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SEPARATION OF ANTHRAQUINONE SULPHONIC ACIDS BY ION-PAIR LIQUID CHROMATOGRAPHY

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SUMMARY

The separation of sulphonic acid derivatives of anthraquinone using reversed-phase high-performance liquid chromatography in the presence of quaternary ammonium salts as ion-pairing agents has been investigated. It is shown that there is a general increase in retention time with increasing size of the quaternary ammonium ion, R_4N^+ , but that the separation depends critically upon the length of the longest carbon chain. Retention of substrates containing ionisable groups such as $-OH$ and $-NH_2$ is strongly affected by the pH of the eluent. Severe tailing and double peaks were observed at pH values close to the pK_a of the solute. At low concentrations of pairing agent the retention time varies directly with R_4N^+ concentration, but saturation effects are observed at higher concentration (*ca.* 0.06 M for tetrabutylammonium bromide). The practical utility of the separation procedure is demonstrated for the separation of (a) a series of anthraquinone mono- and disulphonic acids using cetyltrimethylammonium bromide as pairing agent in acetonitrile–water (50:50, v/v) as eluent and Spherisorb S5 ODS as the stationary phase, and (b) sulphonated and non-sulphonated derivatives of 1-aminoanthraquinone using an isocratic eluent of methanol–water–tetrabutylammonium hydroxide and Spherisorb S5 ODS as the stationary phase.

INTRODUCTION

Anthraquinone sulphonic acids are important in the dyestuff industry both as final dyestuffs for wool and nylon applications and as intermediates for disperse and reactive dyes. They are generally prepared by sulphonation of anthraquinone or 1-aminoanthraquinone. A mixture of mono- and disulphonic acids and also various isomers is obtained, depending on the sulphonation conditions used. Optimisation of a particular product can be achieved by alteration of the reaction conditions. Thus it is important to have a precise and rapid method of analysis. Chromatographic separation of such ionisable compounds present problems in terms of retention and symmetry of peaks. However several groups have successfully applied “soap chromatography” to the separation of similar types of compounds, using, for example, cetrimide as the pairing agent^{1–5}.

In the present study, reversed-phase ion-pair chromatography has been used to separate anthraquinone sulphonic acids. The effect of chain length of the pairing agent, concentration of pairing agent in the eluent and pH of the eluent on retention of certain compounds has been studied.

EXPERIMENTAL

The equipment used was a Perkin-Elmer Series 3 Liquid Chromatograph and a Waters 6000 with a Cecil variable-wavelength detector.

The column packing material used was Spherisorb S5 ODS (Phase Separation, Queensferry, U.K.). Methanol was HPLC grade material (Rathburn Chemicals) and the quaternary ammonium salts were obtained from Eastman Kodak. All UV-VIS spectroscopic measurements were made using a Perkin-Elmer 200 UV-VIS spectrophotometer.

Columns were of 5 mm-bore polished stainless steel; 200 and 250 mm in length with Z.D.V. end-fittings (Phase Separation). They were packed by pumping a slurry of packing material in carbon tetrachloride into the column at a pressure of 5000 p.s.i. Then *ca.* 200 ml of methanol was passed through the column at 5000 p.s.i.

Column efficiencies were assessed by determining the number of theoretical plates (N) and the reduced plate height (h). The unretained volume (V_0) was determined by measuring the retention of the inorganic salts, sodium bromide and sodium dichromate, at a concentration of $1 \cdot 10^{-4} M^{6,7}$.

The solutes used in the study were obtained from the I.C.I. (Organics Division) sample collection (Table I). The ion-pairing agents, the alkylammonium salts were reagent grade (BDH Chemicals). The eluents were prepared by mixing the reagents in the desired proportions and degassed by applying a vacuum.

