

However, in several experiments the migration front was split into two fronts, indicating that the triamine was adsorbed in two different forms. In such cases, the quantity of each species adsorbed was calculated by means of the following equation:

$$Q_{i(st)} = (V_{fi}-V_o)\ [Q_t]_{(m)}\ [h_i/(h_1+h_2)] \qquad (2)$$

where:

$$Q_{t(st)} = Q_{1(st)} + Q_{2(st)} \qquad (3)$$

in which, $Q_{i(st)}$ is the quantity of the form i of chelating agent in the stationary phase (mmol), V_{fi} is the mobile phase volume measured at the inflexion point of front i (mL), h_i is the height of this front (cm) and h_1+h_2 is the total change of refractive index produced by the two fronts and measured in cm.

After each experiment, the column was washed with 60 mL of pure ethanol in order to remove all the adsorbed chelating agent.

Pure methanol was not capable of completely desorbing the adsorbed components.

Standard and Reagents.

Analytical grade methanol and ethanol were from Merck. Water was bidistilled and degassed thoroughly by vacuum. The chelating agent, 4-dodecyldiethylenetriamine (Q), was purchased from Eastman Chemicals. The zinc salts were analytical grade from Merck. The other salts and reagents employed were analytical grade. All chemicals were employed without further purification.

Mobile phases were prepared taking aliquots from concentrated solutions of dodecyldiethylenetriamine, zinc acetate and ammonium acetate and adding the required volume of methanol and water. When necessary, the pH was adjusted with perchloric acid. The eluents used to pre-equilibrate the column were prepared in a similar way but without chelating agent.

The zinc acetate and ammonium acetate concentrated solutions were prepared dissolving the appropriate quantity of the salts in water. The zinc acetate solution was titrated with normalized EDTA using dithizone as indicator of the equivalent point. The ammonium acetate solution was titrated using the formaldehyde method (12).

The triamine concentrated solution was prepared taking about 2.7 mL of the pure reagent and adding methanol to a final volume of 100 mL. An aliquot of this solution was diluted with water and titrated with normalized HCl. From the titration curve (Fig.1a) it is inferred that the pH jump corresponds to the simultaneous neutralization of two basic groups of the triamine (pK_a between 8.5 and 9.5), while the third amino group is a very weak base (pk_a about 3.5 to 4) and the neutralization reaction is not quantitative.

In a separate experiment, the same aliquot of the triamine concentrated solution was first mixed with an equimolar quantity of $ZnCl_2$ and then titrated with HCl. In this case, the characteristics of the titration curve (Fig.1b) consisted of:

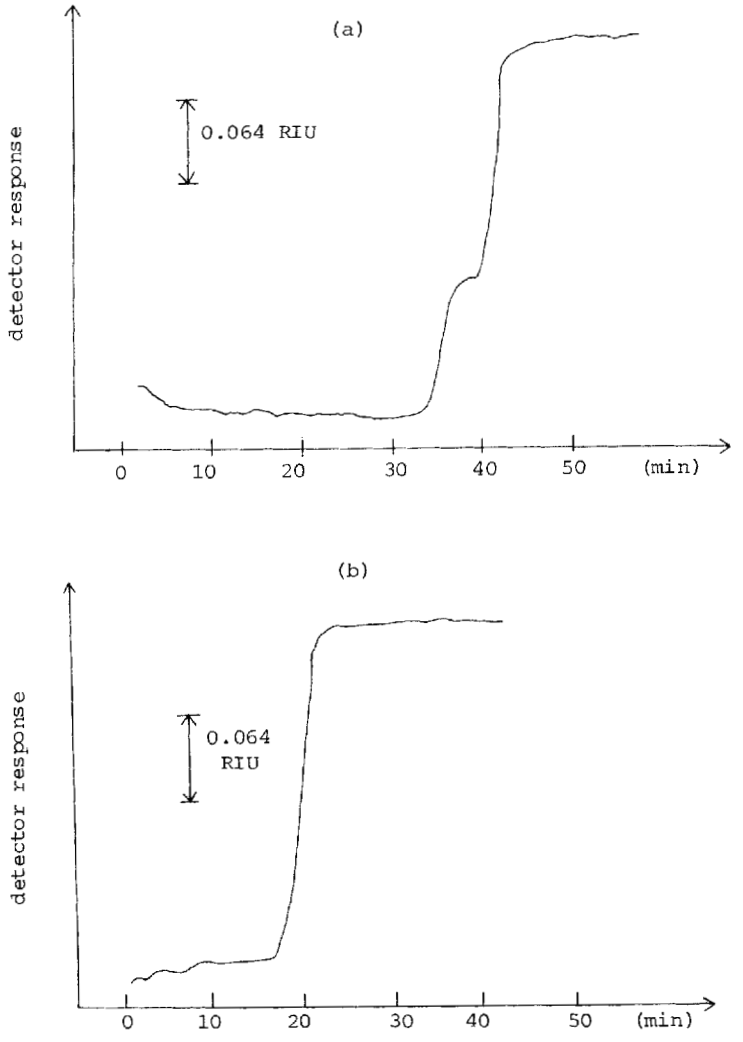

FIGURE 1. Titration Curves of 4-Dodecyldiethylenetriamine with HCl

(a) 5 mL aliquot of a concentrated (0.085 M) triamine solution.
(b) same as (a) but also containing 8 mL of $ZnCl_2$ 0.055 M arrows show the region where the emulsionlike solution exists.

