CHROM. 7808

GRADIENT ELUTION IN LIQUID CHROMATOGRAPHY

V. CATION-EXCHANGE CHROMATOGRAPHY OF N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOYL ESTERS AND AMIDES IN MIXED AQUEOUS–ORGANIC SOLUTIONS —INFLUENCE OF THE NATURE OF THE EXCHANGER ON SEPARATION

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SUMMARY

N,N-Dimethyl-*p*-aminobenzeneazobenzoyl esters and amides are subject to protonation in acidic solutions. The protonated derivatives of N,N-dimethyl-*p*-amino-benzeneazobenzoic acid can be separated by chromatography on strongly acidic sulphonated cation exchangers using aqueous–alcoholic (ethanolic or methanolic) solutions of hydrochloric acid as the mobile phase. The separation is most probably based on a partition mechanism involving the differences in solubility of the individual derivatives in the mobile phase and inside the ion-exchange phase. Cation exchangers with higher degrees of cross-linking offer higher selectivity but longer elution times in comparison with less tightly cross-linked exchangers. The latter make possible the use of less acidic mobile phases but suffer from mechanical instability at higher operating pressures.

INTRODUCTION

Recently, a theory has been developed that can be used as a basis for calculations of the retention characteristics in isocratic and gradient elution chromatography[1,2]. This theory was found to apply well in adsorption chromatography[3,4] and, according to the theoretical assumptions, it should also apply well to many ion-exchange chromatographic applications. This paper deals with the utility of this theory in the cation-exchange chromatography of organic bases in acidic solutions and is concerned with the appropriate choice of the cation exchanger for separations.

THEORETICAL

A simple equation has been shown[1] to describe the relationship between the concentration (mole fraction or molarity) of the more efficient eluting component in the binary mobile phase (*c*) and the capacity ratio of the sample compound (*k'*) in

adsorption chromatography and in the most frequently occurring situations in ion-exchange chromatography:

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

where k_0' and n are constants (k_0' represents the capacity ratio in the mobile phase when $c = 1$).

In chromatography based on simple exchange of ions between the ion exchanger and the mobile phase, eqn. 1 can be expected to apply, provided that a mobile phase containing only one dissolved ionized compound is used in the chromatography of trace amounts of sample ionic species, further that no other equilibria or specific interactions occur in the system, and finally that the activity coefficients of the ions present in the system do not have a great dependence on the concentration of electrolyte in the mobile phase.

It can be shown, however, that eqn. 1 may sometimes be useful, at least over a limited range of mobile phase compositions, even if chemical equilibria take place in the mobile phase, assuming that one ionic form of the sample compound predominates in the mobile phase. Such is the situation if a weak base is chromatographed on the acidic (H^+) form of a cation exchanger using a strong (mineral) acid as the eluent. In this instance, the exchange of all protonated forms of the base, BH^+, $BH_2^{2+} \ldots BH_n^{n+}$, that exist in the system under equilibrium conditions for H^+ ions must be considered. The equilibrium in the mobile phase can be characterized using the protonation constants, K_i ($i = 1 \ldots n$):

$$K_i = \frac{[BH_i^{i+}]}{[B] \cdot [H^+]^i} \tag{2}$$

For each ionized species, a conventional selectivity constant can be defined:

$$K_H^{BH_i} = \frac{(BH_i^{i+}) \cdot [H^+]^i}{[BH_i^{i+}] \cdot (H^+)^i} \tag{3}$$

The parentheses refer to concentrations in the "inner" ion-exchange phase and the square brackets to those in the outer solution (mobile phase). The corresponding activity coefficients are included in the value of the selectivity constant.

Provided that the concentration of the non-protonated form of the base B inside the "inner" ion-exchange (stationary) phase is negligible, the capacity ratio, k', of the base can be expressed as:

$$k' = \frac{V_s}{V_m} \cdot \frac{\sum\limits_{i=1}^{n} (BH_i^{i+})}{[B] + \sum\limits_{i=1}^{n} [BH_i^{i+}]} = \frac{V_s}{V_m} \cdot \frac{\sum\limits_{i=1}^{n} K_H^{BH_i} \cdot K_i \cdot Q^i}{1 + \sum\limits_{i=1}^{n} K_i \cdot [H^+]^i} \tag{4}$$

where V_s and V_m are the total volumes of the stationary and mobile phase, respectively, in the column and Q represents the total exchange capacity of the cation exchanger, which can be assumed to equal the concentration of H^+ ions inside the ion exchanger, if trace amounts of the base are present[5].

The pH of the mobile phase can be assumed to be low enough to allow

$\varSigma\, K_i \cdot [\mathrm{H^+}]^i > 1$, if the capacity ratio of the chromatographed base is maintained within practical limits, making the analysis in reasonable time possible. If the pH range is such that only one protonated form of the base, BH_i^{i+}, predominates in the solution, eqn. 4 can be simplified:

$$k' \approx \frac{V_s}{V_m} \cdot K_{\mathrm{H}}^{\mathrm{BH}i} \cdot Q^i \cdot [\mathrm{H^+}]^{-i} \tag{5}$$

This equation is essentially identical with eqn. 1, where $n = i$, $c = [\mathrm{H^+}]$ and $k_0' = V_s/V_m \cdot K_{\mathrm{H}}^{\mathrm{BH}i} \cdot Q^i$.

The validity of eqn. 5 requires sufficiently large differences between the values of the individual protonation constants. The activity coefficients of the ions in the system must not be much influenced by a change in the composition of the mobile phase.

