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INDIRECT DETECTION IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

RESPONSE DEVIATIONS IN ION-PAIRING SYSTEMS

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SUMMARY

Deviation effects in indirect detection have been studied in ion-pairing systems with several retained components in the eluent. The response extinction or reversal that appears in certain retention ranges have been given a theoretical explanation. Other response disturbances and peak deformations have been explained as due to pH instability and/or protolysis of the detectable eluent component (the probe) in the migrating compound zones. Principles are given for the design of indirect detection systems to optimize the sensitivity and avoid detection disturbances.

INTRODUCTION

Indirect detection is a technique applied in chromatography to visualize compounds with low or no inherent detectable (UV, fluorescence, electrochemical) properties[1–3]. It is technically based on the use of a mobile phase containing a component with a detector response. The detectable component (the probe) can be charged, and it is then distributed to the solid phase as an ion pair(s) and its retention can then easily be regulated by the nature and the concentration of the counter ion(s). Uncharged compounds can also be used as probes[4], but the regulation of the retention must then be made by other means.

The indirect detection effects are due to disturbances of established equilibria that occur in the injection zone on application of a sample. These disturbances will affect all components in the system that are coupled by a common interaction, which in reversed-phase chromatography normally is the competition for the limited binding capacity of the adsorbent. The equilibrium displacements will affect the concen-

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trations of all retained mobile phase components, and all affected compounds will migrate as separate zones through the column. The migrating zones of the solutes injected as well as of the eluent components will contain a mobile phase with a composition different from the normal one and will appear as peaks in the chromatogram, even though only one mobile phase component, the probe, gives detectable response. The peaks representing the analytes (solute peaks) and the mobile phase components (system peaks) can be positive or negative depending on the charge and the retention of the compound relative to that of the probe.

The theoretical background for the indirect detection technique has been presented in recent publications[5–7]. Quantitative expressions for the response in reversed-phase ion-pairing systems have been developed. They are valid for systems where one ion pair of the probe is the dominating competitor for the solute, as demonstrated by the existence of only one system peak with significant retention. Such conditions can occur when the remaining components in the mobile phase are hydrophilic buffering agents and the adsorbent is alkyl- or aryl-bonded silica. The simple relationship between the response and the retention of the solute relative to the probe is not valid when several retained system peaks appear in the chromatogram. This indicates that several ion pairs in the mobile phase compete for the adsorbent, and it can occur, *e.g.*, when the solid phase is highly hydrophobic. A theoretical approach to the response in such systems is presented in this paper.

The indirect detection is due to changes in the baseline absorbance that often are smaller than $\pm$ 1%. Solute and system peaks show good symmetry and have about the same width as in direct detection. However, extra peaks and peak deformation are sometimes observed in ion-pairing systems. The deviations can be combined with changes in retention that depend on the amount of sample, which indicates that the systems are unstable. The background to the disturbances is discussed in this paper and recommendations for the construction of ion-pairing indirect detection systems are given.

EXPERIMENTAL

Butylamine hydrochloride and 1-methylpyridinium hydrogensulphate (MP) were obtained from Eastman-Kodak (Rochester, NY, U.S.A.). Sodium 6-hydroxy-naphthalene-2-sulphonate (60HNS) and 3-hydroxybenzoic acid (30HBA) were obtained from E. Merck (Darmstadt, F.R.G.). They were recrystallized from water before use. All other chemicals were of analytical grade and were used without further purification.

The detector used was a LDC Spectromonitor III. The pump was an Altex 100 A, and the injector a Rheodyne 7125 with a loop volume of 25 μl.

μBondapak Phenyl (10 μm) (Waters Assoc., Milford, MA, U.SA.) and PRP-1 (10 μm) (Hamilton, Bonaduz, Switzerland) were used as the solid phases. They were packed by the slurry technique in columns of sizes 100 mm $\times$ 4.6 mm I.D. or 100 mm $\times$ 3.2 mm I.D. Part of the studies was performed with a PLRP-S (5 μm) prepacked column, 150 mm $\times$ 4.6 mm I.D., from Polymer Laboratories (Amherst, MA, U.S.A.).