- The initial pH and the pH values during the first part of the curve were notably lower than before.
- In the second part, the pH decrease was slower than in the absence of $ZnCl_2$ and, at pH ≈ 5.6 ($[Cl^-]$ ≈ 0.045 M), the initially transparent solution turned to a white, phosphorescent, emulsion-like solution which disappeared suddenly at pH ≈ 4.5 .

In this experiment, it is evident that the formation of the complex Q-Zn provokes a decrease in the chelating agent basicity, but it is not possible to ascertain whether the complex is progressively destroyed by the acid at the beginning of the titration, or if it can resist the addition of a certain amount of HCl before being destroyed

In order to understand the observed phenomena, we repeated the experiment with twice the concentration of $ZnCl_2$. In this case, an emulsion immediately appeared and again dissapeared at pH 4.5; the titration curve was similar to Fig. 1b.

We suggest that, under our experimental conditions, the concentration of hydrophobic chelating agent (of the order of 10^{-2} M) exceeded the Critical Micellar Concentration (CMC) of this compound, either in its free or complexed form. It is known (13,14) that micellar solutions where the counterion concentration exceeds a certain threshold value, characteristic of surfactant species and counterion species, undergo a phase transition with structural changes that give rise to lamellar or liquid crystalline forms. Thus, we suppose that the micelles of Q-Zn undergo this transition, forming the white emulsion when a certain concentration of chloride is present, whereas the micelles of free Q cannot do it under the same conditions.

This also means that the complex can resist the addition of a quantity of acid equivalent to its molar concentration (protonation of an amino group of the triamine) before it begins to be destroyed.

RESULTS AND DISCUSSION

Preliminary experiments using two mobile phases with different amounts of methanol and the same triamine, zinc acetate and ammonium acetate concentrations, showed that depending on the eluent composition, the chelating agent can migrate through the column and be adsorbed on the stationary phase in a unique form (Fig. 2b) or in two different forms (Fig. 2a). It is known that metallic complexes are more stable in solvents of lower dielectric constant. Thus, under the conditions of Figure 2b (80% methanol) the species in the mobile and stationary phases is probably the chelate-metal complex Q-Zn, while in Figure 2a (60% methanol) one front represents the adsorption of the free triamine while the other is due to the adsorption of the complex.

The total change of refractive index when the front(s) exit the column is proportional to the total concentration of chelating agent in the mobile phase. In the experiments of Figure 2, the concentrations are equal and it is observed that the total height of the front(s) is practically the same (no special precautions were taken to thermostat the detector). Therefore, we conclude that where two fronts appear, the fraction of the total height represented by each front is equal to the fraction of the chelating agent concentration adsorbed as free triamine Q, or as the Q-Zn complex. The problem in this case is to determine which front corresponds to which species.

Effect of the Ratio of Metallic Ion to Chelating Agent in the Mobile Phase.

In order to solve the problem mentioned above and to confirm our hypothesis concerning the nature of each front, we decided to progressively change the ratio of zinc acetate to dodecyldietylene triamine in the mobile phase.

Figure 3 shows the migration fronts obtained when this ratio was varied from 0 to 4.25. In all the experiments, the chelating