RESULTS AND DISCUSSION

Effect of tetrabutylammonium salts on the retention of anthraquinone derivatives

A mixture of 65:35 methanol-water was used as the eluent, with the addition of 0.2 g of tetrabutylammonium salt per 100 ml of eluent. The results (Table II) show that an increase in retention time of the aminoanthraquinone monosulphonic acids is effected, but there was no significant increase in retention of the aminoanthraquinone disulphonic acid (compound V).

Effect of chain length of quaternary ammonium salts on the retention of mono- and disulphonic acids

The results shown in Table III and Figs. 1 and 2 suggest that the addition of the same mass of salt per 100 ml of eluent (0.2 g/100 ml) of various quaternary ammonium bromide salts effects an increase on retention of the anthraquinone mono- and disulphonic acids which is related to the increase in chain length of the pairing agent.

It may be seen from Fig. 1 that cetyltrimethylammonium bromide (nineteen carbon atoms) does not fall in line with the other four quaternary ammonium compounds in which the carbon atoms are grouped as tertiary alkyl salts. This suggests that the effect of the salts is not simply determined by the number of carbon atoms,

TABLE I
INDEX OF COMPOUNDS

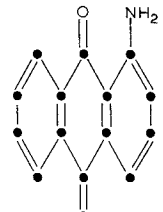
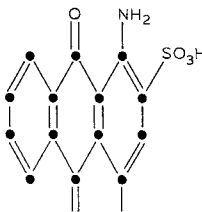
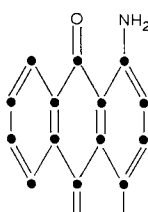
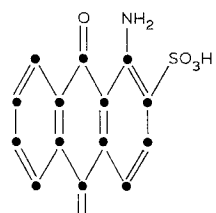
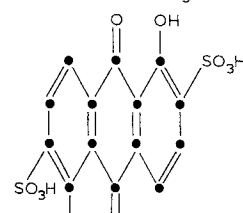
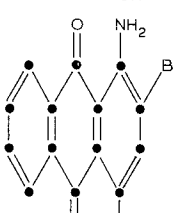
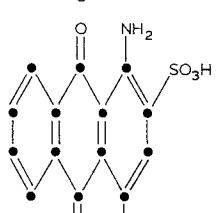
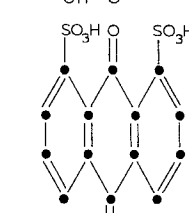
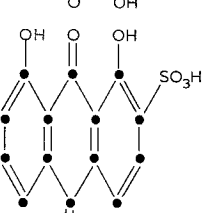
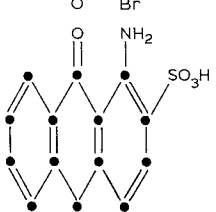
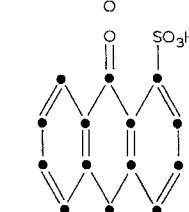
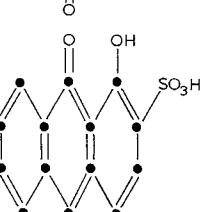
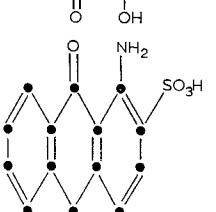
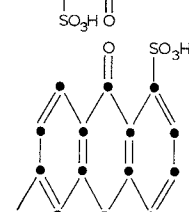
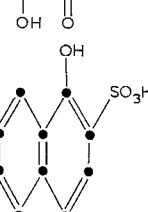
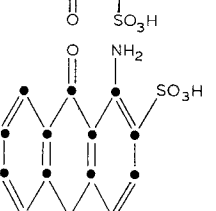
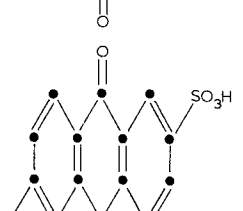
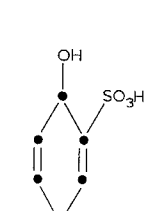
I		VII		XIII	
II		VIII		XIV	
III		IX		XV	
IV		X		XVI	
V		XI		XVII	
VI		XII		XVIII	

TABLE II

RETENTION (k') OF ANTHRAQUINONE DERIVATIVES IN THE PRESENCE OF TETRA-BUTYLAMMONIUM SALTS

Solvent, methanol-water (65:35, v/v).