The mobile phases were aqueous solutions, containing a detectable ion, NP, 60HNS or 30HBA, buffering agents and in some cases an organic solvent in low concentrations. The mobile phase flow-rate was 0.50 ml/min.

The eluent reservoir, injector column and connecting tubes were thermostatted at 25.0°C in a water-bath in order to eliminate the disturbances of temperature fluctuations.

The samples were injected, dissolved in the mobile phase. The capacity factors were calculated from $k' = (V_R - V_m)/V_m$, where V_m, the volume of mobile phase in the column, was obtained from the front peak of the chromatogram.

The detection wavelength was 254 nm when using 60HNS or 30HBA as the probe, 272 nm when using MP and 270 nm when 40HBA was the detectable component.

RESULTS AND DISCUSSION

Theoretical background for indirect detection in ion-pairing systems

Reversed-phase ion-pair chromatography gives many possibilities for adjusting the retention of the ionic compounds in the system. Ionic analytes and mobile phase components compete for the limited binding capacity of the adsorbent. The injection of a solute gives rise to a change in the distribution at the injection zone of the adsorbed compounds. In analytical applications the disturbances are so small that the distribution behaviour of the different compounds can be described by linear expressions, such as

$$\Delta C_{i,s} = C_{i,s} - C^{\circ}_{i,s} = \sum_{j=1}^{n} a_{i,j} \cdot \Delta C_{j,m} \quad (1)$$

where $C_{j,m}$ is the concentration of j in the mobile phase, $C_{i,s}$ is the quotient of the amount of i in the stationary phase to the volume of mobile phase in the column, $\Delta C_{i,s}$ is the change in the concentration of any compound i in the stationary phase, $C^{\circ}_{i,s}$ is the initial concentration and

$$a_{i,j} = \partial C_{i,s}/\partial C_{j,m} \quad (2)$$

for each compound (solute or mobile phase additive) is evaluated for the starting concentration in the mobile phase reservoir, *i.e.*, zero for a solute and differing from zero for a mobile phase component. When n compounds are involved in the same interaction, n^2 $a_{i,j}$ terms are obtained, representing the elements of a quadratic matrix. The diagonal elements in this matrix, *i.e.* $a_{i,i}$, which correspond to the capacity factors of i, are the eigenvalues of the matrix. The relative changes of compounds i and j in a peak are given by the corresponding eigenvectors and can be used to express the relative response[5,6], as discussed below. The background to the interaction in these multicomponent systems has been extensively discussed by Helfferich and Klein[8] and by Riedo and Kováts[9].

In a previous paper[6], response models were developed for a system containing a hydrophobic adsorbent in an aqueous mobile phase with three components: *e.g.*, a cationic probe, Q^+, an anion, X^-, and a further cationic component, B^+, which is so hydrophilic that its distribution to the adsorbent is negligible. A solute injection will give rise to changes in the distribution of Q^+ and X^- at the injection zone, and the resulting chromatogram will show one solute peak and one retained system peak.

The discussion below deals with chromatographic systems in which mobile phase components are distributed to the adsorbent as two different ion pairs, resulting in chromatograms with two retained system peaks. The mobile phase is assumed to contain a buffering agent in excess. A theoretical expression for the response can be developed according to the same principles as previously published[6], if the concentration of one of the buffering components can be considered to be constant.

Constant [H^+] in the mobile phase

The mobile phase contains a cationic probe, Q^+, and a buffering agent, HX. The solute, HA^+, has the same charge as the probe. The buffering anion, X^-, participates in the distribution of Q^+, HA^+ and H^+, and it is likely that [X^-] in the migrating zones deviates from its normal value in the eluent. The most hydrophilic cation is H^+, and it is distributed only as an ion pair with X^-. The assumption of constant [H^+] in the mobile phase is therefore a reasonable approximation.

