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GRADIENT ELUTION IN LIQUID CHROMATOGRAPHY

VI. CATION-EXCHANGE CHROMATOGRAPHY OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOYL ESTERS AND AMIDES IN MIXED AQUEOUS-ORGANIC SOLUTIONS — INFLUENCE OF THE COMPOSITION OF THE MOBILE PHASE ON RETENTION CHARACTERISTICS AND PRACTICAL EXAMPLES OF SEPARATION

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SUMMARY

N,N-Dimethyl-*p*-aminobenzeneazobenzoyl esters and amides can be chromatographed on cation exchangers in the H⁺ form with aqueous–alcoholic solutions of hydrochloric acid as the mobile phase. Characteristic minima of capacity ratios occurring at a certain concentration of alcohol (*ca.* 80 % of ethanol) are due to a complex variety of simultaneously acting phenomena. Solutions containing 80 % of ethanol are best suited to chromatography, as the capacity ratios of chromatographed compounds are the lowest and the retention ratios are the highest in these solutions. Capacity ratios decrease with increasing concentration of hydrochloric acid according to a theoretically assumed relationship. The results of chromatographic and spectroscopic measurements confirmed the protonation of the derivatives to the first step in the mobile phases useful for practical separations. Ion-exchange chromatography can yield more efficient separations of some aliphatic esters and anilides than adsorption chromatography on silica, but excessive analysis times cannot be avoided.

INTRODUCTION

In Parts I–V of this series[1-5], a theory was presented that can be used to correlate retention characteristics (retention volume, peak width, resolution) with the parameters of elution (isocratic, gradient, stepwise and combined two-step elution, isocratic in the first step and gradient in the second step). This theory was found to apply well in adsorption chromatography and can be expected to be useful for numerous ion-exchange applications also.

In this paper, the validity of the basic assumptions in this theory is verified for a complex system of weak organic bases chromatographed on cation exchangers in acidic solutions. N,N-Dimethyl-*p*-aminobenzeneazobenzoyl esters and amides are partially protonated in ethanolic and methanolic solutions of hydrochloric acid and

therefore they are weak organic bases[6]. The influence of the composition of the mobile phase on the retention characteristics of sample compounds in this system is examined thoroughly. On the basis of previous experiments[5], a cation exchanger with a low degree of cross-linking (Dowex 50W-X2 (H$^+$)) was chosen. Some experiments were also performed with a cation exchanger with a higher degree of cross-linking (Ostion LG KS 0804–8% DVB, 20–40 μm).

THEORETICAL

The theoretical treatment of the problem was presented in Part V[5]. The basis of the theory is represented by an equation that describes the relationship between the capacity ratio, k', of the sample compound and the concentration, c (molarity or mole fraction), of the more efficient eluting component in the binary mobile phase:

$$k' \approx k_0' \cdot c^{-n} \tag{1}$$

where k_0' and n are experimental constants.

EXPERIMENTAL

The compounds chromatographed, ion exchangers and columns, solutions used as the mobile phase, instrumentation and operating techniques used in the present work were identical with those described in Part V[5].

RESULTS AND DISCUSSION

Influence of the concentration of alcohol in the mobile phase on sorption
The experimental capacity ratios, k', of N,N-dimethyl-p-aminobenzeneazobenzoyl amides and esters in solutions containing different amounts of hydrochloric acid and ethanol (or methanol) are given in Table I for Dowex 50W-X2 (H$^+$), 200–400 mesh, and in Table II for Ostion LG KS 0804 (H$^+$), 20–40 μm.
The influence of the concentration of methanol and ethanol in the mobile phase on the capacity ratios of N,N-dimethyl-p-aminobenzeneazobenzoyl derivatives on Dowex 50W-X2 was studied at a constant concentration of hydrochloric acid (0.925 M) in the mobile phase (Table I). Fig. 1 shows the relationship between the logarithms of the capacity ratios of methyl, n-amyl and n-nonyl esters of N,N-dimethyl-p-aminobenzeneazobenzoic acid and the logarithm of mole fraction of ethanol (x); the influence of the logarithm of the mole fraction of methanol on log k' of the methyl, n-propyl, n-amyl and n-nonyl esters and the methylamide, n-butylamide, dimethyl-, diethyl-, di(n-propyl)- and di(n-butyl)amide is illustrated in Fig. 2.
In ethanolic mobile phases, the occurrence of minima of log k' is observed at mole fractions of ethanol of about 0.4–0.6 (which corresponds to 60–85%[*]). Only the dimethylamide and diethylamide show distinct minima of k' in methanolic solutions in the concentration range studied, in approximately 80% methanol (mole fraction 0.6–0.7). The relationships between log k' of the other compounds studied and the logarithm of the mole fraction of methanol decrease monotonously over the whole concentration range up to 91.5% methanolic solution.

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[*] All concentrations quoted in percentages are by volume.

