Journal of Chromatography, 648 (1993) 315-324
Elsevier Science Publishers B .V., Amsterdam

CHROM. 25 349

Effects on analyte peak performance by separated system peaks in ion-pair adsorption chromatography

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(First received January 22nd, 1993; revised manuscript received May 28th, 1993)

ABSTRACT

Organic ionic components added to the eluent in ion-pair chromatography often have retention volumes similar to the analytes. The performance of analyte peaks may be influenced by the system peaks derived from such mobile phase components. Earlier work has mainly dealt with situations in which the retention times of analyte and system peaks are close. However, distortions of analyte peaks (peak deformation and peak compression) can be caused by large system peaks even when the two different kinds of peaks are separated. In this work, systematic investigations of this situation were performed. It was found that the analyte peaks were more easily deformed by counter-ion system peaks than by co-ion system peaks. In addition, guidelines are given for the design of general ion-pair chromatographic systems which do not suffer from peak distortion effects.

INTRODUCTION

When a solution with a composition different from the bulk eluent is injected into a chromatographic system, the equilibria at the top of the column will be disturbed. As a result, concentration changes of the eluent components (system zones) will be generated, which will migrate along the column with a speed that is characteristic for the system peak components [1–5]. When the zones containing an excess of shortage of the eluent components are detected at the column outlet, they appear as either positive or negative system peaks.

System peaks will always be induced when samples are introduced in systems containing more than one eluent component, if the sample components interact with one or several of the mobile phase components. If a sample is injected into an equilibrated system, this will give rise to as many migrating system zones as there are interacting components in the eluent, except the main solvent [4]. However, only the retarded eluent components will be discriminated from the front disturbances. In most systems such components do not have UV or fluorescence properties, and an universal detector principle, such as refractive index (RI), may be necessary to visualize the system zone.

A broad spectrum of different kinds of analyte peak shapes may result upon combined elution with system zones. In most cases the analyte peaks will be deformed, but under certain conditions extremely narrow and we&shaped peaks are obtained. The common detectors used are often only selective for the analytes, whereas the presence of system peaks is often indicated only by the distorted analyte peaks. Peak deformation and compression effects due to interferences with large system peaks were first reported in ion-pair adsorption chromatography of metal ions in 1984 [6], and of substituted benzamides 1 year later [7]. Since then, several studies have outlined the parameters of importance for obtaining optimum peak compression effects [8–

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12], as well as those responsible for peak deformations in order to prevent the appearance of such peaks [10–14]. A model system based on alkyl-modified silica and an acidic eluent containing an organic UV-absorbing amine, protriptyline, has been used for the basic investigations [5,10–12]. Since the analytes were cationic amines, the analyte retention volumes were strongly dependent on the concentration of the eluent amine (co-ion). A photodiode array UV detector was used for those studies, which made it possible to obtain independent signals from both eluent and analyte components.

Deformations, which certainly are undesired, will occur more easily than compressions. The risk of deformation increases when the system peaks are large. This is especially true for coupled column systems, in which a large fraction of the mobile phase from a preceding column is introduced into the separation column [14,15]. However, serious deformations may also appear when only a single column is used in the analysis of biological fluids (bioanalysis), because of the complex samples injected [13].

Effects on analyte peaks by separated system peaks have only been mentioned occasionally in the literature [14]. The present study is a systematic investigation of the effects of system peaks that analyte peaks may be subjected to, even when they are separated from the large system peak at the column outlet. More particularly, a comparison has been made of distortions of anionic and cationic analytes in systems containing an organic amine in the eluent,

Parameters of importance in the design of chromatographic ion-pair systems in order to prevent distortions by system peaks will also be discussed.

EXPERIMENTAL

Chemicals

Acetonitrile (LiChrosolv), phosphoric acid (99% crystalline) and 1 *M* sodium hydroxide solution (Titrisol) were obtained from Merck (Darmstadt, Germany). N,N-Dimethyloctylamine (DMOA) was obtained from Janssen Chimica (Beerse, Belgium), and N,N-dimethylnonylamine (DMNA) and N,N-dimethyldecylamine (DMDA) from Ames Labs. (Millford, CT, USA). The amines were distilled before use. Protriptyline (PT) was obtained from Merck, Sharp and Dohme (Haarlem, Netherlands).

The substituted benzamides used as cationic analytes are denoted FLA in combination with a number [5]. They were synthesized at CNS Research and Development, Astra Arcus (Södertälje, Sweden).

Alprenolol, atenolol, metoprolol and propran-0101 hydrochloride also used as cationic analytes were obtained from Astra Hassle (Mölndal, Sweden) and pronethalol hydrochloride from Imperial Chemical Industries (Macclesfield, UK). Sodium naphthalene-2-sulphonate was obtained from Eastman-Kodak (Rochester, NY, USA) and sodium anthraquinone-2-sulphonate was obtained from Fluka (Buchs, Switzerland), and they were both used as anionic analytes.

