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CHROMATOGRAPHY OF AROMATIC ACIDS ON LANTHANUM-LOADED ION-EXCHANGE RESINS

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SUMMARY

Cation-exchange resins loaded with lanthanum ions are selective sorbents for anions of aromatic acids and for dipolar ions like trigonelline and amino acids. Their utility is limited by slow sorption and desorption, and it seems that two different binding mechanisms are involved. Eluents are aqueous acetate buffers of pH 5; columns are run at 65°C. Effects of pH and lanthanum loadings are shown.

INTRODUCTION

The term "ion-moderated partition chromatography" has recently been used to describe the chromatography of non-ionized organic compounds on ion-exchange resins. It implies that the nature of the resin counter-ion influences the retention of these compounds. Thus, iron(III) forms complexes with phenols, and resins loaded with Fe(III) are selective absorbents for phenols^{1,2}. The use of transition-metal cations to promote the retention of amines and amino acids by ion-exchange resins has long been known and forms the basis of "ligand-exchange chromatography."

In 1975 Goulding³ showed that carbohydrates were retained from aqueous solutions on columns of polystyrene-type cation-exchange resins, and that the retention depended on the inorganic counter-ions. Since Goulding's work, such resins loaded with calcium ions have become widely used for the chromatography of sugars⁴. Hydrogen-form resins are used for carboxylic acids, with dilute sulfuric acid eluents⁵ and unspecified "heavy-metal columns," said to contain Pb(II), are sold⁶. Unfortunately the packed columns are sold under proprietary names, and there is little fundamental information about them in the literature.

We have used sulfonated polystyrene cation-exchange resins for the chromatography of polar aromatic compounds, including caffeine and the xanthenes, and we have shown that retention depends in a regular and characteristic manner on the inorganic counter-ions⁷. It is greater with divalent ions than with univalent. We have found that calcium-form resins retain aromatic hydrocarbons more strongly than do sodium-form resins (see Table I). It is hard to believe that calcium ions form coordination complexes with hydrocarbons, and the increased retention may be due to the fact that calcium-form resins are less swollen than sodium-form resins, that is, that

TABLE I

CAPACITY FACTORS OF HYDROCARBONS

Aminex 50-X4 resin, 190 mm × 6.3 mm; temperature, 55°C; solvent, acetonitrile-water (50:50); void volume, 2.4 ml throughout.

Compound	Counter-ion		
	Na ⁺	Mg ²⁺	Ca ²⁺
Anthracene	0.9	1.15	1.3
Fluoranthene	1.8	2.3	2.55
Pyrene	2.3	2.85	3.2

the calcium-form polymer beads have a higher volume fraction of carbonaceous matter.

If divalent ions cause stronger retention than univalent, it is only natural to expect trivalent ions to cause even stronger retention. We have previously used Fe(III)-form resins for the chromatography of aromatic hydrocarbons⁸, but the hydrolysis of Fe(III) requires that the eluents (aqueous methanol, ethanol or acetonitrile) be acid. The best choice of a trivalent ion is lanthanum(III). This ion has the largest radius of any simple triply charged ion, and it is the least hydrolyzed. According to the literature, La(III) is about 1% hydrolyzed at pH 6 (ref. 9).

EXPERIMENTAL

Equipment

Most chromatography was done in high-pressure glass columns (Glenco Corp.), 4.5 mm I.D., with adjustable bed supports; they were water-jacketed and run at 65°C. Stainless-steel columns, 4.5 mm I.D., were also used. Pumps were Models 6000A and M-45 (Waters Assoc.); Rheodyne Model 7125 and Waters U6K injection valves were used; detection was by Altex (254 nm) and Schoeffel (variable-wavelength) UV detectors, and in some cases by refractive index (Waters Assoc.). Certain experiments used fluorescence detection (Kratos-Schoeffel Model FS 970). The recorders were from Linear Instruments Corp.

