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COMPARISON OF RETENTION BEHAVIOUR OF AROMATIC SULPHONIC ACIDS IN REVERSED-PHASE SYSTEMS WITH MOBILE PHASES CONTAINING ION-PAIRING IONS AND IN SYSTEMS WITH SOLUTIONS OF INORGANIC SALTS AS THE MOBILE PHASES

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SUMMARY

The retention of 34 naphthalene and anthraquinone sulphonic acids, which are important as dye intermediates, was measured on C_{18} and C_8 columns in mobile phases containing either an ion-pairing reagent or an inorganic salt in aqueous methanolic solvents. The influence on retention of the length of the bonded alkyl chains, of the nature and concentration of the ion-pairing reagent (an alkylammonium ion), of the inorganic salt (sodium sulphate) and of methanol in the mobile phase was investigated. Systems containing inorganic salts offer distinct advantages for the separation of sulphonic acids with different numbers of sulphonic groups and allow some efficient separations that are not feasible in the mobile phases with ion-pairing reagents. An explanation of the retention mechanism is attempted.

INTRODUCTION

Aromatic sulphonic acids derived from benzene, naphthalene, anthracene and anthraquinone are used largely in the industrial production of dyes. They are prepared by sulphonation of simple aromatic hydrocarbons or their derivatives, which usually yields mixtures of isomers and acids with different numbers of sulphonic groups.

High-performance liquid chromatography (HPLC) offers potential advantages over other analytical methods for the analysis of samples containing mixtures of sulphonic acids, both in the control of dye intermediates and technical products and as a control method in the optimization of reaction conditions in the sulphonation reaction step. Anion-exchange chromatography on anion-exchange resins[1,2], salting-out chromatography on cation-exchange resins[3,4] and chromatography on dextran gels[5,6] were tested for separation of aromatic sulphonic acids, but these methods yield a relatively low efficiency of separation.

Chromatography on porous-layer anion exchangers made possible separations of some simpler mixtures of these acids[7–11]. Columns packed with silica were also

used for separations of sulphonic acids, either with an aliphatic amine[12,13] or a quaternary alkylammonium ion-pairing agent[14-16] as a stationary liquid phase, or with solutions of acetate buffer in aqueous organic solvents[17,18] (also in non-aqueous organic solvents with an addition of crown ethers[19]) as the mobile phase. However, these techniques usually require quite long equilibration times between the stationary and mobile phases used and therefore it is not always easy to accomplish a rapid change in operating conditions.

The use of chemically bonded stationary phases (alkylsilica gels) in reversed-phase systems seems more attractive than the use of other column packing materials. However, if the separation of aromatic sulphonic acids is attempted in mobile phases composed only of water and an organic solvent, the solutes are usually eluted near or even prior to the column void volume, very often as strongly distorted or split peaks of irreproducible shapes, owing to ionic exclusion effects[20]. Occasionally, separations of simple mixtures of a few sulphonic acids may be achieved under these conditions[21]. If an ion-pairing reagent such as a quaternary ammonium salt is added to the mobile phase, it is possible to achieve efficient and rapid separations of a number of aromatic sulphonic acids, azo dyes and their intermediates[20,22-26]. We have reported elsewhere[20,27,28] another possibility for separation of quite complex mixtures of aromatic sulphonic acids, using mobile phases containing an inorganic salt in the concentration range 0.1–0.5 M.

We considered it useful to compare the possibilities of ion-pair reversed-phase chromatography and of reversed-phase chromatography with mobile phases containing inorganic salts for the separation of technically important aromatic sulphonic acids. This is the objective of the present work, together with a systematic study of various factors that influence the retention when the two separation techniques are used.

EXPERIMENTAL

The equipment used included a Model 6000 pump, an U6K injector and a UV detector (254 nm); a differential refractometer R-401 was used for measuring of column dead volumes (all from Waters Assoc., Milford, MA, U.S.A.). Stainless-steel columns (300 × 4.2 mm I.D.) were packed in the laboratory using a slurry-packing technique with an octadecylsilica material (Silasorb C_{18}, 10 μm) and with an octylsilica material (Silasorb C_8, 10 μm), both obtained from Lachema (Brno, Czechoslovakia). The void volumes, V_0, of the packed columns were determined as the retention volumes of D_2O measured with the aid of a differential refractometer and were 2.9 ml for the C_{18} and 3.2 ml for the C_8 columns.

The mobile phases were prepared by mixing water (double distilled in glass with addition of potassium permanganate) with methanol (reagent grade) in the required volume ratios and by dissolving the calculated quantity of sodium sulphate or of an tetraalkylammonium salt [tetramethylammonium sulphate (TMAS), tetraethylammonium sulphate (TEAS) and tetrabutylammonium sulphate (TBAS)] in this mixed solvent. The tetraalkylammonium sulphate salts were prepared from tetraalkylammonium iodides (Lachema) by reaction with silver sulphate. The presence of Ag^+ and I^- ions in the mixture after conversion was controlled by reactions with a saturated solution of silver sulphate and with a solution of potassium iodide, and

the addition of the reaction components was adjusted until the two reactions were negative. Then, the reaction mixture was filtered and concentrated by evaporation in a water bath. The solution was adjusted to a volume of 100 ml with twice-distilled water. The concentrations of tetraalkylammonium ions in these solutions were determined by titration with tetraphenylborate under potentiometric indication using a coated-wire ion-selective indication electrode. The mobile phases were prepared by dissolving a calculated aliquot of this solution in aqueous methanolic solvents.

The samples of aromatic sulphonic acids were obtained from East-Bohemian Chemical Works (Synthesia, Semtín, Czechoslovakia).

The capacity ratios, $k' = (V_R - V_0)/V_0$ (where V_R = retention volume and V_0 = void volume), were measured on the two columns in a number of mobile phases with different concentrations of methanol, sodium sulphate, TMAS, TEAS or TBAS. As the number of k' values measured is high, the experimental data are given rather as the parameters of log k' vs. concentration (or log of concentration) plots than as k' in Tables I-VI.

RESULTS AND DISCUSSION

In the present work, the influence of the concentration of an ion-pairing reagent or of an inorganic salt and of the concentration of methanol in the mobile phase on the retention of aromatic sulphonic acids was investigated, as well as the relationships between the retention and the size of the alkyl chains in quaternary alkylammonium ion-pairing reagents on both octadecylsilica and octylsilica columns.

Influence of the size of alkyl chains in a quaternary ammonium ion-pairing reagent added to the mobile phase

Table I shows the capacity ratios of several naphthalenesulphonic acids on C_{18} and C_8 columns in mobile phases containing tetramethylammonium (TMA), tetraethylammonium (TEA) and tetrabutylammonium (TBA) sulphate in two different concentrations. The log k' values increase with increasing number of carbon atoms (n_C) in ion-pairing reagents (Fig. 1). Generally, these plots are not linear, but the curves apparently become close to straight lines for higher n_C values. Therefore, to have a better comparison between the log $k' = f(n_C)$ plots for the two columns and two concentrations of ion-pairing reagents tested, we approximated these plots as straight lines. The slopes, intercepts and correlation coefficients of regression lines fitted to the experimental data are also given in Table I. The slopes of the regression lines (and k' values) are almost identical on both the C_8 and C_{18} columns for a given mobile phase. The slopes for most acids do not depend significantly on the concentration of the tetraalkylammonium ion in the mobile phase and, for most acids tested, are between 0.1 and 0.2 (except for 1-naphthylamine-8-sulphonic acid).

Linear log $k' = f(n_C)$ plots with similar values of slopes were observed for various homologous series in reversed-phase chromatography. These experimental data suggest that the nature of the alkyl-bonded phase has only a minor importance in the retention mechanism and the lengths of the alkyl chains in the alkylammonium ions contribute to the retention of the sulphonic acids in much a similar way as the alkyl chains in non-polar compounds.