Electroneutrality conditions give:

$$[X^-] = [HA^+] + [Q^+] + [H^+] \tag{3}$$

Ion pairs of HA^+, Q^+ and H^+ compete for the binding capacity of the adsorbent. If they compete according to a Langmuir model, and the surface is homogeneous, the concentrations of Q^+ and HA^+ on the solid phase can be expressed by

$$C_{Q,s} = \frac{A \cdot K_{QX}[Q^+]\,([H^+] + [Q^+] + [HA^+])}{N_1} \tag{4}$$

$$C_{HA,s} = \frac{A \cdot K_{HAX}[HA^+]\,([H^+] + [Q^+] + [HA^+])}{N_1} \tag{5}$$

where

$$N_1 = 1 + (K_{HAX}[HA^+] + K_{QX}[Q^+] + K_{HX}[H^+])\,([HA^+] + [Q^+] + [H^+]) \tag{6}$$

and A is the product of the binding capacity of the solid phase and the phase volume ratio in the column; K_{QX}, K_{HX} and K_{HAX} are the equilibrium constants for the distribution of Q^+, H^+ and HA^+ to the solid phase as ion pairs with X^-. The partial derivatives for the probe, Q^+, and the solute, HA^+ (*cf.*, eqn. 2), then are:

$$a_{HA,HA} = \frac{\partial C_{HA,s}}{\partial C_{HA,m}} = A \cdot K_{HAX}([H^+] + [Q^+])\,(1 - \theta_{Q,H}) = k'_{HA} \tag{7}$$

$$a_{Q,Q} = \frac{\partial C_{Q,s}}{\partial C_{Q,m}} = (A \cdot K_{QX}[H^+] + 2A \cdot K_{QX}[Q^+])\,(1 + K_{HX}[H^+]^2) + A \cdot K_{QX}[Q^+]^2 K_{HX}[H^+](1 - \theta_{Q,H})^2 = k'_Q \tag{8}$$

The fraction of the solid phase that is not covered by Q^+ or H^+ is represented by:

$$(1 - \theta_{Q,H}) = \frac{1}{\{1 + (K_{QX}[Q^+] + K_{HX}[H^+])\,[X^-]\}} \tag{9}$$

Eqns. 7 and 8 represent the eigenvalues of the matrix and correspond to the capacity factors of HA^+ and Q^+, *i.e.*, the k' values of the solute peak and the most retained system peak, respectively.

The interaction term, $a_{Q,HA}$, can be given the form:

$$a_{Q,HA} = -k'_{HA}\theta_Q\left(1 - \frac{1}{K_{HAX}([H^+] + [Q^+])^2}\right) \tag{10}$$

An expression for k'_{HA} is given by eqn. 7. θ_Q, the fractional coverage of the solid phase by Q^+, is defined by:

$$\theta_Q = \frac{C_{Q,s}}{A} = \frac{K_{QX}[Q^+][X^-]}{1 + (K_{QX}[Q^+] + K_{HX}[H^+])[X^-]} \tag{11}$$

The relative changes of the concentrations of HA^+ and Q^+ in the eluted solute peak are given by the corresponding eigenvector, derived from the matrix. The relative response can be expressed as (*cf.*, ref. 6)

$$\frac{\Delta C_Q}{\Delta C_{HA}} = \frac{\varepsilon^*_{HA}}{\varepsilon_Q} = \theta_Q\left(1 - \frac{1}{K_{HAX}([H^+] + [Q^+])^2}\right) \cdot \frac{\alpha_s}{1 - \alpha_s} \tag{12}$$

where ε^*_{HA} isthe apparent molar absorptivity of the slute HA^+, ε_Q is the molar absorptivity of the mobile phase component Q^+ and $\alpha_s = k'_{HA}/k'_Q$. When $[H^+] \gg [Q^+]$, eqn. 12 gives:

$$\frac{\varepsilon^*_{HA}}{\varepsilon_Q} = \theta_Q\left(1 - \frac{1}{K_{HAX}[H^+]^2}\right) \cdot \frac{\alpha_s}{1 - \alpha_s} \tag{13}$$

Eqn. 13 shows that the relative response increases with increased load, *i.e.*, an higher concentration of Q^+ in the mobile phase leads to a larger distribution of Q^+ to the solid phase and a higher response. The response has a maximum when α_s is close to unity, as shown earlier[6].

It should be emphasized that eqn. 13 is an approximation, since changes of $[H^+]$ in the solute zone have not been taken into consideration due to mathematical restrictions.

