Journal of Chromatography, 506 (1990) 61–74
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1791

Studies on system peaks in ion-pair adsorption chromatography

III. Regulation of system peak gradient retention for obtaining analyte peak compression

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SUMMARY

Peak compression can be obtained when an analyte is eluted in the co-ion gradient in a large system peak. Matching of the analyte retention with this gradient was investigated. The chromatographic system was based on silanized silica and an acidic eluent, containing acetonitrile and a probe, which was an UV-detectable hydrophobic amine, protriptyline. Large system peaks, or zones, containing probe deficiencies were induced by injecting high concentrations of a hydrophobic organic anion together with cationic analytes. The back part of these system zones consisted of a steep gradient of increasing probe (co-ion) concentration, giving strongly compressed peaks when low analyte amounts were also eluted with this back-gradient. It was found that variation of the probe concentration was an efficient way of matching the retention of the system peak with analytes of widely varying retentions.

INTRODUCTION

When a solution deviating in composition from the eluent is injected into a chromatographic system, the established equilibria between the solid phase and the mobile phase are disturbed. This results in migrating zones of each eluent component participating in an interaction common with a component in the injected sample. These migrating zones, so-called system zones, can only be visualized if at least one of the equilibrated eluent components can be detected[1]. The direction of the system peak will be positive or negative, depending on the charge and retention volume of the sample component relative to the corresponding eluent component[2,3].

0021-9673/90/$03.50

When a reversed-phase ion-pair adsorption chromatographic system is equilibrated with an organic eluent cation, large system peaks can be induced by the injection of high concentrations of an organic anion. The back part of these negative system peaks consists of a steep gradient of increasing cation concentration. Co-elution of cationic analytes with this system peak gradient (co-ion) resulted in extremely narrow analyte peaks[4,5]. When an analyte had other positions within the system zone, peak deformations were obtained instead[5].

Both peak compression and deformation effects were previously investigated in a reversed-phase ion-pair liquid chromatographic (LC) system, but no systematic changes of the probe concentration in the eluent were performed[5]. The parameters governing the retention volumes of co-ion gradient and analyte have also been investigated[5,6]. When the analyte and the system zone were eluted close to each other, fine adjustments of the retention volumes were made by changing the concentration of the anion in the injection solution in order to obtain peak compression.

The aim of this study was to investigate the effect of different probe concentrations for matching system zone and analyte retention volumes, in order to obtain peak compression for analytes with widely different retention volumes. For this purpose, it was important to investigate carefully the parameters determining the retention of the gradient in the back part of the large system zone or peak.

THEORETICAL

The retention equations used are based on the stoichiometric ion-pair adsorption model[6–10]. Retentions of both the analytes and the eluent component (system peak) are described by the partial derivative of the adsorption isotherm[6]. However, different retention equations will result, owing to the different starting conditions. At the start of elution, the analyte concentration is zero, whereas the concentration of the eluent component is finite.

Eqns. 1–3 describe the net retention volume of a cationic analyte, HA^+, an anionic analyte, Z^-, and the organic eluent component, the probe Q^+. In order to simplify the discussion, the solid phase is assumed to contain only one type of adsorption site. X^- is a buffer component present in the eluent. The subscript m means mobile phase and the concentration unit is molarity.

$$V_{N,HA} = \frac{W_s K_0 K_{HAX}[X^-]_m}{1 + K_{QX}[Q^+]_m[X^-]_m} \tag{1}$$

$$V_{N,Z} = \frac{W_s K_0 K_{QZ}[Q^+]_m}{1 + K_{QX}[Q^+]_m[X^-]_m} \tag{2}$$

$$V_{N,Q} = W_s \cdot \frac{C_{Q,s}}{C_{Q,m}} = \frac{W_s K_0 K_{QX}[X^-]_m}{(1 + K_{QX}[Q^+]_m[X^-]_m)^2} \tag{3}$$

K_0 gives the total adsorption capacity of the solid phase, and K_{HAX}, K_{QX} and K_{QZ} are the adsorption constants. The analyte equations assume symmetrical peaks. For high analyte concentrations, which affect both retention and peak shape, no

quantitative equation is yet available. However, equations containing an additional term in the denominator, including the analyte concentration, can be used for qualitative discussions[6,7]. Eqn. 2, corresponding to the anion Z^-, will then also contain a term in both the numerator and the denominator involving the ion-pair distribution with the cationic buffer component.

The retention equation for the system peak is valid for only infinitesimal changes of the eluent component. In this study, large system peaks or zones were created. The retentions of these large disturbances can only be discussed qualitatively with support of eqn. 3.

At high concentrations all zones affect each other when they initially migrate together at the top of the column. The equations discussed assume that these effects are negligible, *i.e.*, the situation when the migrating zones have separated from each other.