Resins

The following gel-type cation-exchange resins were used: Aminex 50-X4 and Aminex A5, 13 μm, 8% cross-linked, from Bio-Rad Labs.; HC-X7, from Hamilton Corp.; BC-X6, from Benson Co. (the "X6", etc., indicates the degree of cross-linking). A macroporous sulfonated polystyrene resin, Hitachi 3011-S, was kindly given to us by Hitachi Corp., Tokyo, Japan. The particle diameters ranged from 20 μm (Aminex 50-X4 and Hitachi 3011-S) down to 7–10 μm (BCX6). Chelex-100, 200–400 mesh, a polystyrene resin with iminodiacetate functional groups, was supplied by Bio-Rad Labs.

Chemicals

A large number of chemicals were used and obtained from various manu-

TABLE II
RETENTION VOLUMES ON MACROPOROUS HITACHI 3011-S

Corrected retention volumes in ml; column 15 cm × 4 mm, temperature, 65°C; solvent, aqueous 0.01 M acetate, pH 5.0.

Compound type	Compound	Counter-ion			Retention ratio, La:Na
		Na ⁻	Ca ²⁺	La ³⁺	
Non-ionic	Caffeine	3.2	4.3	4.4	1.3
	Acetanilide	8.5	12.2	14.2	1.7
	Phenacetin	14.5	22.3	30.7	2.1
Dipolar ion	Homarine	1.0	1.6	4.4	4.4
	Trigonelline	1.6	3.8	24.9	15
Acid anions	Benzoate	0.4	0.4	11.3	28
	Urate	0.3	0.4	8.5	28

facturers, mainly Aldrich. This company and J. T. Baker Co. supplied lanthanum chloride heptahydrate, which was of 99.99% purity.

RESULTS AND DISCUSSION

Preliminary tests: comparing counter-ions

In these tests, columns were packed with resins in the desired counter-ion form (Na, Mg, Ca, La) and "conditioned" by pumping 0.01–0.10 M acetate solutions whose pH was adjusted by adding acetic acid. The cations of the buffers were the same as those desired in the resin, except that the buffers used with lanthanum-loaded

TABLE III
RETENTION VOLUMES ON HC-X7

Corrected retention volumes in ml; column 15 cm × 4 mm, temperature, 65°C; solvent, aqueous 0.01 M acetate, pH 6.0 (Ca), 5.3 (La).

Compound type	Compound	Counter-ion		Retention ratio, La:Ca
		Ca ²⁺	La ³⁺	
Non-ionic	Caffeine	6.4	5.1	0.8
Dipolar ion	Homarine	2.5	4.5	1.8
	Trigonelline	5.2	20.7	4.0
Amino acid	Glycine	3.2	11.1	3.5
	α -Alanine	2.7	9.6	3.5
	β -Alanine	4.0	21.6	5.4
	<i>p</i> -Amino-benzoic acid	0.0	17.1	v. large
Acid anions	Benzoate	0.1	18.3	v. large
	<i>p</i> -nitrobenzoate	0.6	39.9	v. large

columns were mixtures of sodium acetate with lanthanum chloride or nitrate. Columns were normally run at 65°C; the high temperature facilitated mass transfer. Tables II and III show elution volumes of representative compounds, and show clearly that lanthanum ions promote the retention of certain compound classes, particularly the anions of organic acids. The dipolar ions of trigonelline and amino acids are also retained more strongly by La^{3+} than by Ca^{2+} or Na^+ . It is interesting to compare trigonelline (T) with homarine (H):



Trigonelline has the larger dipole moment and is affected more by La^{3+} . The same comparison can be made with α - and β -alanine.

We noted in a previous paper⁷ that a possible cause for the effect of divalent counter-ions in promoting the retention of trigonelline might be the existence of intense local electrostatic fields, as shown in Fig. 1. The inverse square law of force requires that in a linear array of singly charged fixed negative ions, a doubly charged counter-ion like Ca^{2+} finds its most stable position near *one* of the fixed negative ions, not between two of them. The same argument holds *a fortiori* for triply charged counter-ions like La^{3+} .

The counter-ion effect is minimal, essentially zero, for caffeine. It is appreciable for phenacetin, $p\text{-C}_2\text{H}_5\text{OC}_6\text{H}_4\text{NHCOCH}_3$, and is very large for the anions of carboxylic acids, uric and picric acids. Here we must look for another mechanism than ion-dipole attraction, and the formation of complexes such as LaA^{2+} , where A^- is the acid anion, is likely. This matter will be discussed below.

