Journal of Chromatography, 102 (1974) 333–351
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 7905

SEPARATION OF ACIDIC COMPOUNDS BY HIGH-PRESSURE LIQUID–LIQUID CHROMATOGRAPHY INVOLVING ION-PAIR FORMATION

J. C. KRAAK and J. F. K. HUBER*

Laboratory of Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, Amsterdam (The Netherlands)

SUMMARY

The distribution of acidic compounds in the phase system tri-*n*-octylamine–aqueous perchloric acid was investigated. The distribution equilibrium can be expressed by a simple formula which describes the dependence of the distribution coefficient on the single equilibrium constants, pH and ion concentration in the aqueous phase. The theoretical expression was shown to agree well with the experimental data. The distribution of strong acids was caused mainly by ion-pair formation and for weaker acids at low pH by the liquid–liquid distribution of the acid, while at larger pH ion-pair formation can also be involved in the distribution equilibrium. Special selectivity effects can be obtained by changing the temperature, pH or type and concentration of the anion in the aqueous phase.

It is shown that the tri-*n*-octylamine–aqueous perchloric acid system is very suitable for separation of acidic compounds like sulphonic and carboxylic acids by high-pressure liquid–liquid chromatography.

INTRODUCTION

The acid-binding properties of long-chain aliphatic amines have been used for 25 years in the extraction of anions from aqueous solutions with organic solvents[1]. This type of extraction is based on the formation of an ion pair between the acid and the amine. In most instances, the ion pair is insoluble in aqueous phases but is readily soluble in organic solvents. Schill and co-workers have made extensive studies on the ion-pair extraction of acids and discussed the extraction conditions and side-reactions that can occur[2]. Few papers have been published about ion-pair formation used in high-pressure column liquid chromatography[3–7].

The analytical separation of aromatic sulphonic acids is a problem that has not yet been solved satisfactorily. Most work in this field has involved the use of ion-exchange chromatography[8–10]. Several pairs of aromatic sulphonic acids were separated by liquid–liquid chromatography on columns packed with Chromosorb impregnated with tricaprylylamine (Alamine 336) using aqueous hydrochloric acid for the elution

* Present address: Institut für Analytische Chemie, Universität Wien, Währinger Strasse 38, Vienna, Austria.

of the first acid and aqueous perchloric acid as the mobile phase for the elution of the second acid[11].

The potential of high-pressure liquid chromatography[12–14], which has been developed in recent years, has not previously been exploited in the separation of aromatic sulphonic and carboxylic acids. Also, the distribution equilibrium of this type of compound has not been investigated in liquid–liquid systems consisting of an aqueous and an amine phase. The results of such a study are presented in this paper, including phenols as an example of very weak acids.

EXPERIMENTAL

Apparatus

The liquid chromatographic experiments were carried out on a high-pressure liquid chromatograph (Siemens SP 200, Siemens, Karlsruhe, G.F.R.) with a UV spectrophotometer or refractive index detector fitted on custom-made straight thick-walled glass columns. The feed lines for the eluent were constructed from stainless-steel 316 tubes and Swagelock couplings in order to resist the acidic medium. In addition to a septumless injector (Siemens), a custom-made septum injection port and a 10-μl syringe (Hamilton 701 N) were also used. The thick-walled borosilicate glass columns had an I.D. of 3.0 mm and a length of 185 mm. In order to prevent loss of stationary phase, a stainless-steel pre-column (50 × 1 cm) was used. The wavelength of the detector in most experiments was adjusted to 263 nm.

Materials

The materials used were tri-*n*-octylamine (TOA; Fluka, Buchs, Switzerland); 70% (w/w) aqueous perchloric acid (Merck, Darmstadt, G.F.R.); diatomite (Kieselgur, Merck) washed with 1 *M* aqueous perchloric acid, ground and classified to a particle size range of 5–10 μm by means of an air classifier (Alpine MZR, Augsburg, G.F.R.); low-surface-area silica (Spherosil XOC 005, Rhone-Progil, Neuilly sur Seine, France); sulphonic acids (Merck; Fluka); carboxylic acids (Merck, Fluka); and phenols (Fluka).

Procedures

The chromatographic capacity factor, κ_i, of a component i for a given column was derived from measurements of its retention time, t_{Ri}, and the retention time, t_{R0}, of a non-retarded component. It is determined by the distribution coefficient, K_i, and the volume ratio, q, of the stationary and the mobile phase:

$$\frac{(t_{Ri} - t_{R0})}{t_{R0}} = \kappa_i = K_i \cdot q \qquad (1)$$

The selectivity factor (relative retention), r_{ji}, of two compounds was calculated from their capacity ratios:

$$\kappa_j/\kappa_i = r_{ji} \qquad (2)$$

In order to prepare the columns, the glass tubes were dry-packed with small portions of coated acid-washed diatomite (Kieselguhr) or low-surface-area silica. The pre-column was similarly filled with acid-washed diatomite (100–200 μm) coated with 10% TOA.

The aqueous perchloric acid solutions were prepared by diluting a weighed amount of the 70% (w/w) standard solution of perchloric acid with distilled water. The strength of this solution was determined by titration with aqueous borax solution. The pH of the mobile phase was measured with a pH meter (Radiometer, Copenhagen, Denmark). Perchlorate solutions containing 0.05 *M* ClO_4^- but having different pH values were prepared from 0.05 *M* perchloric acid by adding sodium hydroxide until the required pH was attained. The eluents containing different concentrations of perchlorate or additional anions were prepared by dissolving the sodium salt of the anions in 0.05 *M* perchloric acid.

The samples were dissolved in the mobile phase and injected into the top of the column with a 10-μl syringe through a septum or with the septumless injector.

THEORETICAL

Total distribution coefficient of an acid

The distribution of an acid HX between an aqueous phase containing a strong acid HA and an organic phase consisting of a long-chain aliphatic amine B depends on a number of competing equilibria. By considering only the most important equilibria, a simple expression for the total distribution coefficient can be derived. The following equilibria are considered:

(1) Liquid–liquid distribution of the undissociated acid HX between the stationary and the mobile phases. HX is assumed to be a component of the sample.

$$(HX)_m \leftrightharpoons (HX)_s$$

$$K_{HX} = \frac{[HX]_s}{[HX]_m} \tag{3}$$

where K_{HX} = liquid–liquid distribution coefficient of HX; the square brackets denote the concentration of the component concerned; and the subscripts m and s refer to the mobile (aqueous) and the stationary (organic) phase, respectively.

(2) Dissociation of the acid HX in the mobile phase.

$$(HX)_m \leftrightharpoons (H^+)_m + (X^-)_m$$

$$K_1 = \frac{[H^+]_m [X^-]_m}{[HX]_m} \tag{4}$$

where K_1 = dissociation constant of HX.

(3) Ion exchange of the anion X^- in the mobile phase with the ion pair BHA in the stationary phase.

$$(X^-)_m + (BHA)_s \leftrightharpoons (BHX)_s + (A^-)_m$$

$$K_2 = \frac{[BHX]_s [A^-]_m}{[X^-]_m [BHA]_s} \tag{5}$$

where K_2 = ion-exchange constant for the anion X^- in the mobile phase and the ion pair BHA in the stationary phase.

(4) Ion-pair formation between the dissociated acid HA in the mobile phase and the amine B in the stationary phase. The acid HA is a constituent of the eluent and is assumed to be a strong acid.

$$(B)_s + (H^+)_m + (A^-)_m \leftrightharpoons (BHA)_s$$

$$K_3 = \frac{[BHA]_s}{[B]_s\,[H^+]_m\,[A^-]_m} \tag{6}$$

where K_3 = formation constant of the ion pair BHA in the stationary phase.