EXPERIMENTAL

Apparatus, chromatographic technique and preparation of the eluent

These were as described previously[6,7]. For all injections a 100-μl loop was used.

Chemicals

Analytical-reagent grade chemicals were used unless indicated otherwise.

Acetonitrile (LiChrosolv) and dichloromethane were obtained from Merck (Darmstadt, F.R.G.) and protriptyline (PT) from Merck, Sharp and Dohme (Haarlem, The Netherlands). Sodium octanesulphonate and octylsulphate were obtained from Eastman-Kodak (Rochester, NY, U.S.A.), sodium nonyl- and decylsulphate from Merck and sodium nonane- and decanesulphonate from Fluka (Buchs, Switzerland). Phosphoric acid (99% crystalline) and 1 *M* sodium hydroxide solution (Titrisol) were obtained from Merck.

Desipramine and imipramine hydrochloride used as analytes were obtained from Ciba-Geigy (Basle, Switzerland). The substituted benzamides also used as analytes are denoted FLA combined with a number (*cf.*, ref. 6). They were synthesized at CNS Research and Development, Astra Research Centre (Södertälje, Sweden).

Detection technique

The detection technique has been described previously[5,7]. When the analyte was a benzamide, its signal was measured at a wavelength where only this compound absorbed[6]. For desipramine or imipramine as analytes, their signals were recorded at 252 nm, where PT also absorbs. To compensate for the PT absorbance, 322 nm was used as a reference, as the PT absorbance at 322 nm equals that at 252 nm. The PT signal was recorded at a wavelength where only PT absorbed.

The compensating technique was used only when an analyte was present. In the other chromatographic runs only the PT signal was recorded. At the lowest PT concentration used in the eluent, the PT signal was recorded at the wavelength maximum at 291 nm. With increasing PT concentrations longer wavelengths were chosen, *viz.*, 320, 330 and 337 nm, respectively, resulting in lower absorbances. The reason for this was that the detector used did not measure negative peaks with lower relative absorbance than -0.1 a.u.f.s.

Retention volume and asymmetry factor (asf)

These were determined as described previously[5].

Four different concentrations of the secondary amine protriptyline (PT; the probe) as additive were used in the reversed-phase ion-pair LC system in this study.

Determination of negative zone parameters

Very large negative system peaks had a zone shape (see Fig. 1). The depth of the negative system peak or zone is given in concentration units and is represented as ΔC. The change in PT concentration in the zone relative to the bulk concentration, C_b, was then obtained by taking the ratio $\Delta C/C_b$. This value was used as a measure of the magnitude of the probe equilibrium disturbance and is < 1 only for system peaks, not zones. The width of the zone, $w_{b,z}$, was determined at half-depth of the negative zone as described in Fig. 1.

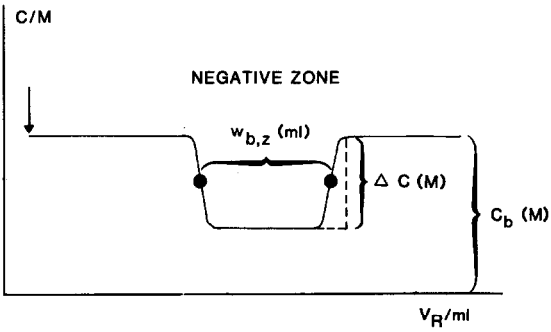

Fig. 1. Principle of measurement of parameters in a negative system zone. C_b (M) is the bulk concentration of probe in the eluent; ΔC (M) is the depth of the zone; the ratio $\Delta C/C_b$ will be the degree of the equilibrium disturbance; $w_{b,z}$ (ml) is the zone width.

RESULTS AND DISCUSSION

Solid phases consisting of silanized silica have often been found to contain two different kinds of adsorption sites with different capacities and affinities[6,7,10,11]. In previous work, the adsorption of PT on Nucleosil C_{18} was studied, indicating a two-site adsorption behaviour of the Langmuir type[6]. According to this, the strong site was covered to 3, 12, 41 and 78%, respectively, at the four different PT concentrations used in this work. At the two highest PT concentrations, the weak site was covered to 5 and 22%, respectively. The adsorption isotherm is assumed to be linear when less than 10% of the adsorption sites are covered with the compound of interest[6,7,10,12]. The two lower PT concentrations used then corresponded fairly closely to the linear part of the adsorption isotherm, whereas the higher PT concentrations corresponded to the non-linear part.

Substituted benzamides[6], desipramine and imipramine were used as cationic analytes.

In reversed phase ion-pair chromatography, equilibrium disturbances are easily obtained by the injection of an organic ion into the system. This results in migrating

zones of the different eluent components that are involved in the disturbances. If at least one of the eluent components is detectable, these zones will appear as positive or negative system peaks[1–3].