Figs. 2 and 3 show typical chromatograms that compare the behaviour of sodium- and lanthanum-form resins. The effect of La^{3+} on retention and selectivity order is clear.

Investigation of lanthanum ions; effects of pH, La^3 and Na^+ concentrations

The normal effect of pH on the retention of organic acids by partition on a non-ionic packing, like neutral polystyrene or bonded reversed-phase silica, is related

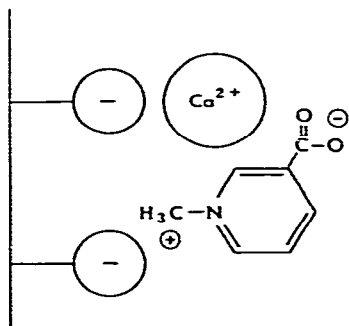


Fig. 1. Retention of a trigonelline molecule by the ion-dipole effect.

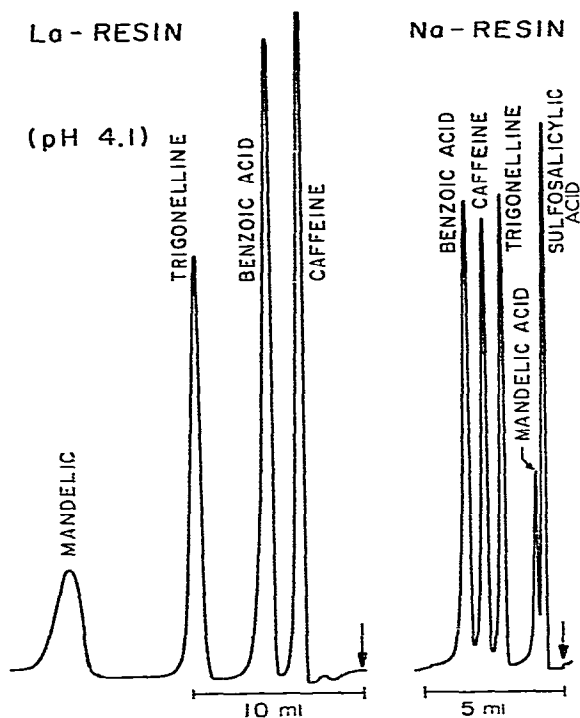


Fig. 2. Chromatograms on Aminex A-5 cation-exchange resin. Column, 180×4 mm; temperature, 65°C ; eluent, 0.05 M sodium acetate buffered to pH 4.1; for the lanthanum column the eluent was 0.01 M in LaCl_3 . Flow-rates, 0.3 (Na) and 0.5 ml/min (La).

to the fact that the uncharged acids, HA , are absorbed while the anions, A^- , are not absorbed, or are absorbed very weakly. Familiar sigmoid curves of retention vs. pH are obtained that have level portions in high and low pH ranges, and whose midpoint, where retention is the mean of that at high and at low pH, comes at $\text{pH} = \text{p}K_a$ (ref. 10).

Similar curves are formed for the retention of weak organic acids on cation-exchange resins in the sodium form⁷. Retention of the acid anions is essentially zero, for they are excluded from the resin by the Donnan equilibrium. This observation was confirmed in the current work.

With lanthanum-loaded resins, on the other hand, the retention of acids does not drop to zero with rising pH. It falls to a finite value, remains constant or rises, depending on the concentrations of La^{3+} and Na^+ in the buffer. A great many graphs of retention vs. pH were obtained in this work. The procedure was to start with a sodium acetate solution of determined concentration, add the desired amount of lanthanum chloride, then add enough pure acetic acid to make the pH between 5 and 5.5. (At higher pH values the buffer capacity of the solution is low, and hydrolysis of La^{3+} becomes appreciable). This buffer was pumped through the resin column and retention volumes found. Then the pH was lowered by adding a few drops of acetic acid, and a new set of retention volumes measured. Then more acetic acid was added