Compound	No pairing agent	Bu_4NOH (0.2 g/100 ml)	Bu_4NBr (0.2 g/100 ml)
I	3.0	3.65	2.4
II	0.13	0.51	0.56
III	0.13	1.04	0.95
IV	0.13	0.22	0.95
V	0.0	0.22	0.19
VI	0.13	0.51	0.58

but that the distribution of the carbon atom among the chains is also important in determining the retention time of the solute. Cetyltrimethylammonium bromide containing nineteen carbon atoms, but with one C_{16} chain, causes considerably larger retention times than tetrapentylammonium bromide which has 20 carbon atoms distributed symmetrically (five to each chain). Thus the chain length of the longest carbon chain is clearly of major importance in determining the observed retention times.

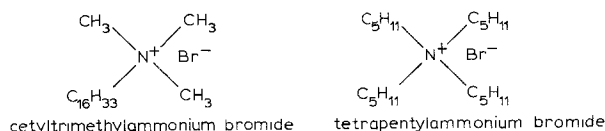


TABLE III

EFFECT OF CHAIN LENGTH OF QUATERNARY AMMONIUM SALTS ON RETENTION (k') OF MONO- AND DISULPHONIC ACIDS

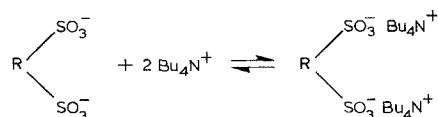
Quaternary alkylammonium bromides in methanol-water (65:35, v/v).

	No pairing agent	Tetra-butyl	Tetra-pentyl	Tetra-hexyl	Tetra-heptyl	Cetyl-trimethyl
Conc./(g/100 ml)	—	0.2	0.2	0.2	0.2	0.2
Conc./M	—	0.0062	0.0052	0.0046	0.0041	0.0055
No. of C atoms	—	16	20	24	28	19
I	3.0	2.4	2.35	2.4	2.78	1.81
II	0.13	0.62	1.11	2.4	8.35	7.27
III	0.13	0.94	1.86	4.67	19.5	11.2
IV	0.13	0.94	1.86	4.62	19.2	11.1
V	0	0.19	0.51	2.50	24.1	36.2
VI	0	0.56	1.05	2.35	8.24	6.3
X	0	0.02	0.08	—	3.32	—
XI	0	0.02	—	—	13.7	—
XII	0	0.02	—	—	18.4	—
IX	0	0.19	0.19	—	5.7	—
VIII	0	0.19	1.7	—	11.3	—
VII	0	0.19	0.30	—	*	—

* Did not elute under the conditions used.

The results in Fig. 2 show that under the eluent conditions used anthraquinone disulphonic acids have only a short retention time with the tetrabutyl pairing agent. With tetraheptyl ammonium bromide there is an increase in retention which depends upon the position of the sulphonic acid groups. Solutes with sulphonic acids in the α -position (compounds IX and X) are less retained than β -substituted derivatives (compound XII). The trisulphonic acid derivative (compound VII) was not eluted from the column with tetraheptylammonium bromide.

The shorter retention times of the disulphonic acids with the tetrabutyl pairing ion are almost certainly due to the fact that complete neutralization of the charge requires association with two cations



The formation of a simple ion pair would still leave a negatively charged entity with a greater affinity for the mobile phase than the stationary phase. With longer and hence more hydrophobic pairing agents, the tendency to form both ion pairs and ion triplets would presumably increase. Once formed, such a bulky "organic" entity as an ion triplet containing two tetrapentyl groups should interact relatively much more