Chromatographic system

Detection was performed by UV photometry (HP 1040A photodiode array or Linear 206 PHD) and by RI (Beckman 156 refractive index detector) coupled in series. The volume between the UV detector and the RI detector was chromatographically determined to be 0.07 ml. An LKB 2150 HPLC pump was used and the sample injector was a Rheodyne 7125, with a 50- or 100-μl loop. A Kipp & Zonen dual-channel recorder (Model BD 112) was used.

Conventionally sized columns with an inner diameter of 4.6 mm and lengths of 100 mm and 150 mm were used. They were either obtained ready-packed from the supplier (Spherisorb S5ODS-1 and Kromasil 100-5-C,,) or packed by a slurry technique (Nucleosil 100-5 C,,). The status of the packed columns was frequently tested with phenolic analytes and water-methanol as eluent. The performance of the columns remained unchanged during this work. The system (column and eluent reservoir) was carefully thermostated in a water bath (25.00 ± 0.01°C).

The eluents were mixtures of acetonitrile and phosphate buffers, with or without the addition of the organic amines DMOA, DMDA or PT. The preparation of the PT eluent was made as

described previously [5]. When the eluent contained PT, UV wavelengths were chosen such that the analytes and the PT system peak could be detected independently. The non-W-absorbing amines were detected with RI detection. The flow-rate was 0.80 ml/min in all experiments. No recirculation of the eluent was used.

Retention volume, asymmetry factor (*asf*) and peak width (w_b), were determined as described previously [10].

When the analyte peak is affected by the system peak, its performance is compared with the corresponding injection when the system peaks are made as small as possible. This so-called "isocratic situation" is achieved by using the eluent as injection solvent; however, even in this case the analyte peak may be distorted by the system peak.

RESULTS AND DISCUSSION

Retention behaviour

Retention equations for these kinds of systems, based on the stoichiometric ion-pair adsorption model, were described earlier [5,16,17]. The adsorption of the eluent amine as ion pair with the anionic buffer component increases at higher eluent amine concentrations, resulting in greater competition for the adsorption sites. Thus the retention of the amine analyte decreases with increasing concentration of the eluent amine, which then acts as a co-ion. On the other hand, the retention of the anionic analyte increases with higher eluent amine concentrations. The anionic analyte is adsorbed to the surface as ion pair with the eluent amine, which in this case acts as a counter-ion.

Equations similar to those for the analytes are valid also for small system peaks [4,5]. The eluent amine often has a high concentration in the eluent, indicating that the capacity factor for its system peak is governed by the position in the non-linear part of the adsorption isotherm at the actual bulk concentration in the eluent. However, when the equilibrium disturbances are very small, the deviation from this position is infinitesimal; therefore, very small system peaks can be treated as linear concentration pulses [1–5,181. On the other hand, the large excess system zone has a higher velocity than the large deficiency system zone, which results in a lower capacity factor for the positive system peak as compared with the negative one. This is also the reason why the large positive system peak shows tailing (the front is steeper than the rear), and why the large negative one shows fronting (the rear is steeper than the front) [10] (see Fig. 1).

Effects of system peaks on co-eluting analytes

The front and rear parts of the large system zones consist of either increasing or decreasing concentration gradients of mobile phase components. In ion-pair adsorption chromatography, the effect on the analyte zone (compression or