EXPERIMENTAL

Instrumentation

The instrument used was essentially the same as that described in Part III[3]. One alteration to this system was that a simple single sapphire plunger pump (MC-300, Mikrotechna, Modřany, Czechoslovakia) was used instead of the two-plunger gradient pump (PPM-68005). Further, the rather agressive mobile phases used in this work (alcoholic solutions of hydrochloric acid) do not permit the direct use of plunger pumps, in which stainless-steel parts are exposed to the liquid being
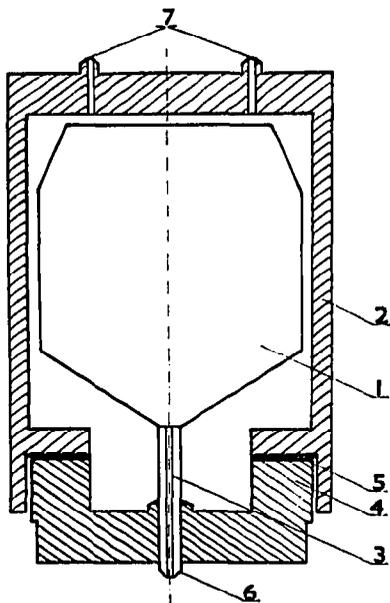
Fig. 1. Transforming device for work with corrosive mobile phases. 1 = Plastic bag; 2 = cylindrical metal vessel; 3 = capillary tubing; 4 = metal cover (screw) of the cylindrical vessel; 5 = sealing plastic insertion foil; 6 = male terminal connection to capillary joining tube to the column; 7 = two male terminal connections to capillary joining tubes to the pump and pressure gauge.

pumped. For this reason, a transforming device of our own construction[6] was employed, which avoided direct contact between the mobile phase and the stainless-steel parts of the pump. This transforming device (Fig. 1) is inserted between the plunger pump and the inlet of the column. It is formed by a flexible plastic bag (1) made from material (polyethylene) resistant to the agressive medium used, which is placed inside a thick-walled cylindrical closed metal vessel (2). The polyethylene bag (1) functions as a reservoir for the agressive mobile phase and is fastened tightly on to a plastic tube (3) leading through the cover (4) of the metal cylindrical vessel (2) (a leak-proof connection is achieved by pressing the tubing into the metal cover, combined with the use of a sealing insertion foil (5). The tube is provided with a male terminal fitting (6) serving as a connection to capillary tubing leading to the column inlet. The metal vessel is provided with a further two outlets (7); one is connected to the pump and the other can be used for connection of a pressure gauge.

The equipment functions as follows.

(1) The pump fills the space in the metal vessel around the plastic bag (1) with a non-agressive liquid (water or ethanol). The plastic bag is empty and the outlet of the capillary tubing (3) is disconnected from the column during this operation.

(2) The pump is stopped and the plastic bag (1) is filled with the mobile phase using a large-capacity (50-ml) syringe through the capillary tubing (3). The corresponding volume of the non-agressive liquid is displaced from the metal vessel (2) through one outlet (7), which is disconnected from the pump during this step.

(3) When the bag (1) has been filled with mobile phase (*ca.* 200 ml total capacity), the outlet (7) from the metal vessel is connected to the pump and the capillary tubing (3) is connected to the inlet of the column. The pump is then started. The non-agressive liquid is pumped into the metal vessel (2) so that hydraulic pressure is developed, which pushes the mobile phase out of the plastic bag on to the column.

The flow-rate of the mobile phase equals the flow-rate of the non-agressive liquid pumped into the transforming device. As the pressure on the inner side of the plastic bag equals that on the outer side, there is little risk of mechanical damage to the plastic material.

As all the parts of the liquid chromatograph that come in contact with the mobile phase (with the exception of the pump) are constructed from PTFE and glass, agressive mobile phases can be used without corrosion of the instrument. The equipment was tested at pressures up to 30 atm and showed good operating capabilities and flow-rate stability.

Ion exchangers

The following polystyrene–divinylbenzene copolymer-based sulphonated strongly acidic cation exchangers were used:

(1) Dowex 50W-X2 (200–400 mesh), Dow Chem., Midland, Mich., U.S.A.

(2) Dowex 50W-X4 (200–400 mesh), Dow Chem.

(3) Dowex 50W-X8 (100–200 mesh), Dow Chem.

(4) Ostion LG KS 0804 (20–40 μm), Spolek pro Chemickou a Hutní Výrobu, Ústí nad Labem, Czechoslovakia.

(5) Macroporous cation exchanger prepared from the macroporous styrene–

divinylbenzene copolymer (6% DVB) according to the procedure of VÚSPL (Research Institute of Synthetic Resins and Lacquers, Pardubice, Czechoslovakia) and fractionated to obtain a fraction with particle diameters in the range 80–90 μm.

The cation exchangers were freed from finely powdered particles by repeated decantation with water and were recycled three times from the Na^+ to the H^+ form before use. Part of the material prepared in this way was used for the determination of ion-exchange capacity, density and interstitial volume; the other part was packed into the column.

The total exchange capacity was determined from the decrease in alkalinity after 100 ml of 0.1 N sodium hydroxide solution had been passed through the column containing a known amount of the cation exchanger in the H^+ form and was related to 1 g of dry ion exchanger[7]. In addition, the exchange capacity for the compounds studied was determined as follows. A 100-ml volume of an ethanolic solution containing *ca.* 0.25 g of the di-(*n*-propyl)amide of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid was passed through the column containing a known amount of the cation exchanger in the H^+ form and the column was washed until a completely colourless eluate was obtained. The eluate and the washing solutions were collected in a calibrated flask and the volume was adjusted to 250 ml with ethanol. The absorbances of this solution and a reference solution were measured and the amount of the amide retained on the column was calculated from the difference and related to 1 g of dry ion exchanger.