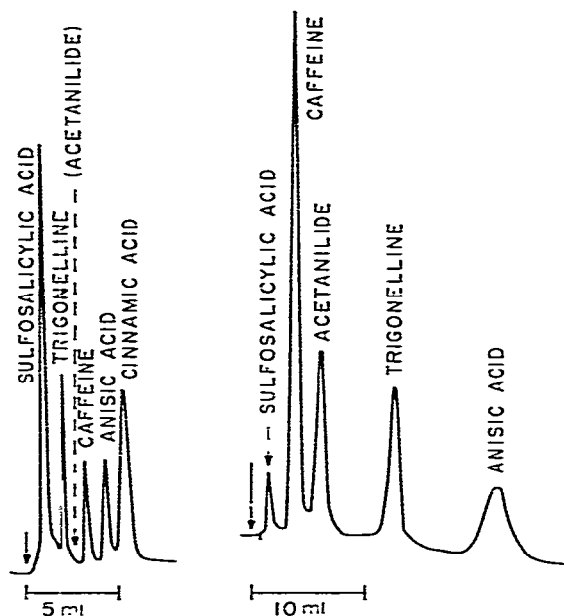


Fig. 3. Chromatograms on Benson BC-X6 cation-exchange resin. Column, 170 mm \times 4 mm; temperature, 65°C; eluent, 0.01 *M* sodium acetate buffered to pH 5.0; for La column, eluent was 0.01 *M* in both sodium acetate and LaCl_3 . Flow-rates, 0.3 and 0.5 ml/min. Sulfosalicylate ions were used as the void marker. Left-hand curve, Na-resin; right-hand curve, La-resin.

and more retentions measured. By this procedure the concentrations of acetate and sodium ions were kept nearly constant.

Using this procedure, curves like those of Fig. 4 were obtained. In Fig. 4 the curves in the absence of La^{3+} are omitted for simplicity, but the maximum retention by the Na^+ -form resin, which occurs below pH 3.5, is shown by a short horizontal line. Retention of trigonelline on the La^{3+} resin, omitted for clarity, was independent of pH and a little greater in 0.02 *M* than in 0.10 *M* sodium acetate.

The effect of sodium acetate concentration could be due to competing complexation of the lanthanum ions by acetate, or it could be due to displacement of lanthanum ions from the resin by sodium ions. To study the effect of lanthanum concentration, a series of experiments was made with eluents 0.01 *M* in sodium acetate, adjusted to pH 5.0 with acetic acid, and containing varying concentrations of added $\text{La}(\text{NO}_3)_3$, starting with low concentrations and working upwards. After each lanthanum addition the column was conditioned by pumping the solution overnight, passing some 500 bulk column volumes. Retentions of four compounds are shown in Fig. 5. These curves are like those obtained by Doury-Berthod *et al.*¹¹ in their study of amino acids on Cu(II)-loaded resins. As the metal-ion concentration increases, its first effect is to form complexes with the solutes (benzoic acids and trigonelline in our case) and carry them into the resin. At higher concentrations the retention drops, because there is now significant complexation with the metal ions in the external solution.

