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RATIONALIZATION OF THE SELECTION OF THE TYPE OF THE ORGANIC MODIFIER(S) FOR SELECTIVITY OPTIMIZATION IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

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SUMMARY

The selection of the type and/or concentration of the organic modifier(s) in reversed-phase ion-pair liquid chromatography (RP-IPC) can be rationalized by considering the nature of the solutes in the sample mixture. The separation of a few typical sample mixtures containing ionic and/or uncharged solutes and requiring the variation or optimization of the type and/or the concentration of the organic modifier(s) is discussed. The examples demonstrate the utility of the sample composition-based parameter selection method and highlight the importance of organic modifier optimization in RP-IPC.

The problems associated with the use of the solvent strength parameters in the determination of the initial eluent compositions prior to mobile phase optimization in RP-IPC are discussed. Eluent compositions which were predicted to lead to identical pairing ion surface concentrations at constant ionic strength (*i.e.*, to create isopotential conditions) agree reasonably with the eluent composition data found experimentally to be isoeluotropic in different RP-IPC systems. Comparison of the different transfer rules reveals that those defined for reversed-phase systems overestimate the isoeluotropic eluent compositions in RP-IPC. When ionized solutes and oppositely charged pairing ions are present, one may need to decrease the predicted organic modifier concentrations by as much as 0.15–0.2 volume fraction units in order to obtain identical retention conditions. The main reasons why transfer rule equations could not be obtained for RP-IPC systems include the different ln k' *vs.* organic solvent concentration behaviors of ionic and uncharged solutes in the regular RP mode and the different adsorption behaviors of the pairing ion(s) as the organic modifier is changed in the eluent systems.

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INTRODUCTION

In reversed-phase ion-pair chromatography (RP-IPC), separation selectivity for ionic–ionizable–uncharged solute mixtures can be usually optimized by the variation of the eluent pH and/or the concentration of the pairing ion and the organic modifier. In contrast to the reversed-phase mode, where the type and concentration of the organic modifiers are the most important selectivity optimization parameters, the general practice in RP-IPC is to use the organic modifier only to set a certain solvent strength in order to control the overall retention of the solutes[1–3]. In a number of studies, the variation of neither the solvent strength (concentration) nor the type of organic modifier was found to result in significant selectivity changes for ionic solute mixtures[3–6].

However, other studies have demonstrated that certain ionic solute mixtures can be separated advantageously by varying the concentration[3,7–9] and/or the type[10–16] of organic modifier(s) in the presence of a pairing ion. Tomlinson and Riley[17] found that for aromatic solutes of similar charge and structure, differing only in their polar functional groups, the separation selectivity could be varied by changing the type and concentration of the organic modifier(s) in the eluent.

The simultaneous variation of the type and/or concentration of the organic modifier and the pairing ion leads to complex eluent systems and more parameters to be optimized. Owing to the greater number and range of optimization variables, finding the selectivity optimum becomes much more difficult, no matter what method (trial-and-error or computer-aided) is used. Therefore, it is imperative to identify the separation problems where the variation of the type and/or concentration of the organic modifier(s) is beneficial and to establish an efficient strategy to select the initial experimental conditions for systematic eluent optimization procedures in RP-IPC.

Previously, a rational approach has been developed by Low *et al.*[18] and Bartha *et al.*[19] for the selection of the primary mobile phase optimization parameters (eluent pH, organic modifier concentration, charge type of the pairing ion) and the secondary mobile phase optimization variables (concentration and hydrophobicity of the pairing ion) by considering the nature (charge type and relative hydrophobicity) of the components in the sample mixture. Until now, the wide choice of other parameters and the limited understanding of the retention behavior of ionic solutes in RP-IPC prevented the systematic investigation of the rules that are involved in the selection of the organic modifier(s). Recently, we have extended the electrostatic theory of ion-pair chromatography to include the simultaneous effects of the organic modifier and the pairing ion on the adsorption of the pairing ion and the retention of charged solutes[20]. Practical conclusions drawn from this theory can be used to rationalize the selection of the type of organic modifier.

As separation selectivity can be improved by changing the type and/or the concentration of the organic modifier(s) in RP-IPC, an efficient strategy is needed to select the composition of the new mobile phase(s). The selection process should make use of the solute retention data measured in the first (unsuccessful) organic modifier–aqueous buffer system. The question is how to estimate the composition of the new binary eluent that will lead to reasonable retention limits (similar to those obtained with the first modifier system) for the least and the most retained components.