In this study, the equilibria of PT were disturbed by injecting a large amount of an organic anion (sulphonate or sulphate) with a retention higher than that of the probe. The injection of such an organic anion increases the distribution of the cationic probe to the solid phase. The anion is eluted together with the excess of the probe (a positive probe peak) (see Fig. 2). The compensating deficiency was visualized as a negative probe peak (the system peak) with lower retention. When larger amounts of the anion were injected, the probe deficiencies in the eluate appeared as negative zones. The depth of the zone, ΔC, approached the level of the probe concentration in the eluent, C_b. At such a large equilibrium disturbance with the ratio $\Delta C/C_b$ close to 1.0, the probe concentration in the zone was close to zero and in the back part of the system zone the probe concentration increased steeply. The retention of this gradient, $V_{R,G}$, was determined according to Fig. 2.

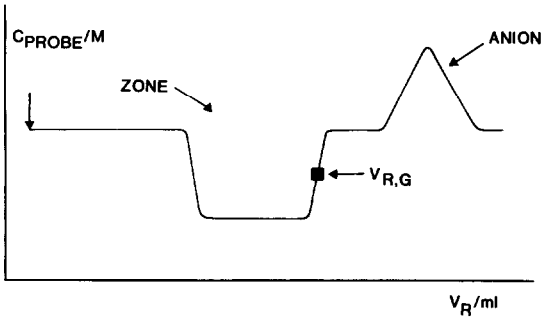

Fig. 2. Schematic representation of the probe signal after the injection of a high concentration of a hydrophobic anion. The gradient retention volume, $V_{R,G}$ (ml), is measured at half-depth of the negative zone.

Peak compression was obtained when a cationic analyte was injected together with a large amount of an organic anion and was eluted together with the back part of the negative probe system zone[4,5]. In this situation the analyte retention volume is greater than that in the isocratic experiment, *i.e.*, analyte injected without organic anion in the injection solution[5].

Retention regulation

The most useful peak compressions occur when the analyte peak is eluted with the gradient of the back part of the system zone or peak. The parameters determining the retention volume of this gradient and the analyte were therefore carefully studied. In this work, the emphasis was on the effects of the probe concentration in the eluent, which was systematically varied. The ionic strength and pH were kept constant.

The regulation of the analyte retention has been described elsewhere[5–7,10,11]. The retention volume of the cationic analyte decreased with increasing concentration of PT, the latter then acting as a co-ion (*cf.*, eqn. 1). If the cationic analyte was injected together with an organic anion, the analyte retention volume increased[5].

TABLE I

RETENTION VOLUMES AND ASYMMETRY FACTORS OF CATIONIC ANALYTES WHEN INJECTED INTO THE SYSTEM EQUILIBRATED WITH ELUENT LACKING A PROBE

Injected sample: $1.0 \cdot 10^{-5}$ *M* analyte in phosphate buffer (pH 2.0). Eluent: phosphate buffer (pH 2.0)–acetonitrile (3:1).

Analyte	V_R *(ml)*	*asf*	*asf*[a]
FLA 870	12.0	2.3	1.6
FLA 965	16.0	2.7	
FLA 659	19.5	2.4	1.2
Desipramine	19.9	2.3	1.1
Imipramine	22.5	2.6	1.1
Protriptyline (PT) (probe)	21.8	2.2	

[a] Eluent contained $9.5 \cdot 10^{-4}$ *M* protriptyline.

The retention volumes of the analytes, including PT, injected as an analyte, when the system was equilibrated with eluent without probe, are given in Table I. The analyte retention volume decreased and the peak symmetry was improved with increasing probe concentration in the eluent.

Elution of imipramine and desipramine at a high PT concentration ($9.5 \cdot 10^{-4}$ *M*) resulted in very symmetrical analyte peaks; the asf value was 1.1 (*cf.*, Table I). The structural similarity between the probe (also a tricyclic antidepressant amine) and these analytes indicates similar affinities for the strong site[10]. At this high probe concentration, the strong sites, probably responsible for the analyte peak asymmetry, were covered to 78%[6].

When the organic anion was injected into the system, the probe acted as a counter ion (*cf.*, eqn. 2)[5,6,9,11]. Hence the anion retention volume increased with increasing probe concentration in the eluent[6]. As mentioned, the buffer ions are also of importance for the retention of an organic ion[7].

The anions were injected in order to obtain large negative system zones or peaks of the probe, thus giving the probe gradient. For this purpose, it is necessary that the anion has a larger retention volume than the probe gradient of interest. The system peak retention volume decreases with increasing probe concentration (*cf.*, eqn. 3). With a low probe concentration in the eluent, it was necessary to inject hydrophobic anions, such as decylsulphate, nonylsulphate or decanesulphonate, in order to obtain separation between the system zone and the anion.