K_{HAX} increases with increasing hydrophobicity of the solute, HA^+. This has a large influence on the relative response, particularly when k'_{HA} is smaller than k'_Q, as summarized in Table I.

When HA^+ is hydrophilic with a low retention, $K_{HAX}[H^+]^2$ is small, and the expression $\{1 - (1/K_{HAX}[H^+]^2)\}$ will then be negative and the relative response will assume a negative value. With increasing K_{HAX}, the quotient, $1/(K_{HAX}[H^+]^2)$ will

TABLE I
DIRECTION OF SOLUTE PEAK WITH INCREASING HYDROPHOBICITY OF HA^+

α_s	$\frac{1}{K_{HAX}[H^+]^2}$	$\frac{\varepsilon^*_{HA}}{\varepsilon_Q}$
<1	>1	Negative
<1	1	0
<1	<1	Positive
>1	<1	Negative

decrease and, when its value is equal to 1, the relative response will be equal to zero. With further increase of K_{HAX} and k'_{HA} the relative response will reach an increasingly positive value until α_s is higher than 1 when the response changes to negative in the same way as in a system with a single system peak.

The concentration $[H^+]$ mainly influences the response for solutes with own K_{HAX}; $\varepsilon^*_{HA}/\varepsilon_Q$ of such solutes increases with increasing $[H^+]$.

The expressions for the capacity factors and the relative response for an uncharged solute, S, derived according to the same general principles are very simple and equal to those obtained in systems with a single system peak:

$$k'_s = a_{S,S} = A \cdot K_S(1 - \theta_{Q,H}) \tag{14}$$

and

$$\frac{\Delta C_Q}{\Delta C_S} = \frac{\varepsilon^*_S}{\varepsilon_Q} = \theta_Q \cdot \frac{\alpha_s}{1 - \alpha_s} \tag{15}$$

Reversal of the response direction between two system peaks

A study of the response in a reversed-phase chromatographic system with two system peaks was made with methylpyridinium ion as the detectable component and cyclamic acid as the buffering agent, using a polystyrene copolymer as the solid phase. The chromatographic system is denoted as No. 1 in Table II.

The response pattern for solutes in a system with one retained system peak has been presented in several previous papers. When the solute is uncharged or has the

TABLE II
CHROMATOGRAPHIC SYSTEMS
HCy = Cyclamic acid; HP = phosphoric acid; Phenyl = μBondapak Phenyl.

System No.	*Detectable component*	*Counter/buffer ions*	*Organic solvent*	*Solid phase*
1	10^{-4} *M* MP	0.01 *M* HCy	4% Methanol	PLRP-S
2	$(0.26–15) \cdot 10^{-5}$ *M* 60HNS	0.01 *M* HP	4% Methanol	PRP-1
3	10^{-3} *M* 30HBA	$3.7 \cdot 10^{-4}$ *M* TPrA Sodium hydroxide to pH 6		Phenyl

same charge as the probe, the solute peak is positive before the system peak and negative after; when the solute and probe have opposite charges, the response pattern is reversed. However, in the present system, cationic solutes, i.e., solutes with the same charge as the probe, show a deviating response pattern.

The peaks originating from the mobile phase components (system peaks) were identified by comparison of the chromatograms obtained for a series of solutes with different hydrophobicities. The k' values are given in Table III. The third system peak was the largest and was found to originate from the probe, methylpyridinium ion. The origin of the remaining three system peaks has not been elucidated.

The response pattern of alkylamines with k' values between k'_{SP2} and k'_{SP3} are demonstrated in Fig. 1A–C. Methylamine and ethylamine, which appear rather close to SP2, give negative peaks, whereas propylamine, which comes just before SP3, gives a positive peak. It should furthermore be noted that, on injection of the same amounts of methylamine and ethylamine, methylamine gave a much larger peak. This is a deviation from the normal response behaviour. Ethylamine is more strongly retained and is eluted closer to SP3 (the system peak of the probe) than methylamine. In a system with one retained system peak this should give an higher response.

Capacity factors and peak directions for these amines as well as for some cations and uncharged solutes are summarized in Table III. The results are in agreement with the theoretical predictions above based on eqns. 13 and 15.