In the reversed-phase mode, solvent polarity scales[21,22], empirical transfer rules[23,24] or partition coefficient-based transfer rules[25] were developed for the common organic modifier (methanol, acetonitrile or tetrahydrofuran)–water binary eluent systems to select the initial isoeluotropic binary eluent compositions for systematic binary, ternary or quaternary eluent optimization. Unfortunately, none of the RP solvent strength parameters or transfer rules proved applicable in the RP-IPC mode[14,15,26,27].

In this paper, we consider the merits of the optimization of the type and/or the concentration of the organic modifiers in RP-IPC. We identify a few typical solute mixtures that require and benefit from the variation of the type of organic modifier. The problem of defining transfer rules for RP-IPC systems is examined on the basis of the extended electrostatic theory of ion-pair chromatography.

EXPERIMENTAL

High-performance liquid chromatographic (HPLC)-grade methanol, acetonitrile (ACN) and tetrahydrofuran (THF) were purchased from Mallinckrodt (Paris, KY, U.S.A.) and Fischer (Fairlawn, NJ, U.S.A.). Distilled, deionized water was prepared with a Milli-Q water purification system (Millipore, Milford, MA, U.S.A.). Gold Label quality triethylamine (TEA), phosphoric acid (85%, w/w) and sodium dihydrogenphosphate were used as buffer components (Aldrich, Milwaukee, WI, U.S.A.). Solutes and ion-pairing reagents (tetrabutylammonium bromide and sodium octylsulfonate) were obtained from Aldrich (Milwaukee, WI, U.S.A.) and used without further purification.

Mobile phases were prepared from various amounts of methanol, acetonitrile or tetrahydrofuran and either 25 m*M* H_3PO_4–25 m*M* NaH_2PO_4 (pH 2.1) buffer with sodium bromide (NaBr) salt and sodium octylsulfonate, or 15 m*M* triethylamine–phosphate buffer (pH 7.5) with tetrabutylammonium bromide. Sample mixtures were dissolved in the eluents and shifting peaks in the successive chromatograms were identified by injecting each solute separately.

A Novapak C_{18} (5 μm) (Waters Assoc., Milford, MA, U.S.A.) column (150 × 4.6 mm I.D.) and an ODS-Hypersil (5 μm) (Shandon Southern, London, U.K.) column (120 × 4.6 mm I.D.) thermostated at 25°C were used with an eluent flow-rate of 1 ml/min.

The chromatographic system consisted of an LC 5560 liquid chromatograph, a Model 4270 integrator, a UV (254 nm) and a refractive index detector (all from Varian, Walnut Creek, CA, U.S.A.) and a Model 7125 six-port injection valve (Rheodyne, Cotati, CA, U.S.A.) with a 20-μl injection loop.

RESULTS AND DISCUSSION

Selectivity effects related to the type of organic modifier(s) in RP-IPC

Recently, we have shown[20] that for mixtures of either ionic solutes or ionic and uncharged solutes the separation selectivity can be changed significantly when the concentrations of the pairing ion and the organic modifier are varied simultaneously. The separation of closely related solutes with similar charge-type and structure especially can benefit from the variation of the type (not only the concentration) of the organic modifier(s) in the presence of a pairing ion.