The density of the dry ion exchanger was determined pycnometrically in *n*-octane[7]. The volume of the mobile phase in the column outside the exchanger beads, V_m, was measured as the volume of the liquid filtered under vacuum from the column of bed volume V_k. Then, the parameter ε was calculated as the ratio V_m/V_k.

ε does not depend significantly on the composition of the mobile phase, bead size, cross-linking of the exchanger or pressure drop across the column. For example, $\varepsilon = 0.316$ in 1 M hydrochloric acid in 76.5% (v/v) ethanol and $\varepsilon = 0.308$ in 0.2 M hydrochloric acid in 76.5% ethanol for Dowex 50W-X2.

Table I surveys the properties of the exchangers used.

Preparation of columns

Ion-exchange columns were packed with the aid of an attached packing vessel of our own construction. A dilute suspension of swollen exchanger is stirred with an electromagnetic stirrer and introduced continuously into the column under pressure by pumping the mobile phase into the packing vessel at a flow-rate about 20% higher than that used in chromatographic operation.

Mobile phase

Ethanolic and methanolic solutions of hydrochloric acid were prepared by mixing hydrochloric acid (density 1.16 g/cm^3, 35%), ethanol (density 0.805 g/cm^3, 94.5%, w/w) or methanol (density 0.792 g/cm^3, 100%) and distilled water in calculated ratios. The exact concentrations of hydrochloric acid in the solutions were determined by titration with standard alkali solution.

Chromatographed compounds

Coloured N,N-dimethyl-*p*-aminobenzeneazobenzoyl amides and esters were

TABLE I

PROPERTIES OF THE CATION EXCHANGERS

Exchanger	Nominal degree of cross-linking (% DVB)	Particle size	Ion-exchange capacity (mequiv./g)		Capacity utilized by the amide* (%)	Density (g/cm³)	ε**
			Total	For amide*			
Dowex 50W-X2	2	200–400 mesh	5.36	1.11	21	1.45	0.316
Dowex 50W-X4	4	200–400 mesh	5.38	—	—	1.55	0.314
Dowex 50W-X8	8	100–200 mesh	4.98	—	—	—	—
Ostion LG KS 0804	8	20–40 μm	5.18	0.83	16	1.42	0.319
Macroporous	6	80–90 μm	4.95	2.31	47	1.38	0.356

* Di-(n-propyl)amide of N,N-dimethyl-p-aminobenzeneazobenzoic acid.

** $\varepsilon = \dfrac{V_m}{V_k}$.

prepared according to methods described by Churáček and co-workers[8-10]. Solutions of individual compounds and artificial mixtures in ethanol were used as samples (10–40 μl in volume).

Detection
Photometric detection at 510 nm was used.

RESULTS AND DISCUSSION

Protonation of the chromatographed compounds
In most of the cation-exchange separations of weak bases, the compounds can be chromatographed in aqueous solutions, have a distinctly basic character and the separations are based essentially on differences in protonation constants. The present separation is different in all these aspects.

N,N-Dimethyl-p-aminobenzeneazobenzoyl amides and esters are non-ionized compounds and are insoluble in water. The dimethylamino group accounts for very weakly basic character of these compounds. Protonation occurs in solutions that contain strong mineral acids and the protonated molecules, carrying a positive charge, can be subject to ion-exchange interactions with cation exchangers. Individual derivatives can be separated by chromatography on cation exchangers in the H⁺ form using solutions of strong acids as the mobile phase[11]. This practical system represents an example of the situation discussed in the theoretical section.

As the compounds studied are insoluble in water, mixed aqueous–organic solutions must be used as the mobile phase. Aqueous ethanol or aqueous methanol is well suited for this purpose.

The differences in the number of carbon atoms in the alkyl substituents could

not be assumed to cause significant differences in the protonation constants of the compounds studied. This assumption was confirmed by spectrophotometric measurements of the protonation constants of selected compounds in ethanolic (80%, v/v) solutions of hydrochloric acid. The results are summarized in Table II. The character of the compounds required measurements to be made in solutions containing both alcohol and acid in high concentrations, so that it was impossible to measure the activity of H^+ ions in solution. For this reason, the concentration of hydrochloric acid was used instead of the activity of the H^+ ions in the calculation of protonation constants. As the purpose of the study was to compare the constants of different compounds rather than to determine the exact thermodynamic values, this approach seems justified.

Before the determination of protonation constants, it was necessary to ascertain how many H^+ ions are attached to one molecule of each N,N-dimethyl-p-aminobenzeneazobenzoyl derivative during the protonation. For this purpose, the spectra

TABLE II

EXPERIMENTAL VALUES OF PROTONATION CONSTANTS, K, AND OF THE RATIOS [B]/[HB$^+$] FOR SELECTED N,N-DIMETHYL-p-AMINOBENZENEAZOBENZOYL AMIDES AND ESTERS IN 80% (v/v) ETHANOLIC SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF HYDROCHLORIC ACID

Compounds: 1, methyl ester; 2, ethyl ester; 3, n-propyl ester; 4, n-amyl ester; 5, n-nonyl ester; 6, isopropyl ester; 7, methylamide; 8, n-butylamide; 9, dimethylamide; 10, di-(n-butyl)amide. The experimental ratios [B]/[HB$^+$] represent the arithmetic means of three values evaluated by measuring the absorbance of a solution containing $1.2 \cdot 10^{-3}$ g/l of the compound studied, at three wavelengths (520, 530 and 540 nm; close to the absorption maxima of the protonated forms) in a 2-cm measuring cell, making use of the equation

$$\frac{[B]}{[HB^+]} = \frac{A_{HB^+} - A_s}{A_s - A_B}$$

where A_B is the absorbance of the non-protonated form (solutions in 0.794 M ammonia), A_{HB}^+ is the absorbance of the protonated form (solutions in 4.745 M HCl for esters and in 1.574 M HCl for amides and A_s is the absorbance of the solution containing the given concentration of hydrochloric acid. $c_H \cdot [B]$ and [HB$^+$] are the concentrations of the non-protonated and protonated forms, respectively, of the compound studied in a solution containing hydrochloric acid of concentration c_H. $K = $ [HB$^+$]/[B]$\cdot$[H$^+$] represents the arithmetic means of the values calculated for each concentration of hydrochloric acid, c_H, assuming that [H$^+$] $\approx c_H$.

