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DISTRIBUTION MECHANISM OF IONIZABLE SUBSTANCES IN DYNAMIC ANION-EXCHANGE SYSTEMS USING CATIONIC SURFACTANTS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The mechanism of distribution of acidic compounds in dynamic anion-exchange systems consisting of a hydrophobic column packing in combination with mixtures of *n*-propanol and water containing cetyltrimethylammonium bromide (cetrimide) was investigated.

Ion exchange, partition towards micelles and physical distribution contribute to the retention of ionizable substances. The influence of the concentration of cetrimide, bromide, buffer and *n*-propanol in the eluent, pH and temperature on the amount of adsorbed cetrimide and the retention behaviour was investigated.

The applicability of dynamic ion-exchange systems to the separation of ionizable substances with biomedical or clinical relevance was demonstrated by using a variety of system parameters to adjust the retention.

INTRODUCTION

Dynamic (solvent generated) ion-exchange chromatography is a form of liquid chromatography in which a column packing with a hydrophobic surface is used in combination with water–organic solvent mixtures containing a small amount of a cationic or anionic surfactant. This type of chromatography, applied by Wittmer *et al.*[1] and later introduced as paired ion chromatography (PIC) by Waters Assoc.[2] and as soap chromatography by Knox and co-workers[3,4], has proved to be very suitable for the chromatography of ionizable organic substances[1,3–7].

For reasons to be discussed in this paper, we term the method dynamic ion exchange. Dynamic ion exchange shares characteristics with conventional ion exchange[8], with liquid–liquid chromatography using liquid ion exchangers as the stationary phase[9,10] and with reversed-phase ion-pair partition chromatography[11–13].

Previously two models have been proposed for the chromatographic retention mechanism in dynamic ion exchange. In one model, it is assumed that first ion pairs are formed in the mobile phase, which can distribute between the mobile and the stationary phase[3,7,11–13]. In the other model, it is assumed that ion exchange

occurs between solute ions and counter ions of the surfactant adsorbed at the interface[4,14].

The aim of this study was to elucidate further the retention mechanism, particularly for acidic compounds in dynamic anion-exchange systems.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatographic (HPLC) experiments were carried out on a high-pressure liquid chromatograph (Siemens SP 200, Siemens, Karlsruhe, G.F.R.) using UV detection (variable wavelength, Zeiss PM 2 DLC, Zeiss, Oberkochen, G.F.R.), a high-pressure sampling valve (Valco, CV-6-UHPa) with a 10-μl loop, a stainless-steel column (length 150 mm, I.D. 3.0 mm, O.D. 6.4 mm) and a linear potentiometric recorder (Goertz, Servogor 542).

All feed lines were constructed from stainless-steel 316 tubes and Swagelok zero dead volume couplings. A wavelength of 235 or 254 nm was selected, depending on the absorption of the eluent. The experiments were performed at 23°.

For the breakthrough curves a differential refractometer (R401, Waters Assoc., Milford, Mass., U.S.A.) was used.

Gas chromatographic (GC) experiments were performed with a Model 900 gas chromatograph (Perkin-Elmer, Norwalk, Conn., U.S.A.) using flame-ionization detection and a stainless-steel column (length 2 m, I.D. 2.0 mm).

Materials

In all experiments double-distilled water was used. Cetryltrimethylammonium bromide (cetrimide) was obtained from BDH (Poole, Great Britain). As the solid column support for HPLC, the hydrophobic material LiChrosorb RP-8, 10 μm (Merck, Darmstadt, G.F.R.), and for GC Porapak QS, 100–120 mesh (Waters Assoc.), was used. All chemicals were of analytical-reagent grade.

Procedures

The HPLC columns were packed by a balanced-slurry technique[15]. The column was washed successively with acetone and the appropiate mixture of *n*-propanol and water (both 100× the column volume) and conditioned with the eluent containing cetrimide (100–200× the column volume).

Before measuring the breakthrough curves of *n*-propanol or cetrimide, the column was washed with methanol and water (100 and 300× the column volume, respectively). Next, the eluent without cetrimide was pumped through and fractions of 0.1 g were collected and their *n*-propanol contents determined by GC using nitrogen as the carrier gas at 200°. Then a fixed amount of cetrimide was added to the eluent and the breakthrough curve was recorded with a differential refractometer using the eluent without cetrimide as a reference. Cetrimide in the eluate can be identified by a colour reaction with bromophenol blue[16]. After subtracting the void volume of the column, the amount of adsorbed *n*-propanol or cetrimide can be calculated as described in the literature[17].

RESULTS AND DISCUSSION

To investigate the mechanism of retention of organic acids in dynamic anion exchange, a C-8 bonded silica was chosen as the hydrophobic column packing, mixtures of *n*-propanol and water containing cetrimide being selected as the eluent.