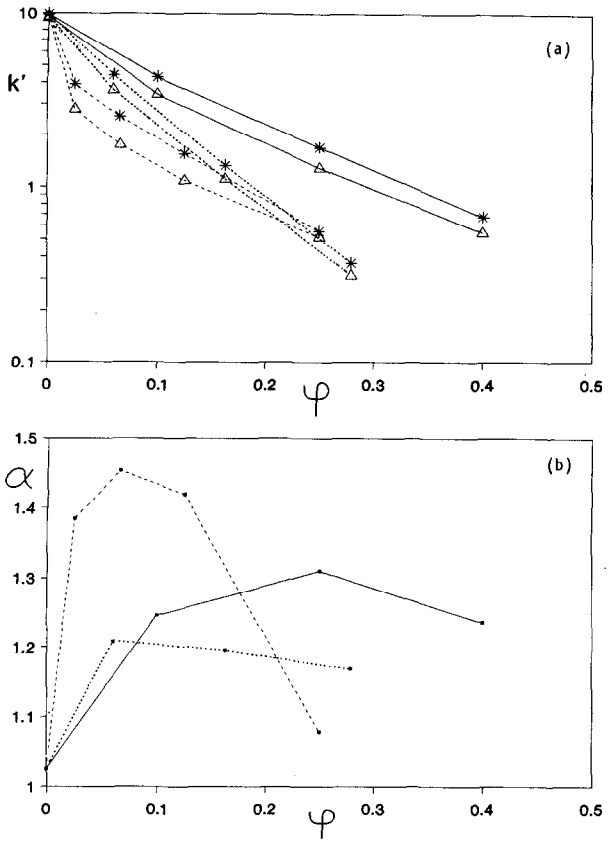

Fig. 1. Variation of (a) the capacity factor (k') and (b) the separation selectivity (α) of adrenaline ($*$) and octopamine ($\triangle$) as a function of the methanol (solid lines), acetonitrile (dotted lines) and tetrahydrofuran (dashed lines) volume fraction concentration (φ) of the aqueous buffer (50 mM phosphate, pH 2.5) eluent, at a constant mobile phase concentration of the sodium octylsulfonate pairing ion (c_A = 40 mM). Column, 5-μm ODS-Hypersil; temperature, 25°C.

A typical example is shown in Fig. 1a, where the capacity factors (k') of two strong bases (adrenaline and octopamine) are plotted against the methanol, acetonitrile and tetrahydrofuran (φ) volume fraction concentration of the aqueous buffer (pH 2.5) eluents. The eluents also contained 40 mM sodium octylsulfonate (a pairing ion) to increase solute retention. Without the negatively charged pairing ion, both solutes are retained too weakly ($k' < 1.2$) and no organic modifier can be added to the aqueous buffer eluent. In Fig. 1b the separation selectivity (α) is plotted against the concentration of the organic modifiers. It can be seen that the separation selectivity changes significantly with the type of organic modifier. The selectivity is much better (1.46) with tetrahydrofuran than with the other two organic modifiers.

Several typical examples are given below to demonstrate that the selection of the type and/or the concentration of the organic modifiers is related to the presence (or absence) of certain solute types. The capacity factor (k') *vs.* pH plots for the samples are used to illustrate the separation problems schematically. The selected combinations of

the mobile phase variables (pH, concentration of the organic modifier and the pairing ion) are shown in a three-dimensional representation. The examples will be discussed according to the procedural strategy described previously[18]. They can be divided into two main groups: (i) separation problems that benefit from the variation of either the concentration (solvent strength optimization) or the type (solvent type optimization) of the organic modifier and (ii) separation problems which benefit from the optimization of the solvent type only.

We do not intend here to evaluate the relative merits of solvent strength *vs.* solvent type optimization, but to demonstrate that selectivity can be altered by variation of either the solvent strength or the solvent type or both. The examples represent satisfactory solutions, not fully optimized separations.

Separation of ionic solutes of similar hydrophobicity. Let us consider a seven-component mixture of catecholamines and related compounds. In an aqueous buffer (pH 2.5) eluent on an ODS-Hypersil column the first component elutes close to the solvent front ($k'_{first} < 0.2$) and components 4 and 5 are not separated (Fig. 2).

This problem can be represented conceptually as the separation of a mixture of hydrophilic strong bases (Fig. 3a). The most important feature of this example is that all components have similar (positive) charges and structures, and are weakly retained between pH 2.5 and 7.5, even in pure aqueous eluents. In order to increase the retention of all solutes and effect their separation, an oppositely (negatively) charged pairing ion must be added to the eluent. A high pairing ion concentration can result in excessive solute retention, which must be (and, fortunately, can be) countered by adding an organic modifier (*e.g.*, methanol) to the eluent. Simultaneous variation of the concentrations of the organic modifier and the pairing ion results in the optimization