TABLE I

CAPACITY RATIOS OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOYL ESTERS AND AMIDES ON DOWEX 50W-X2 (H$^+$), 200–400 MESH, IN VARIOUS AQUEOUS–METHANOLIC AND AQUEOUS–ETHANOLIC SOLUTIONS OF HYDROCHLORIC ACID AS MOBILE PHASE

Column dimensions: length, 240 mm; diameter, 2.68 mm; volume, 1.35 ml. Volume of mobile phase, 0.43 ml. Flow-rate of mobile phase, 0.0642 ml/min.

Compound	HCl concentration (M)											
	1.719	0.925	0.473	0.221	0.925	0.925	0.925	0.925	0.255	0.935	0.937	0.926
	Ethanol (%)									Methanol (%)		
	76.5	76.5	76.5	76.5	95.8	65.0	50.0	35.0	92.3	80.0	65.0	91.5
Methyl ester	9.67	20.3	47.7	100.9	24.9	19.7	36.8		98.3	20.7	35.7	16.4
Ethyl ester		16.6										
n-Propyl ester	7.26	14.1	32.9	63.6	·		33.1 ·			16.9		12.1
n-Butyl ester		12.3										
n-Amyl ester	6.07	10.9	23.6	43.5	13.6	12.0	30.0	191.9		13.6	31.8	10.2
n-Hexyl ester		9.41										
n-Octyl ester		7.33										
n-Nonyl ester	4.05	6.77	13.0	23.1	9.04	7.85	26.3		31.5	10.3	32.0	6.84
n-Decyl ester		6.04										
Isopropyl ester		13.5										
Isobutyl ester		11.5										
Methylamide	12.0	27.1	70.5	165.7						27.3	36.3	24.9
Ethylamide		23.4		137.2								
n-Propylamide		20.7		111.3								
n-Butylamide	8.59	17.7	40.4	94.1						19.8	28.7	17.2
n-Hexylamide		14.9										
Allylamide		20.0										
Dimethylamide	15.9	31.3		161.2						30.4	30.7	34.1
Diethylamide	11.0	20.9	50.6	99.9						20.1	27.4	22.2
Di(*n*-propyl)amide	8.34	14.8	32.9	67.0						16.7	23.6	15.8
Di(*n*-butyl)amide	6.84	11.2	23.8	46.2						13.6	21.1	12.9
Anilide		26.0										
N-Ethylanilide		17.4										
p-Chloroanilide		25.4										
o-Nitroanilide		16.1										
p-Nitroanilide		27.8										
Phenyl ester		26.1										
o-Cresyl ester		22.7										

Analogous minima of selectivity were observed by Pietrzyk[7] for the sorption of various aromatic amines on the hydrogen form of cation-exchange resins in a number of mixed aqueous–organic solvents (alcohols, acetone, acetonitrile, dioxane and tetrahydrofuran) at an organic solvent concentration of about 80%. This phenomenon was explained by the simple concurrence of the following two opposite effects. An increase in the dielectric constant of the solution brought about by the addition of water to the organic solvent accounts for the decrease in the electrostatic forces between the functional groups of the resin and the protonated amine cations. This effect results in a decrease in the distribution coefficients. A similar decrease in sorption

TABLE II

CAPACITY RATIOS OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOYL ESTERS ON OSTION LG KS 0804 (H⁺), 20–40 μm, IN VARIOUS AQUEOUS–ETHANOLIC SOLUTIONS OF HYDROCHLORIC ACID AS MOBILE PHASE

Column dimensions: length, 240 mm; diameter, 2.68 mm; volume, 1.35 ml. Volume of mobile phase, 0.43 ml. Flow-rate of mobile phase: 0.034 ml/min. Ethanol concentration, 76.5%

Compound	HCl concentration (M)			
	4.745	0.922	0.478	0.221
Methyl ester	7.92	57.4	—	—
n-Propyl ester	—	28.9	—	—
n-Amyl ester	3.51	16.3	38.6	—
n-Hexyl ester	—	11.8	23.4	—
n-Octyl ester	—	7.52	13.9	—
n-Nonyl ester	1.78	6.32	10.8	21.8
n-Decyl ester	—	—	8.10	15.6

will be caused, on the other hand, by the better solvation and higher solubility of the organic base in the outer solution after the addition of an organic solvent to water. It can be assumed that the latter effect is more important in dilute aqueous solutions of the organic solvent, while the first effect will predominate in concentrated solutions. As a consequence, minimum sorption will occur at a certain concentration of the organic solvent in the mobile phase.