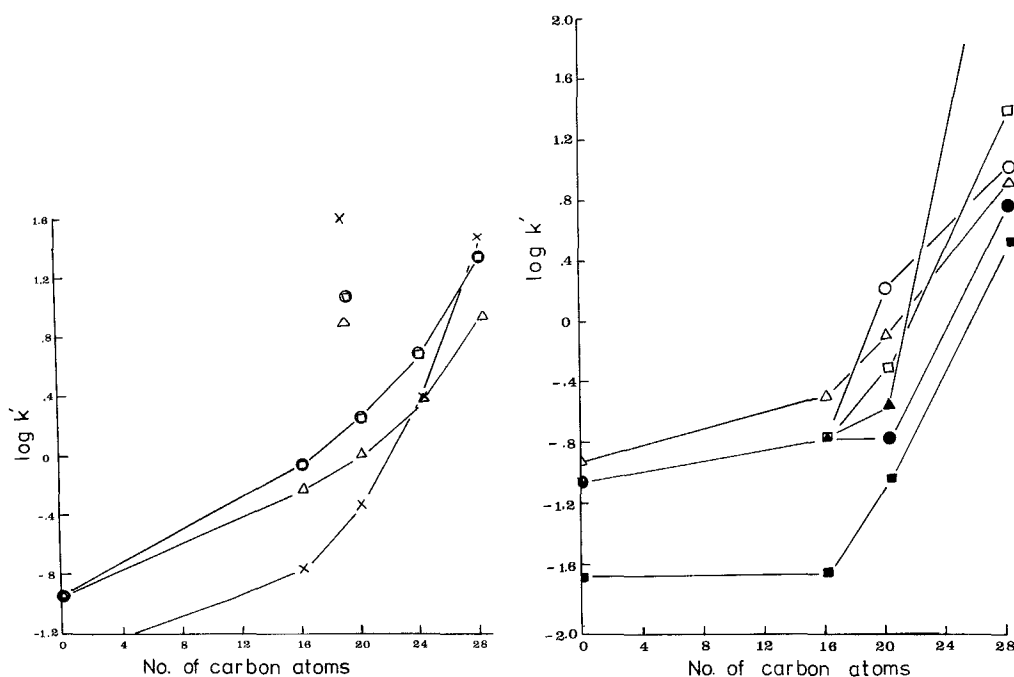


Fig. 1. Effect of number of carbon atoms in the pairing agent on the retention time for anthraquinone sulphonic acids: (Δ) II; (\times) V; (\circ) IV; (\square) III. The points displaced from the curves correspond to cetyltrimethylammonium bromide (C_{19}) as pairing agent.

Fig. 2. Effect of the number of carbon atoms in the pairing agent on the retention times of mono-, di- and trisulphonic acid derivatives of anthraquinone: (\blacksquare) X; (\bullet) IX; (\square) V; (Δ) II; (\circ) VIII; (\blacktriangle) VII.