(5) Exchange of the proton of the acid HX in the stationary phase for the cation M^+ in the mobile phase which is assumed to be a constituent of the eluent.

$$(M^+)_m + (HX)_s \leftrightharpoons (MX)_s + (H^+)_m$$

$$K_4 = \frac{[MX]_s\,[H^+]_m}{[HX]_s\,[M^+]_m} \tag{7}$$

where K_4 = ion-exchange constant for the cation M^+ in the mobile phase and the acid HX in the stationary phase.

Under the usual analytical chromatographic conditions, the sample is present in the mobile phase in very dilute solution, so that it can be assumed that its concentration in the stationary phase is also very low. Further, it is assumed that the components B and BHA of the stationary phase are insoluble in the mobile phase. Under these circumstances, the overall concentration of the stationary phase is given by the relationship

$$[B]_s + [BHA]_s = C \tag{8}$$

where C is constant. According to eqns. 3–8, the total distribution coefficient of X is given by the expression

$$K_X = \frac{[HX]_s + [BHX]_s + [MX]_s}{[HX]_m + [X^-]_m} \tag{9}$$

where K_X = total liquid–liquid distribution coefficient of the constituent X. From eqns. 3–9, an expression can be derived that describes the total distribution coefficient as the sum of three terms:

$$K_X = \Delta K_{X_1} + \Delta K_{X_2} + \Delta K_{X_3} \tag{10}$$

where

$$\Delta K_{X_1} = K_{HX} \cdot \frac{1}{1 + K_1/[H^+]_m}$$

$$\Delta K_{X_2} = K_2\,C \cdot \frac{1}{([A^-]_m + 1/K_3\,[H^+]_m)\,(1 + [H^+]_m/K_1)}$$

$$\Delta K_{X_3} = K_{HX}\,K_4 \cdot \frac{[M^+]_m}{[H^+]_m + K_1}$$

The first term describes the distribution of the acid, the second term the effect of ion-pair formation with the amine and the third term the effect of ion-pair formation with a monovalent cation. Eqn. 10 describes the dependence of the total distribution coefficient on the concentrations of the eluent anion, of the eluent cation and the pH of the mobile phase.

RESULTS AND DISCUSSION

The capacity ratio describes the migration of a compound in a chromatographic system, and can be determined from the retention time of this compound and that of a non-retarded compound. The values of the capacity ratio for different compounds on the same column are proportional to their distribution coefficients, the phase ratio being the proportionality constant. The selectivity of a phase system with respect to the distribution of two compounds is described by the selectivity factor, which is given by the ratio of the capacity ratios of the two compounds. For a number of phenols, carboxylic acids and sulphonic acids, the capacity ratio and the selectivity factor for successively eluting components were determined on a column containing tri-*n*-octylamine as stationary phase and aqueous perchloric acid with the eventual addition of a salt as the mobile phase.

The effects of temperature, perchloric acid concentration, pH and concentration and nature of the salt were investigated.

Influence of temperature

The temperature dependence of the capacity ratio on a given column is determined primarily by the temperature dependence of the distribution coefficient, although a minor effect can arise from a change in the phase ratio with temperature. The influence of temperature on the total distribution coefficient is determined according to eqn. 10 by the temperature dependence of the distribution coefficient K_{HX} of the species HX, and of the chemical equilibrium constants K_1, K_2, K_3 and K_4.

The influence of temperature on the capacity ratio was studied in the phase system tri-*n*-octylamine–0.05 *M* perchloric acid. In this system, the third term in eqn. 10 vanishes, as $[M^+]_m$ is zero. In order to be certain that no stationary phase was stripped from the column during the sequence of measurements at different temperatures, the capacity ratios of some standard compounds were measured on the same column at the same temperature before and after the series of experiments. As shown in Table I, the capacity ratio was found to be constant with high accuracy.

From Table II, it can be seen that the change in the capacity ratio with temperature was found to be insignificant in some instances and to be substantial in others in which the capacity ratio partially decreases and partially increases with temperature. This behaviour can be explained by means of eqn. 10. If the first term in this equation is dominant, then the temperature dependence of the total distribution coefficient will be determined mainly by the partial distribution coefficient K_{HX} of the species HX. If the second term is dominant, then the ion-exchange constant K_2 will determine the temperature dependence of the total distribution coefficients. Depending on the enthalpy change, due to the dominating process, the corresponding equilibrium constant will increase or decrease with temperature and so will the total distribution coefficient. It can be assumed that the distribution coefficient K_{HX} de-

TABLE I

CONTROL OF THE CONSTANCY OF THE CAPACITY RATIO AT 25° BEFORE AND AFTER THE SERIES OF EXPERIMENTS

Phase system: tri-*n*-octylamine–0.05 *M* perchloric acid.

Compound	κ_t	
	Before	*After*
4-Nitrobenzoic acid	1.95	1.93
4-Nitrobenzenesulphonic acid	2.36	2.37
4-Methylbenzoic acid	4.30	4.27

TABLE II

INFLUENCE OF TEMPERATURE ON THE CAPACITY RATIO, κ_t, AND SELECTIVITY FACTOR, r_{jt}, FOR THE PHASE SYSTEM TRI-*n*-OCTYLAMINE–0.05 *M* PERCHLORIC ACID AT pH 1.5

Compound	*25°*		*45°*		*65°*	
	κ_t	r_{jt}	κ_t	r_{jt}	κ_t	r_{jt}
Phenol	1.03	—	0.89	—	0.72	—
3-Methylphenol	3.03	2.94	2.50	2.81	1.92	2.67
4-Methylphenol	3.13	1.03	2.61	1.04	1.99	1.04
2-Methylphenol	3.35	1.07	2.82	1.08	2.13	1.07
4-Nitrophenol	3.49	1.04	2.60	1.08	1.85	1.15
2-Nitrophenol	5.16	1.48	4.08	1.57	2.95	1.59
2,6-Dimethylphenol	6.05	1.17	5.63	1.38	4.41	1.49
2,5-Dimethylphenol	8.33	1.38	7.06	1.25	5.10	1.16
2,4-Dihydroxybenzoic acid	0.49	—	0.37	—	0.27	—
Benzoic acid	0.85	1.73	0.72	1.94	0.57	2.11
4-Nitrobenzoic acid	1.09	1.28	0.98	1.36	0.73	1.28
2-Hydroxybenzoic acid	1.73	1.59	1.43	1.46	1.12	1.53
4-Methylbenzoic acid	2.43	1.40	2.02	1.41	1.50	1.40
2-Amino-4-nitrobenzoic acid	2.89	1.19	1.99	1.01	1.29	1.16
Cinnamic acid	3.03	1.05	2.39	1.20	1.71	1.33
Benzenesulphonic acid	0.42	—	0.62	—	0.82	—
4-Nitrobenzenesulphonic acid	1.34	3.19	1.69	2.73	1.97	2.40
4-Toluenesulphonic acid	1.37	1.02	1.95	1.15	2.40	1.22
2-Naphthol-6-sulphonic acid	2.82	2.06	2.90	1.49	2.92	1.22
1-Naphthol-2-sulphonic acid	3.30	1.17	3.85	1.33	4.00	1.37
1-Naphthol-4-sulphonic acid	3.37	1.02	3.81	1.01	3.95	1.01
1-Naphthol-5-sulphonic acid	4.10	1.22	4.80	1.26	4.97	1.26
1-Naphthol-3-sulphonic acid	10.17	2.48	9.54	1.99	7.33	1.47
1-Naphthol-3,6-disulfonic acid	0.45	—	0.69	—	0.98	—
4,5-Dihydroxy-2,7-naphthalene-disulphonic acid	0.53	1.18	0.75	1.09	1.05	1.07
2-Naphthol-3,6-disulphonic acid	2.00	3.77	3.85	5.13	6.72	6.40
1-Naphthol-3,8-disulphonic acid	15.14	7.57	22.61	5.87	28.37	4.22

creases with temperature whereas the ion-exchange constant K_2 increases with temperature.