It seems, however, that other factors may also have a significant influence on the sorption. The measurements of Rückert and Samuelson[8] showed that the ratio of the concentration of ethanol in the outer solution and in the solution inside the exchanger reaches a maximum at a certain composition of the outer solution. Thus, minimum sorption can be expected in this solution on the basis of considerations that take into account only the simple partition mechanism based on the different solubilities of the compounds studied in both phases. For example, the maximum ratio of the concentration of ethanol in the outer solution to that in the "inner" solution is reached at approximately 80% of ethanol in the outer solution (mole fraction *ca.*
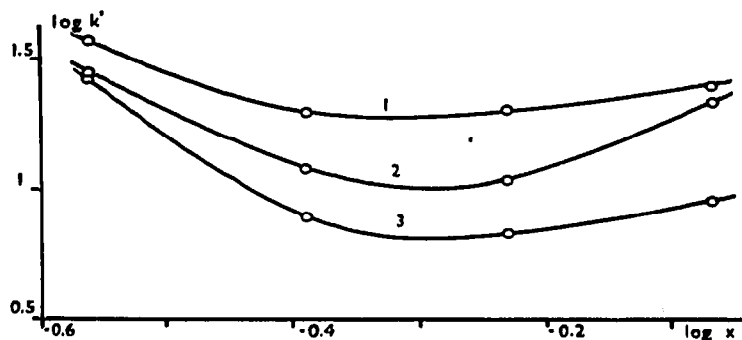

Fig. 1. Relationship between the capacity ratios (*k'*) of N,N-dimethyl-*p*-aminobenzeneazobenzoyl esters and the mole fraction (*x*) of ethanol in 0.925 *M* aqueous–ethanolic solutions of hydrochloric acid on Dowex 50W-X2 (H⁺), 200–400 mesh. 1, Methyl ester; 2, *n*-amyl ester; 3, *n*-nonyl ester. Column dimensions: length, 240 mm; diameter, 2.68 mm; volume, 1.35 ml.
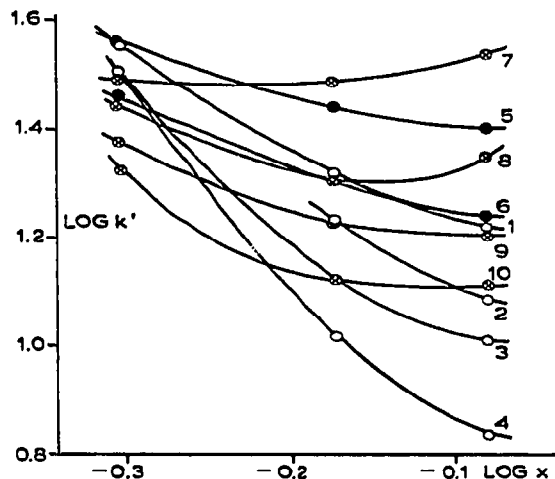
Fig. 2. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzencazobenzoyl esters and amides and the mole fraction (x) of methanol in 0.926–0.937 M aqueous–methanolic solutions of hydrochloric acid on Dowex 50W-X2 (H⁺), 200–400 mesh. 1, Methyl ester; 2, n-propyl ester; 3, n-amyl ester; 4, n-nonyl ester; 5, methylamide; 6, n-butylamide; 7, dimethylamide; 8, diethylamide; 9, di(n-propyl)amide; 10, di(n-butyl)amide. Column dimensions: as in Fig. 1.

0.5–0.65) for aqueous–ethanolic solutions in equilibrium with Dowex 50W-X8 in the form of an alkali metal (Li⁺, Na⁺, K⁺).

On the other hand, an increasing concentration of ethanol in the mobile phase leads to a decrease in the swelling of cation exchangers, which means that the volume of the solution inside the exchanger is decreased. As the number of exchange groups and counter ions in the exchanger remains unchanged, the limited swelling causes a relative increase in ion-exchange capacity in unit volume of the exchanger. The concentration of the counter ions (H⁺) in the inner solution is increased and the ion-exchange equilibrium is thus influenced.

The differences between the content of ethanol in the outer solution and in the solution inside the ion exchanger decrease with decreasing degree of cross-linking (the concentrations are virtually equal in both solutions with Dowex 50-X1 (Na⁺)[9]) and, consequently, the influence of the concentration of ethanol in the mobile phase on the sorption of organic ions depends on the degree of cross-linking of the exchanger. The sorption is also influenced by the ionic strength of the outer solution; higher ionic strengths cause a decrease in the difference between the content of ethanol in the outer solution and that in the solution inside the exchanger[9].

It is obvious that the influence of the concentration of alcohol in the mobile phase on the capacity ratios of chromatographed compounds is complex. A variety of different effects can act simultaneously and it is difficult to establish which mechanism is the most important.