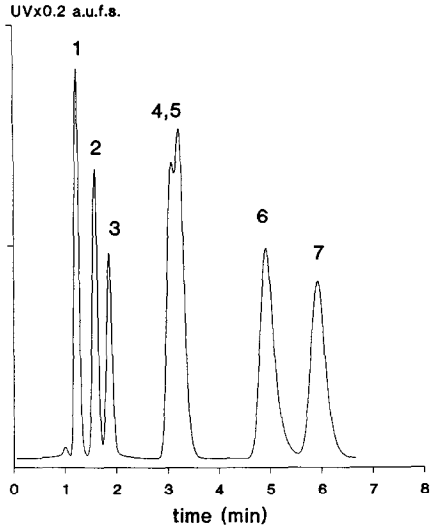

Fig. 2. Chromatogram of a seven-component mixture of catecholamines and related compounds. Column, 5-μm ODS-Hypersil; eluent, 50 mM aqueous phosphate buffer (pH 2.5), constant ionic strength (175 mM) adjusted with sodium bromide. Solutes: (1) noradrenaline; (2) adrenaline; (3) octopamine; (4) 3,4-dihydroxyphenylalanine; (5) dopamine; (6) isoprenol; (7) tyrosine.

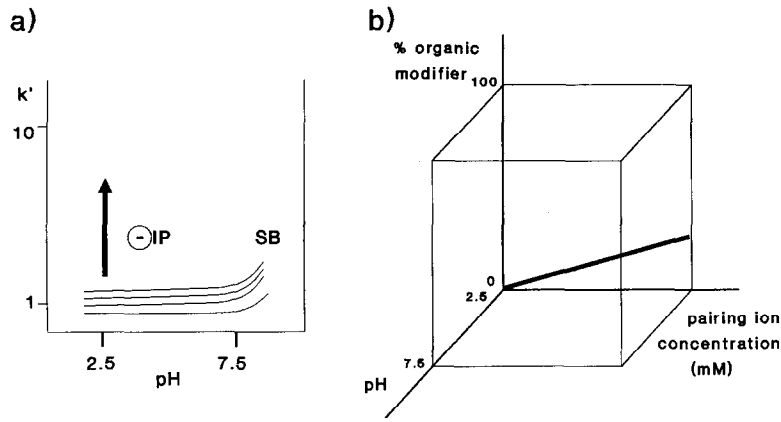

Fig. 3. (a) Schematic k' *vs.* pH behavior of a solute mixture of hydrophilic strong bases (SB); (b) selected combination of mobile phase variables (pH, organic modifier and pairing ion concentration) for selectivity optimization. The arrow indicates the direction of solute retention change as a negatively charged ion-pairing reagent (−IP) is added to the eluent.

parameter space shown in Fig. 3b. According to the electrostatic retention theory of ion-pair chromatography[20], ln k' of the ionic solutes is linearly related to the volume fraction of the organic modifier (φ) and to ln c_A, where c_A is the mobile phase concentration of the pairing ion. Therefore, in order to compensate for their opposite effects on solute retention, a linear increase in the concentration of the organic modifier must be accompanied by a logarithmic increase in the mobile phase concentration of the pairing ion. Whereas in opportune cases the separation selectivity will vary along this composition line, the overall ionic solute retention will remain within reasonable limits. When the solutes cannot be separated by this strategy, one can try to change the type of the organic modifier.

This approach is followed with the seven-component sample shown in Fig. 4a: 2 mM sodium octylsulfonate and 10% (v/v) methanol are added to the eluent. The overall analysis time is comparable to that in Fig. 2, and components 2–3, 4–5 and 6–7 coelute. The retention order is also identical in the chromatograms in Figs. 2 and 4a. Therefore, selection of separation conditions intermediate to these extremes is not likely to improve the separation. An identical elution order and coeluting peaks are observed when methanol is replaced with 2.5% (v/v) tetrahydrofuran (Fig. 4b). On the other hand, the use of 6% (v/v) acetonitrile results in different selectivity (*cf.*, elution order of components 6–7) and a good separation of all components (Fig. 4c).

The separation of mixtures of hydrophilic strong acids or mixtures of weak acids or weak bases represents a similar problem because, owing to their low retention in the ion-suppression mode, no organic modifier can be added to the eluent. As a general rule, when the sample contains very hydrophilic ionic and/or ionizable solutes, the addition of an oppositely charged ion-pairing reagent and the simultaneous variation of the type and/or the concentration of the organic modifier will probably enhance the selectivity of the separation.