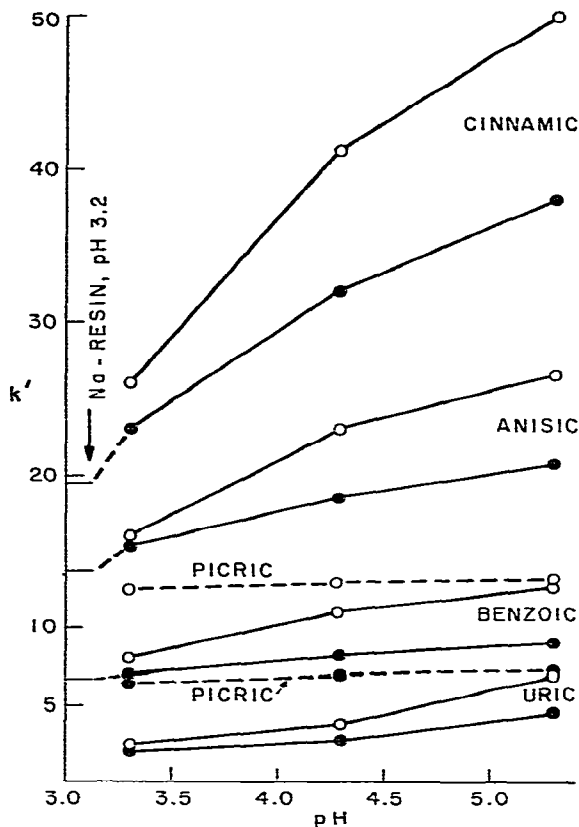


Fig. 4. Retentions on Aminex A-5 cation-exchange resin at 65°C. Eluents were 0.01 *M* in LaCl_3 and 0.10 *M* (closed circles) or 0.02 *M* (open circles) in sodium acetate. Void volumes for calculating the capacity factors (k') were found with sulfosalicylic acid in Na-form column.

Effect of lanthanum loading

The distributions depend on several factors: the La^{3+} loading of the resin, the La^{3+} concentration in the solution, the Na^+ concentration and the ionic strength of the solution, and the acetate concentration, which may affect the lanthanum activity through the formation of lanthanum acetate complexes. The first consideration is the concentration of lanthanum ions in the resin. It would seem easy to measure this concentration by stripping the resin with acid and titrating the lanthanum in the extract. In practice this procedure was very difficult, because the last portions of lanthanum tended to remain in the resin and were extracted very slowly; furthermore, the EDTA titration of lanthanum proved unsatisfactory. What was needed was a rapid method for measuring the lanthanum in the column at various times without having to remove it from the column.

To find the lanthanum content of the column we used cerium(III) as a fluorescent probe. The ions Ce^{3+} and La^{3+} have nearly the same affinity for an ion-exchange resin, but the former is fluorescent while the latter is not. Our procedure was to inject a small amount of $\text{Ce}(\text{NO}_3)_3$, 20 μl of 0.001 *M* solution, into solutions flowing through a column of Benson BC-X6 resin, 40 \times 4 mm, and monitor the

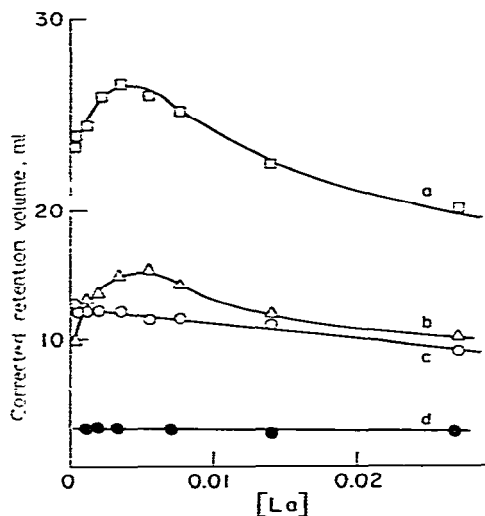


Fig. 5. Retention on Hamilton HC-X7 in 0.01 *M* sodium acetate at pH 5, with lanthanum concentrations in solution adjusted by adding $\text{La}(\text{NO}_3)_3$. Curves: a = *p*-methylbenzoic acid; b = benzoic acid; c = trigonelline; d = caffeine.

fluorescence with excitation at 265 nm, and emission above 370 nm. Sharp fluorescence peaks were observed (see Fig. 6). If Ce and La are retained equally, then:

$$\text{La in column} = (\text{corrected elution volume of Ce}) \times (\text{La concentration in eluent})$$

The lanthanum concentrations in the eluent were determined gravimetrically by precipitating the oxalate, filtering, and igniting it to the oxide in a platinum crucible. The elution volumes were found from the measured flow-rates and the retention times measured from the chart paper.

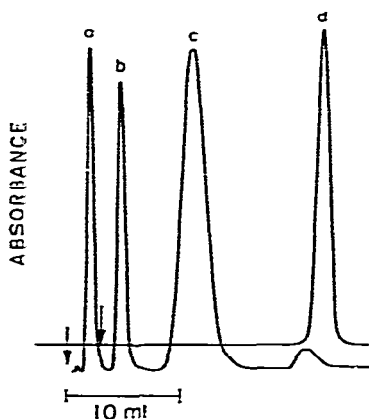


Fig. 6. Chromatograms, including Ce(III), on Benson BC-X6, 40 × 4.5 mm, at 65°C. Eluent, 0.065 *M* sodium acetate, 0.013 *M* LaCl_3 , pH 5.0. Note relative sharpness of the peaks. Peaks: a = caffeine; b = trigonelline; c = cinnamic acid; d = cerium(III). Peak d recorded by fluorescence, others by UV; arrows indicate points of injection. Plate numbers: b, 320; c, 130; d, 1300.