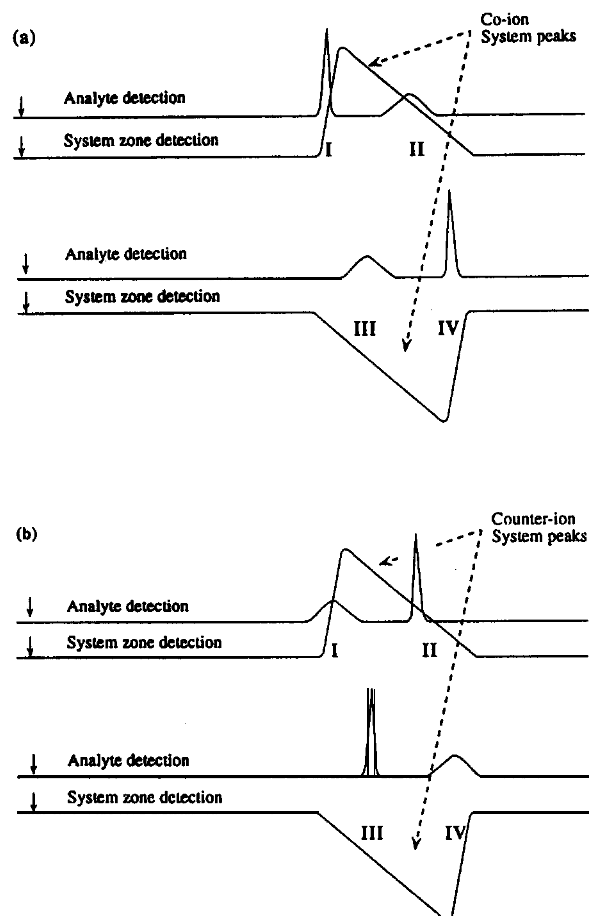

Fig. 1. Schematic illustration of the effects on analyte peaks of elution with different parts of a large positive or negative system peak. (a) The system peak component acts as a *co-ion.* (b) The system peak component acts as a *counter-ion.*

broadening) depends on the position of the analyte peak in the positive or negative system peak, and whether the system peak component is a co-ion or a counter-ion of the analyte. The different situations are schematically depicted in Fig. 1a and b. The effects represented in Fig. 1a result from the interactions with a system peak component acting as a co-ion towards the analytes; the situation in Fig. 1b refers to a system peak component acting as a counter-ion. In this work system peak effects by an organic amine in the eluent were studied. Fig. 1a describes in this case effects on cationic analytes and Fig. 1b effects on anionic analytes.

The analyte zone will be narrower than if eluted isocratically if it migrates in a gradient causing a continuously increasing migration rate; the rear part of the analyte zone will then experience a more accelerated velocity than the front part. These conditions prevail in a gradient of either increasing co-ion concentration or of decreasing counter-ion concentration. The cationic analyte peak is therefore compressed at the front part of the large positive co-ion system peak [9] or at the rear part of the negative one [7,10–12] (Fig. 1a, positions I and IV). On the other hand, the anionic analyte peak is narrower at the rear part of the positive counter-ion system peak [10,13,19] or at the front part of the negative one (Fig. 1b, positions II and III). The mechanisms behind the effects are different. In the increasing co-ion gradient, the cationic analyte experiences increasing competition for adsorption sites, whereas in the decreasing counter-ion gradient the analyte experiences a weaker ion-pair adsorption effect.

Gradients giving a continuously decreasing migration rate for the analyte zone result in broader analyte peaks than if eluted isocratically; the front part of the analyte zone moves under conditions which speed up the migration rate as compared with the rear part. Analyte elution at the rear part of the positive co-ion system peak or at the front part of the negative one [10] will therefore lead to broadening (Fig. 1a, positions II and III), as well as analyte elution at the front part of the positive counter-ion system peak or at the rear part of the negative one (Fig. 1b, positions I and IV).

If the analyte is eluted only partly in a co-ion or counter-ion gradient, peak deformation or splitting occurs [14]. Splitting also occurs at high analyte concentrations in a gradient normally giving a compressed analyte peak [10].

Even if the analyte zone is separated from the system zone at the column outlet, they have been eluted together at an earlier stage of their migration along the column. The distortions may therefore also occur, albeit with less pronounced effects, if the analyte is eluted before or after the actual system peak concentration gradient appearing at the column outlet.

To investigate the effects of system peaks on analyte peaks eluting earlier or later than the system peak, the analytes were injected either in pure buffer (lacking protriptyline), yielding positive system peaks, or in buffer-acetonitrile (i.e. eluent lacking protriptyline) , giving negative system peaks. The two situations were experimentally evaluated in a system with 0.91 m*M* protriptyline in buffer-acetonitrile as eluent. UV detection was performed at 337 nm, giving selective information about protriptyline; for this purpose the non-selective and non-sensitive RI detection could not be used. The initial conditions for the two situations are schematically shown (at the moment after the equilibria disturbance) in Fig. 2a and b. In both cases protriptyline diffuses into the injection zone from the bulk eluent and from the mobile phase ahead of the injection zone. In the case of buffer injection there will be an increased adsorption of protriptyline to the stationary phase as the protriptyline molecules reach the buffer zone (Fig. 2a). The mobile phase shortage of protriptyline in the injection zone is eluted with the non-retained buffer, while the stationary phase excess of protriptyline in the injection zone is eluted as a positive system zone. The zones appear as a negative front peak and a positive system peak on the chromatogram, respectively (Fig. 3a). An increased level of the baseline was observed; this started at the rear part of the negative front peak and continued until the positive system peak (Fig. 3a). This phenomenon could not be explained; it may be connected to the large disturbance of the acetonitrile equilibrium.