Compound	Ratio [B]/[HB$^+$] in solutions containing hydrochloric acid at different concentrations, c_H						Average value of K
	1.574 M	0.922 M	0.478 M	0.220 M	0.0963 M	0.01106 M	
1	0.047	0.080	0.127	0.303	0.726	9.00	13.8
2	0.036	0.092	0.149	0.294	0.697	8.24	14.0
3	0.023	0.078	0.140	0.290	0.694	5.82	16.6
4	0.036	0.078	0.126	0.261	0.646	6.14	16.0
5	0.056	0.099	0.162	0.305	0.698	7.45	12.8
6	0.050	0.074	0.133	0.269	0.656	7.66	14.1
7	0*	0.055	0.116	0.252	0.604	7.87	16.6
8	0*	0.037	0.099	0.213	0.570	7.11	19.8
9	0*	0.051	0.090	0.226	0.624	8.57	17.7
10	0*	0.095	0.173	0.340	0.787	9.42	11.8

* Complete protonation is assumed.
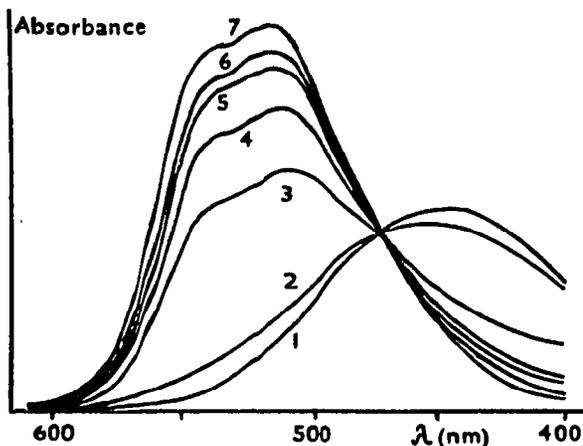
Fig. 2. Spectra of the *n*-butyl ester of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid in solutions containing various concentrations of hydrochloric acid in 80% (v/v) ethanol. Hydrochloric acid concentration (M): 1, 0.01106; 2, 0.0963; 3, 0.220; 4, 0.478; 5, 0.922; 6, 1.574; 7, 4.745.

of three model derivatives (the *n*-butyl ester, methylamide and dimethylamide) were measured in solutions containing different concentrations of hydrochloric acid (0.01–4.74 M) in 80% ethanol in the visible region (360–750 nm). All of the spectral curves for the *n*-butyl ester have a common intersection (isosbestic) point (Fig. 2), which indicates that the protonation to the first step occurs only in the range of acid concentrations studied. All of the spectral curves for the methylamide and dimethyl-amide intersect at the isosbestic point, with the exception of the curve for the most concentrated acid solution, 4.745 M hydrochloric acid (Fig. 3). This means that the protonation of the amides to the second step is involved in solutions with high acid concentrations. In practice, however, these solutions are not used as mobile phases and it can be assumed that the protonation of the amides to the first step predominates in less acidic solutions.
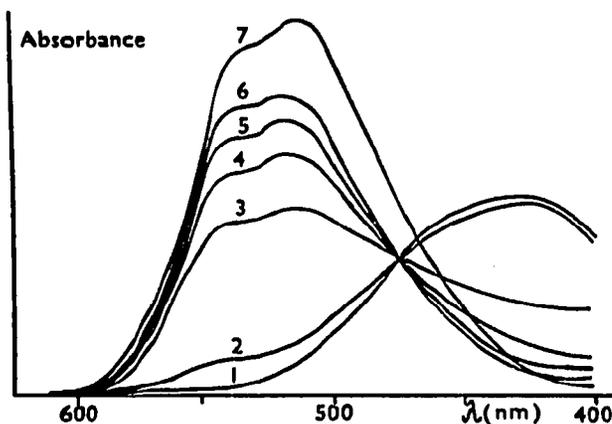
Fig. 3. Spectra of the dimethylamide of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid in solutions containing various concentrations of hydrochloric acid in 80% (v/v) ethanol. Concentrations of hydrochloric acid for curves 1–7 are as in Fig. 2.

In the calculation of protonation constants, the complete protonation of esters in 4.745 M hydrochloric acid was considered, while it was necessary to assume the complete protonation of amides in 1.574 M hydrochloric acid. This assumption probably does not much influence the results. The protonation of amides is unlikely to differ significantly from that of esters, which exceeds 95% in 1.574 M hydrochloric acid, as can be seen from Table II.

The experimental values of the protonation constants for all of the compounds studied (esters and primary and secondary amides) are similar and do not show any systematic shift with increase in the length of the alkyl chains; the differences can be attributed to experimental errors. The experimental results thus confirm the original assumption concerning the separation mechanism. Chromatographic separations of N,N-dimethyl-p-aminobenzeneazobenzoyl amides and esters are obviously not controlled by the differences in protonation of the individual derivatives, but by the differences in the selectivity of the cation exchanger for the individual compounds.