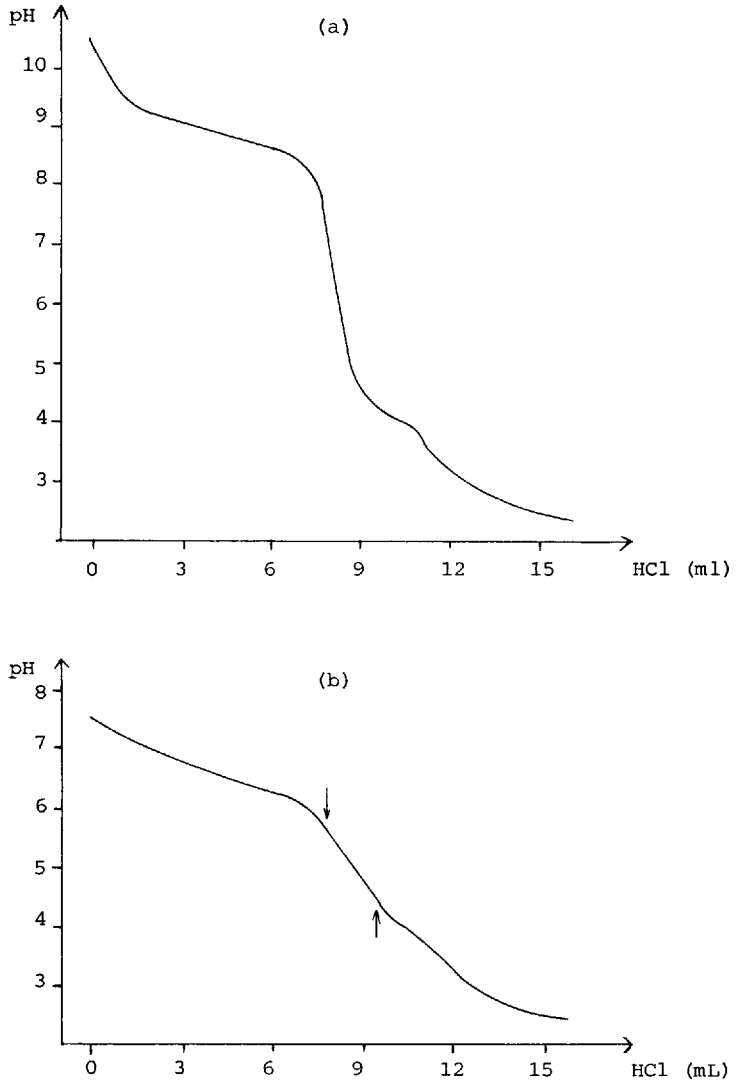

FIGURE 2. Adsorption of the Triamine at Two Different Conditions.

mobile phase: methanol-water (a) 60:40 v/v, (b) 80:20 v/v; with 4-dodecyldiethylenetriamine 0.00508 M, zinc acetate 0.00539 M and ammonium acetate 0.194 M.
detector: refractive index temperature: 40°C

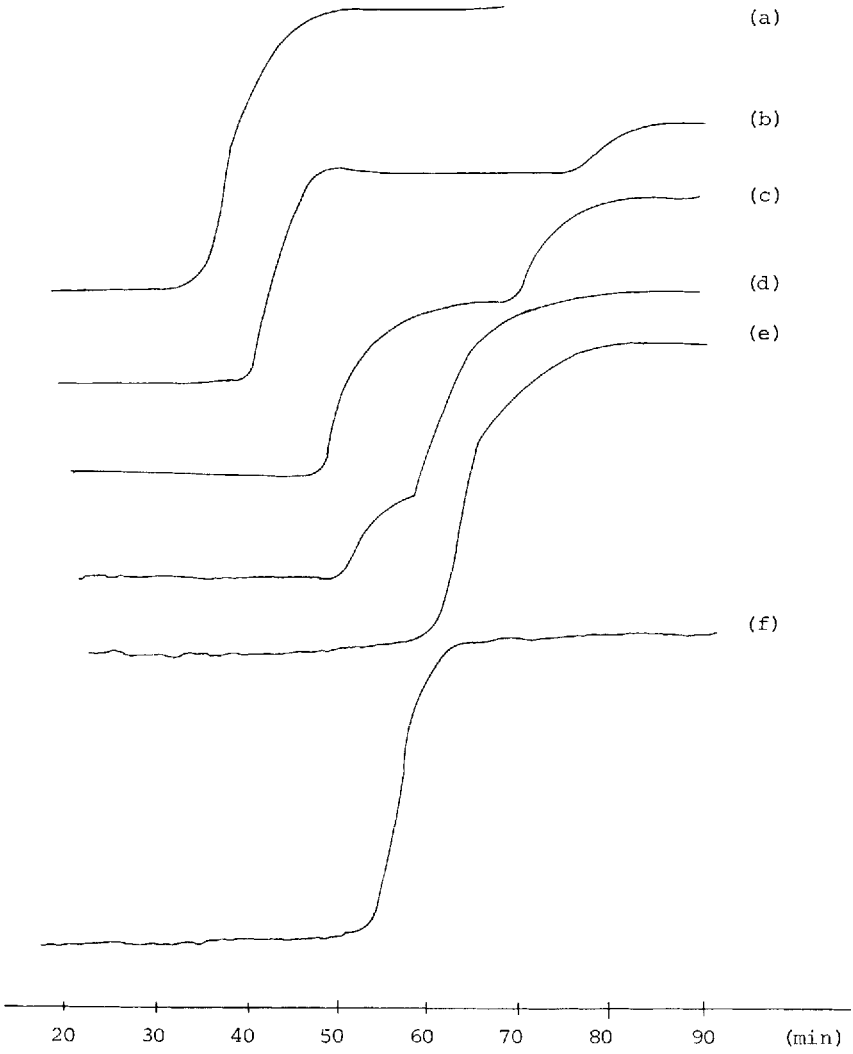

FIGURE 3. Migration Fronts of the Triamine for Different Proportions of Zinc in the Mobile Phase.

Conditions as in TABLE 1.

$Zn_t:Q_t$ (a) 0 (b) 0.42 (c) 0.74 (d) 1.06 (e) 2.12 (f) 4.24

agent, ammonium acetate and methanol concentrations were the same and only the zinc acetate concentration was varied. It can be observed that, even in the absence of zinc, the chelating agent is significatively adsorbed. This result may seem surprising, considering the fact that the methanol content is relatively high (60 %) and that, at the pH of the mobile phase (7.4), two amino groups of Q are protonated. This means that the triamine is highly hydrophobic and, in its protonated form, is adsorbed as an ion pair with acetate as counterion.