To find the total capacity of the column, a solution of $\text{La}(\text{NO}_3)_3$, concentration 0.0278 M as determined gravimetrically, containing a small excess of nitric acid (pH 3.4) to prevent hydrolysis, was pumped through the column and injections of $\text{Ce}(\text{III})$ made until the retention volume had become constant. Then the column was flushed with distilled water. The lanthanum was stripped from the column by pumping 100 ml of 1 M nitric acid, and the amount of lanthanum was measured gravimetrically. The column was again flushed with distilled water; then 0.5 M potassium nitrate solution was passed until the effluent was no longer acid. The acid that had been removed was titrated with standard sodium hydroxide.

Three independent values were thus obtained for the ion-exchange capacity of the column. They were:

La content, by Ce probe elution:

$0.273\text{ mmol} = 0.819\text{ mequiv. as La}^{3+}$

La recovered and found gravimetrically:

$0.276\text{ mmol} = 0.828\text{ mequiv.}$

Hydrogen ions eluted: 0.825 mequiv.

We were thus able to validate the cerium probe method and to convert the cerium retention data to per cent lanthanum loading of the column. From cerium retentions and the retentions of model compounds, Fig. 7 was constructed.

From Figs. 5, 6 and 7 it seems that trigonelline behaves differently from benzoic or cinnamic acid. Its retention is less dependent on the lanthanum concentration. Trigonelline peaks are always sharper than the carboxylic acid peaks (see Fig. 6).

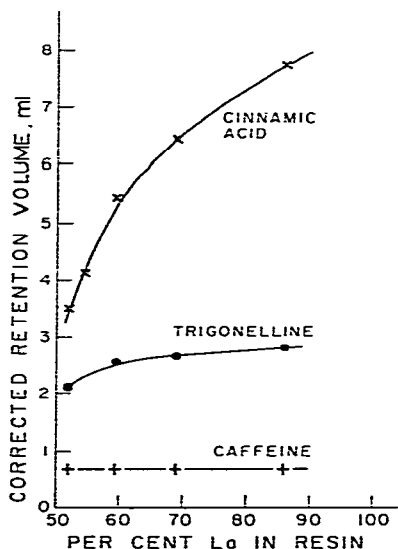


Fig. 7. Retention of model compounds as a function of per cent lanthanum loading of the column (same column as Fig. 5). La concentration was 0.01 M throughout; Na concentration ranged from 0.01 to 0.10 M , thus determining the lanthanum in the resin.

Tentatively we think that trigonelline is retained by the mechanism shown in Fig. 1, while the acids are retained as lanthanum complexes like LaA^{2+} .

Retention by a chelating resin

To test this hypothesis we used a chelating resin, Chelex-100. Lanthanum ions in this resin are bound covalently and probably have a net charge of only +1. The strong electrostatic fields pictured in Fig. 1 would be absent. Trigonelline would not be strongly absorbed, but carboxylate ions, A^- , could presumably still be bound in the form $\text{resin-N}(\text{CH}_2\text{CO}_2)_2 \text{LaA}$.

The sodium form of Chelex-100 swells enormously and is much too soft to pack in a column. Distribution ratios were therefore measured by a batch shaking technique. A quantity of Chelex-100-Na was weighed into a small vial, sodium acetate buffer of pH 5.0 was added and the vial was reweighed, then a small measured volume, about 100 μl , of a stock solution of trigonelline or cinnamic acid was added. The vial was shaken; a portion of the solution was removed and filtered to remove resin particles, then its UV absorbance was measured. From this absorbance and the absorbance of the stock solution, the distribution ratio, D , of the organic compound was calculated. Another portion of Chelex-100-Na was weighed into a second vial and converted to the lanthanum form by shaking with a lanthanum salt solution, followed by washing; distribution ratios were measured, using the same sodium acetate buffer as before.