All di-, tri- and tetrasulphonic acids and some hydroxy- and aminonaphtha-

TABLE I

CAPACITY RATIOS (k') OF NAPHTHALENE SULPHONIC ACIDS IN CHROMATOGRAPHY ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS IN MOBILE PHASES CONTAINING ION-PAIRING REAGENTS OF DIFFERENT ALKYL LENGTHS DISSOLVED IN METHANOL–WATER (35:65) AND PARAMETERS OF THE REGRESSION LINES $\log k' = A + B \cdot n_C$

n_C = Total number of carbon atoms in an ion-pairing tetraalkylammonium ion; R_k = correlation coefficients of the regression lines fitted to the experimental data; c_i = molar concentration of the ion-pairing reagent in the mobile phase. Ion-pairing reagents: tetramethylammonium (TMA, n_C = 4), tetraethylammonium (TEA, n_C = 8) and tetrabutylammonium (TBA, n_C = 16) ions; all as sulphates.

k'

Acid	C_{18}						C_8					
	$c_i = 0.005\ M$			$c_i = 0.01\ M$			$c_i = 0.005\ M$			$c_i = 0.01\ M$		
	TMA	TEA	TBA	TMA	TEA	TBA	TMA	TEA	TBA	TMA	TEA	TBA
2-Naphthalene sulphonic	1.0	1.3	14.0	1.5	1.7	16.3	0.7	1.0	12.6	0.9	1.5	15.4
1-Naphthylamine-8-sulphonic	0.03	0.2	26.2	0.2	0.4	—	0	0.09	33.2	0.07	0.5	52.2
1-Naphthylamine-6-sulphonic	0.01	0.08	2.5	0.1	0.2	3.3	0.03	0.09	2.7	0.15	0.2	3.3
2-Naphthol-1-sulphonic	1.2	1.9	32.7	1.9	2.1	43.2	0.8	1.1	26.0	1.0	1.7	—
1-Naphthol-4-sulphonic	0.1	0.3	5.6	0.3	0.4	7.2	0.1	0.2	4.8	0.2	0.4	6.2
2-Naphthol-6-sulphonic	0.02	0.1	2.8	0.2	0.2	3.4	0.06	0.1	3.1	0.1	0.3	3.7

Parameters of regression lines

| Acid	C_{18}						C_8					
	$c_i = 0.005\ M$			$c_i = 0.01\ M$			$c_i = 0.005\ M$			$c_i = 0.01\ M$		
	A	B	R_k	A	B	R_k	A	B	R_k	A	B	R_k
2-Naphthalenesulphonic	−0.52	0.10	0.92	−0.32	0.09	0.96	−0.69	0.11	0.96	−0.54	0.11	0.98
1-Naphthylamine-8-sulphonic	−2.58	0.25	0.998	−1.76	0.28	—	—	—	—	−2.17	0.24	0.999
1-Naphthylamine-6-sulphonic	−2.75	0.20	0.999	−0.88	0.12	0.99	−2.26	0.17	0.99	−1.38	0.12	0.98
2-Naphthol-1-sulphonic	−0.53	0.12	0.98	−0.37	0.12	0.95	−0.78	0.13	0.95	—	—	—
1-Naphthol-4-sulphonic	−1.50	0.14	0.99	−1.14	0.12	0.97	−1.64	0.14	0.97	−1.31	0.13	0.993
2-Naphthol-6-sulphonic	−2.36	0.18	0.999	−1.37	0.11	0.95	−1.91	0.15	0.95	−1.41	0.12	0.993

log k'
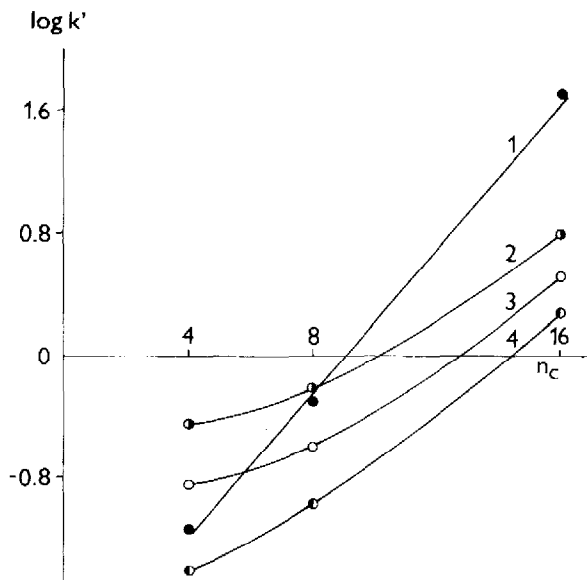
Fig. 1. Plots of the capacity ratios (k') of aromatic sulphonic acids on an octylsilica column as a function of the number of carbon atoms (n_C) in tetraalkylammonium ions used as ion-pairing reagents (in concentration 0.01 M) in methanol–water (35:65) as the mobile phase. Tetramethyl-, tetraethyl- and tetrabutylammonium sulphate were tested as ion-pairing reagents. Compounds: 1 = 1-naphthylamine-8-sulphonic acid; 2 = 1-naphthylamine-7-sulphonic acid; 3 = 1-naphthylamine-6-sulphonic acid; 4 = 1-naphthylamine-4-sulphonic acid.

lenemonosulphonic acids are only slightly (or not at all) retained in mobile phases containing 0.01 M or less tetramethyl- and tetraethylammonium sulphate. Therefore, these acids were not included in Table I. This also means that only mobile phases containing tetrabutylammonium ions in this concentration range can be used for practical separations of aromatic sulphonic acids. TMA or TEA ions perhaps may be useful at higher concentrations, but this possibility was not examined further.

Influence of the concentration of a quaternary ammonium ion-pairing reagent in the mobile phase on the retention of sulphonic acids

Table II shows the capacity ratios of aromatic sulphonic acids on C_{18} and C_8 columns in two mobile phases with different concentrations of TBAS as ion-pairing reagent. Most acids tested are retained slightly more on an octadecylsilica column than on an octylsilica column in a given mobile phase, but the differences appear to be not very significant, which suggests in this case the relatively minor importance of the nature of a bonded alkyl phase on the separation. The retention increases with increasing concentration of TBA ions in the mobile phase (c_i) and the plots of log k' as a function of log c_i are nearly linear (Fig. 2). The parameters of the regression lines fitted to these plots are given in Table III. The intercept (parameter A) represents the hypothetical extrapolated log k' in the mobile phase containing 1 M of TBAS and therefore it hardly has a real meaning. However, the slopes (parameters B) of these plots can be used as a measure of the influence of an ion-pairing agent on retention. A and B depend on the structure of the acids and generally, both A and

TABLE II

CAPACITY RATIOS k' OF NAPHTHALENE AND ANTHRAQUINONE SULPHONIC ACIDS IN CHROMATOGRAPHY ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS IN MOBILE PHASES CONTAINING AN ION-PAIRING REAGENT OR AN INORGANIC SALT IN AQUEOUS METHANOLIC SOLVENTS

Mobile phases: 1 = 0.005 M tetrabutylammonium sulphate in methanol water (35:65); 2 = 0.01 M tetrabutylammonium sulphate in methanol water (35:65); 3 = 0.1 M sodium sulphate in methanol–water (10:90); 4 = 0.4 M sodium sulphate in methanol water (10:90).