Separation of ionic solutes of different hydrophobicity. The separation of a four-component mixture of aromatic sulfonic acids demonstrates another type of

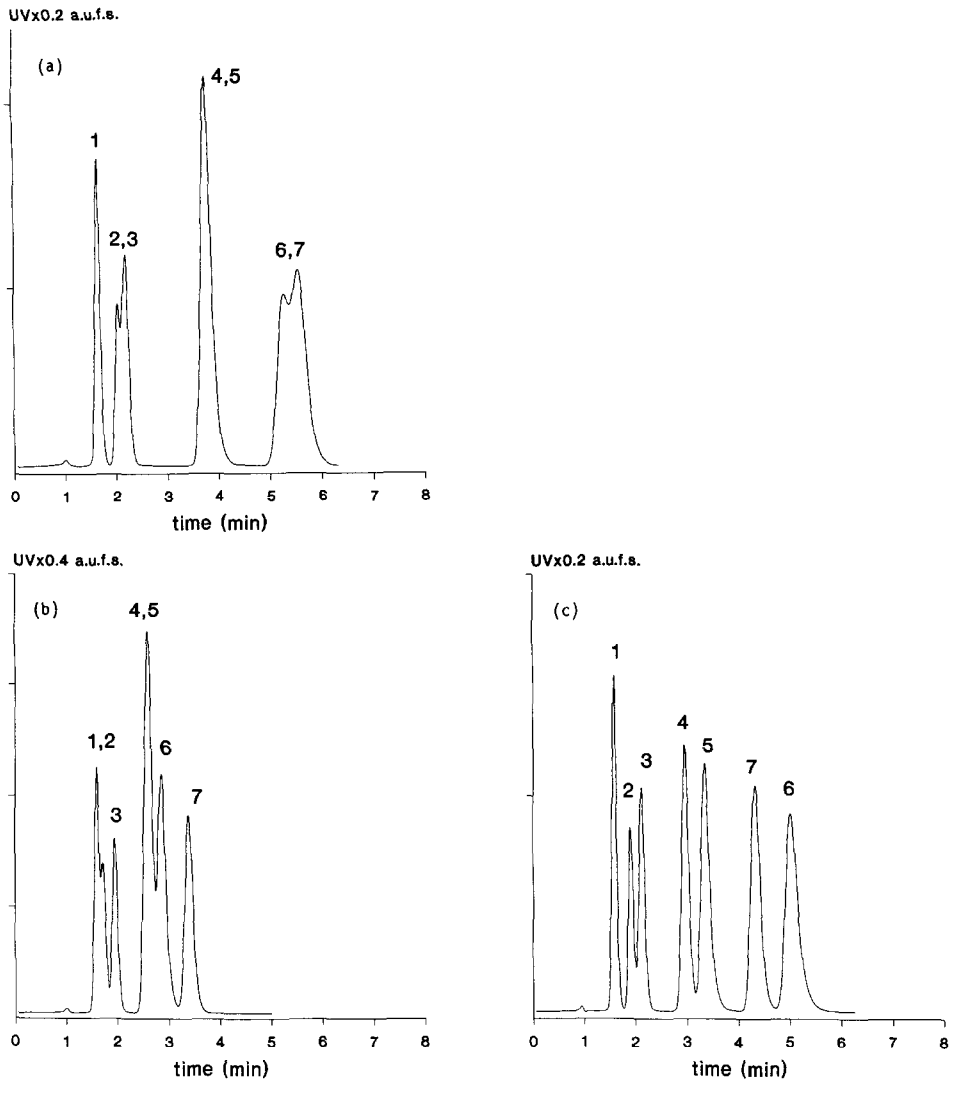

Fig. 4. Chromatograms of the seven-component mixture in Fig. 1 in aqueous buffer eluents which contain 2 m*M* sodium octylsulfonate and (a) 10% (v/v) methanol, (b) 2.5% (v/v) tetrahydrofuran, (c) 6% (v/v) acetonitrile. Solutes and other conditions as in Fig. 2.

separation problem. In the absence of a pairing ion, this sample can be eluted in 10 min using a 20% (v/v) methanol–triethylamine–phosphate buffer (pH 7.5) eluent and a Novapak C_{18} column. However, the first component elutes with the solvent front, components 2 and 3 coelute and the last component elutes far removed from the others (Fig. 5).