When zinc ions are present in the mobile phase, there is a second front whose height increases with the concentration of metallic ion in the eluent while, at the same time, the height of the first front decreases. This fact indicates that the first front corresponds to the adsorption of Q and the second one to the adsorption of Q-Zn. When the ratio of zinc to triamine is 1.06, the first front still represents about 30% of the total height, which indicates that the chelate-metal complex is not very strong under the conditions employed in these experiments. When the metallic ion concentration is twice the triamine concentration, the latter is completely complexed in the mobile and stationary phases.

The confirmation of the existence of two different forms of the triamine in the stationary phase and the fact that all the curves in Figure 3 have approximately the same total height, justify the calculation procedure proposed in the experimental section [equations (2) and (3)]. This allowed us to evaluate the amount of Q, Q-Zn and total chelating agent, Q_t, in the stationary phase. These results are presented in Figure 4 and Table 1.

Figure 4 shows that the adsorption of the complex is stronger than the adsorption of free triamine. Thus, the concentration of total chelating agent in the stationary phase increases with the ratio of metallic ion to chelating agent. However, in the presence of a great excess of zinc, there is a slight reduction in the adsorption of Q-Zn. This effect could be due to the incorporation

TABLE 1.

Effect of the Ratio of Zn(II) to Triamine in the Mobile Phase.

mobile phase: methanol-water (pH=7.4) 60:40 v/v containing dodecyldiethylenetriamine 0.00254 M, zinc acetate variable and ammonium acetate 0.194 M.

$Zn_t:Q_t$ # (m)	$Q_{(st)}$ * ($x10^2$)	$Q\text{-}Zn_{(st)}$ * ($x10^2$)	$Q_{t(st)}$ @ ($x10^2$)
0.00	8.76	-	8.76
0.42	8.30	2.96	11.26
0.74	7.42	5.78	13.20
1.06	3.71	10.20	13.91
2.12	-	14.69	14.69
4.24	-	13.41	13.41

ratio of total zinc to total triamine in the eluent.
* amount of adsorbed species (mmol).
@ total chelating agent in the stationary phase (mmol).

of extra zinc ions in the structure of the complex in solution (binuclear complex formation), which may increase the average positive charge per molecule thus decreasing its hydrophobicity and its adsorption on the reversed-phase packing.

These results indicate that the complex is more hydrophobic than the free triamine. Under our experimental conditions (pH 7.4), the "free" triamine is diprotonated (QH_2^{2+}) while the complex is not protonated but bears the charge afforded by the zinc ion (QZn^{2+}). The charge of the latter might even be lower if some sites in the metal ion coordination sphere were occupied by acetate molecules. It seems reasonable to suggest that the zinc atom confers a higher hydrophobic caracter to the complex.

Adsorption Isotherms of the Chelating Agent.

In this set of experiments, the amount of Q and Q-Zn adsorbed for different concentrations of total chelating agent in the mobile phase was determined. The ratio of metallic ion to

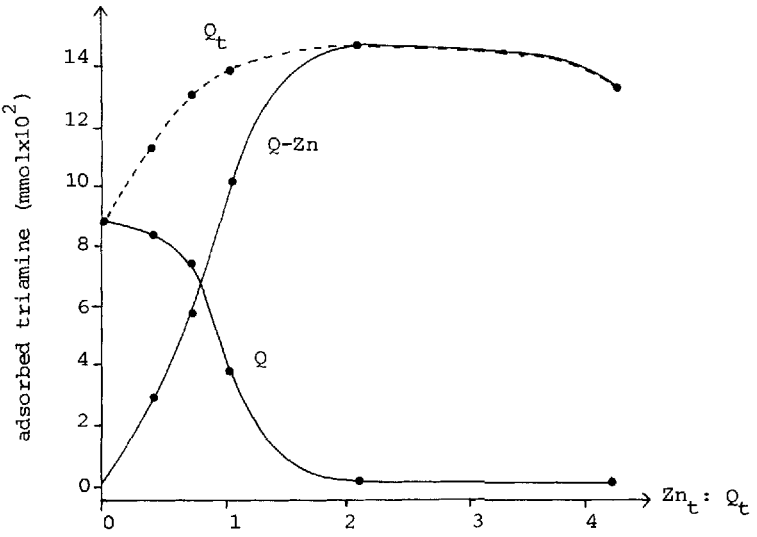

FIGURE 4. Effect of the Ratio of Zinc to Triamine in the Mobile Phase.

Conditions as in TABLE 1.
(——) mmol of Q and Q-Zn adsorbed on the RP-column.
(----) mmol of total chelating agent in the stationary phase.

chelating agent and the organic modifier and ammonium acetate concentrations were kept constant.