Acid	k'							
	Column							
	C_{18}				C_8			
	Mobile phase							
	1	2	3	4	1	2	3	4
2-Naphthalene sulphonic	14.0	16.3	—	—	12.6	15.4	20.1	Large
1,5-Naphthalene disulphonic	3.6	4.8	0.02	0.1	3.4	5.0	0.1	0.15
1,6-Naphthalene disulphonic	5.3	7.4	0.5	0.8	4.9	7.5	0.5	0.8
2,6-Naphthalene disulphonic	3.2	4.1	0.1	0.22	3.1	4.3	0.2	0.3
2,7-Naphthalene disulphonic	4.9	6.6	0.6	0.97	4.6	6.6	0.6	0.9
1,3,5-Naphthalene trisulphonic	16.5	25.7	$\leqslant 0$	$\leqslant 0$	14.7	—	$\leqslant 0$	0.01
1,3,6-Naphthalene trisulphonic	11.4	15.7	$\leqslant 0$	$\leqslant 0$	9.6	14.8	$\leqslant 0$	$\leqslant 0$
1,3,7-Naphthalene trisulphonic	12.8	20.6	$\leqslant 0$	$\leqslant 0$	12.3	20.1	$\leqslant 0$	0.03
1,3,5,7-Naphthalene tetrasulphonic	Large	Large	$\leqslant 0$	$\leqslant 0$	Large	Large	$\leqslant 0$	$\leqslant 0$
1,5-Anthraquinone disulphonic	2.6	3.3	0.1	0.2	2.4	3.0	0.1	0.2
2,6-Anthraquinone disulphonic	11.0	18.4	5.8	7.4	9.8	14.9	3.3	4.0
1,8-Anthraquinone disulphonic	14.8	21.5	14.2	19.0	11.5	16.1	8.2	10.4
1-Naphthylamine-4-sulphonic	1.5	1.9	1.2	1.5	1.2	1.9	1.3	1.6
1-Naphthylamine-5-sulphonic	1.2	1.5	0.9	1.2	1.4	1.7	1.2	1.4
1-Naphthylamine-6-sulphonic	2.5	3.3	3.6	4.5	2.7	3.3	3.9	4.5
1-Naphthylamine-7-sulphonic	5.9	7.8	12.3	14.9	5.2	6.3	8.7	10.4
R-acid	7.4	10.9	0.5	0.7	6.7	10.4	0.4	0.5
G-acid	6.4	8.4	0.6	0.8	6.3	8.9	0.6	0.7

B are at 10–30% higher for the C_8 than for the C_{18} column, even although the retention is slightly lower on the octylsilica column than on the octadecylsilica column.

The slopes B generally increase with increasing number of sulphonic groups in the acid. Di- and trisulphonic acids can probably form associates with a larger average number of tetrabutylammonium ions than monosulphonic acids. The parameters A also increase from mono- to trisulphonic acids. This means that di- and trisulphonic acids are more strongly retained than monosulphonic acids in the mobile phases with high concentrations of ion-pairing agent while the opposite holds true for the mobile phases with low concentrations of TBA ions. Therefore, in certain mobile phases with medium concentrations of the ion-pairing agent, sulphonic acids with different numbers of sulphonic groups may have a similar retention, which may cause problems when the separation of these acids is attempted. An obvious exception from the rule is 1-naphthylamine-8-sulphonic acid which has very large B and A parameters in comparison to the other acids. This is possibly caused by the formation of inner ion pairs between the NH_2 and SO_3H groups of this acid.
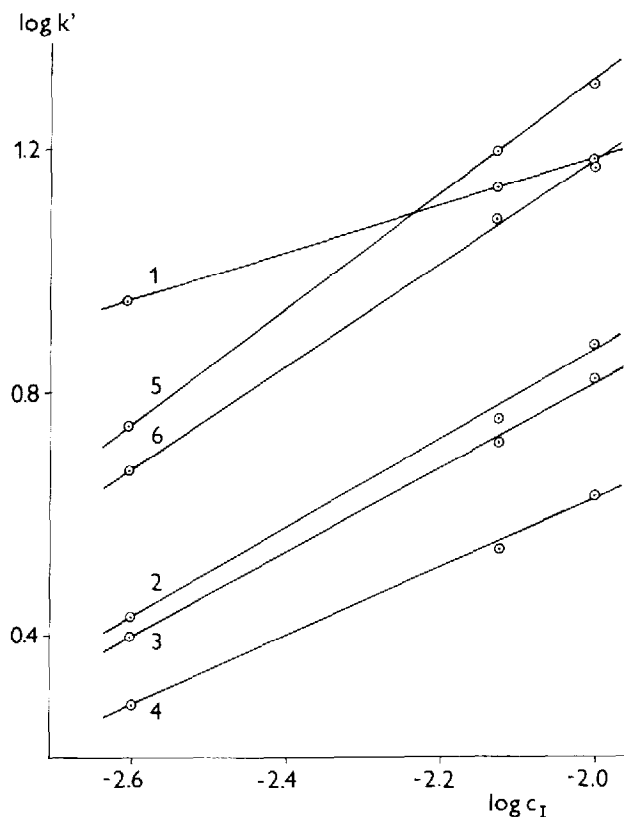
Fig. 2. Plots of the capacity ratios (k') of aromatic sulphonic acids on an octylsilica column as a function of concentration (c_I, M) of tetrabutylammonium sulphate in methanol–water (35:65) as the mobile phase. Compounds: 1 = 2-naphthalenesulphonic acid; 2 = 1,6-naphthalene disulphonic acid; 3 = 2,7-naphthalenedisulphonic acid; 4 = 2,6-naphthalenedisulphonic acid; 5 = 1,3,7-naphthalenetrisulphonic acid; 6 = 1,3,6-naphthalenetrisulphonic acid.

Influence of the concentration of an inorganic salt in the mobile phase on the retention of sulphonic acids

Examples of the capacity ratios of aromatic sulphonic acids on C_{18} and C_8 columns in two mobile phases with different concentrations of sodium sulphate (in methanol–water, 10:90) are shown in Table II. As in the mobile phases containing TBAS, the acids tested are generally retained somewhat more strongly on the octadecylsilica column than on the octylsilica column in a given mobile phase. Here again, the differences between the retention on the two columns are relatively small for most acids.

The retention increases with increasing concentration of sodium sulphate in the mobile phase (c_S). The plots of log k' as a function of log c_S are nearly linear for the C_{18} column, but are slightly curved for the C_8 column (Fig. 3). This is demonstrated by the poorer correlation coefficients for the C_8 column in comparison with the C_{18} column and with the C_8 column in the mobile phase containing tetrabutylammonium sulphate (Tables III and IV). The parameters of the regression lines fitted

P. JANDERA, J. CHURÁČEK, B. TARABA

TABLE III

PARAMETERS OF THE REGRESSION LINES $\log k' = A + B \cdot \log c_l$ FOR AROMATIC SULPHONIC ACIDS ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS

The mobile phases contain different molar concentrations c_l of tetrabutylammonium sulphate in methanol water (35:65). R_k = Correlation coefficients of the regression lines fitted to the experimental data (4 for each compound).