This problem can be represented again in a simplified, abstract form as the separation of hydrophilic and hydrophobic strong acids (Fig. 6a). The salient feature of this example is the large retention gap between the first and last peaks. Initially the

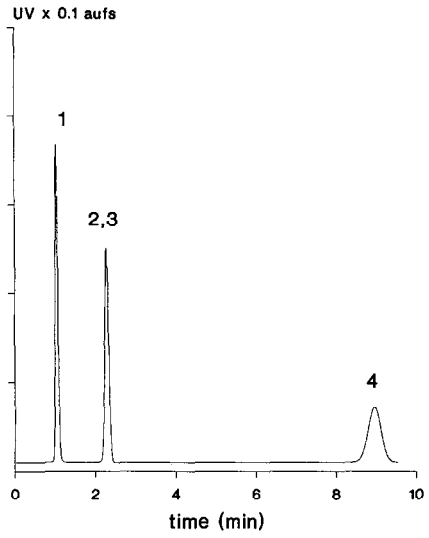

Fig. 5. Chromatogram of a four-component mixture of aromatic sulfonic acids. Column, 5-μm Novapak C_{18}; eluent, 15 m*M* triethylamine–phosphate aqueous buffer (pH 7.5) containing 20% (v/v) methanol. Solutes: (1) 1,5-naphthalenedisulfonic acid; (2) *p*-toluenesulfonic acid; (3) 2,4-dinitrobenzenesulfonic acid; (4) 2-naphthalenesulfonic acid.

organic modifier concentration can be fixed at a level which results in a reasonable retention time for the last peak. The hydrophilic first components, however, elute at the solvent front. As in the previous example, one can try to vary simultaneously the concentrations of the pairing ion and the organic modifier (Fig. 6b) in an attempt to increase the retention of the early eluting components. This also increases the retention

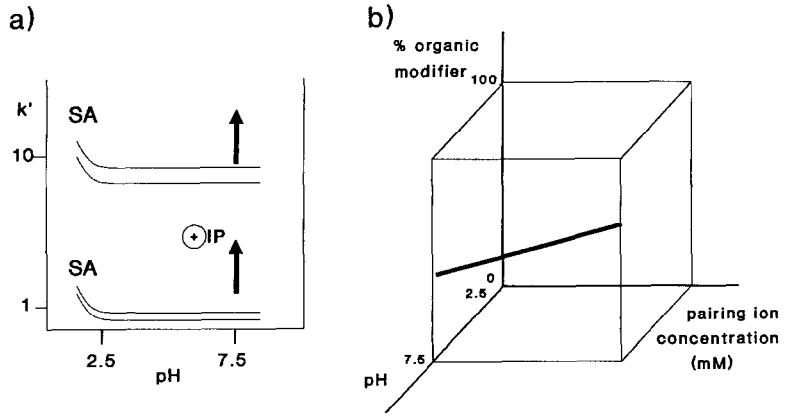

Fig. 6. (a) Schematic *k' vs.* pH behavior of a solute mixture which contains both hydrophilic and hydrophobic strong acids (SA); (b) selected combination of mobile phase variables for selectivity optimization. The arrows indicate the direction of solute retention changes as a positively charged ion-pairing reagent (+IP) is added to the eluent. The heavy line represents the recommended optimization parameter space.

of the last peak(s). In most instances, the retention gap can only be closed by varying the type of the organic modifier.

In our example, a positively charged pairing ion is added to the eluent in order to (i) increase the retention of the first peak, (ii) affect the separation of peaks 2–3, and (iii) close the retention gap between peaks 1–2–3 and 4. The addition of 5 m*M* tetrabutylammonium bromide to the eluent causes a large increase in the retention of the negatively charged strong acids. This can be compensated for by simultaneously increasing the methanol concentration to 42% (v/v) (Fig. 7a). The separation of the