For phenols and carboxylic acids at pH 1.5, it can be concluded that the liquid–liquid distribution of these compounds is the major process, because their capacity ratios decrease with temperature. For sulphonic acids, it can be concluded that ion-pair formation is the major process at pH 1.5 as the capacity ratios of these compounds increase with temperature.

A special case arises with naphtholsulphonic acids, which have both a phenolic group and a sulphonic acid group. These compounds show an insignificant dependence of the capacity ratio on temperature, except for 1-naphthol-3-sulphonic acid, which behaves as a phenol. The behaviour of the other naphtholsulphonic acids suggests that liquid–liquid distribution and ion-pair formation have about the same influence owing to the presence of a phenolic and a sulphonic acid group in the same molecule. The overall effect of temperature on the capacity ratio is small in this instance as the phenolic group causes a decrease in the total distribution coefficient while the sulphonic acid group causes an increase. In the case of naphtholdisulphonic acids, the sulphonic acid function dominates because of the presence of the second sulphonic acid group, and therefore the capacity ratio increases with increase in temperature.

From Table II, it can be seen that in most instances the selectivity factors of successively eluting components within a group of compounds change significantly with temperature.

Influence of pH

The influence of pH can be explained by means of eqn. 10.

The first term in eqn. 10 has a constant value of K_{HX} at $[H^+]_m \gg K_1$. When K_1 is large, this value cannot be achieved because $[H^+]_m$ is limited in practice. In the range where $[H^+]_m$ is of the order of K_1, the first term declines to zero. A further change in the first term is caused by the change in the distribution coefficient of the undissociated compound due to the change in the composition of the liquid phase with pH. According to eqn. 6, the stationary liquid consists predominantly of BHA at low pH and of B at high pH. This prediction was checked by determining the composition of the stationary liquid at low pH.

Tri-*n*-octylamine was mixed with aqueous perchloric acid and the decrease in the amount of perchloric acid in the aqueous phase was determined by titration. At pH 3, it was found that the decrease in the amount of perchloric acid was equivalent to the amount of tri-*n*-octylamine, which supports the prediction of eqn. 6. For sulphonic acids ($K_1 \approx 1$), the first term may be expected to be zero because of the high value of the dissociation constant. For carboxylic acids ($K_1 \approx 10^{-4}$), the first term should have the value of the liquid–liquid distribution coefficient at low pH and should decrease to zero at a pH about equal to the dissociation constant. For phenols ($K_1 \approx 10^{-10}$), the first term should have the value of the liquid–liquid distribution coefficient at low pH, decline in a certain pH range according to eqn. 6 and decrease to zero at a pH about equal to the dissociation constant.

The second term in eqn. 10 has a maximum at pH $= -\frac{1}{2} \log K_1/K_3 [A^-]_m$ and decreases to zero at low and high pH. The exact shape of the curve describing the dependence of the second term on pH depends on the relative magnitude of the

equilibrium constants K_1, K_2 and K_3. Depending on the value of the constants, the second term can have a sharp or a flat maximum.

From Fig. 1b, in which the values of the capacity ratio calculated from eqn. 10 are plotted as function of pH, it can be seen that the experimental curves shown in Fig. 1a can be approached by the theoretical curves (except for phenol), with an appropriate choice of the values for the equilibrium constants and the phase ratio. The decrease in the capacity ratio of phenol which can be seen in Fig. 1a is not described

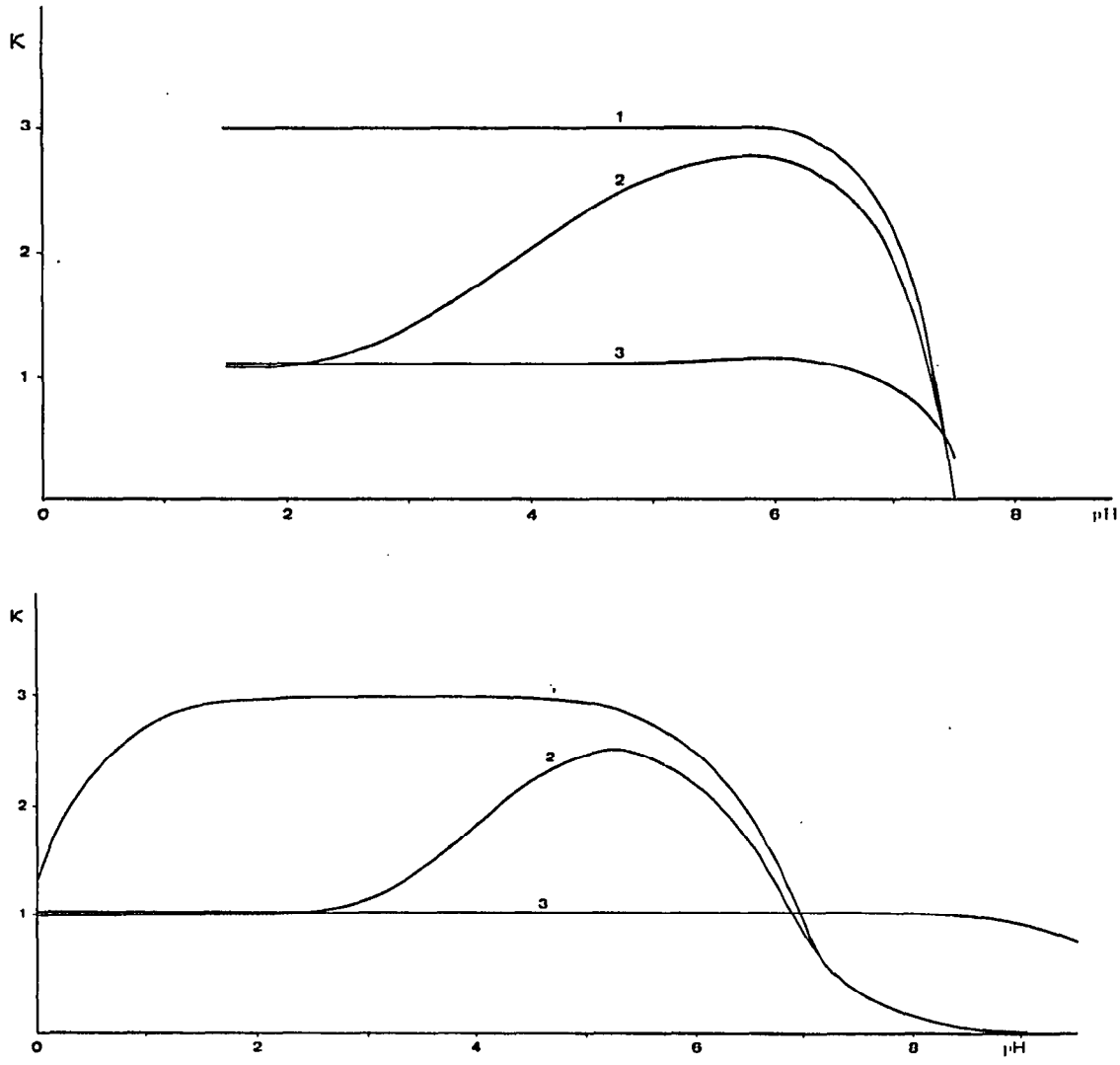

Fig. 1. pH Dependence of the capacity ratio. (a) Experimental results. Sample: (1) 2-naphthol-6-sulphonic acid; (2) 4-nitrobenzoic acid; (3) phenol. Column: TOA, 0.04 g per gram of solid support; 0.05 M $HClO_4$. (b) Calculated according to eqn. 10. (1) $K_1 = 1$, $K_2 = 2$, $K_3 = 10^8$, $K_{HX} = 0$, $q = 0.030$, $C = 2.3$, $[A^-]_m = 0.05$; (2) $K_1 = 10^{-4}$, $K_2 = 2$, $K_3 = 10^8$, $K_{HX} = 36.2$, $q = 0.030$, $C = 2.3$, $[A^-]_m = 0.05$; (3) $K_1 = 10^{-10}$, $K_2 = 0$, $K_3 = 10^8$, $K_{HX} = 37.0$, $q = 0.030$, $C = 2.3$, $[A^-]_m = 0.05$.

by the effect of $K_1/[H^+]_m$ on the value of the first term but by the decrease in the distribution coefficient K_{HX}. Assuming $K_3 = 10^8$, the capacity ratio decreases in the pH range 5–8 according to eqn. 6. Its value beyond pH 8, being the distribution coefficient for tri-*n*-octylamine as stationary phase, is not known, however.