Acid	Column					
	C_{18}			C_8		
	A	B	R_k	A	B	R_k
2-Naphthalene sulphonic	1.816	0.298	0.990	2.004	0.402	0.988
1,5-Naphthalene disulphonic	1.684	0.497	0.995	2.038	0.663	0.956
1,6-Naphthalene disulphonic	2.043	0.582	0.993	2.368	0.739	0.994
2,6-Naphthalene disulphonic	1.526	0.454	0.994	1.793	0.576	0.995
2,7-Naphthalene disulphonic	1.891	0.532	0.992	2.251	0.706	0.990
1,3,5-Naphthalene trisulphonic	3.026	0.800	0.994	4.164	1.302	—
1,3,6-Naphthalene trisulphonic	2.592	0.687	0.983	2.858	0.834	0.990
1,3,7-Naphthalene trisulphonic	2.760	0.721	0.999	3.182	0.929	0.991
1-Naphthylamine-4-sulphonic	0.910	0.321	0.998	0.970	0.346	0.997
1-Naphthylamine-5-sulphonic	0.872	0.344	0.9999	0.973	0.364	0.989
1-Naphthylamine-6-sulphonic	1.240	0.362	0.999	1.272	0.374	0.990
1-Naphthylamine-7-sulphonic	1.637	0.374	0.999	1.624	0.405	0.983
2-Naphthylamine-6-sulphonic	1.854	0.679	0.954	—	—	—
1-Naphthylamine-8-sulphonic	3.367	0.847	—	3.480	0.870	0.990
2-Naphthylamine-1-sulphonic	1.617	0.488	0.993	—	—	—
1-Naphthylamine-3,6-disulphonic	1.031	0.306	0.991	—	—	—
2-Naphthylamine-4,8-disulphonic	1.511	0.465	0.994	—	—	—
2-Naphthylamine-3,6,8-trisulphonic	2.298	0.558	0.991	—	—	—
1-Naphthylamine-4,6,8-trisulphonic	2.364	0.573	0.990	—	—	—
2-Naphthol-1-sulphonic	2.704	0.528	0.991	0.357	0.844	—
1-Naphthol-4-sulphonic	1.872	0.500	0.989	1.893	0.542	0.985
2-Naphthol-6-sulphonic	1.374	0.414	0.991	1.496	0.452	0.970
R-acid	2.334	0.644	0.997	2.687	0.826	0.992
G-acid	1.956	0.510	0.992	2.441	0.733	0.986
2-Amine-5-naphthol-7-sulphonic	0.948	0.332	0.991	—	—	—
1-Amine-8-naphthol-3,6-disulphonic	0.900	0.322	0.9998	—	—	—
2-Amine-5-naphthol-1,7-disulphonic	1.948	0.535	0.995	—	—	—
1-Anthraquinone sulphonic	1.856	0.292	0.997	—	—	—
1,5-Anthraquinone disulphonic	1.311	0.392	0.998	1.380	0.444	0.983
2,6-Anthraquinone disulphonic	2.597	0.669	0.998	2.875	0.839	0.987
1,8-Anthraquinone disulphonic	2.453	0.560	0.9998	2.659	0.714	0.983

to the experimental $\log k'$ vs. $\log c_s$ plots in Table IV have an analogous meaning to the parameters of the $\log k'$ vs. $\log c_l$ plots in Table III.

For most acids, the slopes B and the intercepts A in Table IV do not show a significant dependence on the length of the bonded alkyl chains and the values for the C_8 column are usually close to the parameters of the regression curves on the C_{18} column, or are only slightly higher. These parameters usually are between 30 and 60% of the value of the parameters A and B of the $\log k'$ vs. $\log c_l$ plots in Table III for a given acid and column, but these differences may be partly influenced by
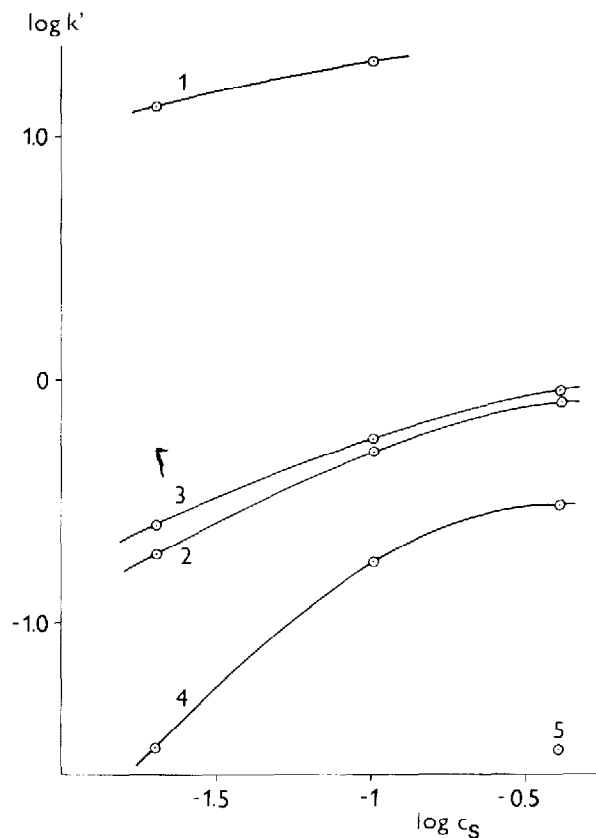
Fig. 3. Plots of the capacity ratios (k') or aromatic sulphonic acids on an octylsilica column as a function of concentration (c_s, M) of sodium sulphate in methanol water (10:90) as the mobile phase. Compounds as in Fig. 2.

the different concentrations of methanol in the mobile phases containing sodium sulphate and those with TBAS.

There is an essential difference between the retention of mono-, di- and trisulphonic acids in the mobile phases containing sodium sulphate and in the mobile phases with an ion-pairing reagent (TBAS). In the solutions of sodium sulphate, the retention of the acids studied decreases in the order mono- > di- > trisulphonic acids (Fig. 3, Tables II and IV). The parameters A (log k' in the mobile phases containing 1 M sodium sulphate) follow generally the same order and therefore, even if the slopes B of the regression lines increase in the order mono- < di- < trisulphonic acids, the acids with different numbers of sulphonic groups can be separated over the whole range of practically possible concentrations of sodium sulphate in the mobile phase. As in the mobile phases containing TBAS, the parameters B and A of 1-naphthylamine-8-sulphonic acids are significantly increased as compared with other monosulphonic acids.

TABLE IV

PARAMETERS OF THE REGRESSION LINES $\log k' = A + B \cdot \log c_S$ FOR AROMATIC SULPHONIC ACIDS ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS

The mobile phases contain different molar concentrations, c_S, of sodium sulphate in methanol–water (10:90). R_k = Correlation coefficients of the regression lines fitted to the experimental data (3 4 for each compound).

Acid	Column					
	C_{18}			C_8		
	A	B	R_k	A	B	R_k
1,6-Naphthalene disulphonic	0.060	0.376	0.999	0.126	0.483	0.988
2,7-Naphthalene disulphonic	0.140	0.371	0.9994	0.143	0.426	0.990
1-Naphthylamine-4-sulphonic	0.248	0.177	0.9997	0.294	0.190	0.993
1-Naphthylamine-5-sulphonic	0.140	0.174	0.9999	0.225	0.170	0.978
1-Naphthylamine-6-sulphonic	0.717	0.155	0.9999	0.740	0.180	0.981
1-Naphthylamine-7-sulphonic	1.240	0.157	0.999	1.112	0.199	0.982
2-Naphthylamine-6-sulphonic	0.625	0.171	0.9999	—	—	—
1-Naphthylamine-8-sulphonic	—	—		1.697	0.256	0.949
1-Naphthol-4-sulphonic	0.809	0.168	0.999	—	—	—
2-Naphthol-6-sulphonic	0.649	0.141	0.9991	—	—	—
R-Acid	−0.040	0.251	0.9994	−0.139	0.310	0.955
G-Acid	0.008	0.267	0.9999	−0.004	0.320	0.949
2-Amine-5-naphthol-7-sulphonic	0.395	0.158	0.9956	—	—	—
1-Amine-8-naphthol-3,6-disulphonic	0.397	0.096	0.969	—	—	—
2-Amine-5-naphthol-1,7-disulphonic	−0.126	0.289	0.9996	—	—	—
2,6-Anthraquinone disulphonic	0.982	0.243	0.991	0.749	0.288	0.960
1,8-Anthraquinone disulphonic	1.404	0.279	0.992	1.168	0.307	0.974

Influence of the concentration of methanol in the mobile phase on the retention of sulphonic acids

The retention of sulphonic acids decreases with increasing concentration of methanol (c) in the mobile phases containing sodium sulphate as well as in the mobile phases with TBAS. The plots of $\log k'$ decrease linearly with increasing c in the mobile phases containing 10% and more of methanol; the plots are slightly curved for mobile phases with less than 10% of methanol (Figs. 4 and 5).