At the two higher probe concentrations, $1.9 \cdot 10^{-4}$ and $9.5 \cdot 10^{-4}$ *M*, the less hydrophobic anions, nonanesulphonate and octylsulphate, could also be utilized, because at these probe concentrations the anions had larger retention volumes than the system peak gradient (see Table II). The hydrophobic anions used at the two lowest probe concentrations could also be used at the higher probe concentrations. However, their retention volumes were then very large (*cf.*, Table II).

Large system peak retention

The validity of the system peak equation has been investigated in a previous study[6]. However, the equation is valid only for infinitesimal changes in the probe

TABLE II

RETENTION VOLUMES OF ORGANIC ANIONS WHEN INJECTED INTO THE SYSTEM EQUILIBRATED WITH A HIGH PROBE CONCENTRATION IN THE ELUENT

Injected sample: $5.0 \cdot 10^{-3}$ *M* organic anion in phosphate buffer (pH 2.0). Eluent: $1.9 \cdot 10^{-4}$ *M* protriptyline in phosphate buffer (pH 2.0)–acetonitrile (3:1).

Anion	V_R *(ml)*[a]
Nonanesulphonate	22.0
Octylsulphate	24.8
Decanesulphonate	43.5
Nonylsulphate	>50
Decylsulphate	>84

[a] System zone back part gradient ($V_{R,G}$) = 13.4–17.2 ml.

equilibrium, *i.e.*, very small system peaks with $\Delta C/C_b$ ratios close to zero. For large system peaks the retention equation can be used only qualitatively and at least two additional effects must be taken into consideration. One of them is that large negative system peaks have higher retention volumes than large positive system peaks, owing to the non-linear adsorption isotherm[6]. The system peak retention volume will further be affected by the organic anion injected, its retention volume increasing with increasing anion hydrophobicity[6].

The decanesulphonate anion was more retained than the probe. It was injected at $5.0 \cdot 10^{-3}$ *M* concentration into systems equilibrated with eluents of different probe concentrations. The retention volumes for half the downslope of the front part and half the upslope of the back part, $V_{R,G}$, of the negative system zone or peak for different eluent concentrations of the probe are shown in Fig. 3. With increasing probe concentration, the retention volumes of both the front and the back parts of the system zone decreased. However, the retention volume of the back-part gradient decreased more than the front part, resulting in a decreased zone width. At the two lower probe

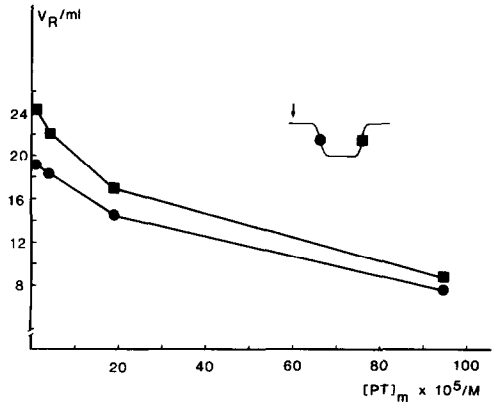

Fig. 3. Retention volumes of the front (●) and back (■) parts of the negative zone or peak, created by the injection of decanesulphonate, *versus* the probe concentration. Sample, $5.0 \cdot 10^{-3}$ *M* anion in phosphate buffer (pH 2.0); eluent, protriptyline in phosphate buffer (pH 2.0)–acetonitrile (3:1).

concentrations, *i.e.*, $7.6 \cdot 10^{-6}$ and $3.8 \cdot 10^{-5}$ *M*, large negative system zones were obtained (*cf.*, Fig. 2), with the ratio $\Delta C/C_b = 1.0$ (see Table III). At the two highest probe concentrations used, large negative system peaks were obtained instead of zones, the $\Delta C/C_b$ ratio being 0.67 and 0.37, respectively. The results indicate that the ability of a certain anion to disturb the probe equilibrium by the magnitude required for a zone to appear instead of a peak decreases at high probe concentrations corresponding to the non-linear part of the adsorption isotherm.

TABLE III

PARAMETERS FOR THE NEGATIVE SYSTEM ZONE OR PEAK OBTAINED BY THE INJECTION OF DECANESULPHONATE INTO THE SYSTEM EQUILIBRATED WITH ELUENTS OF DIFFERENT PROBE CONCENTRATIONS

Injected sample and eluent as in Fig. 3.