Peak deformation

The ideal chromatographic system, designed for indirect detection, shows a stable baseline as well as symmetrical solute and system peaks with the same peak width as those obtained in direct detection. However, peak deformation and extra peaks can appear under certain chromatographic conditions. Some examples of deviations and possible explanations are given below. The discussion will deal only with deformations such as wave-shaped peaks and extra peaks that are specific for the

TABLE III
RETENTION AND PEAK DIRECTION
System 1 (see Table II).

Compound	*k'*	*Peak direction*
Mobile phase components	0.48 1.07 4.9* 5.6	
Methylamine	1.30	Neg.
Ethylamine	1.80	Neg. (small)
Propylamine	4.10	Pos.
Butylamine	13.4	Neg.
Triethylamine	13.3	Neg.
Ethyltrimethylammonium ion	3.7	Pos.
Triethylmethylammonium ion	12.9	Neg.
Diaminobutane	7.5	Neg.
1,5-Pentanediol	3.0	Pos.
1,3-Cyclohexanediol	2.9	Pos.
Acetonitrile	2.5	Pos.

* System peak originating from methylpyridinium ion.

indirect detection systems. Peak asymmetry and related effects that are due to overloading of the solid phase will not be treated.

The indirect detection systems that show deformation of solute or system peaks all have a low buffer capacity and/or a low fractional loading of the probe on the adsorbent. The specific deformations mentioned above, are as a rule, seen only in systems containing an ion-pairing probe, and it is probable that they are due to changes in the ionic composition in the zone of the migrating compound.

Peak deformations have been observed on highly hydrophobic adsorbents such as PRP-1 as well as on alkyl- or aryl-bonded silica. The background to the disturbances may, to some extent, change with the hydrophobicity of the adsorbent and, therefore, examples of both kinds of solid phases are presented.

PRP-1 as adsorbent with low loading of probe. The use of a probe with high molar absorptivity is one important way to achieve high detection sensitivity. However, such a probe can be used only in low concentration in order to avoid a very high baseline absorbance that might give rise to errors due to excessive noise and a non-linear response of the detector. The problems that can occur in such a system are illustrated by the results obtained with 60HNS ($\varepsilon \approx 5400$) as the detectable mobile phase component. It was added as the sodium salt to the mobile phase, which also contained a constant concentration of phosphoric acid and 4% of methanol. The probe concentration varied between $2.6 \cdot 10^{-6}$ and $1.5 \cdot 10^{-4}$ *M*. The chromatographic system is denoted as No. 2 in Table II.

When water or an excess of the probe was injected, the main system peak had good symmetry and its capacity factor was stable, with a relative standard deviation of 1.0–2.4% on repeated injections of the same solution. However, for injected solutes, the retention showed large variations on repeated applications of the same sample solution. The chromatograms of ionic solutes showed deviations from the normal pattern. Injection of butanesulphonate gave a solute peak without disturbing

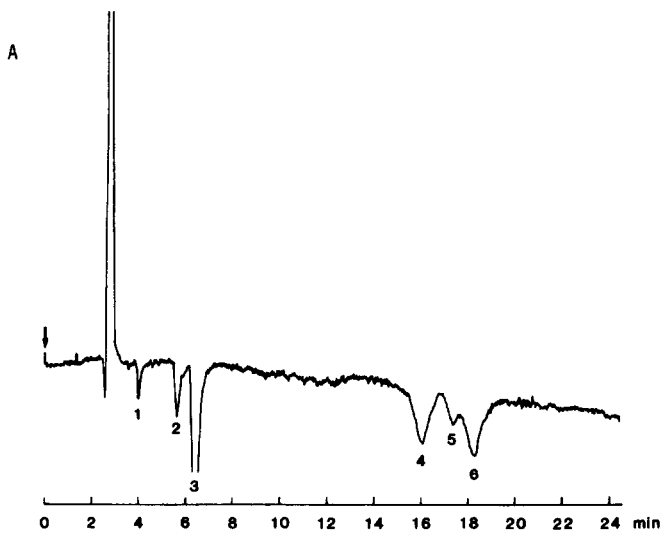


Fig. 1.