Table V shows the parameters of the regression lines fitted to the plots of $\log k'$ vs. c for aromatic sulphonic acids in mobile phases containing a constant concentration (0.005 M) of tetrabutylammonium sulphate. The parameters of analogous regression lines calculated from the experimental data in mobile phases containing a constant concentration (0.4 M) of sodium sulphate are given in Table VI. The slopes B are very close on the C_{18} and on the C_8 phase for most acids, which again suggests only a minor influence of the support on retention. (The slopes B in the mobile phases containing sodium sulphate are slightly higher for the C_{18} column than for the C_8 one.) The same holds true for the intercepts A. It is interesting to note also that the slopes B of the plots in the mobile phases containing sodium sulphate are mostly quite near to the values of B in the mobile phases containing TBAS for a given acid (the differences usually are not more than 20% relative). However, the parameters A are far lower in the mobile phases containing sodium sulphate than in the solutions of TBAS. This is due to the fact that the retention in the mobile
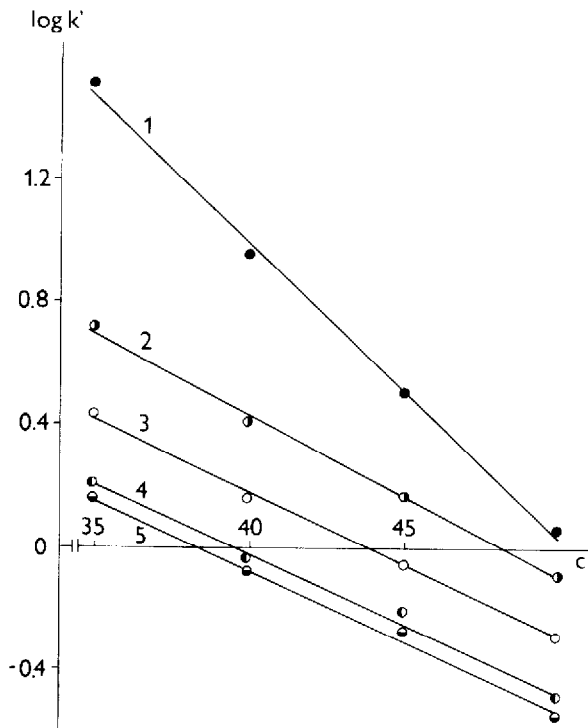
Fig. 4. Plots of the capacity ratios (k') of aromatic sulphonic acids on an octylsilica column as a function of concentration (c, %, v/v) of methanol in the mobile phase containing 0.005 M tetrabutylammonium sulphate in methanol–water mixed solvents. Compounds: 1 = 1-naphthylamine-8-sulphonic acid; 2 = 1-naphthylamine-7-sulphonic acid; 3 = 1-naphthylamine-6-sulphonic acid; 4 = 1-naphthylamine-4-sulphonic acid; 5 = 1-naphthylamine-5-sulphonic acid.

phase containing 0.005 M TBAS is much higher than in the mobile phase containing 0.4 M sodium sulphate (for an equal concentration of methanol in the mobile phase).

    With a few exceptions, the slopes B for all acids and systems (stationary–mobile phase) are in the range 4–8. Both in the mobile phases containing sodium sulphate and those containing TBAS, B generally increases with increasing number of sulphonic groups in the molecule, *i.e.* from mono- to tetrasulphonic acids. Here again, 1-naphthylamine-8-sulphonic acid with high values of B is an exception to the rule. The parameters A decrease with increasing number of sulphonic groups in mobile phases containing sodium sulphate, where the naphthalene tetra- and most trisulphonic acids are eluted with the retention volumes close to (or even lower than) the column void volume. In the mobile phases containing TBAS, the slopes A generally decrease in the order: tetra- > tri- > mono- $\geqslant$ disulphonic acids.

Retention mechanism
    The separation mechanism of the ion-pair reversed-phase chromatography has been explained in different ways. In one model, it is assumed that ion-pair-forming ions are first sorbed on the surface of a non-polar bonded phase by their hydrocar-
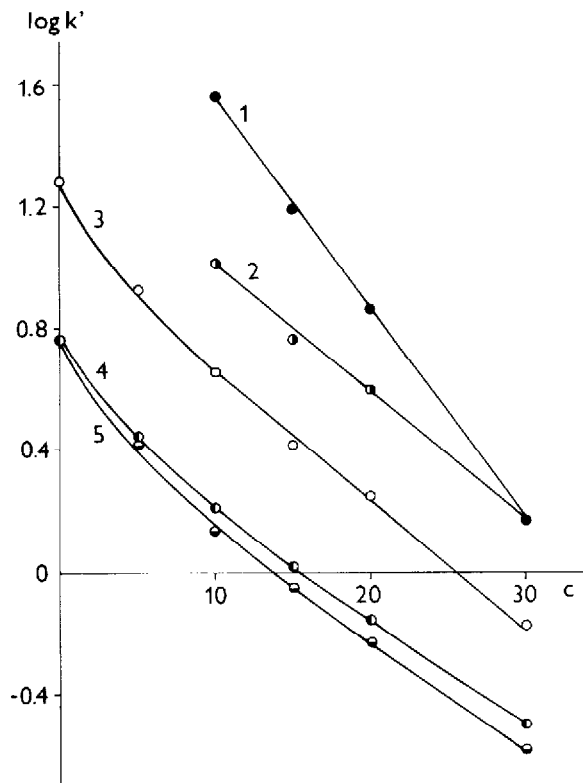
Fig. 5. Plots of the capacity ratios (k') of aromatic sulphonic acids on an octylsilica column as a function of concentration (c, %, v/v) of methanol in the mobile phase containing 0.4 M sodium sulphate in methanol–water mixed solvents. Compounds as in Fig. 4.

bonaceous parts and then the solute ions form ion pairs with the adsorbed ions and are retained (the concept of a dynamically created ion-exchange system)[29]. Another model assumes ion-pair formation between the large organic cations and anions. The ion pairs formed possess a zero overall charge and are retained on the surface of the hydrocarbonaceous-bonded phase by virtue of hydrophobic interactions, in a similar way to organic molecules with large non-polar moieties[22]. Finally, the retention is explained by adsorption of a pair of ions due to a dynamic equilibrium between the solute ions and the ions added to the mobile phase both in a primary adsorbed layer, containing ions with hydrophobic moieties, and in a secondary diffuse ion layer containing counter-ions[30]. The retention in the mobile phases containing inorganic salts has been ascribed to the salting-out effect[27,28].

However, the numerous analogies between the retention of aromatic sulphonic acids in the mobile phases containing an alkylammonium ion and that in the mobile phases with sodium sulphate found in the present work seem to indicate that there is no sharp fundamental difference in using the two mobile phases and, possibly, a retention mechanism can be suggested that would explain the phenomena observed in the mobile phases containing both inorganic and organic ions. For this purpose,

TABLE V

PARAMETERS OF THE REGRESSION LINES $\log k' = A - B \cdot c$ FOR AROMATIC SULPHONIC ACIDS ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS

The mobile phases contain different volume concentrations, c, of methanol in water; all mixed mobile phases contain 0.005 M TBAS. R_k = Correlation coefficients of the regression lines fitted to the experimental data (4 for each compound).