The amount of adsorbed cetrimide was determined for a number of eluent compositions and temperatures by means of breakthrough curves. Fig. 1 shows the detector response as a function of the amount of eluate when the eluent composition is changed from 25% (w/v) of *n*-propanol to 25% (w/v) of *n*-propanol containing 0.4% (w/v) of cetrimide. Two steps in the detector response can be noticed. The first step occurs as a result of the displacement of *n*-propanol at the interface by cetrimide, and the second is caused by the breakthrough of cetrimide. The amount of adsorbed cetrimide for a number of eluent compositions and temperatures is given in Table I.

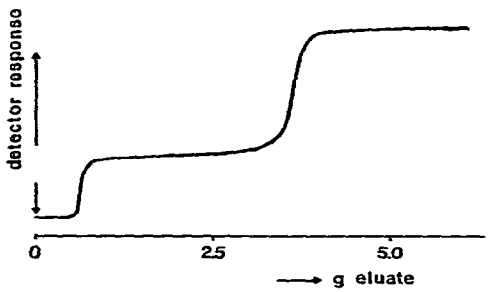


Fig. 1. Breakthrough curve of cetrimide using a refractive index detector. Eluent: 25% (w/v) *n*-propanol, 0.4% (w/v) cetrimide, pH 6.0.

The effect of the presence of cetrimide in the eluent was investigated by measuring the capacity ratios (k_i') of organic acids on a column initially loaded with cetrimide by elution of mixtures of *n*-propanol and water containing 0.4% (w/v) of cetrimide and subsequently eluted with an eluent of the same composition but without cetrimide. Stable phase systems were obtained up to 12.5% (w/v) of *n*-propanol. Table II shows the retention behaviour of some organic acids in the presence and absence of cetrimide in the eluent and when no cetrimide is present in the mobile phase as well as in the stationary phase. It is shown clearly that strong retention occurs when the stationary phase is loaded with cetrimide and no cetrimide is present in the eluent and that the retention is diminished when cetrimide is present in the eluent.

From the results in Table I and particularly in Table II, the following conclusions can be drawn.

Firstly, the very strong retention in the absence of cetrimide in the eluent can be explained by means of an anion-exchange mechanism[4,14] according to

$$(CA)_a + (X^-)_m \rightleftarrows (CX)_a + (A^-)_m \quad (1)$$

where CA = cetrimide, X^- = solute ion, A^- = counter ion and the subscripts a and m refer to the adsorbed and the mobile phase, respectively.

TABLE I

AMOUNT OF CETRIMIDE ADSORBED AS A FUNCTION OF THE ELUENT COMPOSITION AND TEMPERATURE

Variable	*Value*	*Eluent**	*Cetrimide adsorbed (mg)*	*Eluent**	*Cetrimide adsorbed (mg/g)*	*Eluent**	*Cetrimide adsorbed (mg/g)*
Cetrimide concentration (%, w/v)	0.1	A	12.0	B	13.3	C	19.2
	0.2		16.7		22.3		26.3
	0.5		27.8		33.3		37.8
	1.0		37.0		40.7		43.7
	2.0		47.9		47.9		49.6
NaBr concentration (*m*)	0	D	13.3				
	0.025		20.8				
	0.050		26.6				
	0.075		30.9				
	0.100		32.7				
Phosphate concentration (*m*)	0	D	13.3				
	0.010		17.7				
	0.025		21.3				
	0.050		22.6				
	0.075		23.9				
pH	1.8	E	24.1				
	4.0		24.7				
	6.0		24.1				
	7.5		22.9				
Temperature (°C)	25	F	21.2				
	30		19.3				
	35		18.0				
	40		16.7				
	45		16.0				
n-Propanol concentration (%, w/v)	10	G	140.8(75.7**)				
	15		92.4(114.0**)				
	20		51.9(158.4**)				
	25		33.7(202.9**)				

* Eluents:
A = 25% (w/v) *n*-propanol, 0.055 *M* phosphate buffer, pH 6.0.
B = 25% (w/v) *n*-propanol, 0.055 *M* phosphate buffer, 0.055 *M* total bromide, pH 6.0.
C = 25% (w/v) *n*-propanol, 0.055 *M* total bromide, pH 1.6.
D = 25% (w/v) *n*-propanol, 0.4/%(w v) cetrimide, pH 6.0.
E = 25% (w/v) *n*-propanol, 0.4% (w/v) cetrimide, 0.036 *M* total bromide.
F = As E, pH 6.0.
G = 0.4% (w/v) cetrimide, 0.111 *M* total bromide, pH 6.0.

** Adsorbed *n*-propanol (mg/g)

Secondly, the presence of cetrimide in the eluent causes a significant decrease in retention. From this result, it can be concluded that the retention model of adsorption of ion pairs formed in the mobile phase[3,7,11–13] according to

$$(C^+)_m + (X^-)_m \rightleftarrows (CX)_s \qquad (2)$$

can be excluded. This model should predict no retention in the absence of cetrimide and an increase in retention in the presence of cetrimide.

TABLE II

EFFECT OF THE PRESENCE OF CETRIMIDE IN THE ELUENT ON THE CAPACITY RATIO (k_i')

n-Propanol concentration: 10% (w/v).