The molecular (non-ionic) sorption on ion exchangers caused by interactions of the skeleton of the ion exchanger with the hydrocarbon part of the organic molecule is suppressed in the presence of an organic solvent in the outer solution[12]. The differences in chromatographic behaviour between the individual N,N-dimethyl-p-aminobenzeneazobenzoyl derivatives can most probably be explained on the basis of a partition mechanism between the outer solution and the liquid inside the ion-exchange beads. These two solutions differ in the content of alcohol. Structural differences between the individual derivatives can be accompanied by significant differences in polarities. The resulting differences in partition between the two liquid phases enable chromatographic separations to be achieved.

The distribution coefficients of N,N-dimethyl-p-aminobenzeneazobenzoyl esters and amides depend on the following factors:

(a) The chemical structure of the derivative.

(b) The concentration of the strong acid (pH) in the outer solution.

(c) The nature and concentration of the organic solvent (alcohol) in the outer solution. These factors influence the swelling of the exchanger and the ratio of the concentration of the organic solvent in the outer solution to that in the liquid inside the particles of the exchanger.

(d) The properties of the cation exchanger (structure of the skeleton, nature of the functional (exchange) groups, ion-exchange capacity, porosity and degree of cross-linking). All of these properties significantly influence the swelling of the exchanger and the solvation of the exchange groups and thus control the concentration of the alcohol and of H^+ ions inside the ion-exchange phase. An appropriate choice of the last three parameters is decisive for successful chromatographic separations.

Choice of the cation exchanger

The properties of the cation exchangers used in the present work are given in Table I. Both the selectivity and the efficiency of separation on different exchangers were compared. Experimental values of distribution coefficients, capacity ratios, numbers of theoretical plates and plate heights of some derivatives on the cation exchangers tested are given in Table III. The mobile phase consisted of 0.922–0.935 M hydrochloric acid in 76.5–80% (v/v) ethanol in most experiments.

TABLE III

ELUTION PARAMETERS OF THE N,N-DIMETHYL-*p*-AMINOBENZENEAZOBENZOYL ESTERS AND AMIDES IN CHROMATOGRAPHY ON DIFFERENT CATION EXCHANGERS USING ETHANOLIC SOLUTIONS OF HYDROCHLORIC ACID AS THE MOBILE PHASE

Cation exchangers: A, Dowex 50W-X8, 100–200 mesh: B, Ostion LG KS 0804 (8% DVB), 20–40 μm; C, macroporous (6% DVB), 80–90 μm; D, Dowex 50W-X4, 200–400 mesh; E, Dowex 50W-X2, 200–400 mesh (all in H$^+$ form). Mobile phases: I, 0.922–0.935 M HCl in 80% (v/v) ethanol; II, 0.925 M HCl in 50% (v/v) ethanol; III, 0.921 M HCl in 95.8% (v/v) ethanol; IV, 0.221 M HCl in 80% (v/v) ethanol; V, 0.935 M HCl in 80% (v/v) methanol. Chromatographed derivatives of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid: 1, methyl ester; 2, ethyl ester; 3, *n*-propyl ester; 4, *n*-butyl ester; 5, *n*-amyl ester; 6, *n*-hexyl ester; 7, *n*-octyl ester; 8, *n*-nonyl ester; 9, *n*-decyl ester; 10, dimethylamide; 11, diethylamide; 12, di-(*n*-propyl)amide; 13, di-(*n*-butyl)amide. u = flow-rate of mobile phase; $D_v = V'_R/V_k$ = volume distribution coefficient; V_k = volume of exchanger bed in column; h = plate height; N = plate number; k' = capacity ratio. Column dimensions: length, 240 mm; I.D., 2.68 mm; volume (V_k), 1.35 ml.

Cation exchanger	Mobile phase	u (ml/min)	Deriv-ative	D_v	k'	N	h(mm)	$h \cdot \dfrac{(1+k')^2}{k'}$	$h \cdot \dfrac{(1+k')^2}{k'} \cdot \dfrac{1}{u}$
A	I	0.0361	1	17.1	54.1	30	8.0	449	12.4
			5	4.7	14.8	13	18.5	312	8.6
			8	1.3	4.1	5	50.0	318	8.8
B	I	0.0340	1	18.1	57.4	310	0.8	46	1.4
			3	9.2	28.9	185	1.3	40	1.2
			5	5.2	16.3	98	2.4	45	1.3
			6	3.7	11.8	56	4.3	60	1.8
			8	2.0	6.3	26	9.1	77	2.3
C	I	0.0479	1	10.5	33.3	35	6.8	241	5.0
			3	6.1	19.2	21	11.2	238	5.0
			5	4.2	13.1	20	12.2	185	3.9
			8	2.1	6.5	14	16.9	147	3.1
D	I	0.0361	1	10.0	31.5	104	2.3	77	2.1
			3	6.2	19.6	72	3.3	72	2.0
			5	4.3	13.7	57	4.2	67	1.8
			8	2.1	6.8	34	7.1	63	1.7
E	I	0.01604	1	6.4	20.3	138	1.7	39	2.4
			3	4.5	14.1	114	2.1	34	2.1
			5	3.5	10.9	117	2.1	27	1.7
			8	2.1	6.8	111	2.2	19	1.2
	I	0.0642	2	5.2	16.6	58	4.1	77	1.2
			4	3.9	12.3	48	5.0	72	1.1
			5	3.5	10.9	43	5.6	72	1.1
			6	3.0	9.4	40	6.0	69	1.1
			7	2.3	7.3	37	6.5	62	1.0
			9	2.1	6.8	29	8.3	74	1.2
	II	0.1245	1	11.6	36.8	41	5.9	227	1.8
			3	10.5	33.1	37	6.5	228	1.8
			5	9.5	30.0	36	6.6	211	1.7
			8	8.3	26.3	28	8.5	239	1.9
	III	0.0311	1	7.9	24.9	95	2.5	68	2.2
			5	4.3	13.6	67	3.6	56	1.8
			8	2.9	9.0	52	4.6	51	1.7
E	IV	0.1245	1	31.9	100.9	96	2.5	257	2.1
			3	20.1	63.6	110	2.2	143	1.2
			5	13.8	43.5	64	3.8	171	1.4
			8	7.3	23.1	47	5.1	128	1.0
	V	0.0311	1	6.6	20.7	155	1.5	35	1.1
			5	4.3	13.6	92	2.6	41	1.3
			8	3.3	10.3	78	3.1	38	1.2
			10	9.6	30.4	162	1.5	48	1.5
			11	6.4	20.1	101	2.4	53	1.7
			12	5.3	16.7	82	2.9	54	1.7
			13	4.3	13.6	106	2.3	36	1.1