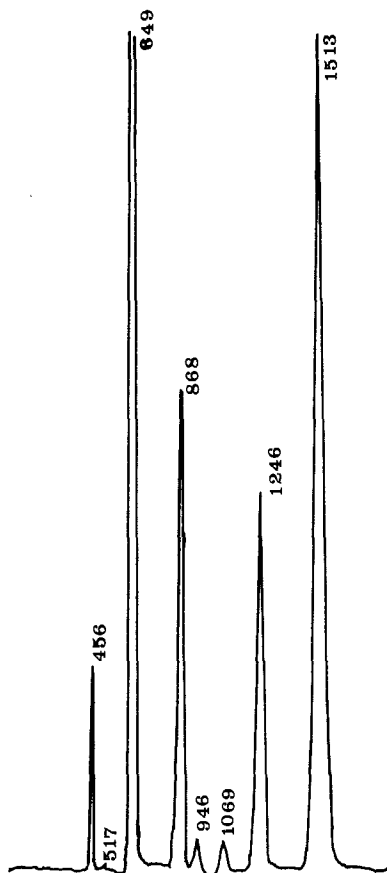


Fig. 3. Chromatographic separation of anthraquinone mono- and disulphonic acids. Chromatographic conditions: column, 200×5 mm I.D.; packing, Spherisorb S5 ODS; eluent, acetonitrile-water (50:50, v/v), cetyltrimethylammonium bromide, 0.8 g/100 ml; flow-rate, 2 ml mm^{-1} ; wavelength, 254 nm. Peaks and retention times (10^{-2} min): 1 = anthraquinone-1-sulphonic acid (456); 2 = anthraquinone-2-sulphonic acid (517); 3 = anthraquinone-1,5-disulphonic acid (649); 4 = anthraquinone-1,6-disulphonic acid (868); 5 = anthraquinone-2,6-disulphonic acid (946); 6 = anthraquinone-2,7-disulphonic acid (1069); 7 = anthraquinone-1,7-disulphonic acid (1246); 8 = anthraquinone-1,8-disulphonic acid (1513).

favourably with the stationary phase than with the mobile phase. The effect of the position of the sulphonic acid groups might be explained in terms of a greater difficulty of formation of ion triplets for α -sulphonic acids because of steric hindrance due to the adjacent carbonyl groups. It is, however, also possible that with a sufficiently bulky pairing cation, an ion pair, despite its overall negative charge, might interact quite favourably with the stationary phase.

In practical terms, longer chain pairing agents provide a means of separating isomers of anthraquinone disulphonic acids, as shown in Fig. 3.

Effect of increasing concentration of the pairing agent on k'

The results of varying the concentration of tetrabutylammonium bromide in the range 0.001 g/100 ml to 5.0 g/100 ml are shown in Table IV and Fig. 4. They

TABLE IV

EFFECT OF CONCENTRATION OF Bu_4NBr ON THE RETENTION (k') OF ANTHRAQUINONE DERIVATIVES

Solvent, methanol-water (65:35, v/v).

$[\text{Bu}_4\text{NBr}]$ (g/100 ml)	$[\text{Bu}_4\text{NBr}]$ (mol dm ⁻³)	Compound No.		
		V	II	III
0.00	0.00	0	0.15	0.15
0.001	$3.1 \cdot 10^{-5}$	0	0.11	0.22
0.002	$6.2 \cdot 10^{-5}$	0	0.15	0.26
0.004	$1.2 \cdot 10^{-4}$	0	0.17	0.28
0.008	$2.4 \cdot 10^{-4}$	0	0.19	0.32
0.01	$3.1 \cdot 10^{-4}$	0.034	0.24	0.39
0.02	$6.2 \cdot 10^{-4}$	0.068	0.59	0.94
0.05	$1.5 \cdot 10^{-3}$	0.17	0.82	1.30
0.1	$3.1 \cdot 10^{-3}$	0.39	1.28	2.03
0.2	$6.2 \cdot 10^{-3}$	0.69	1.99	3.25
0.5	$1.5 \cdot 10^{-2}$	1.50	2.91	4.90
1.0	$3.1 \cdot 10^{-2}$	2.08	3.64	6.02
2.0	$6.2 \cdot 10^{-2}$	2.27	3.80	6.38
5.0	$1.5 \cdot 10^{-1}$	2.24	3.75	6.27

show that the introduction of an ion-pairing agent, even at a concentration as low as 0.001 g/100 ml (*i.e.* $3.1 \cdot 10^{-5} M$), increases the retention of the monosulphonic acid derivatives. The retention of both mono- and disulphonic acids increases almost linearly with concentration of pairing agent until a concentration of *ca.* 0.5 g/100 ml (0.015 *M*). Beyond this stage levelling off begins, and above a concentration of *ca.* 2 g/100 ml (0.062 *M*) no further increase was detected. An increase in concentration to 5 g/100 ml (0.15 *M*) actually leads to a slight decrease in retention time. This is