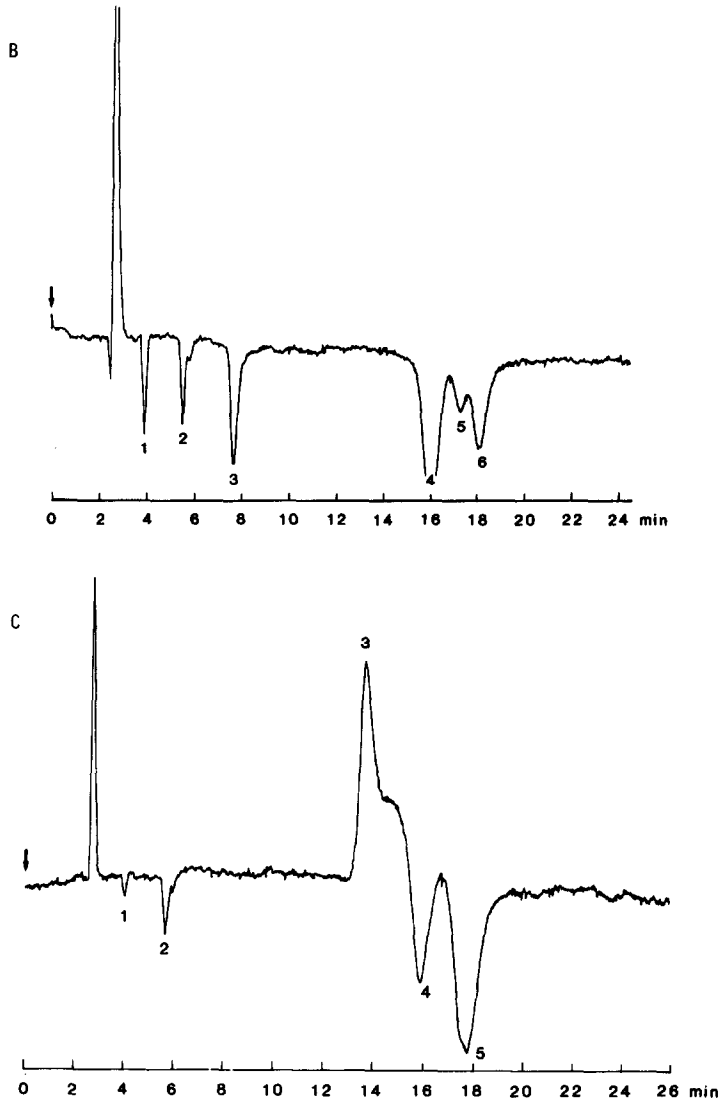


Fig. 1. (A) Reversed response of solute. Solid phase: PLRP-S 5 μm. Mobile phase: 10^{-4} *M* methylpyridinium–10^{-2} *M* cyclamic acid–4% methanol. Solute: methylamine. Peaks: 1, 2, 4 and 6 = system peaks; 3 = methylamine; 5 = unidentified impurity. (B) Solute: ethylamine (peak 3); other peaks as in (A). (C) Normal response of solute. Solute: propylamine. Peaks: 1, 2, 4 and 5 are system peaks; 3 = propylamine.

asymmetry but a wave-shaped system peak at a probe concentration of $5 \cdot 10^{-6}$ *M* (Fig. 2A). At a probe concentration of $1.5 \cdot 10^{-4}$ *M* there is also a tendency for the solute peak to assume a wave shape (Fig. 2B). Peak deformation was also observed on injection of butylamine. On the other hand, acetonitrile as a solute gave a normal chromatogram.

A low fractional coverage, θ_Q, of the probe on the adsorbent is a possible cause of the peak deformation. Low-fractional-coverage systems are known to be less stable[2],