Compound	k_i'		
	A^*	B^{**}	C^{***}
1,2,3-Benzenetricarboxylic acid	0	5.78	6.31
Benzoic acid	0.44	7.48	10.00
6-Hydroxy-1-naphthalenesulphonic acid	0.31	19.40	44.14
Salicylic acid	0.78	22.07	50.24
1-Naphthol-4-sulphonic acid	0.72	29.84	131.09
8-Amino-1-naphthol-3,6-disulphonic acid	0	30.68	42.57

* Unloaded column, eluent + 0.1 *M* NaBr, pH 6.0.
** Loaded column, eluent + 0.4% (w/v) cetrimide, 0.1 *M* NaBr. pH 6.0.
*** Loaded column, eluent + 0.1 *M* NaBr, pH 6.0.

The decrease in the retention in the presence of cetrimide in the eluent can be explained by interactions between cetrimide associates and solute ions. In this respect, it can be noticed that micelles of cetrimide will be present in the eluent in all of the experiments because of the low critical micelle concentration (*CMC*) ($CMC = 10^{-3}$–10^{-4} $M \approx 0.04$–0.004%, w/v)[18]. This does not imply that micelles are adsorbed at the interface. It has been reported that organic anions can be incorporated into the core of cetrimide micelles, which can occur without the loss of bromide ion[19]. The partition coefficient of aromatic sulphonic acids between the aqueous solution and cetrimide micelles was found to be of the order of $1 \cdot 10^4$–$2 \cdot 10^4$ (ref. 19). On the basis of these data, it seems reasonable to assume that under the experimental conditions used here the solute ions in the mobile phase are completely incorporated by the cetrimide micelles.

The expression for the overall distribution constant (K_X) of the acid HX can be derived from the following equilibria in which three phases are involved, *viz.*, the adsorbed phase, a, the mobile phase, m, and the micelle phase, micel:

$$(HX)_m \overset{K_{HX}}{\rightleftarrows} (HX)_a \tag{3}$$

$$(H^+)_m + (X^-)_m \overset{K_1}{\rightleftarrows} (HX)_m \tag{4}$$

$$(CA)_a + (X^-)_m \overset{K_2}{\rightleftarrows} (CX)_a + (A^-)_m \tag{5}$$

$$(X^-)_m \overset{K_3}{\rightleftarrows} (X^-)_{micel} \tag{6}$$

where

K_{HX} = partition coefficient of the undissociated acid HX between the adsorbed and mobile phase;

K_1 = formation constant of the acid HX;
K_2 = ratio of the formation constants of the adsorbed associates CX and CA (selectivity coefficient);
K_3 = partition coefficient of the anion X^- between the micelle and mobile phase.

The overall distribution constant of the acid HX at a constant concentration of *n*-propanol in the eluent is given by

$$K_X = \frac{[HX]_a + [CX]_a}{[HX]_m + [X^-]_m + [X^-]_{micel}} = K_{X1} + K_{X2} \tag{7}$$

where

$$K_{X1} = K_{HX} \cdot \frac{1}{(1 + 1/K_1[H^+]_m + K_3/K_1[H^+]_m)} \tag{8}$$

$$K_{X2} = K_2[CA]_a \cdot \frac{1}{[A^-]_m} \cdot \frac{1}{(1 + K_1[H^+]_m + K_3)} \tag{9}$$

The first term, K_{X1}, describes the physical distribution and the second term, K_{X2}, the anion-exchange process. At $1/K_1\,[H^+]_m \ll 1$, the first term in general predominates and then $K_X = K_{HX}$. At $K_1\,[H^+]_m \ll 1$, the second term will predominate and then $K_X = K_2\,[CA]_a/(1 + K_3)\,[A^-]_m$. As is known from conventional ion exchange[8], for *n*-basic acids an approximate expression for the ion exchange contribution can be derived:

$$K_{X2} = \frac{K_2}{(1 + K_3)} \cdot [CA]_a^n \cdot \frac{1}{[A^-]_m^n} \tag{10}$$

Eqn. 10 predicts a proportional relationship between K_X (and hence the capacity ratio, k_i') and $[CA]_a^n$ and $[A^-]_m^{-n}$, in which n is the charge on the solute.

The influence of the concentration of adsorbed cetrimide on k_i' was measured for different cetrimide concentrations in the eluent at pH 1.6 and 6.0 and constant concentrations of the counter ion, buffer and *n*-propanol. As shown in Fig. 2, up to a concentration of adsorbed cetrimide of 40 mg/g for all solutes the dependence of k_i' on $[CA]_a$ is in good agreement with eqns. 7–10. The slopes of the log–log correlations equal the charge on the solute when ion exchange predominates, as predicted by eqn. 10. At higher cetrimide concentrations in the eluent deviations occur for most solutes. These are probably caused by an increase in K_3 as a result of an increase in the number, size or charge of the micelles[18,20]. Some solutes show a correlation with eqn. 10 over the whole range of cetrimide concentrations investigated. This is in agreement with Table II, from which it can be concluded that these solutes have little tendency to be incorporated in the core of cetrimide micelles and hence K_3 will be small.