In practice, it is advantageous to choose a concentration of alcohol such that the sorption is near to the minimum, in order to make possible the use of a mobile phase with a lower content of acid. In solutions containing less than 50% of ethanol or methanol, the solubility of the derivatives studied is decreased to such an extent
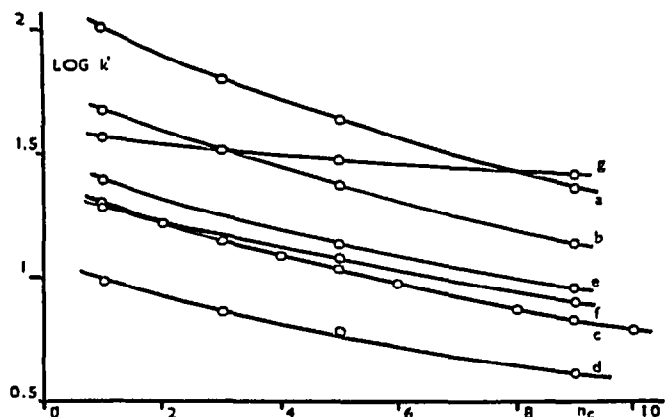
Fig. 3. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and the number of carbon atoms (n_c) in the alkyl chains on Dowex 50W-X2 (H$^+$), 200–400 mesh, using aqueous–ethanolic solutions of hydrochloric acid with different concentrations of the acid and ethanol as the mobile phase. a, 0.221 M HCl in 76.5% ethanol; b, 0.473 M HCl in 76.5% ethanol; c, 0.925 M HCl in 76.5% ethanol; d, 1.719 M HCl in 76.5% ethanol; e, 0.925 M HCl in 95.8% ethanol; f, 0.925 M HCl in 65.0% ethanol; g, 0.925 M HCl in 50% ethanol. Column dimensions: as in Fig. 1.

that, in addition to very high capacity ratios, the elution peaks are extremely broad. The use of such solutions as the mobile phase is completely unsuitable.

Not only the capacity ratios of the individual organic ions, but also their retention ratios, depend on the concentration of alcohol in the mobile phase. The influence of the concentration of ethanol on the ratio of sorption of the individual aliphatic N,N-dimethyl-p-aminobenzeneazobenzoyl esters on Dowex 50W-X2 (H$^+$) in solutions with a constant concentration of hydrochloric acid (0.925 M) is shown in Fig. 3, and the influence of the concentration of methanol on the sorption of aliphatic esters and amides is illustrated in Fig. 4. These figures show curves illustrating the relationship between the logarithms of the capacity ratios of the derivatives studied and the
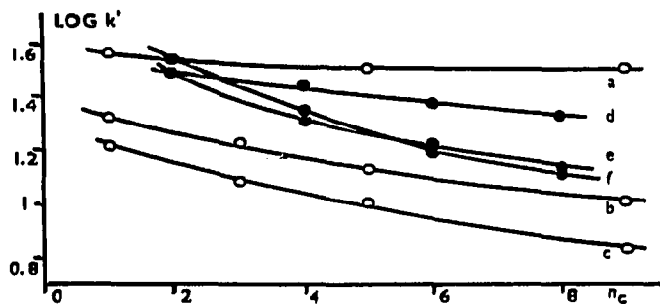

Fig. 4. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl esters (curves a–c) and amides (curves d–f) and the number of carbon atoms (n_c) in the alkyl chains on Dowex 50W-X2 (H$^+$), 200–400 mesh, using aqueous–methanolic solutions of hydrochloric acid with different concentrations of methanol as the mobile phase. a and d, 0.937 M HCl in 65% methanol; b and e, 0.935 M HCl in 80% methanol; c and f, 0.926 M HCl in 91.5% methanol. Column dimensions: as in Fig. 1.

number of carbon atoms in the alkyl chains in different mobile phases. Comparison of the curves for different concentrations of ethanol (Fig. 3) shows that curve c for the 76.5 % solution is the steepest, which means that the greatest differences in sorption can be achieved in this solution. Curve e, corresponding to the 95.8 % solution, is only slightly less steep, while the decrease in steepness is more distinct with curve f for the 65 % solution. The differences between the capacity ratios of the individual compounds are considerably lower in 50 % ethanolic solution (curve g) than in more concentrated ethanolic solutions. This behaviour probably can be explained by a decrease in the difference between the concentration of ethanol in the outer solution and that in the solution inside the exchanger at lower concentrations of ethanol, which is connected with the decrease in the differences in ratios of solubility in the two phases between the individual chromatographed compounds.

The dependence of the differences between the sorption of individual homologous esters and amides on Dowex 50W-X2 (H$^+$) in methanolic solutions is analogous to that in ethanolic solutions. The differences increase with increasing concentration of methanol (from 65 % to 91.5 % solution). It is interesting that the change in concentration of methanol shows a greater influence on the sorption of esters than on that of amides (Fig. 4).

The relationship between log k' and the length of the alkyl chain is steepest in 91.5 % methanol. Its slope corresponds approximately to the slope of the analogous relationship for 65 % ethanol. Over the whole concentration range of methanol, the differences in sorption (selectivity) are lower than in 80 % ethanolic solutions.

Solutions of hydrochloric acid in 80 % ethanol were chosen for further study of the influence of the concentration of acid in the mobile phase on the capacity ratios of N,N-dimethyl-p-aminobenzeneazobenzoyl derivatives (verification of eqn. 1), as these solutions show the greatest selectivity differences among all the alcoholic (ethanolic and methanolic) solutions studied.