The analytes are adsorbed more strongly in

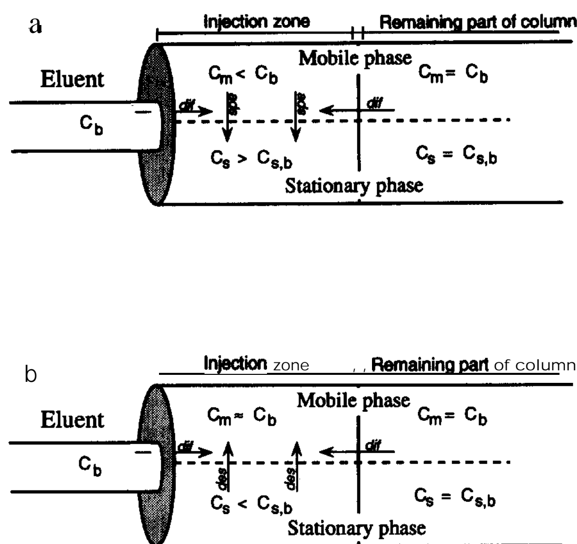

Fig. 2. Schematic illustration of the conditions in the injection zone after injection of (a) buffer and (b) buffer–acetonitrile into a system equilibrated with protriptyline in buffer–acetonitrile. C_m and C_s are the actual mobile phase and stationary phase concentrations of protriptyline, respectively. C_b is the bulk concentration of protriptyline and $C_{s,b}$ the stationary phase concentration at equilibrium with the bulk. The arrows indicate the diffusion (dif), adsorption (ads) and desorption (des) processes.

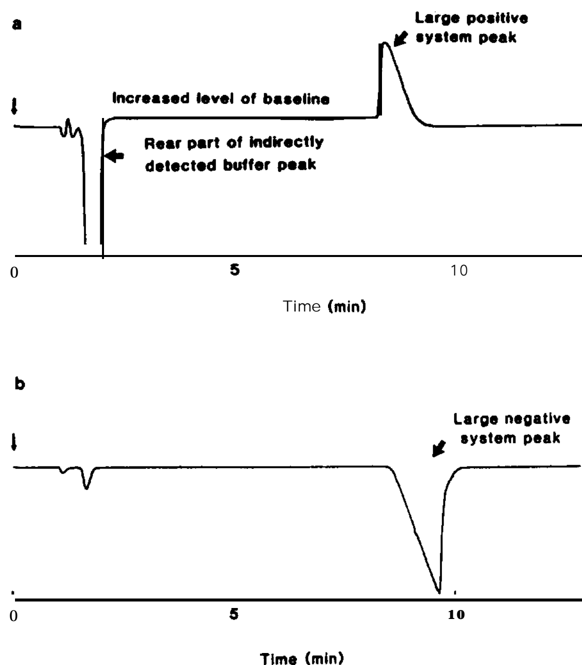

Fig. 3. Injection of (a) buffer or (b) buffer-acetonitrile into a chromatographic system equilibrated with protriptyline. Sample: 100 μl of (a) buffer or (b) eluent without protriptyline. Eluent: 0.91 mM protriptyline in phosphate buffer (pH 1.9, ionic strength = 0.05)–acetonitrile (3:1). Column: Nucleosil C_{18} (100 x 4.6 mm). UV detection was performed at 337 nm.

the buffer zone than in the other regions in the column; this causes enhanced enrichment of the incoming analyte solution, but also a gradient effect caused by the rear part of the buffer zone on the analyte zone before resolving. In Fig. 3a, the buffer zone is indirectly detected as a negative protriptyline peak in the front. The rear part of the buffer zone will consist of a steeply increasing organic amine gradient as well as a steeply decreasing buffer gradient; both gradients would have narrowing effects on co-eluting cationic analytes, but for anionic analytes the opposite effect would be caused by the amine (counter-ion) gradient. However, the closer the anionic analytes were eluted to the buffer zone, the narrower the analyte peak was compared with the injection in eluent. This indicates that the decreasing buffer gradient, causing a narrower analyte zone, dominates in effect over the increasing counter-ion gradient in this case.

When eluent lacking protriptyline was injected, some of the protriptyline molecules adsorbed to the stationary phase will desorb and diffuse to the mobile phase in the injection zone (Fig. 2b). This leaves a shortage of protriptyline in the stationary phase in the injection zone to elute as a negative system peak, which can be seen on the chromatogram in Fig. 3b.