Figure 5 and Table 2 show the results of this study for a twenty-fold increase of the total triamine concentration in the eluent. The ratio of metallic ion to the chelating agent (approximately one to one) corresponds to conditions where the free triamine and the complex coexist in both phases.

The curves in Figure 5a only represent an approximation of the adsorption isotherms because in the x-axis we are not reporting the concentrations of Q or Q-Zn in the mobile phase, but the total triamine concentration. In fact, those concentrations cannot be calculated from the chromatographic experiments alone and it would be necessary to first determine the Q-Zn complex dissociation constant, under our experimental conditions, in order to evaluate them.

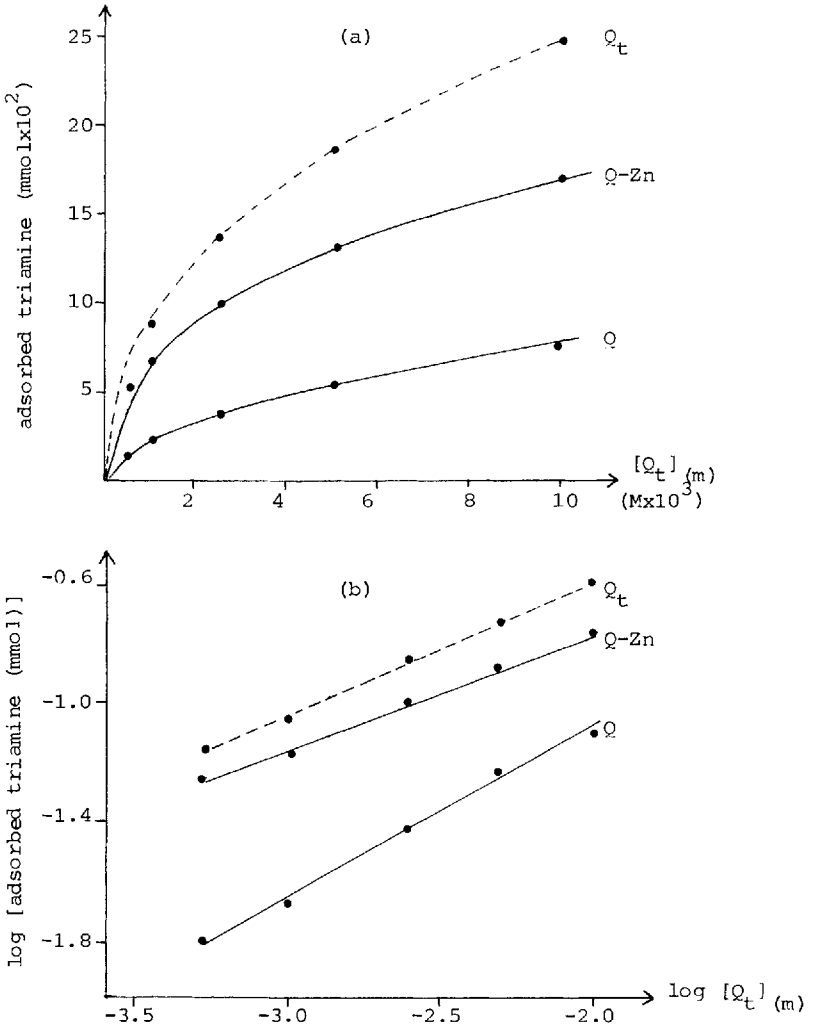

FIGURE 5. Adsorption Isotherms of Q and Q-Zn.

Conditions as in TABLE 2
(a) Freündlich isotherms. (b) linearized isotherms.
(———) mmol of Q and Q-Zn adsorbed on the RP-column.
(----) mmol of total chelating agent in the stationary phase.

TABLE 2.

Effect of the Chelating Agent Concentration in the Mobile Phase.

mobile phase: methanol-water (pH 7.4) 60:40 v/v containing ammonium acetate 0.194 M, zinc acetate and dodecyldiethylenetriamine variable. Ratio of Zn_t/Q_t = 1.06

$[Q_t]_{(m)}$ # ($x10^3$)	$Q_{(st)}$ * ($x10^2$)	$Q\text{-}Zn_{(st)}$ * ($x10^2$)	$Q_{t(st)}$ @ ($x10^2$)
0.51	1.60	5.60	7.20
1.02	2.06	6.83	8.89
2.54	3.71	10.20	13.91
5.08	5.62	13.07	18.69
10.16	7.56	17.18	24.74

total chelating agent in the mobile phase (M).
* amount of adsorbed species (mmol).
@ total chelating agent in the stationary phase (mmol).