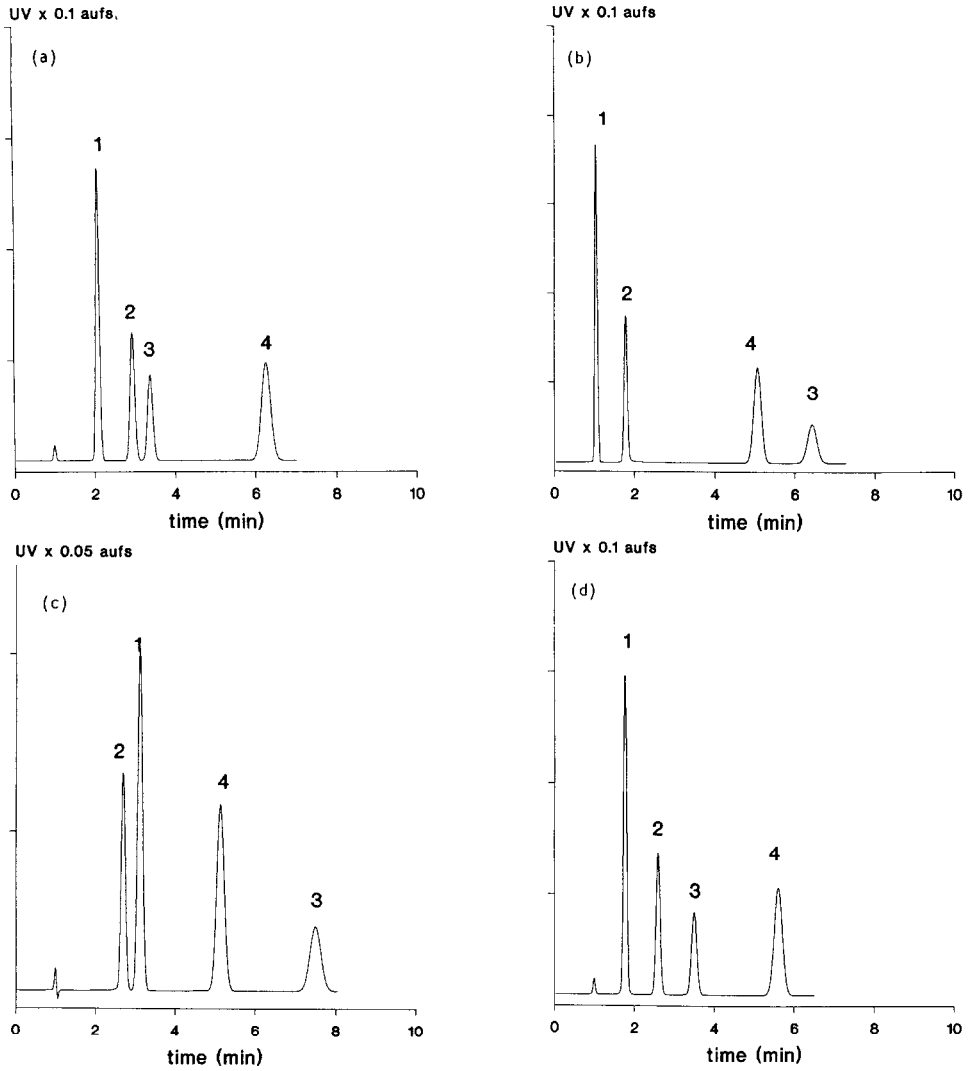

Fig. 7. Chromatograms of the four-component sample mixture in Fig. 5 in aqueous buffer eluents which contain 5 m*M* tetrabutylammonium bromide and (a) 42% (v/v) methanol, (b) 14% (v/v) tetrahydrofuran, (c) 28% (v/v) acetonitrile and (d) 38% (v/v) methanol and 1.4% (v/v) tetrahydrofuran. Solutes and other conditions as in Fig. 5.

sample has improved: the first component elutes at $k' = 1$, components 2–3 are separated and the retention gap between components 2–3 and 4 is decreased.

Replacing methanol with 14% (v/v) tetrahydrofuran or 28% (v/v) acetonitrile (see Fig. 7b and c, respectively), with 5 m*M* tetrabutylammonium bromide in the eluent, results in separations with comparable analysis times but different separation selectivities (compare the elution order reversal of components 1–2 and 3–4). Obviously, mixing the 42% (v/v) methanol eluent with any of the other (28% ACN or 14% THF) eluents would result in improved separation of components 2–3 and in a better spread of the peaks. This prediction is verified by the chromatogram shown in Fig. 7d. The eluent contains 5 m*M* tetrabutylammonium bromide, 38% (v/v) methanol, 1.4% (v/v) THF and 60.6% (v/v) aqueous buffer. This last chromatogram represents a satisfactory solution for the separation problem: all components are well resolved both from each other and the solvent front, the peaks are evenly distributed and the analysis time is reasonable.