The influence of the counter-ion concentration on k_i' was measured for different bromide concentrations at pH 1.8 and 6.0 and constant concentrations of

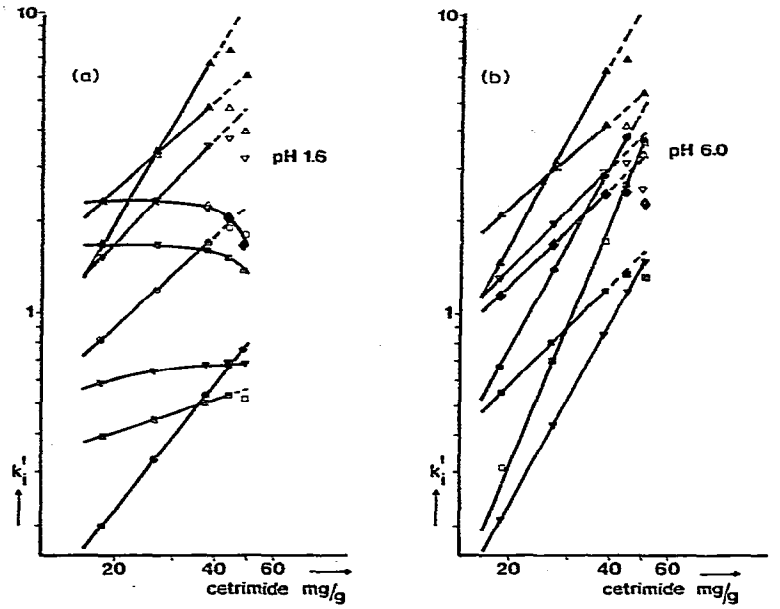


Fig. 2. Dependence of the capacity ratio on the concentration of adsorbed cetrimide at different cetrimide concentrations and pH of the eluent. (a) Eluent: 25% (w/v) *n*-propanol, 0.055 *M* total bromide, pH 1.6, 0.1–2.0% (w/v) cetrimide. (b) Eluent: 25% (w/v) *n*-propanol, 0.055 *M* phosphate buffer, 0.055 *M* total bromide, pH 6.0, 0.1–2.0% (w/v) cetrimide. See Table I for concentration of adsorbed cetrimide. ▲, 1-Naphthol-3,8-disulphonic acid; △, 2-naphthalenesulphonic acid; ▽, 1-naphthol-4-sulphonic acid; ○, 6-hydroxy-1-naphthalenesulphonic acid; ◆, salicylic acid; ■, benzoic acid, ●, 8-amino-1-naphthol-3,6-disulphonic acid; ▼, 5-aminoisophthalic acid; □, 1,2,3-benzenetricarboxylic acid.

cetrimide and *n*-propanol in the eluent. Fig. 3 shows that the dependence of k_i' on $[Br^-]_m$, accounting for the change in $[CBr]_a$ (see Table I), agrees with eqns. 7–10. The slopes of the log–log correlations are in good agreement with the charge on the solutes when ion exchange is the dominating distribution process. At pH 6.0, however, deviations are observed at low bromide concentrations (*e.g.*, no extra bromide addition). This must be attributed to the significant changes in the size and charge of the micelles, which occur on adding extra bromide[18,20]. The results confirm that K_3 is constant at higher bromide concentrations and that K_3 increases sharply when no extra bromide is present. Moreover, it was noticed that the stability and performance of the phase system improved significantly on increasing the ionic strength of the eluent.

It should be borne in mind that buffer ions might also act as counter ions and influence the capacity ratio, as can be seen in Table III. Although the general effect of the buffer concentration (with HPO_4^{2-}) fits the picture well, a more precise description is complex because of the presence of bromide ions, which are still involved in the ion-exchange process.

The influence of pH on k_i' is dependent on the relative magnitudes of the physical distribution and ion-exchange terms given by eqns. 7–9. Fig. 4 shows the