Fig. 4 shows the relationship between the logarithm of the capacity ratio and the number of carbon atoms in the alkyl chain in the homologous series of N,N-dimethyl-*p*-aminobenzeneazobenzoates of lower aliphatic alcohols on cation exchangers with different degrees of cross-linking (X2, X4 and X8). The relationship for Dowex 50W-X8 (100–200 mesh) is virtually identical with that for Ostion LG KS 0804 with the same degree of cross-linking and the relationship for the macroporous exchanger (6% DVB) is very similar to the curve for Dowex 50W-X4, and therefore these relationships have been omitted from Fig. 4.
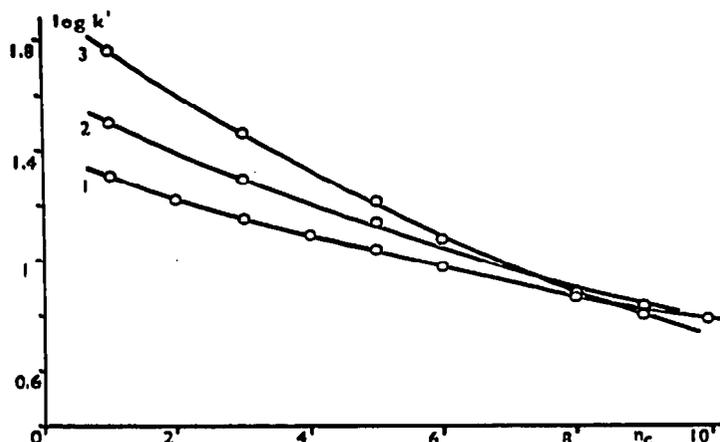
Fig. 4. Relationship between the logarithms of the capacity ratios, k', of aliphatic esters of N,N-dimethyl-*p*-aminobenzeneazobenzoic acid and the number of carbon atoms, n_c, in the alkyl chains for cation exchangers with different degrees of cross-linking. 1, Dowex 50W-X2 (H^+), 200–400 mesh; 2, Dowex 50W-X4 (H^+), 200–400 mesh; 3, Ostion LG KS 0804 (8% DVB) (H^+), 20–40 μm. Column: length, 240 mm; I.D., 2.68 mm; volume, 1.35 ml. Mobile phase: 0.922–0.935 M HCl in 76.5% (v/v) ethanol.

Table III and Fig. 4 show that there are no large differences between the capacity ratios of the esters with higher alkyl groups (C_8–C_{10}) on different cation exchangers and that the capacity ratios are low in comparison with those of lower homologous esters. The decrease in the number of carbon atoms in the alkyl chain is related to an increase in the distribution coefficients. The relative value of this increase is related to the degree of cross-linking of the exchanger, so that the greatest differences in selectivity between the individual homologues are found for cation exchangers with the highest degree of cross-linking (Ostion LG KS 0804 and Dowex 50W-X8), while the opposite occurs for the exchanger with the lowest degree of cross-linking (Dowex 50W-X2).

An explanation of the relationships observed can be attempted by considering that the increasing length of the non-polar alkyl chain will decrease the polarity of the protonated ester or amide. This effect is connected with increasing solubility of a particular derivative in the outer solution which contains a higher amount of ethanol and, consequently, a decrease in sorption should be expected. Provided that the number of carbon atoms in the alkyl chains increases, the relative contribution of each further carbon atom to the decreasing polarity and distribution coefficients of

the protonated derivatives becomes less significant, so that the greatest differences in polarity and sorption are observed with the first members of the homologous series.

A possible explanation of the differences in sorption behaviour of the protonated derivatives on cation exchangers with different degrees of cross-linking is as follows. An increase in the degree of cross-linking is connected with a decrease in the ratio of the concentration of ethanol in the solution inside the ion exchanger to the concentration in the outer solution[7]. This effect favours a decrease in sorption with increasing degree of cross-linking. On the other hand, an increase in the degree of cross-linking restricts the swelling of the exchanger. Table I demonstrates that neither the exchange capacity per gram of dry exchanger nor the percentage of the useful capacity for the derivatives studied depends significantly on the degree of cross-linking (with the exception of the macroporous exchanger). Therefore, the concentration of H^+ ions inside the exchanger and, consequently, the sorption of the derivatives studied should increase with increasing degree of cross-linking. The two effects described act in the opposite manner and probably compensate one the other for the esters with higher alkyl chains (C_8–C_{10}), so that the sorption on different cation exchangers is approximately equal, regardless of the degree of cross-linking (Fig. 4). The influence of the higher concentration of H^+ ions inside the exchanger will probably predominate if more polar esters with lower alkyl chains are sorbed and their capacity ratios increase with increasing degree of cross-linking.