Influence of the concentration of acid in the mobile phase on the capacity ratios of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and amides

Tables I and II give a survey of the capacity ratios of N,N-dimethyl-p-aminobenzeneazobenzoyl derivatives on Dowex 50W-X2 (H$^+$) and Ostion LG KS 0804 (H$^+$) in ethanolic solutions that differ in their content of hydrochloric acid. The relationships between the logarithms of the capacity ratios and the number of carbon atoms in the alkyl chains of aliphatic esters and amides in solutions (76.5 % of ethanol) with different concentrations of hydrochloric acid (0.221–4.765 M) are shown in Fig. 3 (aliphatic esters on Dowex 50W-X2, curves a–d), Fig. 5 (primary amides, curves e and f; secondary amides, curves a–d; on Dowex 50W-X2) and Fig. 6 (aliphatic esters on Ostion LG KS 0804).

On Dowex 50W-X2, the relationships have almost identical slopes in solutions with different concentrations of hydrochloric acid. The slope increases slightly with decreasing acidity of the mobile phase, but this change is not very significant. The homologous series of esters shows a steeper relationship between log k' and the number of carbon atoms in the alkyl chains than the series of primary amides, and a less steep relationship than that for secondary amides (Figs. 3 and 5).

The increase in the slope of the relationship between log k' and the number of carbon atoms in alkyl chains with decreasing concentration of the acid in the mo-
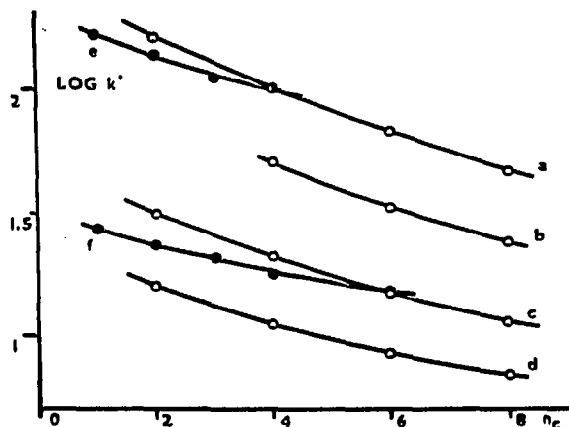
Fig. 5. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl amides and the number of carbon atoms (n_c) in the alkyl chains on Dowex 50W-X2 (H$^+$), 200–400 mesh, using aqueous–ethanolic solutions containing hydrochloric acid with different concentrations as the mobile phase. a and e, 0.221 M HCl in 76.5% ethanol; b, 0.473 M HCl in 76.5% ethanol; c and f, 0.925 M HCl in 76.5% ethanol; d, 1.719 M HCl in 76.5% ethanol. Column dimensions: as in Fig. 1. Curves a–d relate to the homologous series of secondary amides and curves e and f to the series of primary homologues.
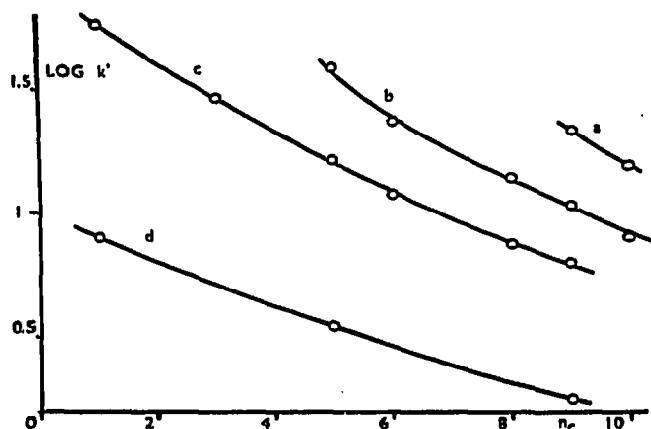

Fig. 6. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and the number of carbon atoms (n_c) in the alkyl chains on Ostion LG KS 0804 (H$^+$), 20–40 μm, using aqueous–ethanolic solutions containing hydrochloric acid with different concentrations as the mobile phase. a, 0.221 M HCl in 76.5% ethanol; b, 0.478 M HCl in 76.5% ethanol; c, 0.922 M HCl in 76.5% ethanol; d, 4.745 M HCl in 76.5% ethanol. Column dimensions: as in Fig. 1.

bile phase is more distinct on Ostion LG KS 0804 (H$^+$) (Fig. 6) than on Dowex 50W-X2 (H$^+$).

Figs. 7–9 illustrate the relationships between the logarithms of the capacity ratios of the derivatives studied and the logarithm of the molar concentration of hydrochloric acid. The relationships are approximately linear on both exchangers and the slopes are almost identical for different individual derivatives studied on both cation exchangers and are close to the value -1.