Acid	Column					
	C_{18}			C_8		
	A	B	R_k	A	B	R_k
2-Naphthalene sulphonic	3.049	5.482	0.999	3.154	5.906	0.999
1,5-Naphthalene disulphonic	2.624	5.852	0.998	2.731	6.236	0.998
1,6-Naphthalene disulphonic	2.864	6.071	0.996	2.886	6.266	0.9997
2,6-Naphthalene disulphonic	2.552	5.832	0.9998	2.531	5.828	0.996
2,7-Naphthalene disulphonic	2.850	6.209	0.9993	2.837	6.242	0.998
1,3,5-Naphthalene trisulphonic	3.614	6.948	0.9992	3.978	8.058	0.9994
1,3,6-Naphthalene trisulphonic	3.488	6.947	0.9994	3.431	7.044	0.998
1,3,7-Naphthalene trisulphonic	3.372	6.566	0.999	3.710	7.556	0.998
1,3,5,7-Naphthalene tetrasulphonic	4.684	8.340	0.9999	—	—	—
1-Naphthylamine-4-sulphonic	1.871	4.818	0.998	1.785	4.524	0.996
1-Naphthylamine-5-sulphonic	1.788	4.818	0.993	1.741	4.524	0.996
1-Naphthylamine-6-sulphonic	2.134	4.952	0.999	2.080	4.750	0.998
1-Naphthylamine-7-sulphonic	2.578	5.196	0.999	2.562	5.318	0.998
2-Naphthylamine-6-sulphonic	2.192	5.224	0.999	—	—	—
1-Naphthylamine-8-sulphonic	4.561	8.914	0.999	4.842	9.588	0.998
2-Naphthylamine-1-sulphonic	2.462	5.522	0.998	—	—	—
1-Naphthylamine-3,6-disulphonic	2.492	5.944	0.990	—	—	—
2-Naphthylamine-4,8-disulphonic	2.453	5.605	0.996	—	—	—
2-Naphthylamine-3,6,8-trisulphonic	3.348	6.605	0.9999	—	—	—
1-Naphthylamine-4,6,8-trisulphonic	3.423	6.729	0.9999	—	—	—
2-Naphthol-1-sulphonic	3.706	6.284	0.9992	3.751	6.722	0.999
1-Naphthol-4-sulphonic	2.481	5.049	0.998	2.524	5.310	0.999
2-Naphthol-6-sulphonic	2.236	5.175	0.998	2.342	5.306	0.999
R-Acid	3.078	6.312	0.9997	3.165	6.746	0.998
G-Acid	2.941	6.086	0.9998	3.054	6.520	0.997
2-Amine-5-naphthol-7-sulphonic	1.890	4.869	0.9984	—	—	—
1-Amine-8-naphthol-3,6-disulphonic	2.150	5.488	0.988	—	—	—
2-Amine-5-naphthol-1,7-disulphonic	3.040	6.488	0.998	—	—	—
1-Anthraquinone sulphonic	3.141	5.601	0.9997		—	—
2-Anthraquinone sulphonic	3.932	6.530	0.9996	—	—	—
1,5-Anthraquinone disulphonic	2.362	5.483	0.998	2.358	5.596	0.982
2,6-Anthraquinone disulphonic	3.440	6.892	0.9996	3.534	7.364	0.997
1,8-Anthraquinone disulphonic	3.545	6.830	0.9997	3.595	7.312	0.998
1-Amine-2-anthraquinone sulphonic	4.132	6.335	0.9997	2.731	6.236	0.998

various ionic and hydrophobic interactions should be considered. We attempted to classify them as follows.

(1) Sorption of the counter-ion (cation B), added to the mobile phase, onto a surface of an alkyl-bonded stationary phase. Small inorganic (and organic) ions are not sorbed, but rather excluded by virtue of the Donnan effect (the ionized non-silanized silanol groups in the packing material can contribute to this effect). Organic

TABLE VI

PARAMETERS OF THE REGRESSION LINES $\log k' = A - B \cdot c$ FOR AROMATIC SULPHONIC ACIDS ON OCTADECYLSILICA (C_{18}) AND OCTYLSILICA (C_8) COLUMNS

The mobile phases contain different volume concentrations, c, of methanol in water; all mixed mobile phases contain 0.4 M sodium sulphate. R_k = Correlation coefficients of the regression lines fitted to the experimental data (4–6 for each compound).

Acid	Column					R_k
	C_{18}			C_8		
	A	B	R_k	A	B	
2-Naphthalene sulphonic	2.084	4.784	0.997	1.779	3.919	0.9998
1,5-Naphthalene disulphonic	−0.294	6.906	0.995	−0.395	4.334	0.995
1,6-Naphthalene disulphonic	0.613	6.788	0.993	0.412	5.363	0.998
2,6-Naphthalene disulphonic	0.018	6.612	0.9993	0.075	6.377	0.984
2,7-Naphthalene disulphonic	0.594	5.303	0.991	0.482	5.388	0.996
1-Naphthylamine-4-sulphonic	0.752	4.855	0.985	0.682	4.140	0.990
1-Naphthylamine-5-sulphonic	0.686	5.213	0.987	0.666	4.442	0.990
1-Naphthylamine-6-sulphonic	1.351	5.784	0.978	1.190	4.745	0.992
1-Naphthylamine-7-sulphonic	1.631	4.729	0.999	1.414	4.147	0.998
2-Naphthylamine-6-sulphonic	1.012	4.590	0.9998	—	—	—
1-Naphthylamine-8-sulphonic	2.455	7.916	0.9998	2.251	6.970	0.9999
2-Naphthol-1-sulphonic	—	—	—	1.826	3.980	0.9997
1-Naphthol-4-sulphonic	1.143	3.874	0.999	1.055	3.656	0.995
2-Naphthol-6-sulphonic	1.065	4.586	0.998	1.170	4.476	0.997
R-Acid	0.557	6.856	0.999	0.346	6.150	0.998
G-Acid	0.558	6.265	0.998	0.409	5.451	0.998
2-Amine-5-naphthol-7-sulphonic	0.872	5.163	0.999	—	—	—
1-Amine-8-naphthol-3,6-disulphonic	1.038	5.988	0.989	—	—	—
2-Amine-5-naphthol-1,7-disulphonic	0.552	8.062	0.992	—	—	—
1-Anthraquinone sulphonic	2.516	5.972	0.9992	—	—	—
1,5-Anthraquinone disulphonic	0.145	8.388	0.998	−0.290	4.357	0.999
2,6-Anthraquinone disulphonic	1.653	7.390	0.998	1.338	6.599	0.991
1,8-Anthraquinone disulphonic	1.860	5.894	0.9998	1.571	5.381	0.997

ions with larger hydrocarbonaceous moieties are adsorbed on the surface and it has been shown that this adsorption can be described either by the empirical Freundlich isotherm or by the modified Langmuir isotherm, both in the form[31]

$$[B]_{sorb} = a \cdot [B]_{mob}^b \tag{1}$$

where $[B]_{sorb}$ and $[B]_{mob}$ are the concentrations of the univalent counterion B in the mobile aqueous organic phase (mob) and in the stationary phase (sorb)-adsorbed layer.