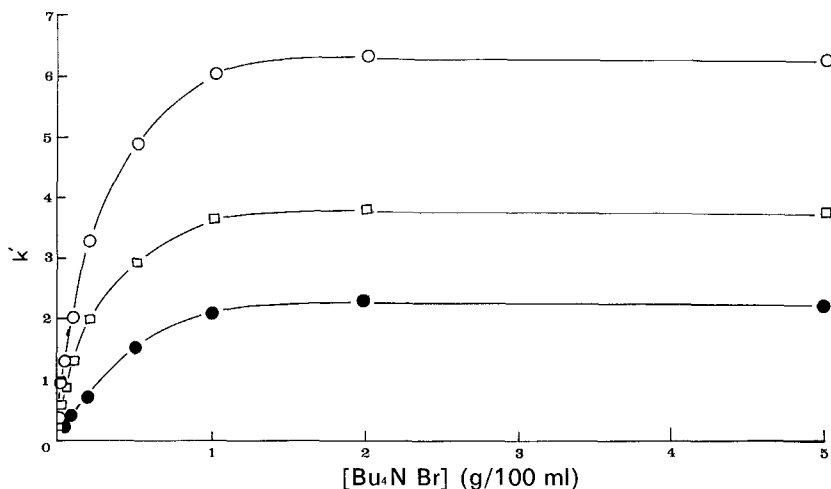
Fig. 4. Effect of $[\text{Bu}_4\text{NBr}]$ on the retention of III (○), II (□) and V (●).

TABLE V

ION-PAIR FORMATION AND RETENTION (k') OF ANTHRAQUINONE DERIVATIVES IN Bu_4NBr SOLUTIONS

Solvent, methanol-water (65:35, v/v).

$[\text{Bu}_4\text{NBr}]$ (g/100 ml)	$[\text{Bu}_4\text{NBr}]$ (mol dm ⁻³)	Ion-pair* formation (%)	k' (II)	k' (III)
0.05	$1.5 \cdot 10^{-3}$	6.5	0.82	1.30
0.10	$3.1 \cdot 10^{-3}$	13.4	1.28	2.03
0.20	$6.2 \cdot 10^{-3}$	23.7	1.99	3.25
0.50	$1.5 \cdot 10^{-2}$	42.8	2.91	4.90
1.0	$3.1 \cdot 10^{-2}$	60.8	3.64	6.02
2.0	$6.2 \cdot 10^{-2}$	75.6	3.80	6.38
5.0	$1.5 \cdot 10^{-1}$	88.2	3.75	6.27

* Calculated from eqn. 3 with $K_{\text{IP}} = 55 \text{ M}^{-1}$ (ref. 11).

in broad agreement with the findings of Knox⁸, Melander and Horváth⁹ and Knox and Hartwick¹⁰.

The variation in retention time with increasing concentration of P^+ may be understood in terms of ion-pair formation and extraction, according to eqns. 1 and 2 (ref. 11), in which A^- refers to the sulphonic acid derivative



It may be readily shown that for a given concentration of P^+ in the aqueous phase, the fraction of A^- in the form of ion-pairs is given by eqn. 3 (ref. 11), where $[\text{A}^-]_{\text{aq, total}}$ is the total concentration of A^- in the aqueous phase. Thus at low $[\text{P}^+]$, such that $K_{\text{IP}}[\text{P}^+]_{\text{aq}} \ll 1$, the

$$\frac{[\text{A}^-\text{P}^+]_{\text{aq}}}{[\text{A}^-]_{\text{aq, total}}} = \frac{K_{\text{IP}}[\text{P}^+]_{\text{aq}}}{1 + K_{\text{IP}}[\text{P}^+]_{\text{aq}}} \quad (3)$$