PT (M)	ΔC *(M)*	$\Delta C/C_b$	$\frac{V_{R,anion}}{V_{R,G}}$	$\Delta Cw_{b,Z}$ *(mol PT adsorbed)*
$7.6 \cdot 10^{-6}$	$7.6 \cdot 10^{-6}$	1.0	1.2	$0.4 \cdot 10^{-7}$
$3.8 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	1.0	1.4	$1.4 \cdot 10^{-7}$
$1.9 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	0.67	2.6	$3.4 \cdot 10^{-7}$
$9.5 \cdot 10^{-4}$	$3.5 \cdot 10^{-4}$	0.37	10.3	$4.1 \cdot 10^{-7}$

The depth of the negative system peak, ΔC, increased with increasing eluent probe concentration (Table III)[6]. The product of depth and the width of the zone ($\Delta Cw_{b,Z}$) reflects the extra amount of probe (in moles) adsorbed due to the injection of the anion. This value also increased (Table III) with increasing eluent probe concentration; however, at high probe concentrations the increase was relatively smaller.

The smaller amount of PT adsorbed relative to the eluent concentration at high probe concentrations and the smaller equilibrium disturbances are probably due to both the existence of a non-linear adsorption isotherm and increased competition between the anion and the phosphate ion as counter ions to PT (see Theoretical). However, at low probe eluent concentrations, competition is also low, and the anion injections result in ion-pair adsorption of more or less all the content of the probe in a certain region of the eluent.

The separation between the anion and the back gradient increased with increasing probe concentration (see Table III). This is a result both of the increased anion retention volume and the decreased system peak retention volume.

A comparison was made between large system zones or peaks induced by injections of anions of different hydrophobicity. The anions injected were, in order of increasing hydrophobicity (based on retention volumes in the same eluent), decanesulphonate, nonylsulphate and decylsulphate (*cf.*, Table II). The anion concentration was $5.0 \cdot 10^{-3}$ *M* and injections were made at different eluent probe concentrations (see Table IV).

For all three anions, the injections resulted in the appearance of zones at the two lowest probe concentrations. However, at the higher probe concentration, the

TABLE IV

PARAMETERS OF THE NEGATIVE SYSTEM ZONE OR PEAK OBTAINED BY INJECTION OF ANIONS OF DIFFERENT HYDROPHOBICITY

Injected sample and eluent as in Fig. 3. A = Decanesulphonate; B = nonylsulphate; C = decylsulphate.

Zones or peaks	*PT (M)*	$V_{R,G}$ *(ml)*			$w_{b,Z}$ *(ml)*		
		A	*B*	*C*	*A*	*B*	*C*
Zones	$7.6 \cdot 10^{-6}$	24.5	25.6	27.6	5.0	5.6	6.3
	$3.8 \cdot 10^{-5}$	22.2	23.0	23.8	~4	~4	~4
Peaks	$1.9 \cdot 10^{-4}$	16.9	16.9	17.2	~2.5	~2.5	~2.5
		(0.7)[a]	(0.7)[a]	(0.6)[a]			

[a] $\Delta C/C_b$ values for the peaks.

disturbances on the probe equilibrium were smaller, and so peaks appeared instead. The retention volumes of the zone or peak, especially that of the back-part gradient, increased with increasing anion hydrophobicity, the effect being largest at the lowest probe concentrations (Table IV). At the high probe concentration, $1.9 \cdot 10^{-4}$ *M*, corresponding to the non-linear part of the adsorption isotherm, the gradient retention volumes were similar, despite the different anion hydrophobicities. However, the extents of probe equilibrium disturbances were also of the same magnitude.

At the lowest probe concentration the zone width increased with increasing hydrophobicity of the injected anion, while the zone widths were approximately the same at the higher probe eluent concentrations for all anions injected (*cf.*, Table IV). This was probably due to the increased competition at high probe concentrations. Effects of anion hydrophobicity when injecting anion concentrations higher than $5.0 \cdot 10^{-3}$ *M* were not studied.

When nonylsulphate was injected in increasing concentrations at a probe concentration of $1.5 \cdot 10^{-5}$ *M*, an increasingly deeper negative system peak appeared and the retention of the back part increased[5]. However, at a certain anion concentration, the back-part retention began to decrease with a further increase in the anion concentration, probably an effect of the closely eluted anion.

At the two lowest probe concentrations used in this study, similar results were obtained with injections of the hydrophobic anions decanesulphonate and nonylsulphate. At these probe concentrations the more hydrophobic anion, decylsulphate, did not show the tendency for decreasing back-part retention when the anion concentration injected was increased to $5.0 \cdot 10^{-3}$ *M*. The retention volume of decylsulphate was larger than those for the other two anions (*cf.*, Table II), resulting in an improved anion separation from the system zone gradient.