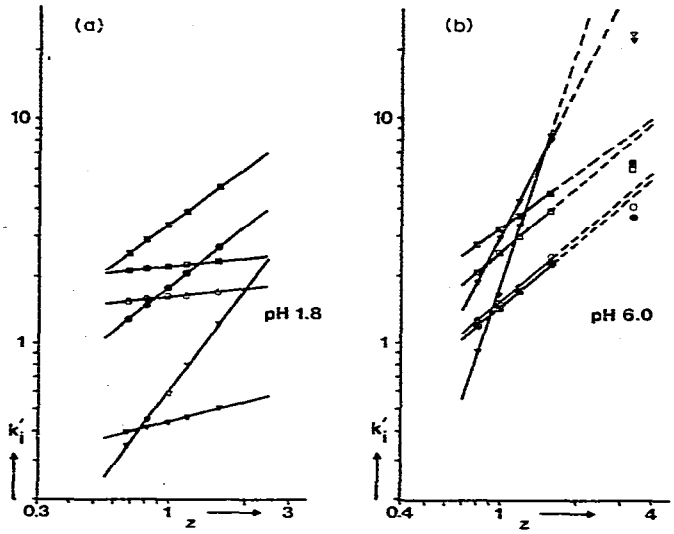


Fig. 3. Dependence of the capacity ratio on the bromide concentration at different pH, accounting for the change in the concentration of adsorbed cetrimide with the bromide concentration. $z = [CBr]_s^n/[Br^-]_m^n$. (a) Eluent: 25% (w/v) *n*-propanol, 0.4% (w/v) cetrimide, 0.025 *M* HBr, pH 1.8, 0–0.1 *M* NaBr. (b) Eluent: 25% (w/v) *n*-propanol, 0.4% (w/v) cetrimide, pH 6.0, 0–0.1 *M* NaBr. See Table I for concentration of adsorbed cetrimide. ■, 1-Naphthol-4-sulphonic acid; ●, 6-hydroxy-1-naphthalenesulphonic acid; □, salicylic acid; ▽, 8-amino-1-naphthol-3,6-disulphonic acid; ○, benzoic acid; ▼, 1,2,3-benzenetricarboxylic acid.

TABLE III

INFLUENCE OF THE PHOSPHATE BUFFER CONCENTRATION ON THE CAPACITY RATIO (k_i')

Mobile phase D (see Table I).

Compound	k_i'				
	Phosphate concentration (M)				
	0	*0.01*	*0.025*	*0.050*	*0.075*
3-Aminobenzoic acid	2.25	1.49	1.13	0.84	0.69
6-Hydroxy-1-naphthalenesulphonic acid	3.79	2.74	2.25	1.73	1.55
Benzoic acid	4.07	2.93	2.31	1.95	1.64
Salicylic acid	6.09	4.47	3.96	3.22	2.78
1-Naphthol-4-sulphonic acid	6.41	4.98	4.36	3.64	3.21
5-Aminoisophthalic acid	10.57	5.17	2.88	1.63	1.09
1,2,3-Benzenetricarboxylic acid	22.94	19.35	9.70	4.25	2.47
8-Amino-1-naphthol-3,6-disulphonic acid	23.05	12.26	8.06	4.88	3.52

results when the pH was varied at constant concentrations of cetrimide and bromide. For monovalent acids a retention behaviour is found, as was shown previously in liquid–liquid systems using a liquid ion exchanger as the stationary phase[9,10]. For weak multivalent acids a large pH effect is found according to the increase in the contribution of the ion-exchange process with increasing charge on the solute.

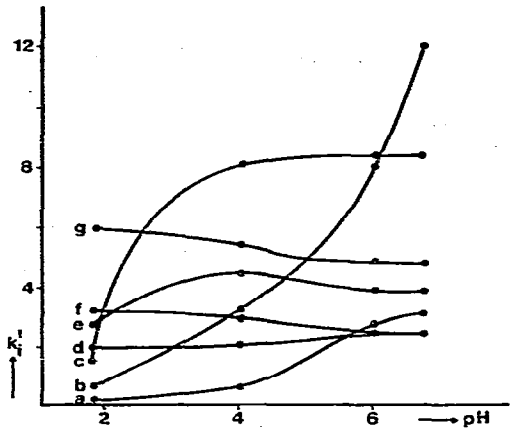


Fig. 4. Influence of pH on the capacity ratio. Eluent E (see Table I). a, 5-Aminoisophthalic acid; b, 1,2,3-benzenetricarboxylic acid; c, 8-amino-1-naphthol-3,6-disulphonic acid; d, benzoic acid; e, salicylic acid; f, 6-hydroxy-1-naphthalenesulphonic acid; g, 1-naphthol-4-sulphonic acid.

The influence of temperature on k_i' is shown in Table IV. Accounting for the decrease in $[CA]_a$ with increasing temperature (see Table I), a residual decrease in k_i' is still found with increasing temperature, as is commonly found in chromatography[21,22]. As expected[5,9], the increase in temperature improved the column efficiency and pressure drop. However, almost no significant shifts in the selectivity factor (r_{ji}) were found, as might be expected when only one distribution mechanism occurs (*i.e.*, ion exchange)[9,10].

TABLE IV

INFLUENCE OF TEMPERATURE ON THE CAPACITY RATIO (k_i') AND SELECTIVITY FACTOR (r_{ji}) OF ACIDS FOR A DYNAMIC ANION EXCHANGE SYSTEM AT pH 6

Mobile phase F (see Table I).