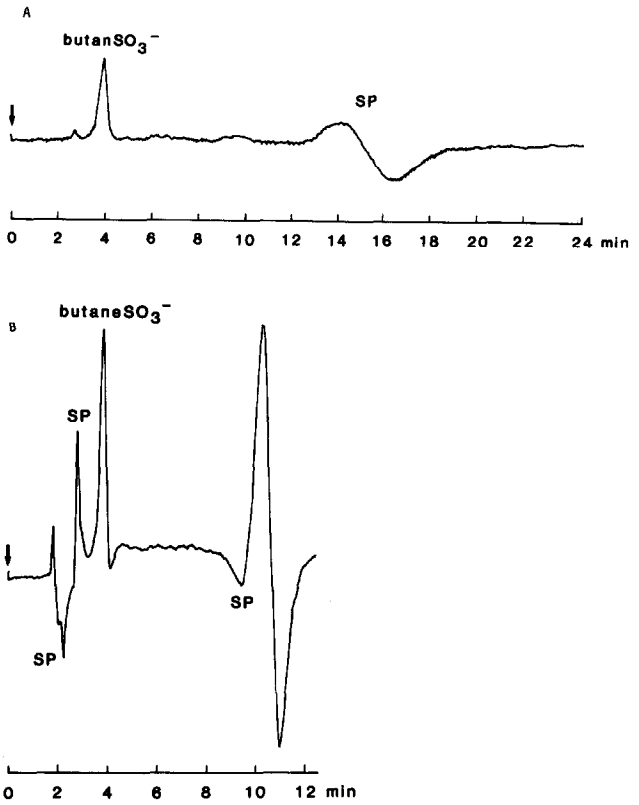


Fig 2. Wave-shaped system peaks. Solid phase: PRP-1. Solute: butanesulphonate. Mobile phases: (A) $5 \cdot 10^{-6}$ *M* 60HNS–0.01 *M* phosphoric acid–4% methanol; (B) $1.5 \cdot 10^{-4}$ *M* 60HNS–0.01 *M* phosphoric acid–4% methanol. SP = system peaks.

and this may result in multipeak chromatograms. However, a low θ_Q does not seem to be the main reason for the disturbances in this particular example. Wave-shaped system peaks appear at probe concentrations of $5 \cdot 10^{-6}$ as well as at $15 \cdot 10^{-4}$ *M* and there is no indication of less deformation of system peaks at probe concentrations as high as 30 times the lower probe concentration.

A low buffer capacity, β, of the chromatographic system can also be the reason for deformation of the peaks. The total concentration of phosphoric acid is 0.01 *M*, and the resulting pH is close to the first pK_a value of phosphoric acid, which gives $\beta = 0.006$. However, this low buffer capacity is not sufficient to prevent a pH change within the system peak zone if the retention of the probe is high and the aprotic anion of 60HNS is bound as ion pair with H^+ or exchanged for $H_2PO_4^-$ in the stationary phase. For example, when the concentration of the probe, 60HNS, is $1.5 \cdot 10^{-4}$ *M*, the system peak has $k' = 3.8$. Approximate calculations based on $\Delta C_{H_2PO_4}/\Delta pH = \beta = 0.006$ give $\Delta pH = 0.002$, which will affect the distribution of 60HNS.

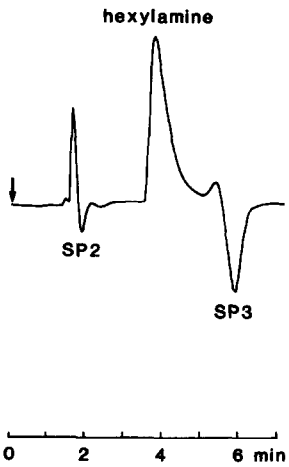
Previous studies[10,11] have shown that the solid phase, PRP-1, is highly hydrophobic and can bind hydrophilic buffer components. Fig. 2A and B show that at least two system peaks appear in the chromatograms. This indicated that the buffer

components, H^+, and dihydrogenphosphate, p^-, are bound. If the peak distortion is due to a pH change, the disturbances should be different, depending on the nature of the buffer components. The disturbances would also increase with decreasing concentration of the buffer and increasing binding of the buffer to the adsorbent.

Experiment at a concentration of 60HNS of $5 \cdot 10^{-5}$ *M* showed that the systems became more unstable when the concentration of phosphoric acid decreased from 10^{-2} to 10^{-3} *M*. Increasing the pH from 2.3 to 6.9 at a constant analytical concentration of phosphoric acid also resulted in a less stable system.