With the higher probe concentration, $1.9 \cdot 10^{-4}$ *M*, the separation between decanesulphonate and the back-gradient was improved (*cf.*, Table III). Injections of decanesulphonate at concentrations up to 0.01 *M* did result in continuously increasing back-part retention volumes (Fig. 4). However, a zone was never developed, and the gradient retention increase was accompanied by an increase in the equilibrium disturbances, *i.e.*, larger $\Delta C/C_b$ values. At this probe concentration the decrease in the back-part system peak retention volumes induced by the octylsulphate anion began at

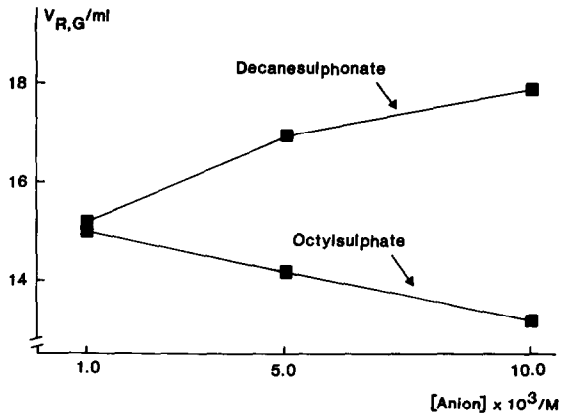

Fig. 4. Back gradient retention volumes, $V_{R,G}$, of system peaks induced by decanesulphonate and octylsulphate at high probe concentration, *versus* the injected anion concentration. Sample, anion in phosphate buffer (pH 2.0); eluent, $1.9 \cdot 10^{-4}$ *M* protriptyline in phosphate buffer (pH 2.0)–acetonitrile (3:1).

the low anion concentration of $1.0 \cdot 10^{-3}$ *M* (Fig. 4). This was also the case for nonanesulphonate, which is similar to octylsulphate in hydrophobicity (*cf.*, Table II). These decreases in gradient retention volume were accompanied by distortions of the system peak shapes (see below). However, when the probe concentration was increased to $9.5 \cdot 10^{-4}$ *M*, the nonanesulphonate concentration could be increased to 0.01 *M* before the decrease in the back-part retention volume started.

The results shown above indicate that it is possible to increase the gradient retention by increasing the anion concentration at different probe eluent concentrations. However, a prerequisite is an adequate anion separation from the gradient, especially when injecting high concentrations of less hydrophobic anions at high probe concentration in the eluent.

It may be assumed that the increase in the gradient retention volume with increasing anion concentration was due mainly to the increased ion-pair distribution with the anion in the start zone at low probe concentration. At high-probe concentrations the increased gradient retention volume may be a consequence of non-linear adsorption behaviour and thus be due mainly to the low concentration of probe in the system peak. In that event, however, it is assumed that the anion concentrations do not reach the levels resulting in distortion of the system peak (see below).

Distortion of anion and system peak

Odd-shaped anion peaks, showing diffuse and deformed front parts, appeared at high anion concentrations injected at all probe concentrations. A prerequisite for this kind of distortion to occur was that the indirectly detected anion peak was eluted after the system peak. At high probe concentrations, the injections of high concentrations of less hydrophobic anions also resulted in odd-shaped system peaks. Unusually shaped large sample and system peaks have also been described by Golshan-Shirazi and Guiochon[13,14].

When a high concentration of the hydrophobic anion decylsulphate was injected

at low probe concentration, the system zone was well shaped and the late-eluted anion peak was tailing (Fig. 5a). When the same concentration of the less hydrophobic decanesulphonate was injected, the anion peak, which eluted very close to the system zone gradient, showed a leading effect, *i.e.*, a diffuse front side and a sharper back side (Fig. 5b). This effect decreased when the probe concentration was increased, and at an adequately high probe concentration the separation was further improved and the decanesulphonate peak showed tailing. If, on the other hand, lower decanesulphonate concentrations were injected, the anion peaks showed tailing. The less hydrophobic anions nonanesulphonate and octylsulphate resulted in very broad and diffuse front parts of the anion peaks when injected at higher probe concentrations in the eluent, $1.9 \cdot 10^{-4}$ *M* (see Fig. 5c). The diffuse front part of the anion peak was extremely broad