(2) Sorption of the solute ion (acid anion A) on the stationary bonded phase, either by virtue of hydrophobic interactions as in (1) or of ionic interactions with ionized residual silanol groups:

$$A_{mob} \leftrightarrows A_{sorb}$$
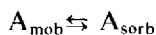
An equilibrium constant (partial distribution coefficient) K_{DA} can be defined for this process:

$$K_{DA} = \frac{[A]_{sorb}}{[A]_{mob}} \tag{2}$$

(3) Sorption of the ion pair (ion associate) AB_n formed in the mobile phase on to the stationary bonded phase due to the hydrophobic effect:
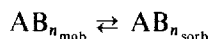
$$AB_{n_{mob}} \rightleftarrows AB_{n_{sorb}}$$

The equilibrium constant K_{DAB_n} is given as

$$K_{DAB_n} = \frac{[AB_n]_{sorb}}{[AB_n]_{mob}} \tag{3}$$

(4) Formation of ion pairs (ion associates) in the mobile phase. This formation is understood as a dynamic process, where the free ions A, B and the ions in an associate AB_n are very rapidly interchanged:
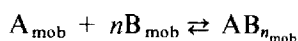
$$A_{mob} + nB_{mob} \rightleftarrows AB_{n_{mob}}$$

The equilibrium constant is

$$K_{IP_{mob}} = \frac{[AB_n]_{mob}}{[A]_{mob} \cdot [B]_{mob}^n} \tag{4}$$

(5) Association of the acid anions A on to the cations B sorbed on the surface of the bonded phase (formation of ion associates in the stationary phase):

$$A_{mob} + nB_{sorb} \rightleftarrows AB_{n_{sorb}}$$

$$\tag{5}$$

$$K_{IP_{sorb}} = \frac{[AB_n]_{sorb}}{[A]_{mob} \cdot [B]_{sorb}^n}$$

(6) The salting-out effect that is non-specific to the ions and is observed at high salt concentrations in the mobile phase both for ionized and non-ionized solutes. The increased retention with increasing concentration of the salt ($[B]_{mob}$) is here due to an increased polarity of the mobile phase that increases the hydrophobic effect. This effect was observed for non-ionized solutes both on ion-exchange resins and on chemically bonded alkyl phases. Increasing the concentration of a salt in the mobile phase has a similar effect on the retention as increasing the concentration of water in the aqueous organic mobile phases currently used in reversed-phase chromatography and, to a first approximation, it can be described by the equation

$$\log K_D = \log K_{0D} + m \cdot [B]_{mob} \tag{6}$$

where K_D and K_{OD} are the distribution coefficients of a solute in the presence and in the absence, respectively, of a salt in the mobile phase. (An empirical equation in this form was found to apply for salting-out chromatography of various organic solutes on ion-exchange resins[32].)

(7) The Donnan effect: an increase in ionic strength helps to overcome the repulsion forces between the negatively charged adsorbed ions [and, eventually, negatively charged ionized unreacted silanol groups in the bonded phase (SiO^-)[33]] on one side and the negatively charged anions of the acids A in the mobile phase on the other side. This effect is specific for ionized solutes and the influence of the concentration of a salt in the mobile phase on K_D can be described by an equation either formally similar to the eqn. 6 or by another equation:

$$\log K_D = f([B]_{mob}) \tag{7}$$

(8) All the above equations are valid at a constant concentration of the organic solvent in the mobile phase, c_{org}. If this concentration is changed, the capacity ratios k' of all the compounds would follow (to a first approximation) the equation[34–36]

$$\log k' = \log a_1 - (m_1 \cdot c_{org}) \tag{8}$$

(9) There are also the effects of co-ions in the mobile phase on retention; these are, however, difficult to predict theoretically.

Thus, assuming a constant c_{org}, we can write for the capacity ratio k' and for the distribution coefficient K_D of an acid

$$k' = \varphi \cdot K_D = \varphi \cdot \frac{[A]_{sorb} + [AB_n]_{sorb}}{[A]_{mob} + [AB_n]_{mob}} \tag{9}$$

Here, φ is the phase ratio V_S/V_0 (V_S = volume of the stationary phase; V_0 = column dead volume) in the column.

From eqns. 1–5, it follows that

$$[A]_{sorb} = K_{DA} \cdot [A]_{mob}$$

$$[AB_n]_{mob} = K_{IP_{mob}} \cdot [A]_{mob} \cdot [B]^n_{mob}$$

$$[AB_n]_{sorb} = K_{DAB_n} \cdot [AB_n]_{mob} + K_{IP_{sorb}} \cdot [A]_{mob} \cdot a^n \cdot [B]^n_{mob}$$

and

$$k' = \varphi \cdot \frac{K_{DA} + K_{DAB_n} \cdot K_{IP_{mob}} \cdot [B]^n_{mob} + K_{IP_{sorb}} \cdot a^n \cdot [B]^{n \cdot b}_{mob}}{1 + K_{IP_{mob}} \cdot [B]^n_{mob}} \tag{10}$$

Using eqn. 10, it could be possible to describe the retention in the mobile phases containing both the inorganic and organic ions.

(1) If the mobile phase contains a small concentration of an organic ion with

non-polar alkyl substituents, such as a tetraalkylammonium cation, it can be assumed that the retention of the acid anions is negligible in comparison with the retention of ion-associates, $i.e.$ $[AB_n]_{sorb} \gg [A]_{sorb}$. If we further assume, in agreement with ref. 30, that the ion associates are formed in the stationary phase rather than in the mobile phase, $[A]_{mob} > [AB_n]_{mob}$ and $K_{IP_{sorb}} \cdot [A]_{mob} \cdot a^n \cdot [B]_{mob}^{n \cdot b} > K_{DAB_n} \cdot [AB_n]_{mob}$, then

$$k' \approx \varphi \cdot K_{IP_{sorb}} \cdot a^n \cdot [B]_{mob}^{n \cdot b} \qquad (11)$$

(2) If the mobile phase contains a considerable concentration of a simple inorganic ion, such as sodium, lithium, potassium cation, $etc.$, the salting-out effect plays a significant role. The amount of this ion adsorbed on the surface of the bonded phase can be neglected, $[B]_{sorb} = 0$ (these ions are rather excluded from the pores of the stationary phase). If we further assume that the concentration of free acid ions in the mobile phase predominates over the concentration of the ion pairs, $[A]_{mob} > [AB_n]_{mob}$, we obtain for the capacity ratio

$$k' \approx \varphi \cdot (K_{ODA} + K_{ODAB_n} \cdot K_{IP_{mob}} \cdot [B]_{mob}^n) \cdot 10^{m[B]_{mob}} \qquad (12)$$

Because the retention of the free acid ions in the mobile phases which do not contain salts is very small for most acids (and some are even excluded), it seems realistic to neglect K_{ODA} in the eqn. 12. If we further use a functional development for the term with $[B]_{mob}$ in the exponent, eqn. 12 can be rewritten as follows:

$$k' \approx \varphi \cdot K_{ODAB_n} \cdot K_{IP_{mob}} \cdot [B]_{mob}^n + 2.31 \varphi \cdot K_{ODAB_n} \cdot \varphi \cdot K_{IP_{mob}} \cdot [B]_{mob}^{n+1} + \ldots \qquad (13)$$

In a certain region of concentration, the first term of this equation prevails over the higher-order terms and the plots of log k' as a function of log $[B]_{mob}$ are nearly linear with slopes close to n, in agreement with the experimental data in the present work. This would also explain why the slopes of these plots increase with increasing number of sulphonic groups in the molecule of the acid. It should be noted that the exponent n, which has a meaning of the average number of the cations associated to the anion of a sulphonic acid, is lower than 1. From Table IV it follows that approximately three to six acid molecules should be associated to one Na^+ ion. Of course, such associates can be understood only as those corresponding to an average number of sulphonic acid molecules with decreased mobility in the electric field of an ion (in a dynamic equilibrium). If such associates are formed, the sulphonic groups of the acids are oriented towards the inorganic metal cation and the hydrophobic hydro-carbonaceous parts of the molecules are directed to the environmental solution, which could explain an increased retention of such an associate on the surface of an alkyl-bonded phase.