From the dependence of the capacity ratio on pH, it can be concluded that the effect of ion-pair formation is predominant for sulphonic acids, whereas distribution of the undissociated compound is predominant for phenols. In the case of carboxylic acids, both processes are significant.

For compounds with small dissociation constants, such as phenols, the capacity ratio approaches a constant value at low pH, as can be seen from Table III

TABLE III

pH DEPENDENCE OF THE CAPACITY RATIOS, κ_i, AND SELECTIVITY FACTORS, r_{ji}, OF PHENOLS, CARBOXYLIC ACIDS AND SULPHONIC ACIDS ON A COLUMN CONTAINING TOA AS STATIONARY PHASE AND 0.05 *M* PERCHLORIC ACID AS MOBILE PHASE AT 25°

Compound	*pH*									
	1.5		*2.5*		*4.1*		*6.0*		*7.5*	
	κ_i	r_{ji}	κ_i	r_{ji}	κ_i	r_{ji}	κ_i	r_{ji}	κ_i	r_{ji}
Phenol	1.07	—	1.07	—	1.07	—	1.11	—	0.34	—
3-Methylphenol	3.03	2.83	3.05	2.85	3.06	2.86	3.16	2.85	0.94	2.76
4-Methylphenol	3.18	1.05	3.21	1.05	3.26	1.06	3.33	1.05	0.93	1.01*
2-Methylphenol	3.34	1.05	3.35	1.04	3.37	1.03	3.51	1.05	1.28	1.38
4-Nitrophenol	3.41	1.02	3.41	1.02	3.48	1.03	4.29	1.22	0.31	4.12*
2-Nitrophenol	5.15	1.51	5.17	1.52	5.12	1.47	5.52	1.29	0.15	2.07*
2,6-Dimethylphenol	6.57	1.28	6.57	1.27	6.62	1.29	6.93	1.26	1.82	12.13
2,5-Dimethylphenol	8.50	1.29	8.53	1.30	8.59	1.30	9.03	1.30	3.25	1.79
2-Aminobenzoic acid	0	—	0.11	—	0.14	—	0.02	—	0	
2,4-Dihydroxybenzoic acid	0.53	—	0.58	5.27	0.80	5.71	0.83	—	0	
Benzoic acid	0.85	1.60	0.92	1.59	0.85	1.06	0.77	1.08*	0	
4-Nitrobenzoic acid	1.05	1.24	1.17	1.27	2.07	2.43	2.74	3.56	0	
2-Hydroxybenzoic acid	1.69	1.61	3.12	2.67	5.62	2.71	5.48	2.00	0	
4-Methylbenzoic acid	2.43	1.44	2.47	1.26*	2.53	2.22*	2.04	2.69*	0	
Cinnamic acid	3.15	1.30	3.12	1.26	3.37	1.33	3.20	1.57	0	
Benzenesulphonic acid	0.43	—	0.43	—	0.46	—	0.42	—	0	
1-Naphthol-3,6-disulphonic acid	0.44	1.02	0.42	1.02*	0.46	1.00	0.40	1.05*	0	
4-Toluenesulphonic acid	1.25	2.84	1.18	2.81	1.45	3.15	1.30	3.25	0	
3-Nitrobenzenesulphonic acid	1.36	1.09	1.31	1.11	1.44	1.01	1.35	1.04	0	
2-Naphthol-3,6-disulphonic acid	2.04	1.50	1.98	1.51	2.29	1.59	1.92	1.42	0	
2-Naphthol-6-sulphonic acid	2.94	1.44	2.96	1.49	2.97	1.30	2.97	1.55	0	
1-Naphthol-2-sulphonic acid	3.64	1.24	3.70	1.25	3.68	1.24	3.65	1.23	0	
1-Naphthol-4-sulphonic acid	3.68	1.01	3.61	1.02*	3.68	1.00	3.63	1.01*	0	
1-Naphthol-5-sulphonic acid	4.56	1.24	4.55	1.26	4.67	1.27	4.71	1.30	0	
1-Naphthol-3-sulphonic acid	11.08	2.43	10.53	2.31	10.65	2.28	10.22	2.17	0	
1-Naphthol-3,8-disulphonic acid	14.53	1.31	14.51	1.38	15.23	1.43	14.75	1.44	0	

* Successive capacity ratios are reversed.

and Fig. 1a. At higher pH values, the capacity ratio was found to decrease sharply. For compounds with medium dissociation constants, such as carboxylic acids, it was found that the capacity ratio approaches a constant value at low pH, has a maximum value at medium pH and decreases to zero at higher pH, as can be seen from Table III and Fig. 1a. For compounds with large dissociation constants, such as sulphonic acids, it can be seen from Table III and Fig. 1a that the capacity ratio is about constant at low pH and decreases sharply to zero at higher pH.

Influence of the nature and concentration of salts

In principle, the addition of a salt can influence the distribution equilibrium of an acidic compound between an organic and an aqueous liquid phase in two ways. On the one hand, the anion of the salt will compete with the anion of the sample with respect to protonation and ion-pair formation, while on the other hand, the cation of the salt can also form an ion pair with the anion of the sample. As a result of the addition of a salt, one can therefore expect a change in the total distribution coefficient if ion-pair formation is involved (or if the salt is derived from a weak acid or base). A number of salts of strong acids and bases were added to a liquid–liquid system consisting of TOA and 0.05 *M* perchloric acid. The influence of these additions on the capacity ratio can be seen from Table V.

The addition of sodium perchlorate causes a significant decrease in the capacity ratio with increasing sodium perchlorate concentration if a strong acid group (*e.g.*, sulphonic acid) is present in the sample molecule. A plot of the capacity ratio as a function of the reciprocal of the anion concentration gives a linear relationship for monovalent acids according to eqn. 10, as shown in Table IV. For divalent acids, a linear relationship is obtained if the total distribution coefficient is plotted as a function of the reciprocal of the square of the anion concentration, as can be seen from Table IV. This suggests, according to eqn. 10, that ion-pair formation with the amine dominates and the liquid–liquid distribution of the protonated anion and ion-pair formation with the sodium cation play a minor role so far as strong acids are concerned. For phenols or carboxylic acids, the change in the capacity ratio was found to be insignificant. For these weak acids, the partition of the undissociated molecules must be assumed to be the predominant process. The effect of the variation of the type of anion at constant concentration is shown in Table V. It can be seen that the capacity ratio changes

TABLE IV

DEPENDENCE OF THE CAPACITY RATIOS, κ_i, OF SULPHONIC ACIDS ON THE PERCHLORATE CONCENTRATION IN THE MOBILE PHASE

a = intercept; b = slope; r = regression coefficient.