Nevertheless, the relation between the amount of each species in the stationary phase and the concentration of total chelating agent in the solvent follows a Freündlich type isotherm as demonstrated by the linear log-log plots (Figure 5b). The regression analysis gives the following equations:

$$\log Q_{(st)} = -0.027 + 0.543 \log [Q_t]_{(m)} \qquad r = 0.997 \qquad (4)$$

$$\log Q\text{-}Zn_{(st)} = -0.012 + 0.379 \log [Q_t]_{(m)} \qquad r = 0.998 \qquad (5)$$

$$\log Q_{t(st)} = 0.239 + 0.423 \log [Q_t]_{(m)} \qquad r = 0.998 \qquad (6)$$

We previously found that other hydrophobic ions (of the type employed in ion pair chromatography) are also adsorbed on reversed phase columns following a Freündlich isotherm (10,11). This implies that, as a general rule, all hydrophobic ions have the same type of adsorption isotherm in RP-columns independently of their structure.

TABLE 3.

Effect of the Salt Concentration in the Mobile Phase.

mobile phase: methanol-water (pH 7.4) 60:40 v/v containing dodecyl diethylenetriamine 0.00254, zinc acetate 0.00269 M and ammonium acetate variable.

[salt] (M)	$Q_{(st)}$* ($x10^2$)	$Q\text{-}Zn_{(st)}$* ($x10^2$)	$Q_{t(st)}$@ ($x10^2$)
0.291	4.17	8.75	12.92
0.194	3.71	10.20	13.91
0.097	1.40	13.14	14.54
0.048	0.33	15.65	15.98

* amount of adsorbed species (mmol).
@ total chelating agent in the stationary phase (mmol).

Effect of the Salt Concentration in the Mobile Phase

Next, the concentration of ammonium acetate in the eluent was varied, keeping other parameters constant. Figure 6 and Table 3 summarize the effect of salt concentration on the adsorption of each species.

Figure 6 shows a remarkable increase in the adsorption of chelate-metal complex as the salt concentration decreases. This is an unexpected result because we previously found (11) that the adsorption of hydrophobic ions is slightly favoured by high salt concentrations.

In this case, the difference arises from the fact that ammonium acetate plays an important role on the formation of the Q-Zn complex in solution. It is known (15) that ammonia is a good complexing agent of transition metal ions and acetate also has a weak complexing ability. Thus, these species compete with the triamine to occupy a site in the tetracoordination sphere of zinc. On the other hand, dodecyldiethylenetriamine can occupy, at most, only three sites in the metal coordination sphere; this means that in the Q-Zn complex there is always at least one site occupied by

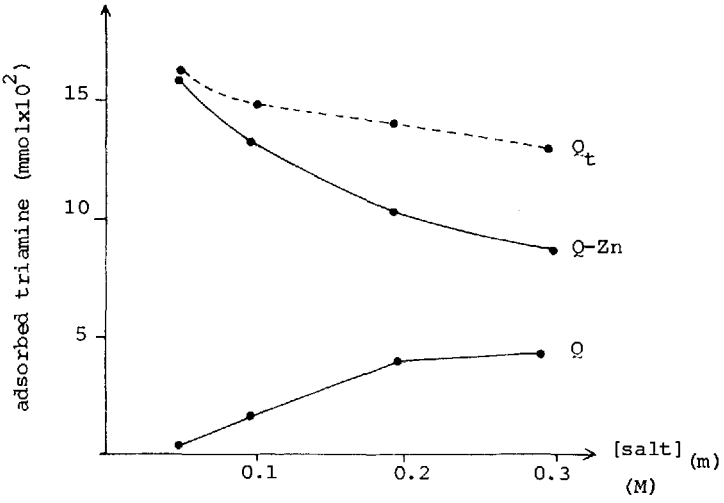

FIGURE 6. Effect of the Salt Concentration in the Eluent.

Conditions as in TABLE 3.
(———) mmol of Q and Q-Zn adsorbed on the RP-column.
(----) mmol of total chelating agent in the stationary phase.

a salt component. These phenomena can be summarized by the following equilibrium:

$$ZnP_4 + Q \rightleftharpoons Q\text{-}ZnP_x + (4-x)\ P \qquad (7)$$

where, P represents ammonia or acetate and x = 1, 2 or 3, depending on the experimental conditions, especially pH and ammonium acetate concentration. To simplify, we omit the protons in the species with acid-base properties (Q, $Q\text{-}ZnP_x$ and P) but it is evident that the equilibrium constant is highly dependent on the pH of the mobile phase.

When the salt concentration decreases, the equilibrium is displaced to the right, the chelate-metal complex formation is easier and the number of salt components in its molecule decreases (x decreases). The result is a strong increase in the proportion of Q-Zn in the mobile and stationary phases (to simplify we will

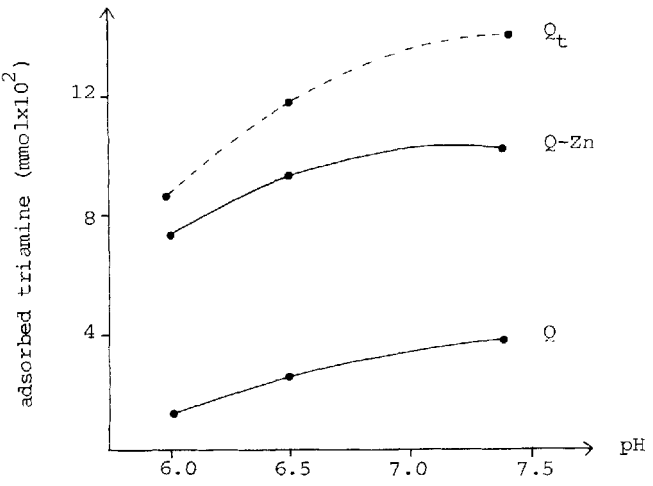

FIGURE 7. Effect of the pH of the Mobile Phase.