These distribution ratios were found (in ml/g dry Na-resin; means of 2-3 experiments):

Cinnamic acid, Na-resin 95, La-resin 450 ($\pm 10\%$);
Trigonelline, Na- and La-resins, 0.5 ± 0.5 .

Lanthanum complexes in solution

It remained to investigate the association of carboxylate anions and trigonelline with lanthanum ions in solution. It had to be weak; UV absorption spectra gave no evidence of association. By adding a tenfold excess of LaCl_3 to dilute solutions of the carboxylic acids, however, changes in pH were observed that allowed formation constants to be calculated. For example, 15 ml 0.010 M benzoic acid in 0.6 M KNO_3 (to control the ionic strength) had pH 3.05. After adding 15 ml 0.10 M LaCl_3 , the pH of the mixed solution was 2.86. From many data of this kind, the following formation constants of binary complexes, LaA^+ , were found:

Acetic acid,	$K = 37$
Formic acid,	$K = 18$
Benzoic acid,	$K = 34$
Mandelic acid,	$K = 130$
Trigonelline,	$K = 16$

The probable error was 10%. The smaller formation constant for formate, compared to acetate, agrees with our observation that formate buffers give stronger retentions of aromatic compounds than acetate buffers.

These formation constants are quite small, and hardly seem large enough to account for the observed chromatographic retentions. The higher lanthanum-ion

concentration in the resin, compared with the solution, would stabilize the complexes in the resin, but this effect is offset by the Donnan exclusion of the anions A^- . Another effect must enter to make the LaA^+ complexes much more stable in the resin than they are in the solution. Probably it is π -bonding between the aromatic anions A^- and the resin polymer.

CONCLUSIONS

The binding of aromatic acid anions by lanthanum-loaded resins is a complex phenomenon that involves several equilibria in the resin and in the solution, and a quantitative treatment is not possible as yet. From the practical viewpoint, lanthanum-loaded resins are selective absorbents for aromatic acids and the dipolar ions of trigonelline and amino acids. Evidence seems conclusive that carboxylate anions and the dipolar ions of trigonelline are retained by different mechanisms. The most convincing evidence is the theoretical-plate heights; carboxylate anions give broader bands than trigonelline, and this compound in turn gives broader bands than the lanthanum-cerium ion exchange (Fig. 6). Formation and dissociation of complexes within the resin is evidently slower than ionic or molecular diffusion.

To make the lanthanum-resin column useful in chromatography, we must somehow speed up the mass transfer and sharpen the bands. Attempts to do this by using resins of lower cross-linking and by using pellicular resins were unsuccessful. Perhaps higher temperatures would improve column performance.

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REFERENCES

- 1 J. Masłowska and W. Piętek, *J. Chromatogr.*, 201 (1980) 293.
- 2 B. M. Petronio, A. Lagana and M. V. Russo, *Talanta*, 28 (1981) 215.
- 3 R. W. Goulding, *J. Chromatogr.*, 103 (1975) 229.
- 4 M. R. Ladisch, A. L. Huebner and G. T. Tsao, *J. Chromatogr.*, 147 (1978) 185.
- 5 D. N. Buchanan and J. G. Thoene, *J. Liquid Chromatogr.*, 4 (1981) 1219.
- 6 *Ion-Moderated Partition Chromatography: When Ion Exchange Isn't Enough*, Bio-Rad Labs., Richmond, CA, 1980.
- 7 H. F. Walton, G. A. Eiceman and J. L. Otto, *J. Chromatogr.*, 180 (1979) 145.
- 8 D. M. Ordemann and H. F. Walton, *Anal. Chem.*, 49 (1977) 764.
- 9 A. E. Martell and R. M. Smith, *Critical Stability Constants*, Plenum Press, New York, 1974.
- 10 D. J. Pietrzyk and C.-H. Chu, *Anal. Chem.*, 49 (1977) 757, 860.
- 11 M. Doury-Berthod, C. Poitrenaud and B. Tremillon, *J. Chromatogr.*, 131 (1977) 73.