The efficiency of separation on different cation exchangers can be compared using Table III. The plate number (N) decreases (the plate height h increases) with increasing length of the alkyl chains in the homologous series of N,N-dimethyl-*p*-aminobenzeneazobenzoyl esters (with decreasing distribution coefficients) for all of the exchangers and flow-rates of the mobile phase tested, with the exception of Dowex 50W-X2 with the lowest flow-rate of the mobile phase. This effect is more pronounced at higher degrees of cross-linking of the exchanger.

The relationship between the plate height, h, and the flow-rate of the mobile phase, u, in ion-exchange chromatography can be written as[13]:

$$h = h_m + h_e + h_{tr} + h_{tf} =$$

$$= \frac{d_0 \cdot \varepsilon}{\sqrt{2}} \cdot \frac{1}{u} + 1.64 r_p + \frac{D_v}{(D_v + \varepsilon)^2} \cdot \frac{0.142 r_p^2}{d_r} \cdot u +$$

$$+ \left(\frac{D_v}{D_v + \varepsilon}\right)^2 \cdot \frac{0.266 r_p^2}{d_0 \cdot (1 + 70 r_p \cdot u)} \cdot u \qquad (6)$$

where D_v is the distribution coefficient of the chromatographed compound, ε is the ratio of the interstitial volume, V_m, to the column bed volume, V_k, r_p relates to the radius of ion-exchange beads and d_r, d_0 to the diffusion coefficients in the ion-exchange phase and in the outer solution, respectively. The partial terms in this equation correspond to the contributions of molecular diffusion (h_m), eddy diffusion (h_e) and the mass-transfer resistance in the ion exchanger (inner diffusion, h_{tr}) and in the thin film layer of liquid surrounding each bead (film diffusion, h_{tf}) to the total plate height, h.

According to eqn. 6, the most distinct dependence on the distribution coefficient can be expected for the term h_{tr}. This indicates that the influence of inner

diffusion on the plate height predominates in the systems studied. In order to demonstrate the influence of inner diffusion more clearly, Table III includes the values of plate height multiplied by the factor $(1 + k')^2/k'$, which is proportional to the term $(D_v + \varepsilon)^2/D_v$:

$$\frac{(D_v + \varepsilon)^2}{D_v} = \varepsilon \cdot \frac{(1 + k')^2}{k'} \tag{7}$$

The values of $h \cdot (1 + k')^2/k'$ for the different derivatives studied fall within a considerably narrower range than the values of h. This is in agreement with the assumption of the predominant influence of inner diffusion on the broadening of the elution zones. Chromatography on Dowex 50W-X2 at the lowest flow-rate of the mobile phase (0.01604 ml/min) is an exception, where the agreement between the individual values of h is better than that between the terms $h \cdot (1 + k')^2/k'$, which show a constant shift with increasing D_v (or k'). In this instance, the elution zones probably move down the column slowly enough to allow equilibrium conditions to be established and inner diffusion no longer controls the plate height. A similar effect was observed in the mobile phase containing hydrochloric acid in low concentration (0.221 M), where the elution zones also move slowly due to the high values of k'. Here, the plate height, h, increases with increasing length of the alkyl chains (decreasing k') to approximately the same extent as the values of $h \cdot (1 + k')^2/k'$ decrease, even at a relatively high flow-rate of the mobile phase. This indicates that here the inner diffusion contributes only partially to h. A similar trend, although with a higher contribution from inner diffusion, was observed with the macroporous cation exchanger.

The term $h \cdot (1 + k')^2/k' \cdot u$ ought not to depend on the nature of the chromatographed compounds, on the degree of cross-linking of the ion exchanger or on the flow-rate of the mobile phase, provided that inner diffusion predominates and the values of this term for different exchangers should be related to each other in the ratio of the squares of the particle radii. The experimental values in Table III are in rough agreement with these assumptions. The decrease in particle diameter will thus cause a very significant increase in the efficiency of the chromatographic process.

The efficiency of separation is strongly influenced by the flow-rate of the mobile phase. Fig. 5 shows the increase in plate height, h, with flow-rate for the n-amyl ester of N,N-dimethyl-p-aminobenzeneazobenzoic acid on Dowex 50W-X2, 200–400 mesh, using 0.925 M hydrochloric acid in 80 % ethanol as the mobile phase. As mentioned, inner diffusion controls h at all flow-rates of the mobile phase except the lowest, and this influence increases as the flow-rate is increased. This effect is most probably responsible for the curvature of the experimental graph. To obtain a higher efficiency of separation, it is necessary to use low flow-rates of the mobile phase.