Effects of counter-ion system peaks

Anionic analytes were dissolved in buffer and injected into a chromatographic system containing 1.4 mM DMOA (counter-ion) in the eluent (Fig. 4a). The positive DMOA system peak was detected by RI detection. The first anion peak had a smaller retention volume than the system peak and was deformed owing to its initial elution with the increasing counter-ion concentration in the front part of the system peak (Fig. lb, position I). When the eluent was used as injection solvent, the positive system peak was much smaller and the first anion peak became well shaped (Fig. 4b). The second anion peak

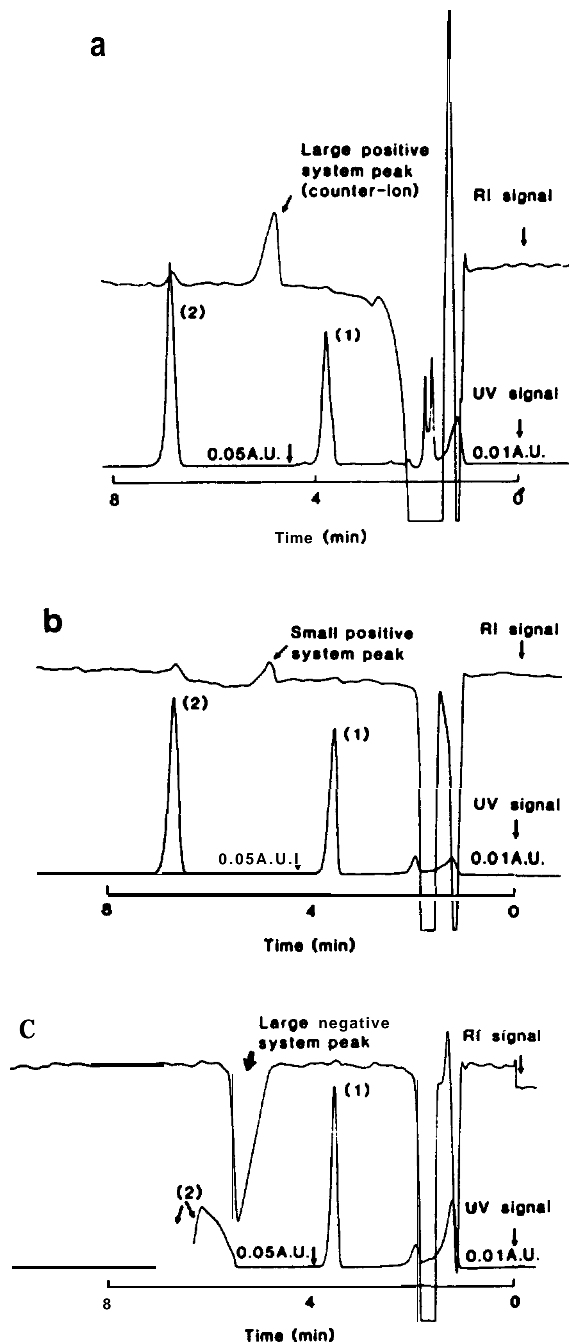

Fig. 4. Effects on anionic analyte peaks at elution before or after a counter-ion system peak. Sample: 100 μl of 10 μM (1) sodium naphthalene-2-sulphonate and (2) sodium anthraquinone-2-sulphonate in (a) buffer, (b) eluent, (c) eluent without N,N-dimethyloctylamine (DMOA). Eluent: 1.4 mM DMOA in phosphate buffer (pH 2.6, ionic strength = 0.1)-acetonitrile (3:1). Column: Spherisorb ODS-1 (100 × 4.6 mm).

had a longer retention time than the system peak, thus the anion had initially eluted at the decreasing counter-ion concentration in the rear part of the system zone (Fig. 1b, position II). The anion peak width was narrower when the buffer was used as injection solvent (Fig. 4a) as compared with the isocratic conditions (Fig. 4b). However, the reduction in peak width was not stronger than that expected, because of the initial enrichment effect on the column top caused by the buffer.

The anions were also dissolved in eluent lacking DMOA prior to injection, which resulted in a negative DMOA system peak (Fig. 4c). Now, the first anion peak is compressed compared with the eluent as injection solvent (Fig. 4b). In this case the anion is not adsorbed more strongly in the injection zone than in the other regions in the column. The reduction in peak width was only due to the initial elution of the anion with the decreasing counter-ion concentration in the front part of the negative system zone (Fig. 1b, position III).

The more retained anion was eluted partly with the increasing counter-ion concentration gradient in the rear part of the negative system zone and partly after the system zone and the anion peak was severely deformed and even split (Figs. 4c and 1b, position IV).

Effects of positive co-ion system peaks

Cationic analytes, which were eluted before or after the positive co-ion system peak (created by injecting the analytes dissolved in buffer), were affected only if they were eluted very close to the system peak. When the analytes were eluted before the system peak, the increased adsorption in the buffer zone also resulted in narrower analyte peaks compared with the injection in the eluent and it was not possible to discriminate between the two effects. Therefore, another approach, resulting in basically the same situation, was used to investigate the effects.