Phenyl-bonded silica as adsorbent with a protolytic probe. Unstable systems giving wave-shaped system and solute peaks as well as extra peaks were also observed when alkyl- or aryl-bonded silica were used as the adsorbents. Examples of such chromatograms are seen in Figs. 3 and 4. The chromatographic system is denoted as No. 3 in Table II. It has a low buffer capacity, and the fractional coverage of the probe is probably fairly low. The probe is protolytic, and the system contains no further buffering agent.

The mobile phase contains three ions, Na^+, 30HBA and tetrapropylammonium, TPrA, and gives two retained system peaks, SP2 originating from 30HBA and SP3 from TPrA. The two chromatograms show distortions but to different extents. The sample peaks are asymmetric but the hexanoic acid peak (Fig. 4) is less distorted than the hexylamine peak in Fig. 3, which ends in an extra peak just before SP3. SP2 is more wave-shaped in Fig. 3. The peak distortion is due to the fact that the probe has a buffering function in the system, and a change in probe concentration can change the pH of the mobile phase significantly, since the buffer capacity is very low. The large distortion on injection of hexylamine could be due to the fact that 30HBA acts as a counter ion in the distribution of the cationic sample.



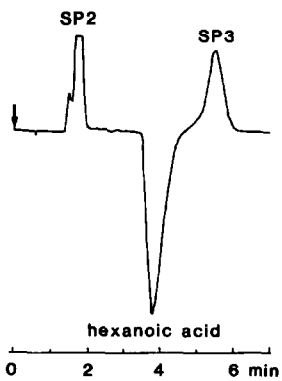


Fig. 3. Unstable chromatographic system. Solid phase: μBondapak Phenyl. Mobile phase: 10^{-3} *M* 30HBA–$3.7 \cdot 10^{-4}$ *M* TPrA–sodium hydroxide to pH 6. Solute: hexylamine. From ref. 2. Reprinted with permission.

Fig. 4. Unstable chromatographic system as in Fig. 3 but solute was hexanoic acid. From ref. 2. Reprinted with permission.

The peak distortions due to low system stability, *e.g.*, low buffer capacity, could be initiated at the injection zone, but it seems likely that they are amplified and, in fact, mainly created during the migration of the compound zone in the column.

Direction of extra peaks

The extra peaks appearing close to the main peaks in insufficiently buffered solutions can have the same or the opposite direction as the main peak (*cf.*, Fig. 2B). A discussion may be based on the basic expression for the indirect response, given below. It is valid for a solute HA^+ in a chromatographic system containing two components, the probe, Q^+, and the counter ion, X^-, that can be distributed to the adsorbent. The third component, B^+, is so hydrophilic that its distribution is negligible (*cf.*, ref. 6)

$$\frac{\varepsilon^*_{HA}}{\varepsilon_Q} = L - M \tag{16}$$

where

$$L = \frac{\alpha_s}{1 - \alpha_s} \cdot \theta_Q \tag{17}$$

and

$$M = \frac{1}{1 - \alpha_s} \cdot \frac{C_Q}{2C_Q + C_B} \tag{18}$$

The M term is usually negligible when C_B is much larger than C_Q. If this is not the case and C_B changes within the solute zone, due to, *e.g.*, protolysis, it can have a significant effect on the response. An increase in C_B will increase the absolute value of the response, and a shoulder or second maximum may appear in the peak. A decrease in C_B has the opposite effect, and if L and M are of similar magnitude, it can even change the direction of the response, which may result in a wave-shaped peak.

Design of ion-pairing systems for indirect detection

From the basic expression for indirect detection in ion-pairing systems (eqn. 16) it follows that optimum sensitivity is obtained when the retention of the solute is close to that of the probe (α_s close to 1) and when the detector response of the probe, *e.g.*, the molar absorptivity is high. For solutes that are uncharged or have the same charge as that of the probe, the sensitivity increases with the fractional loading of the probe on the adsorbent, θ_Q.

To avoid disturbances in sensitivity and peak shape, it is important to use mobile phases which contain only a few components: if possible only the probe and hydrophilic buffer. More than one hydrophobic component in the mobile phase may result in several retained system peaks and decrease the detection sensitivity for the components that are eluted between them.

The probe should be aprotic to avoid detection disturbances due to protolysis.

The buffer should have a capacity and concentration such that changes in the distribution of the probe are avoided.

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