Work at higher flow-rates is also limited by the mechanical properties of the bed of ion-exchanger. As illustrated in Fig. 6, the bed of the cation exchanger with a low degree of cross-linking (Dowex 50W-X2, 200–400 mesh) is subject to such a large contraction, even when moderate flow-rates of the mobile phase are used (*ca.* 30 ml/h with the 240 mm × 1.34 mm I.D. column) that the pressure drop across the column increases sharply and blockage of the column can eventually result. The
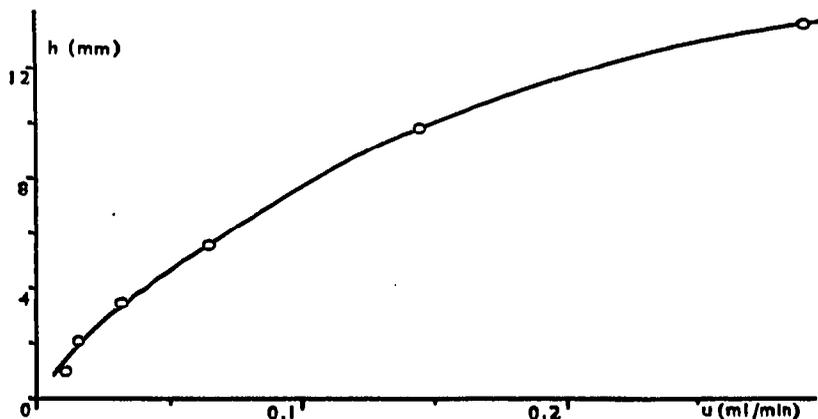
Fig. 5. Influence of the flow-rate of the mobile phase (u) on plate height (h) in the chromatography of the n-amyl ester of N,N-dimethyl-p-aminobenzeneazobenzoic acid. Cation exchanger: Dowex 50W-X2 (H$^+$), 200–400 mesh. Column: length, 240 mm; I.D. 2.68 mm; volume, 1.35 ml. Mobile phase: 0.925 M HCl in 80% (v/v) ethanol.
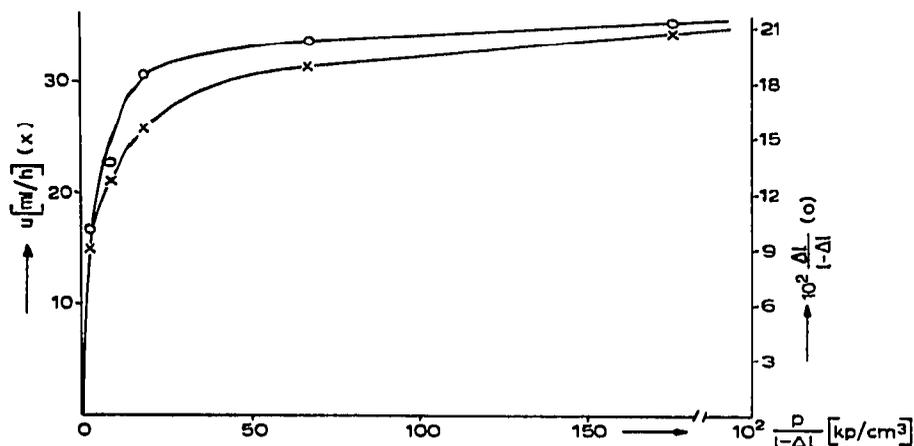
Fig. 6. Influence of the flow-rate of the mobile phase (u) on the properties of the ion-exchange bed, Dowex 50W-X2 (H$^+$), 200–400 mesh. Column dimensions and composition of the mobile phase as in Fig. 5. p = pressure drop across the column; l = initial length of the column (240 mm); Δl = contraction of the ion-exchange bed (mm).

flow of the mobile phase is then stopped or the system could even be damaged. On the other hand, the use of low flow-rates of the mobile phase is disadvantageous, as long analysis times are required.

CONCLUSIONS

The choice of an appropriate cation exchanger for the chromatographic separation of the derivatives of N,N-dimethyl-p-aminobenzeneazobenzoic acid in mixed aqueous–organic solutions of acids should respect the following conditions:

(a) A sufficiently small particle diameter of the cation exchanger is essential if a satisfactory efficiency of separation is to be achieved;

(b) Cation exchangers with a higher degree of cross-linking (X8) yield higher differences in selectivity and the best resolution in a given mobile phase. A disadvantage of these exchangers is the long elution times necessary with the lower homologues. Mobile phases with high concentrations of acid must be used in order to achieve satisfactory elution. Therefore, these exchangers are more suitable for separations of the complete homologous series. The resolution of the higher homologues will be better than when cation exchangers with low degrees of cross-linking are used.

(c) Cation exchangers with low degrees of cross-linking show smaller differences in selectivity for the individual homologues than the highly cross-linked exchangers and the elution of the homologous series can be effected more rapidly. The separation can be achieved using solutions with a lower concentration of acid, which offers a better possibility for the control of selectivity by changing the composition of the mobile phase. The exchangers have larger pores in comparison with the more highly cross-linked exchangers; influence of the inner diffusion can thus be better suppressed and the separation is nearer to equilibrium conditions. The mechanical stability of these exchangers is relatively smaller, which means that higher flow-rates of the mobile phase cannot be used. The exchangers with lower degrees of cross-linking will be more suitable for the separation of amides and esters with shorter alkyl chains or for the separation of samples containing lower homologues in admixture with higher homologues.

REFERENCES

1 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
2 P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 223.
3 P. Jandera and J. Churáček, *J. Chromatogr.*, 93 (1974) 17.
4 P. Jandera and J. Churáček, *J. Chromatogr.*, 104 (1975) 9.
5 P. Jandera and J. Churáček, *J. Chromatogr.*, 98 (1974) 1.
6 P. Jandera and J. Churáček, *Czech. Pat.*, No 161.380 (1974), Appl. No. PV8200-72 (1972).
7 O. Samuelson, *Ion Exchange Separations in Analytical Chemistry*, Almqvist & Wiksell, Stockholm, Göteborg, Uppsala, 1963.
8 J. Churáček, J. Říha and M. Jureček, *Z. Anal. Chem.*, 249 (1970) 120.
9 J. Churáček, *J. Chromatogr.*, 48 (1970) 241.
10 J. Churáček, M. Hušková, H. Pechová and J. Říha, *J. Chromatogr.*, 49 (1970) 511.
11 J. Churáček and P. Jandera, *J. Chromatogr.*, 53 (1970) 69.
12 C. W. Davies and B. D. R. Owen, *J. Chem. Soc.*, (1956) 1681.
13 J. Inczédy, *Analytische Anwendungen von Ionenaustauschern*, Akadémiai Kiadó, Budapest, 1964.