Compound	*Linear regression of κ_i versus $([A^-])^{-n}$*			
	n	*a*	*b*	*r*
2-Naphthol-3,8-disulphonic acid	2	0.0135	0.0054	0.9971
1-Naphthol-3,8-disulphonic acid	2	−0.0051	0.0375	0.9999
2-Naphthol-6-sulphonic acid	1	−0.0038	0.2035	0.9990
1-Naphthol-2-sulphonic acid	1	0.0759	0.2530	0.9994
1-Naphthol-4-sulphonic acid	1	−0.0026	0.2581	0.9993
1-Naphthol-3-sulphonic acid	1	−0.1669	0.5098	0.9991

TABLE V

INFLUENCE OF THE NATURE AND CONCENTRATION OF SALTS ON THE CAPACITY RATIO, κ_i

Compound	*Mobile phase*	*$NaClO_4$ (mole/l)*				
		0	*0.025*	*0.050*	*0.100*	*0.200*
Phenol	0.05 *M* $HClO_4$ + $NaClO_4$ (pH ≈1.5)	1.78	1.82	1.78	1.90	1.91
2,4-Dihydroxybenzoic acid		0.85	0.84	0.81	0.84	0.85
Benzoic acid		1.45	1.42	1.41	1.43	1.55
2-Methylbenzoic acid		3.91	3.87	3.74	4.04	4.17
3-Methylbenzoic acid		5.04	5.06	4.88	5.26	5.46
4-Methylbenzoic acid		4.48	4.42	4.34	4.87	5.07
2-Naphthol-3,8-disulphonic acid		2.22	0.88	0.58	0.32	0.08
2-Naphthol-6-sulphonic acid		4.03	2.74	2.11	1.29	0.80
1-Naphthol-2-sulphonic acid		5.10	3.46	2.69	1.75	1.04
1-Naphthol-4-sulphonic acid		5.14	3.42	2.68	1.66	1.02
1-Naphthol-3,8-disulphonic acid		15.00	6.71	3.61	1.62	0.61
1-Naphthol-3-sulphonic acid		10.17	6.44	4.85	3.25	1.99

Compound	*Mobile phase*	*$X=ClO_4^-$ **	*$X=NO_3^-$*	*$X=Br^-$*	*$X=Cl^-$*	*$X=SO_4^{2-}$*
Phenol	0.05 *M* $HClO_4$ + 0.25 *M* NaX (pH ≈1.5)	1.91	2.96	2.76	2.42	2.33
2,4-Dihydroxybenzoic acid		0.85	3.34	2.09	1.72	1.48
Benzoic acid		1.55	2.25	1.98	2.03	2.13
2-Methylbenzoic acid		4.22	6.85	5.99	5.98	6.65
3-Methylbenzoic acid		5.56	8.76	7.76	7.80	8.67
4-Methylbenzoic acid		5.07	7.80	6.83	6.82	7.67
1-Naphthol-3,6-disulphonic acid		0	0.09	0.21	0.29	0.39
4,5-Dihydroxynaphthalene-2,7-disulphonic acid		0	0.10	0.21	0.31	0.45
2-Naphthol-3,8-disulphonic acid		0.08	0.32	0.82	1.08	1.54
Benzenesulphonic aicd		0.11	0.18	0.36	0.51	0.64
1-Naphthol-3,8-disulphonic acid		0.61	2.32	4.69	7.80	10.00
2-Naphthol-6-sulphonic acid		0.80	2.10	3.50	4.26	3.85
1-Naphthol-4-sulphonic acid		1.02	2.94	4.56	5.20	6.07
1-Naphthol-2-sulphonic acid		1.04	2.98	4.56	5.11	5.92
1-Naphthol-3-sulphonic acid		1.99	4.64	10.11	10.18	13.87

(Continued on p. 344)

TABLE V *(continued)*

Compound	*Mobile phase*	$M = Li^+$	$M = Na^+$	$M = K^+$
Phenol	0.05 *M* $HClO_4$	2.53	2.42	2.64
Benzoic acid	+ 0.25 *M* MCl	2.10	2.03	2.11
2-Methylbenzoic acid	(pH ≈ 1.5)	5.81	5.98	5.82
3-Methylbenzoic acid		7.54	7.80	7.62
4-Methylbenzoic acid		6.72	6.81	6.79
2,6-Dihydroxybenzoic acid		0	0	0
3,4,5-Trihydroxybenzoic acid		0.06	0.10	0.13
3,5-Dihydroxybenzoic acid		0.09	0.10	0.10
3,4-Dihydroxybenzoic acid		0.11	0.14	0.12
2,5-Dihydroxybenzoic acid		0.64	0.67	0.73
4-Hydroxybenzoic acid		0.65	0.68	0.64
2,4-Dihydroxybenzoic acid		1.47	1.55	1.63
2-Hydroxybenzoic acid		5.21	5.33	5.42
4,5-Dihydroxynaphthalene-2,7-disulphonic acid		0.28	0.30	0.38
1-Naphthol-3,6-disulphonic acid		0.35	0.29	0.39
Benzenesulphonic acid		0.45	0.51	0.52
2-Naphthol-3,8-disulhonic acid		1.07	1.08	1.21
4-Toluenesulphonic acid		1.44	1.61	1.69
2-Naphthol-6-sulphonic acid		3.43	4.26	4.00
1-Naphthol-2-sulphonic acid		4.52	5.11	5.55
1-Naphthol-4-sulphonic acid		4.64	5.20	5.60
1-Naphthol-3,8-disulphonic acid		6.60	7.84	8.69
1-Naphthol-3-sulphonic acid		9.72	10.18	11.22

* $X = [ClO_4^-] = 0.20\ M$.

considerably with the nature of the anion for strong acids, where the effect of ion-pair formation prevails. It increases in the order $ClO_4^- < NO_3^- < Br^- < Cl^- < HSO_4^-$. This sequence is only partially correlated with the sequence of the dissociation constants of the corresponding acids, which is $HClO_4 > HBr > HCl \approx H_2SO_4 > HNO_3$. For weak acids (phenols, carboxylic acids), where the ion-pair formation was found to occur to a minor extent, the change in the capacity ratio with the type of anion is significantly smaller, as can be seen from Table V. In this case, the change in the activity coefficients in the aqueous phase due to the presence of different types of anions must be considered to be responsible for the change in the partition coefficient of the undissociated acid, which dominates the total distribution equilibrium.

The change in the capacity ratio that results from the variation of the type of

cation at constant concentration can be seen in Table V. Only a small effect can be found, which shows a uniform tendency only for strong acids. The distribution coefficient increases in the order Li < Na < K. For weak acids, a slightly irregular variation was noted, which can be explained in the same manner as the effect of the variation of the type of anion.

To summarize, it can be concluded that the influence of the addition of salts, like the influence of temperature and pH, indicates that strong ion-pair formation with a predominant influence on the total distribution coefficient occurs only for strong acids. The distribution process for weak acids is determined by the partition of the neutral molecules and dissociation in the aqueous phase.