Conditions as in TABLE 4.
(———) mmol of Q and Q-Zn adsorbed on the RP-column.
(----) mmol of total chelating agent in the stationary phase.

continue to represent the chelate-metal complex by Q-Zn). In fact, when the ammonium acetate concentration is lower than 0.048 M, all the chelating agent in the eluent is probably forming the Q-Zn complex (see Table 3).

The strong effect of ammonium acetate on the phenomena occurring in solution masks the weak influence of the salt concentration, or ionic strength, on the adsorption process.

Effect of the pH of the Mobile Phase.

The pH of the mobile phase was varied over a small range, from 7.4 to 6.0, keeping all other parameters constant. The effect of these changes on the adsorption of both forms of the chelating agent is presented in Table 4 and Figure 7.

Table 4 shows that the adsorption of Q and Q-Zn decreases as the pH decreases. This effect is stronger for the free triamine,

TABLE 4.

Effect of the pH of the Mobile Phase.

mobile phase: methanol-water 60:40 v/v, containing dodecyldiethylenetriamine 0.00254 M, ammonium acetate 0.194 M and zinc acetate 0.00269 M.

pH	$Q_{(st)}$* (x10^2)	Q-$Zn_{(st)}$* (x10^2)	$Q_{t(st)}$@ (x10^2)	Q-$Zn_{(st)}/Q_{(st)}$
7.4	3.71	10.20	13.91	2.75
6.5 #	2.45	9.35	11.80	3.82
6.0 #	1.34	7.23	8.57	5.40
6.5 &	-	15.55	15.55	-

* amount of adsorbed species (mmol).
@ total chelating agent in the stationary phase (mmol).
# pH adjusted with $HClO_4$
& acetic acid/sodium acetate 0.194 M instead of $NH_4CH_3CO_2$

resulting in a net increase of the ratio of Q-Zn/Q in the stationary phase.

The decrease in the amount of adsorbed species as the pH decreases might be attributed to an increase in their degree of protonation which lowers their hydrophobicity. However, the titration curves in Figure 1 show that pH variations over this range only produce minor changes in the proportion of protonated triamine. On the other hand, although the change in the proportion of protonated complex depends on its stability, it is always stronger than the change for the free triamine.

From the previous discussion, it is evident that the increase in the ratio of Q-Zn/Q in the stationary phase as the pH of the eluent is varied from 7.4 to 6 is not due to the protonation of these species, but it probably arises from a similar increase in the ratio of chelate-metal complex to free triamine in the mobile phase. It is known (15) that in the pH range studied, the capacity of the salt components (especially ammonia) to form metal-ligand

complexes drastically decreases as the pH decreases. Thus, under our experimental conditions, the chelate-metal complex formation is easier at lower pH.

It is also interesting to compare the results of the two experiments at pH 6.5 (Table 4), one with ammonium acetate and the other with sodium acetate. In the latter, all the chelating agent in the stationary phase is in the form of Q-Zn complex, while in the former, only 79% of the adsorbed triamine is complexed. This fact is again related to the displacement of the complex formation equilibrium in the mobile phase. Our results indicate that in the experiments with ammonium acetate, the stronger ligand competing with the triamine to occupy the coordination sites of the metallic ion is probably ammonia; thus the formation of the chelate-metal complex is favoured when sodium acetate is dissolved in the eluent.

This hypothesis is confirmed by the fact that the amount of Q-Zn adsorbed in the experiment with sodium acetate is higher than the maximal quantity adsorbed in the presence of the same concentration of ammonium acetate, even at higher pH (compare with experiment 5 in Table 1). In the precedent section we mentioned that a salt component occupies at least one site in the structure of the Q-Zn complex; when this component is acetate, the positive charge of zinc is in part neutralized, the hydrophobicity of the complex increases and its adsorption on the reversed-phase packing is higher. Therefore, we conclude that in the experiments with ammonium acetate this site is preferentially occupied by ammonia.

From the precedent discussion, the behavior observed in the adsorption of the free triamine is easily explained. When the pH decreases, in the range studied, the concentration of free triamine in the eluent decreases while, at the same time, its degree of protonation slightly increases. Then, the logical result is a strong reduction in the amount of Q in the stationary phase.

Effect of the Organic Modifier Content in the Mobile Phase.

Figure 8 and Table 5 show the variation of the amounts of Q and Q-Zn in the stationary phase as the methanol content in the eluent is increased from 50% to 80% .