Compound	*Temperature (°C)*									
	25		*30*		*35*		*40*		*45*	
	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}	k_i'	r_{ji}
3-Aminobenzoic acid	1.12		0.99		0.89		0.80		0.75	
6-Hydroxy-1-naphthalenesulphonic acid	2.25	2.00	1.91	1.93	1.66	1.87	1.46	1.83	1.34	1.79
Benzoic acid	2.29	1.02	2.05	1.07	1.76	1.06	1.54	1.05	1.42	1.05
5-Aminoisophthalic acid	2.69	1.17	2.36	1.15	2.07	1.18	1.84	1.20	1.72	1.21
Salicylic acid	3.72	1.38	3.24	1.37	2.81	1.36	2.45	1.33	2.25	1.31
1-Naphthol-4-sulphonic acid	4.33	1.16	3.64	1.12	3.05	1.09	2.57	1.05	2.29	1.02
8-Amino-1-naphthol-3,6-disulphonic acid	7.29	1.68	6.20	1.75	5.11	1.68	4.24	1.65	3.83	1.67
1,2,3-Benzenetricarboxylic acid	7.73	1.06	6.72	1.08	5.49	1.07	4.67	1.10	4.16	1.08

The influence of the concentration of *n*-propanol on k_i' was measured at pH 6.0 and constant concentrations of cetrimide and bromide. The results are given in Table V and can be compared with the capacity ratios that were found when no cetrimide was present in the mobile phase or in the stationary phase. According to Table I, the adsorption of cetrimide and *n*-propanol is strongly affected by the con-

TABLE V

DEPENDENCE OF THE CAPACITY RATIO (k') ON THE CONCENTRATION OF *n*-PROPANOL AT pH 6.0

Compound	*n-Propanol concentration (%, w/v)*							
	10		*15*		*20*		*25*	
	k'_{11}*	k'_{12}**	k'_{11}*	k'_{12}**	k'_{11}*	k'_{12}**	k'_{11}*	k'_{12}**
1,2,3-Benzenetricarboxylic acid	0	5.78	0	3.43	0	1.41	0	0.56
Benzoic acid	0.44	7.48	0.24	4.16	0.21	2.06	0.19	1.13
6-Hydroxy-1-naphthalenesulphonic acid	0.31	19.40	0.18	7.78	0.08	2.86	0.03	1.19
Salicylic acid	0.78	22.07	0.43	10.47	0.24	4.45	0.14	1.96
1-Naphthol-4-sulphonic acid	0.72	29.84	0.36	15.83	0.18	6.54	0.10	2.52
8-Amino-1-naphthol-3,6-disulphonic acid	0	30.68	0	13.43	0	4.94	0	2.03
2-Naphthalenesulphonic acid	2.94	35.93	1.31	20.96	0.55	8.96	0.29	3.50

* 1 = Unloaded column, eluent + 0.1 *M* NaBr, pH 6.0.
** 2 = Loaded column, eluent + 0.4% (w/v) cetrimide, 0.1 *M* NaBr, pH 6.0.

centration of *n*-propanol. When the concentration of *n*-propanol influences only $[CA]_a$, the slope of the correlation between k'_i and $[CA]_a$ should be equal to the charge (n) on the solute on the basis of eqn. 10. However, because of the changes in K_2 and K_3 on changing the *n*-propanol concentration, such a correlation was not found.

The applicability of dynamic ion-exchange systems to the analysis of peptides, vitamins, drugs and food additives, which can be found in body fluids or foods, is demonstrated in Figs. 5–11. The separation of dipeptides detected by means of a ninhydrin reactor[5] is shown in Fig. 5. The determination of quinine in lemon tonic

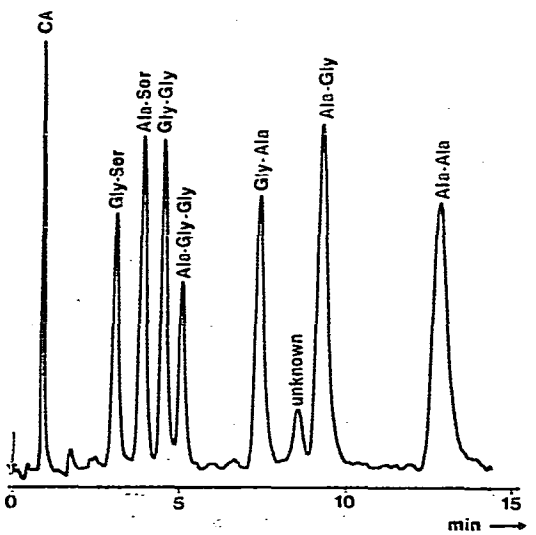


Fig. 5. Analysis of a test mixture of dipeptides using an anionic surfactant.Eluent: 2% (w/v) *n*-propanol, 0.3% (w/v) sodium dodecylsulphate, 0.01 *M* sodium ciyrate, pH 3.25. Column length, 15 cm; Injection volume, 20 μl; detection by reaction with ninhydrin; CA = cysteic acid.