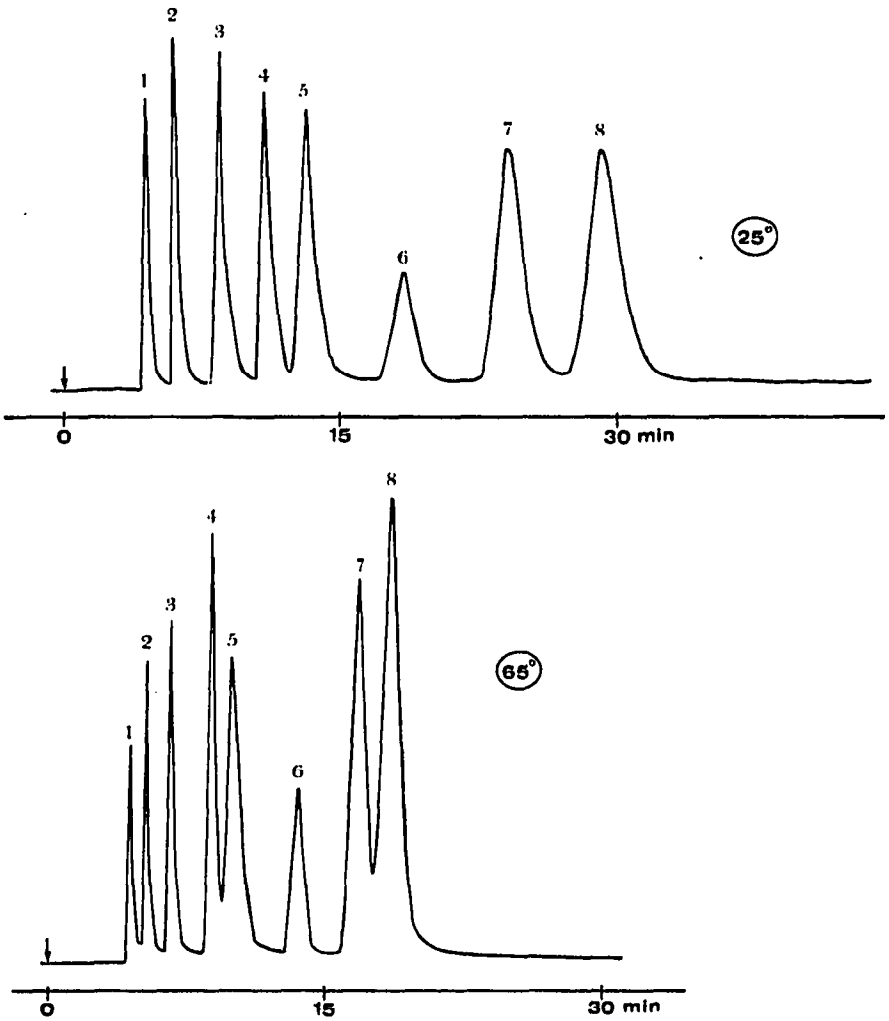

Fig. 2. Influence of temperature on the separation time and resolution of aromatic carboxylic acids at constant flow-rate: (a) 25°; (b) 65°. Sample: (1) 4-aminobenzoic acid; (2) 4-hydroxybenzoic acid; (3) 2,4-dihydroxybenzoic acid; (4) benzoic acid; (5) 4-nitrobenzoic acid; (6) 2-hydroxybenzoic acid; (7) 4-methylbenzoic acid; (8) cinnamic acid. Column: TOA, 0.04 g per gram of solid support; 0.05 *M* $HClO_4$, pH 1.5. Flow-rate: 0.7 mm/sec.

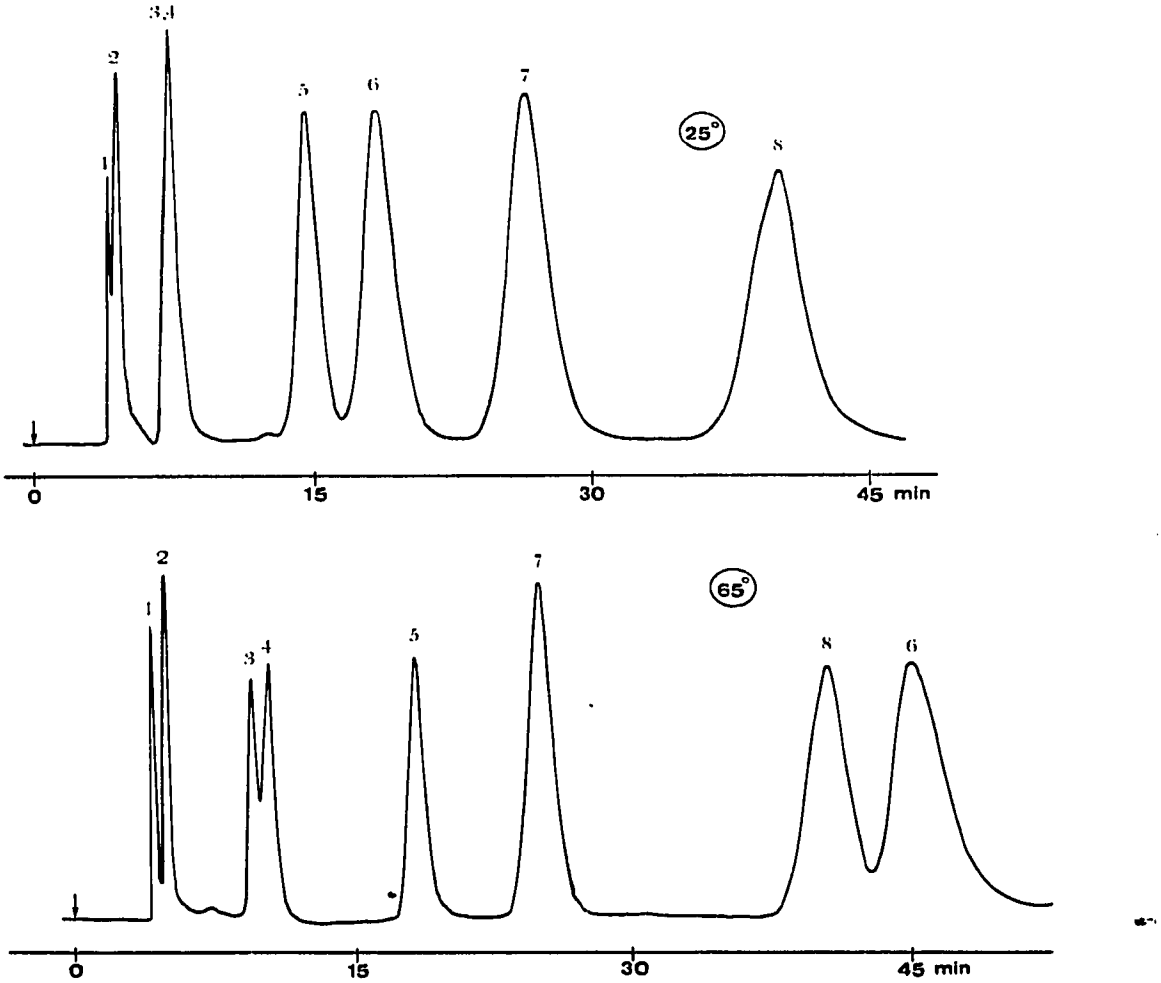

Fig. 3. Influence of temperature on the separation time and resolution of aromatic sulphonic acids at constant flow-rate: (a) 25°; (b) 65°. Sample: (1) 3-aminotoluenesulphonic acid; (2) 2-aminobenzenesulphonic acid; (3) benzenesulphonic acid; (4) 4,5-dihydroxy-2,7-naphthalenedisulphonic acid; (5) 4-nitrobenzenesulphonic acid; (6) 2-naphthol-3,6-disulphonic acid; (7) 2-naphtholsulphonic acid; (8) 1-naphthol-5-sulphonic acid. Column: as in Fig. 2.

Separation of test mixtures

In order to demonstrate the potential of the tri-*n*-octylamine–aqueous perchloric acid system and to show the effect of temperature and perchlorate concentration on the resolution and separation time, a number of separations were carried out.

Fig. 2 shows the influence of temperature on the separation time and resolution of carboxylic acids. The flow-rate was adjusted to the same value in both instances. It can be seen that the separation time decreases by one third without much resolution being lost when the temperature is raised from 25° to 65°. This change in the separation characteristics with temperature is typical of liquid–liquid chromatography when chemical equilibria are not predominant. It is caused essentially by the decrease in