With Kromasil C_{18} as the stationary phase the amine analytes showed peak tailing to a lesser degree than with Nucleosil C_{18} and Spherisorb ODS-1. It was possible to separate the analytes with good peak symmetries without adding an organic amine to the eluent (Fig. 5a). When 2.0

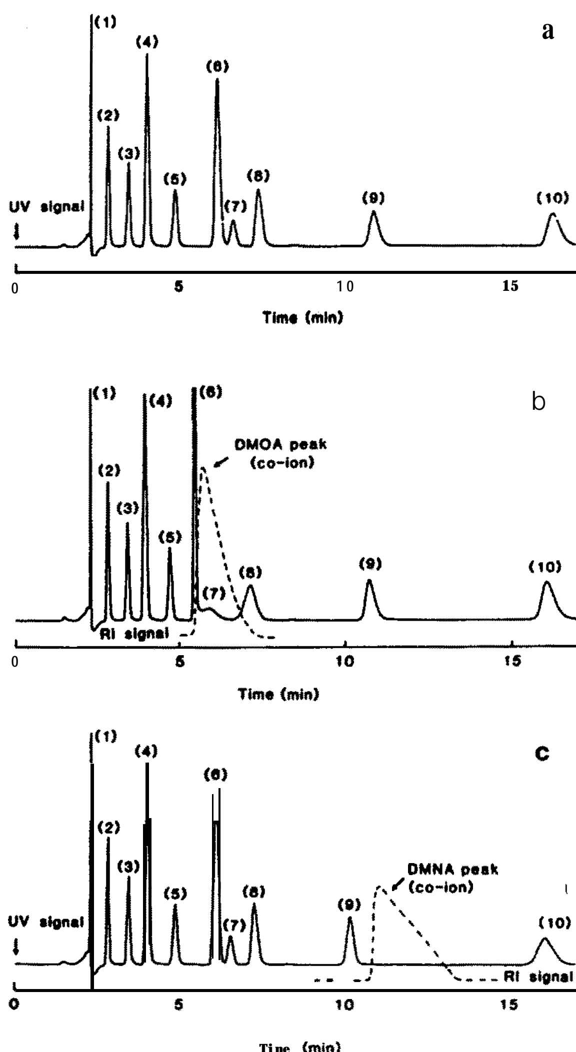

Fig. 5. Effects on cationic analyte peaks upon elution with a large amount of a hydrophobic co-ion added to the sample. Sample: 100 μl of 10 μM analytes in (a) buffer (reference rune), (b) buffer with 2.0 mM N,N-dimethyloctylamine (DMOA), (c) buffer with 2.0 mM N,N-dimethylnonylamine (DMNA). Analytes; 1 = atenolol; 2 = metoprolol; 3 = FLA 731; 4 = pronethalol; 5 = FLA 913; 6 = propranolol; 7 = alprenolol; 8 = FLA 797; 9 = FLA 870; 10 = FLA 659. Eluent: phosphate buffer (pH 2.9, ionic strength = 0.05)–acetonitrile (7:3). Column: Kromasil C_{18} (150x4.6 mm). The RI signal is slightly delayed compared with the UV signal because of the dead volume between the two detectors.

mM DMOA was added to the injection solution, propranolol was eluted with the steeply increasing co-ion concentration in the DMOA front; hence the propranolol was compressed (Fig. 5b). Even if the injection conditions differ from the situation with a positive system peak, the conditions for propranolol were basically the same as shown in Fig. la, position I. This situation has been described for two-component mixtures by Katti and Guiochon [20] and the effects have been utilized to increase the production rate of especially the first-eluting component in preparative chromatography.

The large DMOA peak was detected by RI detection; its signal was incorporated from the RI trace as a dotted line alongside the UV signal. Alprenolol was eluted directly after propranolol, together with the steeply decreasing co-ion concentration in the initial rear part of the large DMOA peak, and its peak was largely deformed. FLA 797 was eluted with the last rear part of the DMOA peak; and this peak was also broadened but to a smaller extent (*cf.* Fig. 5a). The cationic analytes which were eluted after the DMOA peak were not affected. Of the cationic analytes that were eluted before the large DMOA peak, only the two peaks closest to the DMOA front were affected, i.e. pronethalol and FLA 913. They were slightly narrower and higher than in the run without addition of DMOA in the injection solution (Fig. 5a).

The more hydrophobic DMNA (2 mM) was added to the injection solution and its large peak had a position between FLA 870 and FLA 659 (Fig. 5c). The FLA 870 peak was narrower and the FLA 659 peak was broader and both their retention volumes were decreased in comparison with the experiment without addition of DMNA in the injection solvent (Fig. 5a). The cationic analytes eluting earlier than FLA 870 were not significantly affected. In this case FLA 659 was closer to the rear part of the large amine peak than FLA 870 was in the earlier example (Fig. 5b), when no effects on its peak shape were observed.