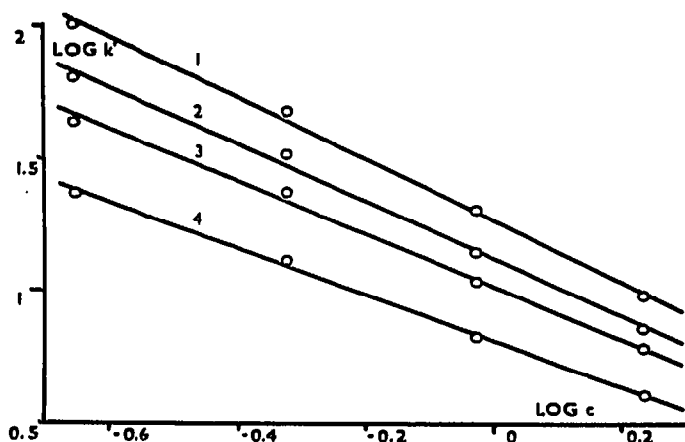Table III gives a survey of the parameters of eqn. 1, n (slopes of the lines) and

Fig. 7. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and the molar concentration of hydrochloric acid (c) in aqueous–ethanolic solutions containing 76.5 % of ethanol on Dowex 50W-X2 (H$^+$), 200–400 mesh. Compounds: 1, methyl ester; 2, n-propyl ester; 3, n-amyl ester; 4, n-nonyl ester. Column dimensions: as in Fig. 1.
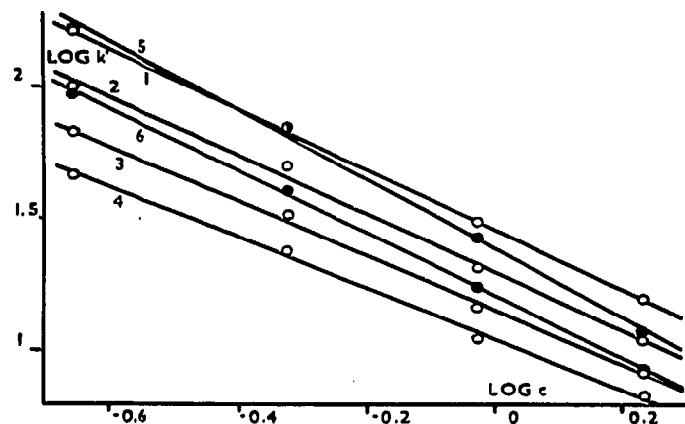
Fig. 8. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl amides and the molar concentration of hydrochloric acid (c) in aqueous–ethanolic solutions containing 76.5 % of ethanol on Dowex 50W-X2 (H$^+$), 200–400 mesh. Compounds: 1, dimethylamide; 2, diethylamide; 3, di(n-propyl)amide; 4, di(n-butyl)amide; 5, methylamide; 6, n-butylamide. Column dimensions: as in Fig. 1.

k'_0 on Dowex 50W-X2 (H$^+$) and Ostion LG KS 0804 (H$^+$). The linear decrease in the experimental values of log k' with increase in the logarithm of the concentration of hydrochloric acid in the mobile phase is in good agreement with theoretical assumptions in Part V[5] and provides evidence that eqn. 1 can be used to describe the influence of the concentration of acid on the sorption of protonated N,N-dimethyl-p-aminobenzeneazobenzoyl esters and amides on cation exchangers in the H$^+$ form. Spectroscopic measurements described in Part V[5] showed that the protonation to the first step predominates in the concentration range studied and that approximately 80% of the total amount of the derivatives exists as the protonated form in 0.221 M hydro-
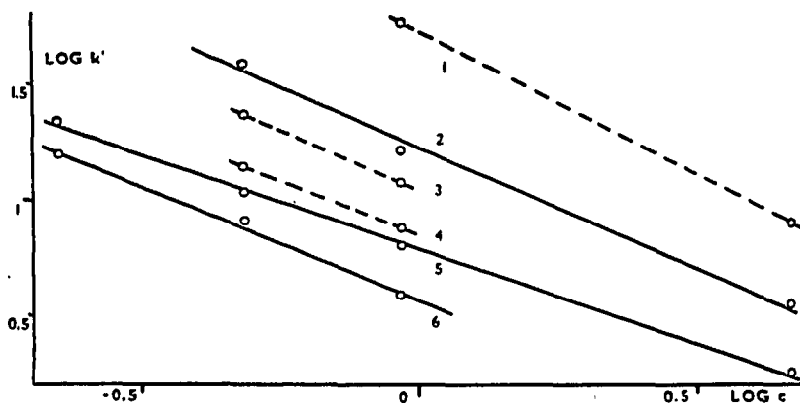
Fig. 9. Relationship between the capacity ratios (k') of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and the molar concentration of hydrochloric acid (c) in aqueous–ethanolic solutions containing 76.5% of ethanol on Ostion LG KS 0804 (H$^+$), 20–40 μm. Compounds: 1, methyl ester; 2, n-amyl ester; 3, n-hexyl ester; 4, n-octyl ester; 5, n-nonyl ester; 6, n-decyl ester. Column dimensions: as in Fig. 1.

chloric acid in 80% ethanol, while the derivatives are protonated to the extent of *ca.* 90% in 0.478 *M* acid and virtually complete protonation can be assumed in more acidic solutions. Thus, the protonated form of the derivatives studied will predominate over the non-protonated form in the mobile phases used in chromatography.