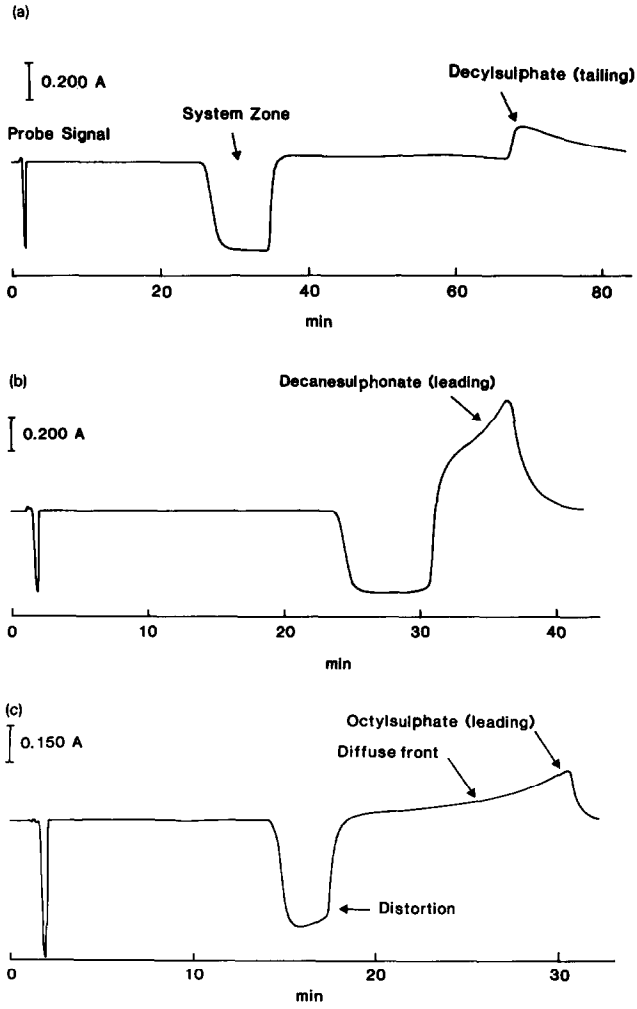

Fig. 5. Shapes of negative system zones and positive anion peaks with injected hydrophobic anions. Sample, $5.0 \cdot 10^{-3}$ *M* anion in phosphate buffer (pH 2.0); eluent, protriptyline in phosphate buffer (pH 2.0)–acetonitrile (3:1). (a) Anion, decylsulphate; PT concentration, $7.6 \cdot 10^{-6}$ *M*. (b) Anion, decanesulphonate; PT concentration, $7.6 \cdot 10^{-6}$ *M*. (c) Anion, octylsulphate; PT concentration, $1.9 \cdot 10^{-4}$ *M*.

and partly overlapped the system peak, also being distorted. A distortion of the system peak is always seen when the diffuse front of the anion peak partly overlaps the back part of the system zone.

Hence for a certain anion injected the deformation of its front decreased with increasing probe concentration. This was probably due to the improved anion separation from the system zone gradient. On the other hand, the high probe concentration increased the risk of deformation for a poorly separated anion. The distortions may be a result of the initial co-migration of the anion zone with the system zone gradient. The probe deficiency in the system zone then gives a lowering of the retention volume of the anion front, resulting in a diffuse front part of the eluted peak (*cf.*, eqn. 2).

Comparison of large and small system peak retentions

Injections of very low analyte concentrations into the system resulted in small system peaks, owing to the low degree of equilibrium disturbances. The retention equation (eqn. 3) is quantitatively valid for these so-called infinitesimal system peaks. The back-part gradient retention volumes of the system zones or peaks, induced by injections of $5.0 \cdot 10^{-3}$ *M* decanesulphonate, were plotted together with the retention volumes of the corresponding infinitesimal system peaks at the different probe concentrations (Fig. 6). In line with eqn. 3, both the gradient retention volume and the infinitesimal system peak retention volume decreased with increasing probe concentration. However, the gradient retention volumes were larger at all probe concentrations. This is partly due to the fact that the retention volumes for the small system peaks were measured at the peak minimum (see ref. 5).

The larger retention volumes for the large system peaks or zones in comparison with the small ones may be explained in the following way. The retention volume of the gradient (large negative system zone or peak) is determined qualitatively by eqn. 3 in combination with the presence of the hydrophobic anion and a low concentration of

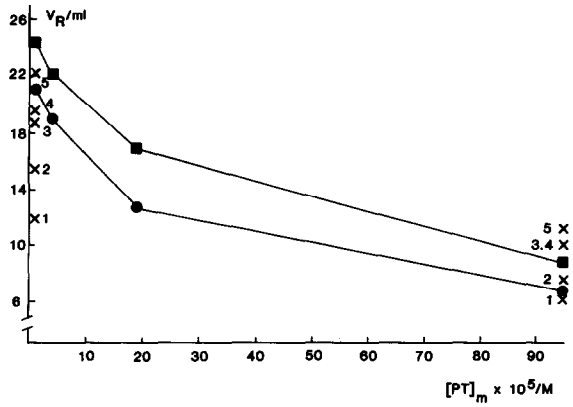

Fig. 6. Gradient retention volumes (■) (zone created by decanesulphonate, see Fig. 3) and retention volumes of infinitesimal system peaks (●) *versus* the probe concentration in the eluent. Retention volumes of some analytes at lowest and highest probe concentrations are also plotted. 1 = FLA 870; 2 = FLA 965; 3 = FLA 659; 4 = desipramine; 5 = imipramine.