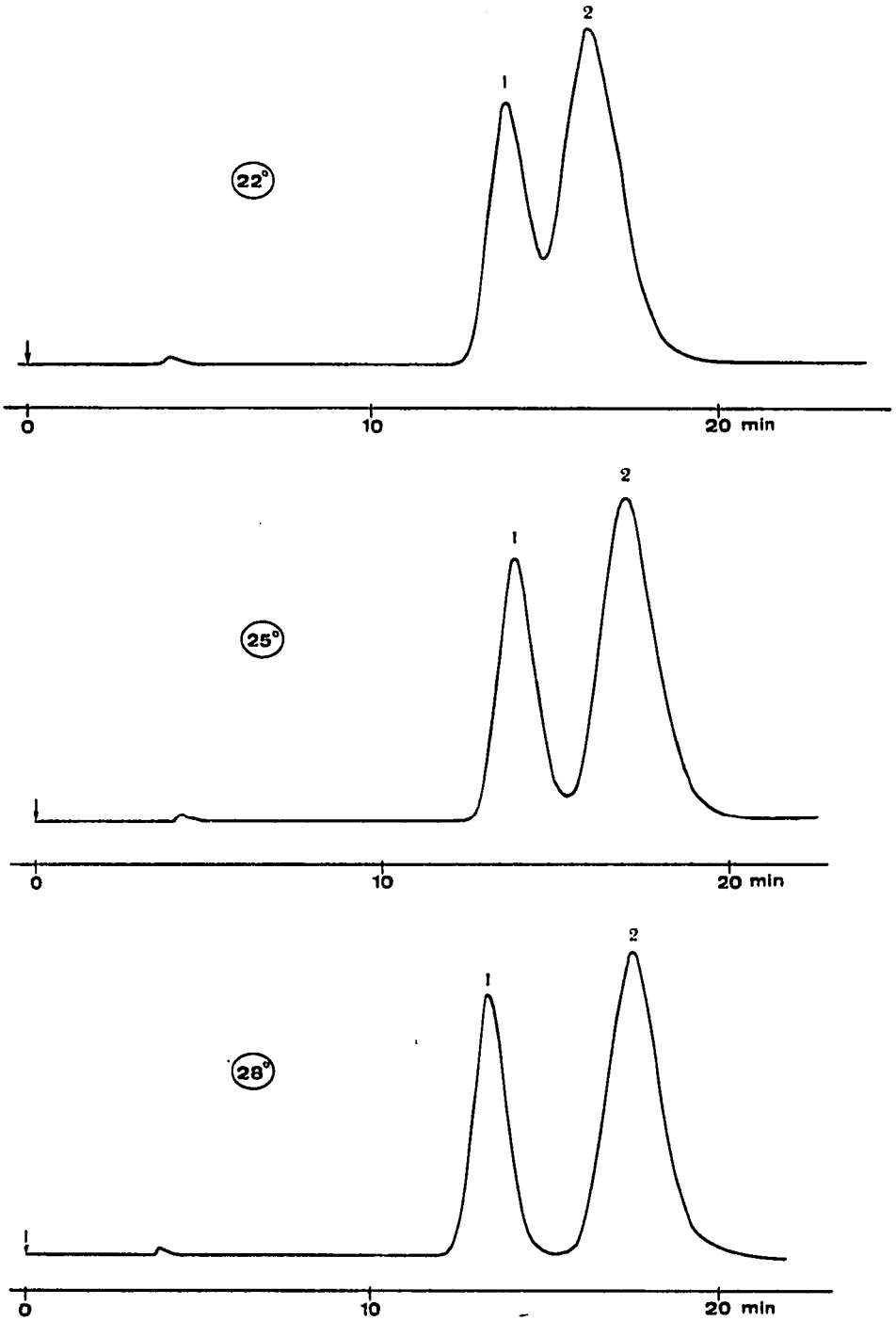

Fig. 4. Extreme example of the influence of temperature on the resolution of two aromatic sulphonic acids: (a) 22°; (b) 25°; (c) 28°. Sample: (1) 4-nitrobenzenesulphonic acid; (2) 2-naphthol-3,6-disulphonic acid. Column: as in Fig. 3.

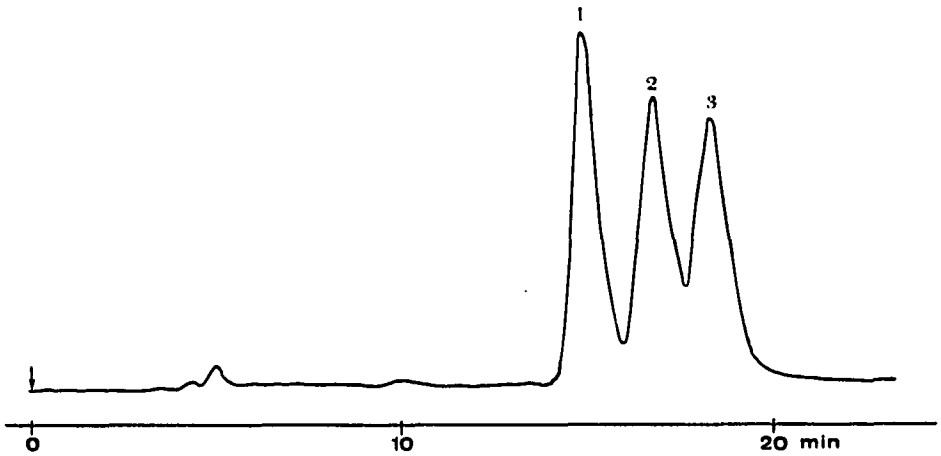

Fig. 5. Separation of methylbenzoic acid isomers. Sample: (1) 2-methylbenzoic acid; (2) 4-methylbenzoic acid; (3) 3-methylbenzoic acid. Column: as in Fig. 3. Temperature: 65°.

the capacity ratio and selectivity factor with temperature, as can be concluded from Table II.

Fig. 3 shows the influence of temperature on the resolution and separation time at constant flow-rate for sulphonic acids. It can be seen that the resolution changes considerably with temperature in some instances, whereas the separation time remains approximately constant. The resolution of benzenesulphonic acid and 4,5-dihydroxy-2,7-naphthalenedisulphonic acid, in particular, is not sufficient to show two peaks at 25°, and at 65° the two peaks are substantially resolved. This behaviour with respect to temperature changes is typical of liquid–liquid chromatography when ion-pair

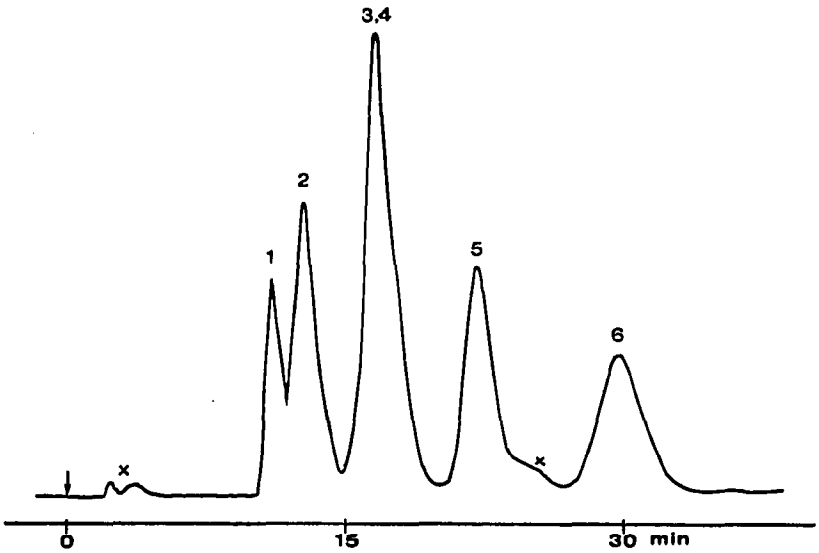

Fig. 6.