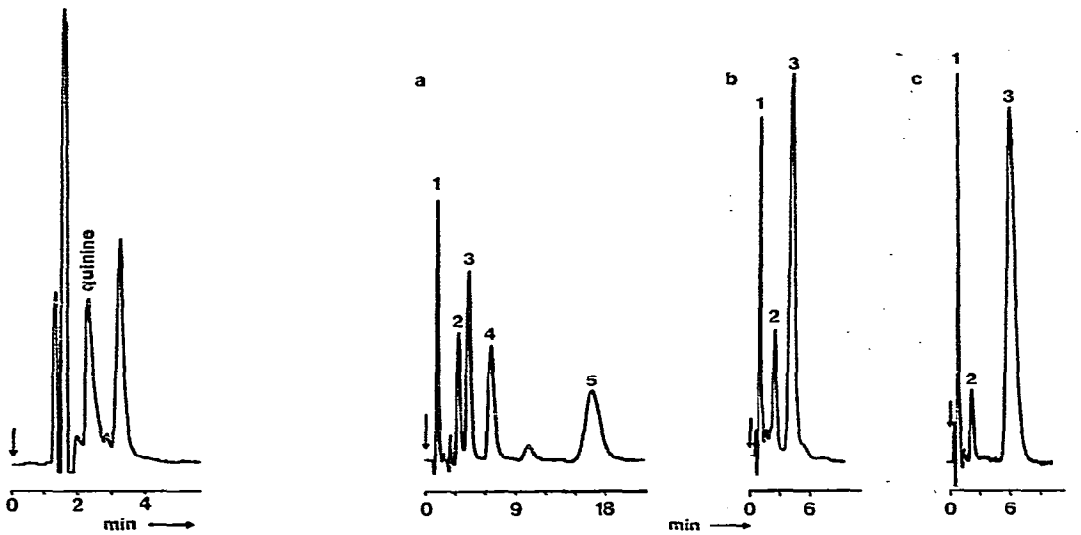


Fig. 6. Analysis of quinine in lemon tonic using an anionic surfactant. Eluent: 25% (w/v) *n*-propanol, 0.01% (w/v) sodium dodecylsulphate, 0.025 *M* Na_2SO_4, pH 6.4. Column length, 15 cm; injection volume, 10 μl; wavelength, 243 nm; 1 ml of tonic containing 45 mg/l of quinine was diluted with 2 ml of eluent.

Fig. 7. Analysis of dyes in a test mixture and in sugared chocolate candies using cetrimide. (a) Eluent: 25% (w/v) *n*-propanol, 0.5% (w/v) cetrimide, 0.01 *M* NaBr, 0.05 *M* NaH_2PO_4, pH 3.5. Column length, 15 cm; injection volume, 10 μl; wavelength, 243 nm. Peaks: 1 = brilliant green (C.I. 420420); 2 = patent blue (C.I. 42051); 3 = indigotine carmine (C.I. 73015); 4 = fast yellow (C.I. 13015); 5 = amaranth red (C.I. 16185). (b) Eluent: 25% (w/v) *n*-propanol, 0.5% (w/v) cetrimide, 0.05 *M* NaBr, pH 6.0. Column length, 15 cm; injection volume, 100 μl; wavelength, 243 nm. The sugar layer of a brown candy was dissolved in 2.5 ml of eluent. Peaks: 1 = background; 2 = indigotine carmine (C.I. 73015); 3 = sunset yellow (C.I. 15895). (c) Conditions as in (b). The sugar layer of a green candy was dissolved in 2.5 ml of eluent. Peaks: 1 = background; 2 = patent blue (C.I. 42051); 3 = tartrazine (C.I. 19140).

with no pre-treatment is shown in Fig. 6. Fig. 7a shows the separation of some dyes that might be used as food additives. As shown in Fig. 7b and c, some of these dyes (those which are allowed by law for colouring foods) can be determined in sugared chocolate candies. The separation of vitamins is illustrated for B vitamins in coated tablets in Fig. 8a and for vitamin A and D in drops in Fig. 8b. Fig. 9 shows the separation of a mixture of sulpha drugs in tablets. The determination of adrenaline in eye-drops is shown in Fig. 10. Fig. 11 illustrates the determination of indomethacin and salicylic acid by direct injection of deproteinized, diluted plasma.

CONCLUSIONS

The results indicate that the mechanism of retention of organic acids in dynamic anion-exchange systems using cetrimide is governed by anion exchange, partition towards the micelles and physical distribution. The concentrations of cetrimide, counter ion and *n*-propanol, pH and temperature, or a combination of these parameters, can be used to adjust the order and degree of retention.