In Part V[5], it was shown that the relationship between the capacity ratio, k',

TABLE III

EXPERIMENTAL CONSTANTS, n AND k_0, OF EQN. 1 DESCRIBING THE RELATIONSHIP BETWEEN THE LOGARITHMS OF THE CAPACTITY RATIOS OF N,N-DIMETHYL-p-AMINOBENZENEAZOBENZOYL ESTERS AND AMIDES AND THE LOGARITHM OF CONCENTRATION (MOLARITY) OF HYDROCHLORIC ACID IN 76.5% ETHANOLIC SOLUTIONS USED AS THE MOBILE PHASE ON COLUMNS OF DOWEX 50W-X2 (H$^+$), 200–400 MESH, AND OSTION LG KS 0804 (H$^+$), 20–40 μm

Column dimensions: length, 240 mm; diameter, 2.68 mm; volume, 1.35 ml. Volume of mobile phase, 0.43 ml.

Compound	Dowex 50W-X2		Ostion LG KS 0804	
	n	k_0'	n	k_0'
Methyl ester	1.15	18.6	—	—
n-Propyl ester	1.07	13.3	—	—
n-Amyl ester	0.98	10.4	1.02	16.7
n-Nonyl ester	0.86	6.5	0.81	6.1
n-Decyl ester	—	—	0.98	3.7
Methylamide	1.29	24.8	—	—
n-Butylamide	1.17	16.3	—	—
Dimethylamide	1.13	29.1	—	—
Diethylamide	1.10	20.1	—	—
Di(n-propyl)amide	1.03	14.4	—	—
Di(n-butyl)amide	0.95	11.1	—	—

of a weak organic base on a cation exchanger in the H^+ form and the concentration of H^+ ions, $[H^+]$, in the mobile phase can be expressed as

$$k' \approx \frac{V_s}{V_m} \cdot K_{H+}^{BH_i^{i+}} \cdot Q^i \cdot [H^+]^{-i} \qquad (2)$$

assuming that only one protonated form of the base, BH^{i+}, predominates in the solution (V_s and V_m are the total volumes of the stationary and mobile phase, respectively, in the column, Q is the total exchange capacity of the cation exchanger and $K_{H+}^{BH_i^{i+}}$ is the conventional selectivity constant describing the sorption of the BH_i^{i+} form on the exchanger in the H^+ form. In agreement with this equation, the experimental values of n in Table III are near to unity (the exponent i in eqn. 2 is identical with the exponent n in eqn. 1 and $i = n = 1$ if protonation to only the first step takes place under the experimental conditions).

Slightly higher values of n for primary amides can be caused by possible interference of protonation to the second step, which takes place in acidic solutions of these compounds, as indicated by spectroscopic measurements[5], but the variation of the experimental values does not permit significant conclusions to be drawn (Fig. 8).

The experimental results confirm the validity of the theoretical assumptions[5] concerning the sorption of N,N-dimethyl-p-aminobenzeneazobenzoyl amides and esters on cation exchangers in alcoholic solutions of acids and show that eqn. 1 is suitable for describing the relationship between the capacity ratios and the concentration of the efficient eluting component (hydrochloric acid) in the mobile phase for this practical example of the chromatography of weak bases on cation exchangers in the H^+ form.

Examples of separation

The separation of N,N-dimethyl-p-aminobenzeneazobenzoyl esters of lower aliphatic alcohols on cation exchangers was more successful than separations by adsorption chromatography on silica using a mobile phase consisting of ethyl acetate
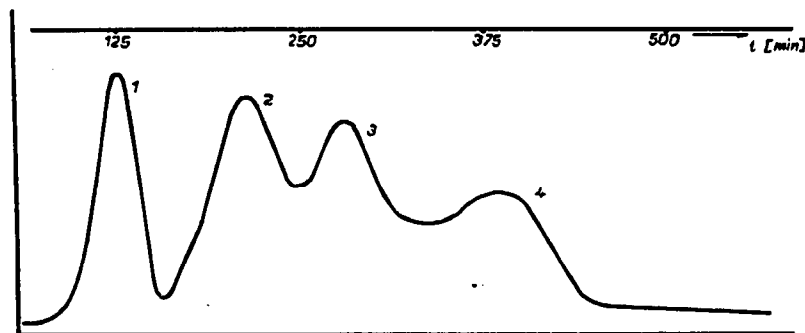