The proposed retention mechanism understands ion-pair reversed-phase chromatography and salting-out reversed-phase chromatography as two limiting instances in the complex multiplicity of phenomena involved when a salt is added to the mobile phase. However, this seems to explain, at least qualitatively, the experimental data, such as the dependence of the slopes of log k' = f(log c_S) and log k' = f(log c_I) plots on the structure of acids (above all on the number of sulphonic groups in

an acid molecule). The similarities of the behaviour of acids in the two systems can be understood if we consider that an associate of the acid with a small alkali metal ion would probably "feel" the hydrophobic effect in much a similar way as a free acid itself, which does not necessarily hold true for the associates of an acid with bulky tetraalkylammonium ions. The increase in retention with increasing size of the tetraalkylammonium ions in the mobile phase can be understood, because the amount of the ions adsorbed on the surface of the alkyl bonded phase increases in the same direction[33]. This can also explain the relatively minor influence of the nature of the alkyl-bonded phase on retention. The effect of the co-ions in the mobile phase (such as phosphate, sulphate, etc. anions) on retention probably consists in competition with solute acid anions for the cation to form the ion-associates. The influence of the concentration of the organic solvent (methanol) in the mobile phase on retention is explained in point (8) of the above discussion.
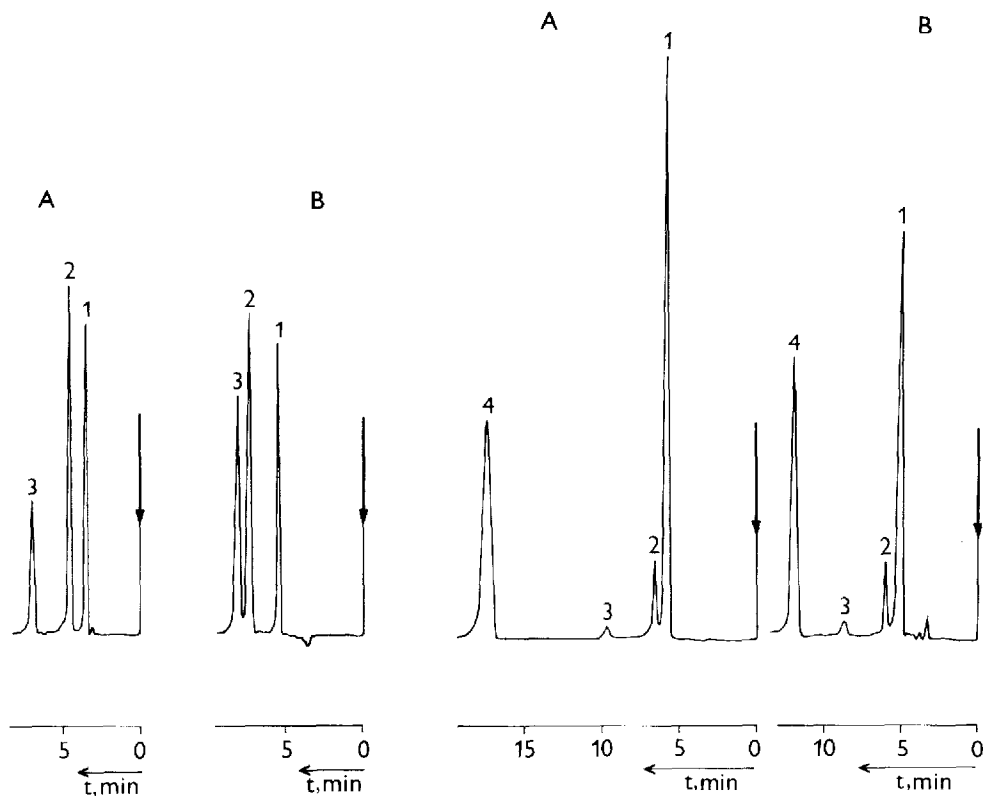

Fig. 6. Separation of isomeric anthraquinone disulphonic acids on an octylsilica column (Silasorb C_8; 7.5 μm; 300 × 4.2 mm I.D.; V_0 = 3.21 ml). Mobile phases: A = 0.4 M sodium sulphate in methanol–water (30:70); B = 0.01 M tetrabutylammonium sulphate in methanol–water (1:1). Flow-rate: 1 ml/min. Detection: UV, 254 nm; 0.32 a.u.f.s. Compounds: 1 = 1,5-anthraquinonedisulphonic acid; 2 = 2,6-anthraquinonedisulphonic acid; 3 = 1.8-anthraquinonedisulphonic acid.

Fig. 7. Separation of isomeric naphtholsulphonic acids on an octylsilica column (Silasorb C_8; 7.5 μm; 300 × 4.2 mm I.D.; V_0 = 3.21 ml). Mobile phases: A = as in Fig. 6A; B = as in Fig. 6B; other conditions as in Fig. 6. Compounds: 1 = 2-naphthol-6-sulphonic acid; 2 = 1-naphthol-4-sulphonic acid; 3 = unknown impurity; 4 = 2-naphthol-1-sulphonic acid.

   Finally, it should be kept in mind that the aromatic sulphonic acids are strong acids, similar to mineral acids, and so are fully dissociated even at pH ≈ 5–7 and therefore the effect of pH on separation can be neglected, which of course is not true with other acids such as carboxylic acids.

Separation selectivity

   In spite of essential similarities in the retention behaviour of aromatic sulphonic acids in mobile phases containing a tetraalkylammonium cation and in the solutions of sodium sulphate, there are certain minor differences in selectivities between the isomeric acids which can be sometimes utilized for a fine tuning of resolution. Figs. 6 and 7 show two examples of the separation of isomeric anthraquinone disulphonic and naphthol monosulphonic acids in the two systems, where the order of elution is identical but where minor differences in selectivities can be clearly seen.

   However, the situation is different when the acids are separated according to the number of sulphonic groups. In the systems with tetraalkylammonium cations, the retention of naphthalene mono- and trisulphonic acids is similar, which can cause difficulties if a separation is attempted (see Fig. 2). However, in the mobile phases containing sodium sulphate, the acids are eluted in the order tetra-, tri-, di- and mononaphthalenesulphonic acids with a clear separation between the acids with different numbers of sulphonic groups. Fig. 8 shows the separation of a few naphthalene di- and trisulphonic acids. Naphthalene monosulphonic acids are quite strongly retained under the isocratic conditions used, but a fine separation of complex mixtures containing naphthalene mono-, di-, tri- and tetrasulphonic acids can be achieved using gradient elution with increasing concentrations of methanol in the mobile phase[28].
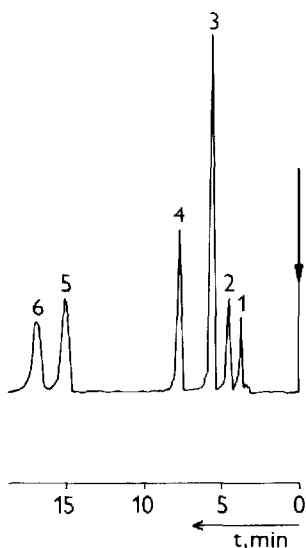

Fig. 8. Separation of naphthalenedi- and trisulphonic acids on an octylsilica column (Silasorb C_8; 7.5 μm; 300 × 4.2 mm I.D.; V_0 = 3.21 ml). Mobile phase: 0.4 M sodium sulphate in water. Flow-rate: 1 ml/min. Detection: UV, 254 nm; 0.16 a.u.f.s. Compounds: 1 = 1,3,6-naphthalenetrisulphonic acid; 2 = 1,3,5-naphthalenetrisulphonic acid; 3 = 1,5 naphthalenedisulphonic acid; 4 = 2,6-naphthalenedisulphonic acid; 5 = 1,6 naphthalenedisulphonic acid; 6 = 2,7-naphthalenedisulphonic acid.

CONCLUSIONS

The retention mechanism proposed is intended to explain numerous similarities in the retention of aromatic sulphonic acids in mobile phases containing sodium sulphate and in the systems with tetraalkylammonium ions.

It may perhaps offer a better understanding of the way in which numerous factors may influence the retention in these systems. Reversed-phase chromatography using aqueous organic solutions of simple inorganic salts, such as sodium sulphate, as the mobile phase offers an alternative method to reversed-phase ion-pair chromatography with the mobile phase containing tetraalkylammonium salts. In addition to a much lower price for the mobile phase with inorganic salts, the separation of naphthalenesulphonic acids is superior to that in reversed-phase ion-pair systems.

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