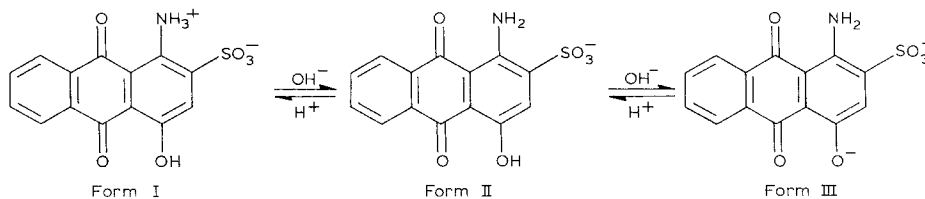
fraction of A^- which is paired increases directly with $[\text{P}^+]_{\text{aq}}$, but levels off at higher $[\text{P}^+]_{\text{aq}}$ such that $K_{\text{IP}}[\text{P}^+]_{\text{aq}} \gg 1$. This is illustrated in Table V for $K_{\text{IP}} = 55 \text{ M}^{-1}$, and is discussed fully in ref. 11. Thus it is suggested that the major reason for the levelling off effect at $[\text{P}^+] \geq 0.03 \text{ M}$ is that by this stage most of the solute in the aqueous phase is already paired.

Effect of pH of the eluent on hydroxyanthraquinones

The results in Table II show that very different k' values are observed for compound IV, 1-amino-4-hydroxyanthraquinone-2-sulphonic acid, in the presence of tetrabutylammonium bromide and tetrabutylammonium hydroxide. This was shown to be due to the difference in pH of solutions of the two pairing agents. The

conclusion is substantiated by the results in Table VI for the effect of varying eluent pH on the retention of a number of hydroxyanthraquinone derivatives.

Solutions covering the pH range 1.8–12.1 were prepared by adding varying amounts of phosphoric and nitric acids to the eluent containing 0.2 g/100 ml of *tert.*-butylammonium hydroxide. Over this range the solutes containing OH and NH₂ substituents can exist in three different ionisation states, *e.g.*



At pH 12.1 compound IV has a very short retention time ($k' = 0.02$), whereas compound III is more strongly retained ($k' = 0.51$). The $\text{p}K_{\text{a}}$ of IV (1-amino-4-hydroxyanthraquinone-2-sulfonic acid) was determined as 9.5, and hence it will be fully ionised (Form III) at pH 12.1. The negatively charged O^- will tend to be repelled from the organic stationary phase, and thus it will have a short retention time. At this pH, compound III, when ion-paired, will behave as a neutral molecule and hence be more retained. It should be noted, however, that elution at $\text{pH} \geq 12$ is not a practical proposition as gradual dissolution and shrinkage of the column occurs.

At pH values 10.8, 8.7 and 7.8, double peaks and severe tailing were observed for compound IV, with its average retention becoming longer. This behaviour correlates with the presence in these solutions of both the free and ionized forms of the OH substituent.

At pH values of 4.3 and 3.0 compound IV will be fully converted into the monoanionic state (form II) and hence, in the presence of the pairing agent, gives a sharp peak with a retention time similar to that of compound III. At these lower pH values, however, compound VI gives badly tailed peaks which sharpen up again by pH 1.8. This presumably results from the ionisation of the NH₂ group (to give form I), as this compound, which has *para* diamino groups, should be significantly more basic than the other compounds studied.

Recently a study of the effect of symmetrically substituted tetraalkylammonium pairing agents in methanol–water mixtures on the retention of a number of naphthalene and anthraquinone sulphonic acids has been reported¹². The effects of chain length and concentration of pairing agent were found to be similar to those described here, although the maximum concentration of pairing agent used (0.01 M) was sufficiently low such that a linear increase in retention time with pairing agent concentration was observed over the whole range studied. The authors also show that mobile phases containing inorganic salts (sodium sulphate) may in some cases offer advantages over ion-pairing reagents.

Králóvský *et al.*¹³ have reported the use of mobile phase containing inorganic salts (sodium sulphate) for the separation of sulphonic acid derivatives of 1-aminoanthraquinone but states that a gradient eluent is required to obtain a separation

TABLE VI
EFFECT OF pH ON THE RETENTION (k') OF ANTHRAQUINONE DERIVATIVES
Solvent, methanol-water (65:35, v/v).