probe in the system peak. When the bulk concentration is low, being on the more or less linear part of the adsorption isotherm, the gradient retention volume is mainly determined by the adsorption of PT as an ion pair with the anion. At these probe concentrations, zones were developed and an increased hydrophobicity or concentration of the injected anion resulted in increased adsorption of PT from the bulk and, therefore, a larger gradient retention volume. For a qualitative description, eqn. 3 might than be modified as below, containing an additional term (corresponding to the hydrophobic anion) in the numerator and with the denominator simplified to 1:

$$V_{N,Q} = W_s K_0 (K_{QX}[X^-]_m + K_{QZ}[Z^-]_m)$$

At high probe bulk concentrations, being on the non-linear part of the adsorption isotherm, the increased gradient retention volume is due to the lower concentration of probe in the system peak. At these high probe concentrations, the contribution from the anion to the gradient retention volume is low. Predictions of gradient retention volumes by use of the unmodified eqn. 3 will then be more adequate.

Matching

To obtain peak compression it is necessary that the analyte and the gradient be eluted together. In this situation the analyte retention is larger than in the isocratic experiment. A prerequisite is that the isocratic retention volume of the analyte peak must be lower than the retention volume of the gradient. As a consequence, it was not possible to compress the imipramine peak in this system (*cf.*, Table I). The isocratic retention volumes of different analytes are given at the lowest and highest probe eluent concentrations (Fig. 6). The analyte retention volumes decreased less than the gradient retention volumes when the probe concentration was increased (*cf.*, eqns. 1 and 3). Hence the gradient was eluted later than all analytes at the lowest probe concentration but was eluted only after two of the analytes at the highest probe concentration (*cf.*, Fig. 6). This resulted in peak compression for more hydrophobic analytes at low probe

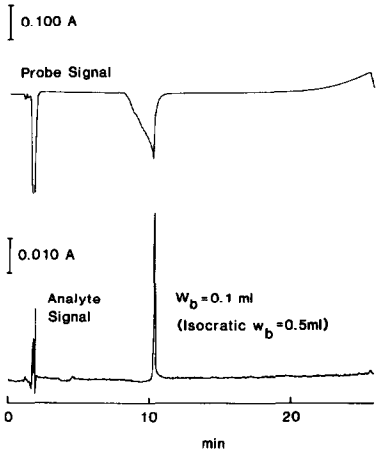

Fig. 7. Peak compression of FLA 870 at high probe concentration in the eluent. The peak width, w_b (ml), is measured at the baseline. Sample, $1.0 \cdot 10^{-5}$ *M* FLA 870 and $5.0 \cdot 10^{-3}$ *M* octanesulphonate in phosphate buffer (pH 2.0); eluent, $9.5 \cdot 10^{-4}$ *M* protriptyline in phosphate buffer (pH 2.0)–acetonitrile (3:1).

concentrations, whereas less hydrophobic analytes were compressed at high probe concentrations. When a poorly retarded analyte, FLA 870, was injected with a large amount of an organic anion at low probe concentration, the analyte peak was deformed and was eluted with the front part of the zone (ref. 6, Fig. 13a). However, at the highest probe concentration, FLA 870 was eluted with the gradient and was compressed (see Fig. 7). At this probe concentration analytes more hydrophobic than FLA 965 were eluted after the gradient.

CONCLUSIONS

The retention of the back-part gradient in a system peak or zone can be regulated by both the probe and the injected anion concentrations. When anion regulation is used, at low probe concentrations the gradient retention is influenced by a linear ion-pair distribution behaviour with the anion in starting and unresolved zones. The gradient retention can then also be regulated by the anion hydrophobicity. At high probe concentrations the retention is mainly influenced by the non-linear adsorption behaviour.

An adequate separation between the anion and the gradient is necessary primarily to avoid distortions of the shape of the back-part gradient, but also in order to allow regulation of the retention of the gradient by use of the anion concentration. Such conditions can be achieved by using an adequately high probe concentration.

Changing the probe concentration is an efficient way of obtaining elution of the analyte with the gradient and thus to achieve analyte peak compression. The less hydrophobic the analyte, the higher is the necessary probe concentration.

ACKNOWLEDGEMENTS

We thank the Department of CNS Research and Development, Astra Research Centre, for making substituted benzamides available. We are also grateful for grants given by the Swedish Academy of Pharmaceutical Sciences (T.F.) and from the I.F. Foundation of Pharmaceutical Research (A.S.). This project has been financially supported by Pharmacia and the Swedish Natural Science Research Council (NFR).

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