Fig. 10. Separation of a mixture containing the n-nonyl ester (1), n-amyl ester (2), n-propyl ester (3) and methyl ester (4) of N,N-dimethyl-p-aminobenzeneazobenzoic acid on Dowex 50W-X2 (H^+), 200–400 mesh, using 0.505 M hydrochloric acid in 76.5 % ethanol as the mobile phase. Column dimensions: length, 315 mm; diameter, 3.00 mm; volume, 1.73 ml. Flow-rate of mobile phase: 0.069 ml/min. Pressure: 10 atm. Sample: 33 μl of ethanolic solution containing 0.5 g/l of each component. Detection: spectrophotometric at 510 nm.
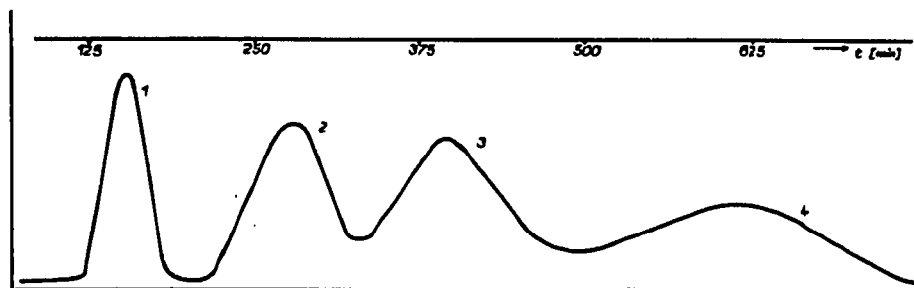
Fig. 11. Separation of a mixture containing the *n*-nonyl ester (1), *n*-amyl ester (2), *n*-propyl ester (3) and methyl ester (4) of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid on Dowex 50W-X2 (H$^+$), 200–400 mesh, using 0.199 M hydrochloric acid in 76.5% ethanol as the mobile phase. Column dimensions: as in Fig. 10. Flow-rate of mobile phase: 0.09 ml/min. Pressure: 11 atm. Sample: 40 μl of ethanolic solution containing 0.5 g/l of each component. Detection: spectrophotometric at 510 nm.

and cyclohexane. The separation of methyl, *n*-propyl, *n*-amyl and *n*-nonyl esters by adsorption chromatography was poor owing to the low retention of these compounds over the whole concentration range of ethyl acetate in the mobile phase. Chromatograms of this mixtue showed one slightly split peak common to the three earlier eluted components, overlapping considerably with the second peak of the methyl ester, even when very low concentrations of ethyl acetate in the mobile phase or gradients of ethyl acetate with a very low slope of the gradient function were used.

The chromatography of lower esters on cation exchangers was more successful, although the separation time was considerably longer. Fig. 10 shows the separation of the above four esters on Dowex 50W-X2 (H$^+$), 200–400 mesh, with 0.505 M hydrochloric acid containing 76.5% of ethanol. The use of a mobile phase with a lower content of acid (0.199 M hydrochloric acid in 76.5% ethanol) results in an improved separation, even with a relatively higher flow-rate of the mobile phase (Fig. 11). This separation can be used for quantitative analysis, but takes a considerable time (*ca*. 12 h).

Chromatography on Dowex 50W-X2 (H$^+$), 200–400 mesh, with 0.955 M hydrochloric acid in 76.5% ethanol gives separations of some N,N-dimethyl-*p*-aminobenzeneazobenzoyl anilides that could not be resolved by chromatography on silica owing to their very similar capacity ratios. Fig. 12 shows the separation of a mixture of the *o*-nitroanilide and *p*-nitroanilide, which takes about 5 h. A mixture of the anilide and N-ethylanilide can also be well resolved under these conditions, in contrast to adsorption chromatography on silica.

The chromatography of N,N-dimethyl-*p*-aminobenzeneazobenzoyl derivatives on cation exchangers in aqueous–ethanolic solutions of hydrochloric acid can be used as a method complementary to adsorption chromatography on silica.

The best concentration range of hydrochloric acid for practical chromatographic separations is between 0.1 and 1 M. Adsorption chromatography, as far as the separation is possible, is to be preferred because of the very long analysis times required by ion-exchange chromatography.

The experiments with gradient-elution ion-exchange chromatography were omitted, partly due to long analysis times and partly due to the fact that the plate number is not constant for different compounds as was assumed in the derivation of the
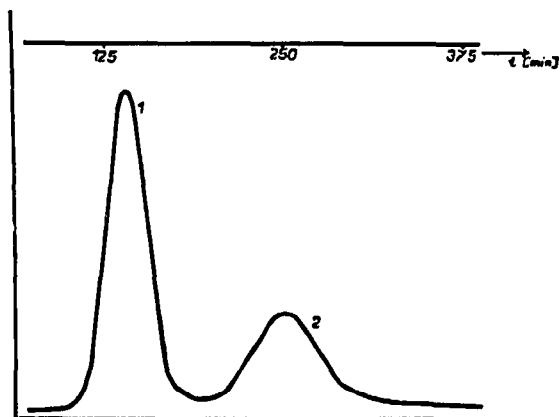
Fig. 12. Separation of a mixture containing the o-nitroanilide (1) and p-nitroanilide (2) of N,N-dimethyl-p-aminobenzeneazobenzoic acid on Dowex 50W-X2 (H⁺), 200–400 mesh, using 0.955 M hydrochloric acid in 76.5% ethanol as the mobile phase. Operating conditions as in Fig. 10.

theoretical relationships for retention characteristics in gradient elution, but depends strongly on the capacity ratio. This latter effect is probably caused by the influence of the "inner diffusion" on the broadening of the elution zones[5].

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