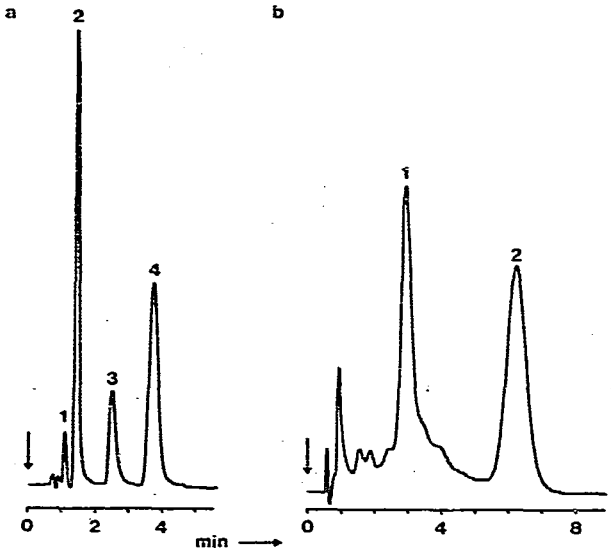


Fig. 8. (a) Analysis of B vitamins in dragées using an anionic surfactant. Eluent: 6% (w/v) *n*-propanol, 0.03% (w/v) sodium dodecylsulphate, 0.02 *M* malic acid, 0.08 *M* total Na^+, pH 2.5. Column length, 15 cm; injection volume, 20 μl; wavelength, 280 nm. A 12-mg amount of a dragée was diluted with 10 ml of eluent. Peaks: 1 = vitamin B_6; 2 = nicotinamide; 3 = vitamin B_1; 4 = vitamin B_2. (b) Analysis of vitamins A and D in drops using cetrimide. Eluent: 40% (w/v) *n*-propanol, 0.2% (w/v) cetrimide, 0.1 *M* NaBr, pH 6.0. Column length, 15 cm; injection volume, 100 μl; wavelength, 287 nm). A 280-mg amount of drops containing 9000 and 3000 IU/ml of vitamin A and D, respectively, was diluted with 3 ml of eluent. Peaks: 1 = vitamin A; 2 = vitamin D.

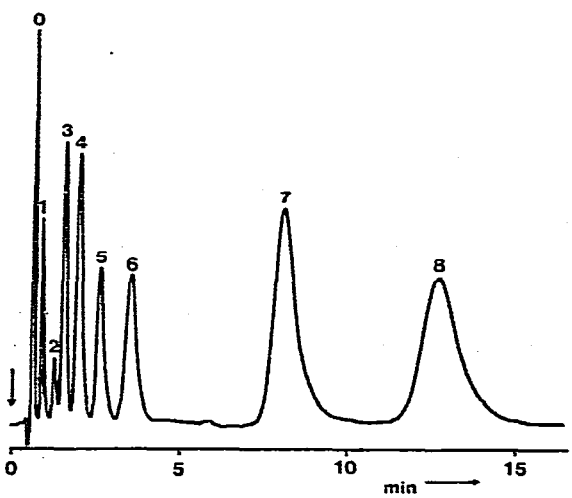
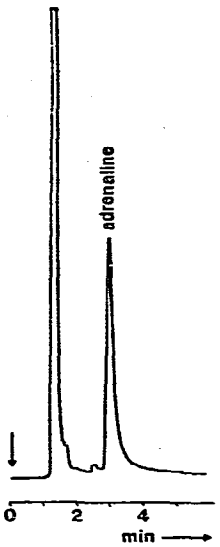


Fig. 9. Separation of a test mixture of sulpha drugs using cetrimide. Eluent: 10% (w/v) *n*-propanol, 0.5% (w/v) cetrimide, 0.025 *M* phosphate, pH 6.0. Column length, 15 cm; injection volume, 10 μl; wavelength, 243 nm. Peaks: 1 = sulphanilamide; 2 = sulphapyridine; 3 = sulphadiazine; 4 = sulphamethoxypyridazine; 5 = sulphacetamide; 6 = sulphamethazine; 7 = sulphamethoxazole; 8 = sulphamethizole.



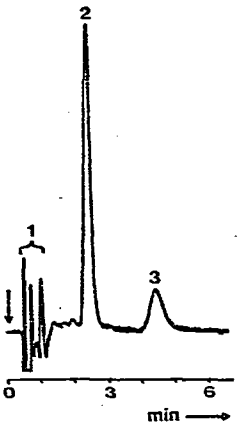


Fig. 10. Analysis of adrenaline in eye-drops using an anionic surfactant. Eluent: 20% (w/v) *n*-propanol, 0.05% (w/v) sodium dodecylsulphate, 0.01 *M* Na_2SO_4, pH 6.4. Column length, 15 cm; injection volume, 10 μl; wavelength, 280 nm. A 120-mg amount of drops containing 10 mg /ml of adrenaline were diluted with 5 ml of eluent.

Fig. 11. Direct injection of deproteinized serum containing salicylic acid and indomethacin using cetrimide. Eluent: 25% (w/v) *n*-propanol, 0.4% (w/v) cetrimide, 0.025 *M* NaBr, 0.05 *M* phosphate, pH 5.0. Column length ,15 cm; injection volume, 100 μl; wavelength, 280 nm. A 0.2-ml volume of plasma containing 50 μg/ml of salicylic acid and 5 μg/ml of indomethacin was deproteinized with *n*-propanol and 20-fold diluted up to eluent composition. Peaks: 1 = serum background; 2 = salicylic acid; 3 = indomethacin.

The stability and performance of the phase system is significantly improved under conditions of high ionic strength. Dynamic anion-exchange systems show excellent kinetic properties. Together with the pressure-stable column packings, this allows for highly efficient columns with high separation speeds. Moreover, the type and capacity of the ion exchanger can be varied without changing the column packing.

Additional experiments with dynamic cation-exchange systems showed a retention mechanism similar to that found for dynamic anion-exchange systems presented in this paper.

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