Effects of negative co-ion system peaks

Cationic analytes are deformed at the front part of the negative co-ion system peak and compressed at the steeply increasing co-ion concentration in the rear part (Fig. la, positions III and IV). In a system containing 0.91 mM protriptyline in the eluent and Nucleosil C_{18} as

stationary phase, 100 μl of 10 μM FLA 797 were injected dissolved in eluent lacking protriptyline. FLA 797 was eluted before the negative system peak and was broader than in the experiment in which it was dissolved in eluent (containing protriptyline), despite a rather large separation factor of 1.7. The peak width increased from 0.32 to 0.39 ml.

When a cationic analyte was eluted after the negative system peaks (created in this way), it was only affected if it was eluted very soon after the negative system peak. In systems containing about 1 mM protriptyline or DMOA the separation factor between the analyte peak and the system peak had to be 1.3 or less, in order to give reductions in analyte peak widths.

An example of how confusing these effects on the analyte peaks can be, when only the analytes are detected, is shown in the chromatogram in Fig. 6a; the second peak is broader than the third peak. Four different cations (substituted benzamides) at a concentration of 10 μM were injected dissolved in eluent lacking PT in a system with 0.94 mM PT in the eluent. The reason for the anomalous peak-width behaviour is revealed when the position of the negative system peak is made visible by the detector (Fig. 6b).

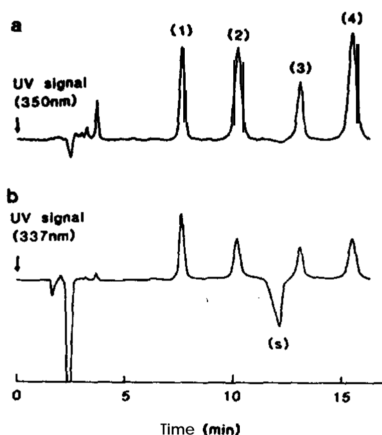

Fig. 6. Weak peak-broadening and peak-narrowing effects on cationic analytes due to elution slightly before and slightly after the negative protriptyline (co-ion) system peak, respectively. (a) The UV signal detected only the analytes (350 nm). (b) The system zone (s) was also visualized (337 nm). Sample: 100 μl of 10 μM (1) FLA 797, (2) FLA 870, (3) FLA 965 and (4) FLA 659 in eluent lacking protriptyline. Eluent: as in Fig. 3 (but 0.94 mM PT). Column: Nucleosil C_{18} (150 × 4.6 mm).

Comparison between the effects of co-ion and counter-ion system peaks

In the chromatographic systems described above, the magnitudes of the analyte peak-broadening and -deforming effects were generally larger than the analyte peak-width reductions. In addition, the deformations were much larger in situations when the analytes were affected by a counter-ion gradient compared with 'a co-ion gradient. The initial adsorption of the analytes was stronger when injected in buffer than when eluent lacking the amine was used as injection solvent (compare Figs. 2 and 3). This fact, in combination with the larger sizes of the negative system peaks compared with the positive ones, is probably the explanation for the stronger effects when analytes were eluted close to the negative system peaks. This is valid for analytes with similar separation coefficients to the different system peaks in the chromatographic systems.

Parameters determining the degree of the distortions

The distortions of analyte peaks which are resolved from large system peaks are especially serious at short separation distances, at large injection volumes and analyte loads and at high bulk concentrations of the organic ion in the eluent. The effects on the analyte peaks from the initial migration together with the large system peak decreased generally with increasing separation at the column outlet. This tendency is evident from the chromatograms in Fig. 5b and c.

In case of a larger injection volume containing either buffer or eluent without the organic amine (or any other equilibrium disturbance agent), an increased amount of the mobile phase components will be redistributed in the injection zone. The system zone of the organic amine will contain a larger deviation from the eluent and its interaction with the analyte zones will consequently be increased.

The effects of increasing the amount of analyte injected were also important. A 100-μl aliquot of 0.01 mM anionic analyte dissolve in buffer was

injected into a system with 0.9 m*M* DMDA in the eluent. The anion peak seemed to be well shaped and had a much smaller retention volume than the positive system peak of the counter-ion (Fig. 7a). When a ten-fold higher analyte concentration was injected, again with the analyte dissolved in the buffer, the analyte peak was broad and deformed (Fig. 7b). The interactions between the analyte and the counter-ion system peak increased because not only the system peak but also the analyte peak involved a large concentration deviation from the eluent. The same tendency was observed when a hydrophobic counter-ion was injected together with the analytes instead of having the counter-ion incorporated in the eluent [12].