Compound	k' (measured in 65% aqueous methanol)							
	12.1	10.8	8.7	7.8	4.2	3.0	1.8	
II	2.89	3.32	2.89	2.72	2.56	2.92		
II	0.24	0.24	0.51	0.72	1.77	1.64	1.32	
III	0.51	0.72	1.08	1.37	2.78	2.56	2.19	
IV	0.02	0.6, 0.72*	1.08, 1.21**	1.37, 1.54*	2.78	2.56	2.19	
V	-0.02	-0.13	-0.02	0.13	0.83	1.0	0.62	
VI	0.70	0.4	0.51	0.78	1.43**	1.48**	1.48	
XIII	***	***	***	***	***	-	-	
XIV	***	***	***	***	***	-	-	
XV	-0.08	0.02	0.38**	0.49**	0.27	-	-	
XVI	-0.08	0.14	0.49**	0.57**	0.13	-	-	
XVII	0.13	0.3	0.41	0.51	0.51	-	-	
XVIII	-0.13	0.02	0.02	0.14	0.46	-	-	

* Double peaks.

** Badly tailed peaks.

*** Did not elute under the conditions used.

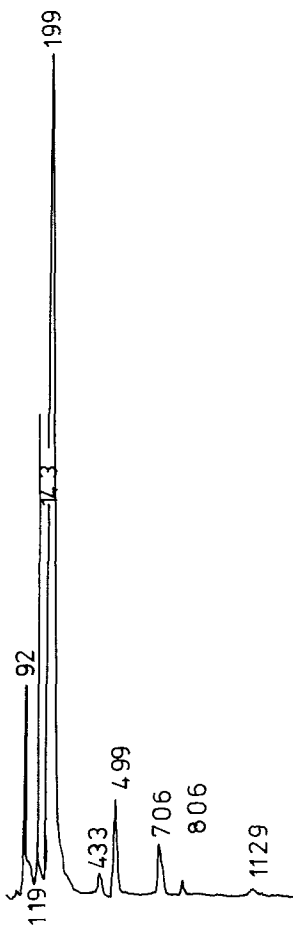


Fig. 5. Chromatographic separation of derivatives of 1-aminoanthraquinone. Chromatographic conditions: column, 250 × 5 mm I.D.; packing, Spherisorb SSC6; eluent, methanol-water (55:45) with tetrabutylammonium hydroxide (0.2 g/100 ml). Peaks and retention times (sec): 1 = 1-aminoanthraquinone-2,4-disulphonic acid (92); 2 = 1-amino-4-hydroxyanthraquinone-2-sulphonic acid (119); 3 = 1-aminoanthraquinone-2-sulphonic acid (145); 4 = 1-aminoanthraquinone-4-bromo-2-sulphonic acid (199); 5 = 1-aminoanthraquinone (433); 6 = 2-aminoanthraquinone (499); 7 = 1-amino-2-bromoanthraquinone (706); 8 = 1-amino-4-bromoanthraquinone (806); 9 = 1-amino-2,4-dibromoanthraquinone (1129).

of both sulphonated and non-sulphonated species. It is shown here (Fig. 5) that these compounds can be separated using an isocratic eluent containing an ion-pairing agent (tetrabutylammonium hydroxide).

CONCLUSIONS

Ion-pair chromatography provides an effective means of separating sulphonic acid derivatives of anthraquinone. The total number of carbon atoms in the chain has a significant effect on the retention of the solutes. There is a general increase in retention time with increase in size of R_4N^+ , but, for a given number of carbon

atoms, the retention time increases with the length of the longest chain. The pH of the eluent has a significant effect on the retention of solutes containing ionisable substituents such as $-\text{NH}_2$ and $-\text{OH}$. Severe tailing was observed when the pH of the eluent was close to the $\text{p}K_a$ of the solute. The retention of solutes increased with increased concentration of the pairing agent until a concentration of *ca.* 0.06 M tetrabutylammonium bromide was reached, when the effect levelled off.

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