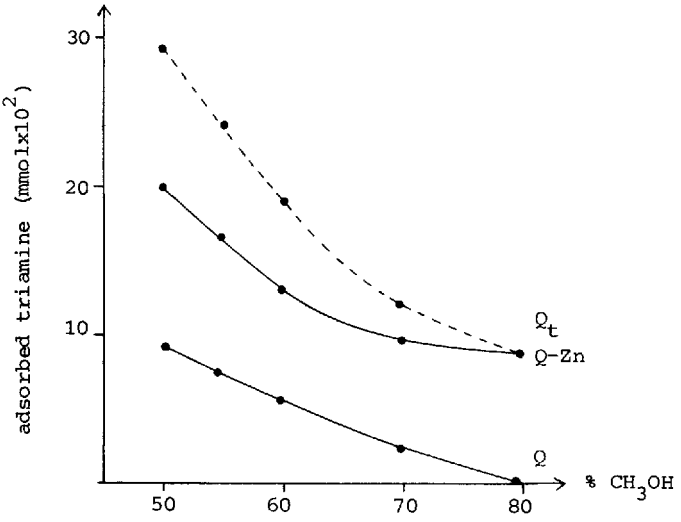

FIGURE 8. Effect of the Organic Modifier Content in the Eluent.

Conditions as in TABLE 5.
(——) mmol of Q and Q-Zn adsorbed on the RP-column.
(----) mmol of total chelating agent in the stationary phase.

TABLE 5.

Effect of the Methanol Content in the Mobile Phase.

mobile phase: methanol-water (pH=7.4) variable, containing dodecyl diethylenetriamine 0.00508 M, zinc acetate 0.00539 M and ammonium acetate 0.194 M.

CH_3OH (%)	$Q_{(st)}$* (x10^2)	$Q\text{-}Zn_{(st)}$* (x10^2)	$Q_{t(st)}$@ (x10^2)
50	9.45	19.76	29.21
55	7.16	16.89	24.05
60	5.62	13.07	18.69
70	2.46	9.43	11.89
80	-	8.64	8.64

@ total chelating agent in the stationary phase (mmol).
* amount of adsorbed species (mmol).

In these experiments, the behavior observed in the adsorption of Q and Q-Zn is the result of two effects: first, the increase in the amount of methanol in the mobile phase provokes a decrease of hydrophobic effects on the species in solution and therefore a lower adsorption on the reversed-phase column, at the same time, the dielectric constant of the solvent decreases and the stability of the chelate-metal complex increases.

The combination of both effects produces a strong decrease in the amount of free triamine adsorbed, especially at higher organic modifier concentrations. On the other hand, the reduction of hydrophobic effects on the Q-Zn complex is in part counterbalanced by the parallel increase of its concentration in the mobile phase. In fact, for a methanol content of 80% v/v all the chelating agent in the eluent is probably complexed with the metallic ion. Thus, the increase in the organic modifier content only produces a moderate effect on the amount of Q-Zn adsorbed.

When the quantity of total chelating agent adsorbed is higher than ≈ 0.3 mmol (methanol content lower than 50% v/v under our experimental conditions), the column suffers a drastic and irreversible loss of efficiency. We cannot explain this phenomenon but we have observed it, systematically, in the RP-adsorption of hydrophobic ions such as dodecylsulphonate [limit of surcharge ≈ 0.3 mmol/g (10)] and tetrabutilammonium [limit of surcharge ≈ 0.1 mmol/g (11)].

CONCLUSIONS

The RP adsorption of hydrophobic chelating agents in the presence of transition metal ions in the mobile phase is a complicated process because it involves, not only, the adsorption phenomenon itself but, also, the phenomena associated to the formation and stability of the chelate-metal complex in the eluent.

In the system examined in this work (dodecyldiethylentriamine and zinc ions) we demonstrated that two different forms of the

hydro- phobic chelating agent can coexist in the stationary phase: the free triamine (Q) and the chelate-metal complex (Q-Zn). The variations in the ratio of these species in the stationary phase reflect the changes in the stability of the complex in the mobile phase.

The stability of the chelate-metal complex in the mobile phase is favoured by the following conditions:

- Excess of metallic ions relative to chelating agent.
- Low concentrations of competitive ligands (salts or buffers).
- Salts or buffers composed by species with weak complexing properties.
- An optimum pH where the chelating agent can form a complex with the metallic ion but the complexing properties of competitive ligands are minimized.
- High content of organic modifier.

These conditions, deduced from chromatographic experiments, are completely congruent with predictions on the displacement of complex formation equilibria, issued from the chemistry of solutions.

On the other hand, the conditions that favour the adsorption of both species on the reversed-phase packing are the same as those for other hydrophobic ions:

- Low organic modifier content.
- High concentration of the species in the mobile phase.
- Reduction of the charge of the species.

The interrelations between the adsorption process and the complex formation in solution do not allow us to find a simple relationship to predict the amount of chelating agent adsorbed for different compositions of the mobile phase or to calculate, a priori, the time required to equilibrate the column. However, the results of this study should make possible a better understanding of the retention mechanism in RP ligand exchange chromatography. A study on this topic is currently in course.

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