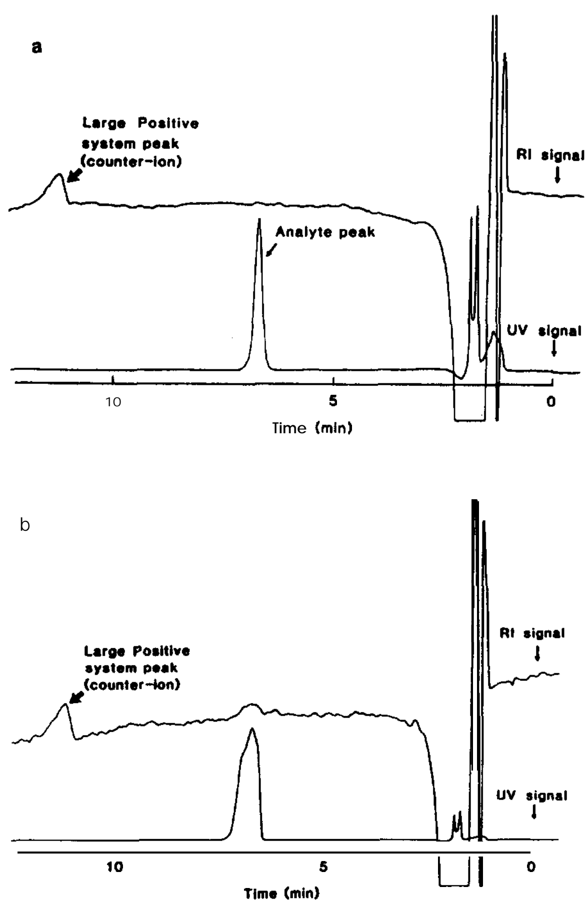

Fig. 7. Effects on an anionic analyte peak at elution well separated in front of a positive counter-ion system peak (DMDA) at (a) low and (b) high analyte concentration. Sample: 100 μl of sodium naphthalene-2-sulphonate in buffer: (a) $C_{analyte}$ = 10 μM; (b) $C_{analyte}$ = 0.1 m*M*. Eluent and column: as in Fig. 4 but 0.9 m*M* N,N-dimethyldecylamine (DMDA) was added to the eluent.

Larger positive or negative system peaks are obtained with increasing bulk concentration of the actual eluent component when the same concentration of an equilibrium disturbance agent is injected [1,2,5,11]. This is because of the comparatively larger amounts of adsorbed and/or desorbed eluent component in the injection zone. Anionic analyte peaks were therefore more affected by the system peak of the counter-ion at higher bulk concentrations of the counter-ion. As an example, distortion of the anionic analyte peaks demonstrated in Fig. 4 was found at a concentration of the counter-ion (DMOA) in the eluent of 1.4 m*M*. At a three times lower counter-ion concentration in the eluent, no analyte peak distortions could be observed at otherwise similar conditions.

Design of systems avoiding peak deformations

System peaks will develop at all times when a sample deviating from the eluent is injected. Large deviations result in large system peaks, which means that the introduction of complex matrices, such as biological fluids or extracts thereof, may cause disturbances of analyte peak shapes due to interfering system peaks. It is therefore essential to be aware of such risks, and the use of RI detectors is recommended in order to reveal the existence of such peaks during the development of assay methods for complex matrices [13].

Parameters determining the selectivity between the analyte and the system peak have been outlined earlier; essential factors are the pH and the ionic strength of the buffer [8,13,21]. The concentration and character of the organic ion are very important parameters, especially in its role as a counter-ion towards the analytes, but may also be important when it acts as a co-ion [11]. Large effects on the selectivity due to batch variations of the solid phase have also been observed [9,13].

For the more common positive system peaks, the conditions chosen should be such that the system peak is eluted before the analytes, since the effects on analyte elutions after the positive

system peaks are rather weak. The injection volume should be as small as possible and the analytes should be dissolved in the eluent whenever possible. In quantitative determinations, peak-area measurements are to be preferred to peak-height measurements to avoid errors due to slight changes in the widths of the analytes or of the internal standard [13].

Peak deformation of a cationic analyte using an organic amine as co-ion in the eluent can often be easily eliminated by changing to a more hydrophilic co-ion. The retention of the system peak will decrease and the retention of the analytes will increase. Then the co-ion concentration can be tuned until suitable analyte retentions are obtained.

When analytes are separated using an organic ion as counter-ion, it is often necessary to have a hydrophobic counter-ion that may elute rather close to the analytes. Serious disturbances due to the risk of more severe deformations in such systems may arise even when the analytes are separated from the system peak in the column outlet. If peak deformation occurs, a change in the concentration of the counter-ion should be made to improve the analyte separation from the system peak. A positive system peak should preferably be regulated to elute before the analyte, whereas a negative system peak should elute after the analyte. The retention of the analyte will increase at an increasing counter-ion concentration and the retention of the system peak is decreased, giving a higher separation factor between the anion and the system peak.

ACKNOWLEDGEMENTS

We thank Dr. Anders Sokolowski for fruitful discussions. This project was financially supported by The Swedish Natural Science Research Council (NFR) .

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