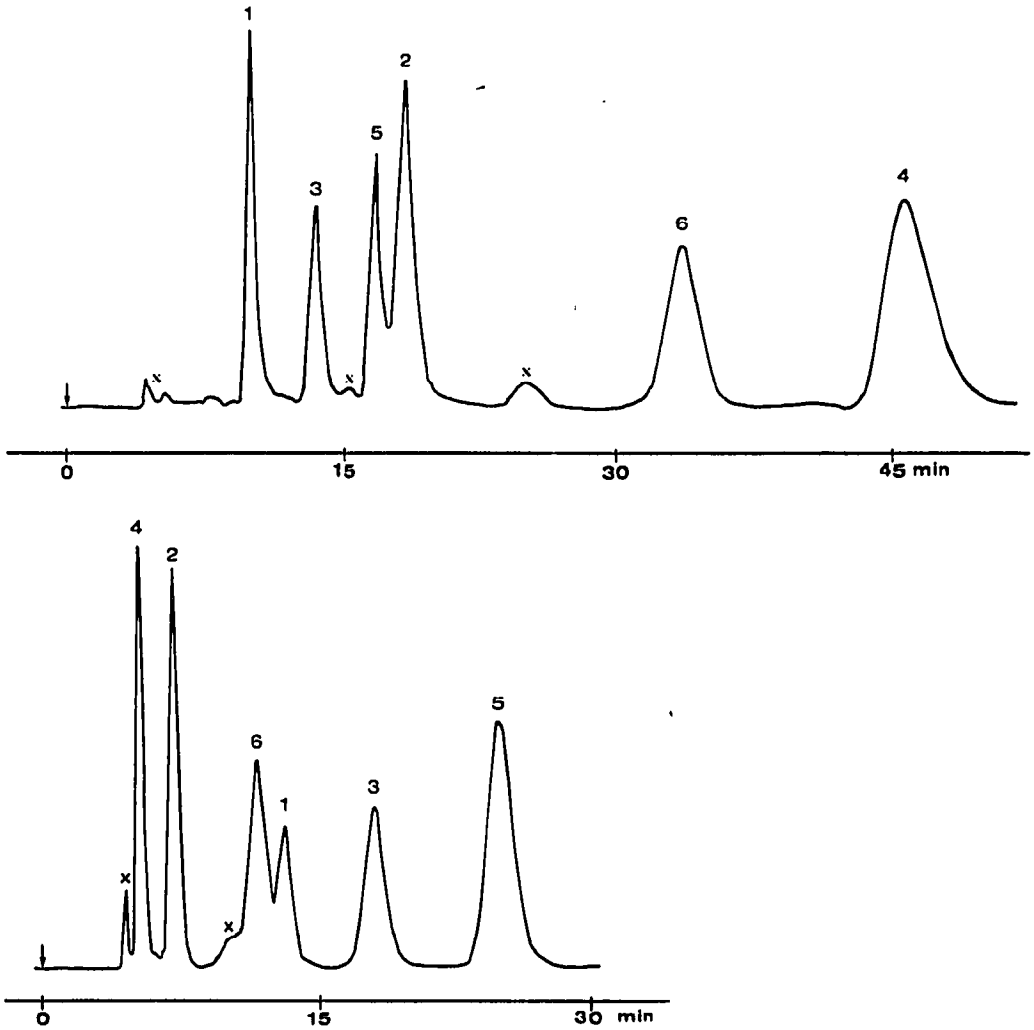

Fig. 6. Influence of temperature and anion concentration in the mobile phase on the group separation of aromatic sulphonic and carboxylic acids. Column: (a) TOA, 0.05 *M* $HClO_4$, pH 1.5; temperature, 25°. (b) TOA, 0.05 *M* $HClO_4$, pH 1.5; temperature, 65°. (c) TOA, 0.20 *M* $HClO_4$, pH 1.5; temperature, 25°. Sample: (1) 4-nitrobenzoic acid; (2) 4-nitrobenzenesulphonic acid; (3) 2-hydroxybenzoic acid; (4) 2-naphthol-3,6-disulphonic acid; (5) 4-methylbenzoic acid; (6) 1-naphthol-2-sulphonic acid. Impurities are marked with crosses.

formation is predominant. According to Table II, it can be explained mainly by the change in the capacity ratio and the selectivity factor with temperature.

An extreme example of the influence of temperature on the resolution is demonstrated in Fig. 4. It can be seen that a temperature increase of 6° causes an increase in resolution of about 80%.

The difficult separation of methylbenzoic acid isomers is shown in Fig. 5. Satisfactory resolution was achieved.

A group separation of carboxylic and sulphonic acids can be of practica importance and is demonstrated in Fig. 6. It can be seen that at 25° the different types of acids are eluted in an irregular sequence and at 65° they are separated in groups, the carboxylic acids being eluted first. An analogous group separation can be achieved by increasing the perchlorate concentration in the mobile phase, when the sulphonic acids are eluted first, as shown in Fig. 6c.

Fig. 7 shows a rapid separation of a five-component mixture of sulphonic acids in which the flow-rate was increased to the limit possible with the glass columns used.

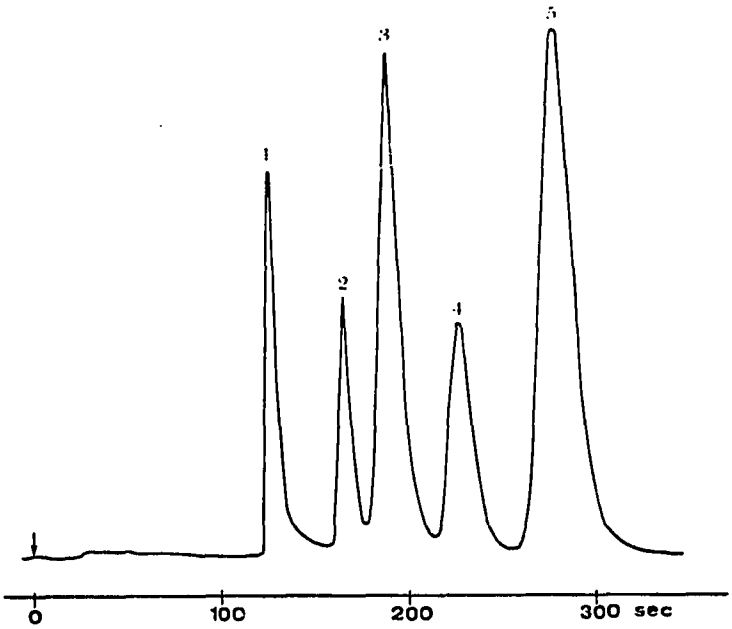

Fig. 7. Rapid separation of five aromatic sulphonic acids. Sample: (1) 3-aminotoluenesulphonic acid; (2) benzenesulphonic acid; (3) 4-nitrobenzenesulphonic acid; (4) 2-naphthol-3,6-disulphonic acid; (5) 2-naphthol-6-sulphonic acid. Column: TOA, 0.20 *M* $HClO_4$, pH 1.5. Flow-rate: 1.5 mm/sec. Temperature: 65°.

ACKNOWLEDGEMENTS

The authors are grateful for the assistance given by A. W. L. de Jong, K. Terwey-Groen and G. Szepesi.

REFERENCES

1 E. L. Smith and J. E. Page, *J. Soc. Chem. Ind., London*, 67 (1948) 48.
2 R. Modin, B.-A. Persson and G. Schill, in J. G. Gregory, B. Evans and P. C. Weston (Editors), *Proceedings of the International Solvent Extraction Conference 1971*, Vol. II, Society of Chemical Industry, London, 1971, p. 1211.
3 B.-A. Persson, *Acta Pharm. Suecica*, 8 (1971) 193.
4 S. Eksborg and B.-A. Persson, *Acta Pharm. Suecica*, 8 (1971) 605.
5 S. Eksborg, P. O. Lagerström, R. Modin and G. Schill, *J. Chromatogr.*, 83 (1973) 99.

6 S. Eksborg and G. Schill, *Anal. Chem.*, 45 (1973) 2092.
7 B.-A. Persson and B. L. Karger, *J. Chromatogr. Sci.*, 12 (1974) 521.
8 M. W. Scoggins and J. W. Miller, *Anal. Chem.*, 40 (1968) 1155.
9 R. H. Stehl, *Anal. Chem.*, 42 (1970) 1802.
10 J. A. Schmitt and R. A. Henry, *Chromatographia*, 3 (1970) 497.
11 J. S. Fritz and R. K. Gillette, *Anal. Chem.*, 40 (1968) 1777.
12 J. F. K. Huber, *Chimia*, Suppl. (1970) 24.
13 J. F. K. Huber and J. A. R. J. Hulsman, *Anal. Chim. Acta*, 38 (1967) 303.
14 J. F. K. Huber, J. C. Kraak and H. Veening, *Anal. Chem.*, 44 (1972) 1554.