This example highlights the selectivity optimization potential that one can realize by varying the type of organic modifier(s) in RP-IPC. As a general rule, when the charge type of the first and last components is the same and the retention gap is excessive, it is advantageous to try a different organic modifier. When the retention gap remains unacceptably large, one must consider either gradient elution or a change in the phase system. If such a solute combination is present in a sample [*i.e.*, weakly and strongly retained solute(s) of identical charge type], this rule will apply irrespective of the nature of all the other components. The presence or absence of other solute types may constrain the selection of the other optimization parameters, *e.g.*, charge type of the pairing ion, eluent pH.

Separation of solutes of different charge types and relative hydrophobicities. There are a number of solute combinations which pose separation problems that preclude any change in the concentration of the organic modifier. Additionally, the sample may contain closely related compounds which cannot be separated by optimizing the eluent pH or the concentration of the pairing ion.

Generally, the presence of hydrophilic and/or hydrophobic uncharged solutes will restrict the (upper and/or lower) concentration limits of the organic modifier. The sample mixture shown in Fig. 8a contains both hydrophilic and hydrophobic uncharged solutes and hydrophilic strong bases. Clearly, the organic modifier concentration must be fixed at a level which results in reasonable retention of both the least and the most retained uncharged solutes. The retention of the weakly retained strong bases can be increased by the addition of an oppositely (negatively) charged ion-pairing reagent. As there are no weak acids or bases present, the eluent pH can be fixed at any convenient level (*e.g.*, at low pH, which usually provides a better peak shape for strong bases). The resulting combination of the optimization variables is shown in Fig. 8b. Although the retention of the strong bases can be shifted in the chromatogram almost at will by changing the concentration of the pairing ion, there is no guarantee that either the charged or the uncharged solutes will be separated with a given organic modifier–aqueous buffer eluent. After exploring the composition line assigned in Fig. 8b, one must change the type of organic modifier and, if necessary, carry out a solvent-type (*e.g.*, ternary eluent) optimization.

However, it is not only the presence of the uncharged solutes which can constrain the concentration of the organic modifier. The concentration of the organic modifier is

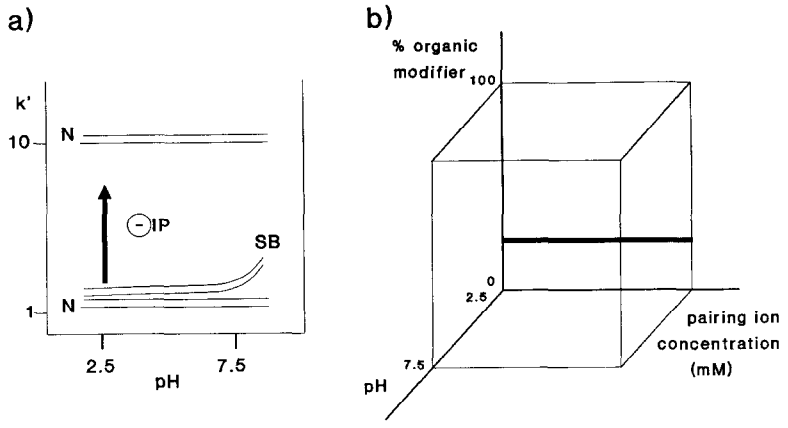

Fig. 8. (a) Schematic k' *vs.* pH behavior of a solute mixture which contains hydrophilic strong bases (SB) and both hydrophilic and hydrophobic uncharged (N) solutes; (b) recommended optimization parameter space represented by the heavy line.

fixed by the presence of hydrophilic and hydrophobic weak acids in Fig. 9a and by strong bases in Fig. 9b. In both instances, a negatively charged ion-pairing reagent must be used to increase the retention of the early eluting strong bases. The suggested optimization parameter space is similar to that shown in Fig. 8b, except that an eluent of high pH is used for the sample mixture in Fig. 9b. Again, if the separation of all solutes cannot be achieved with a given organic modifier along the composition line proposed, one should try a different organic modifier. Separation problems identical with those in Fig. 9 have been extensively analysed elsewhere[19,27].

The examples discussed in the above three sections represent only a few of the cases when the type and/or concentration of the organic modifier(s) must be varied in the presence of a pairing ion in order to separate ionic–ionizable–uncharged solute mixtures. However, these problems clearly demonstrate that the selection of the mobile phase variables is constrained by the presence (or absence) of certain solute types and their particular combinations.

As also shown above, general rules can be formulated which help the analyst to

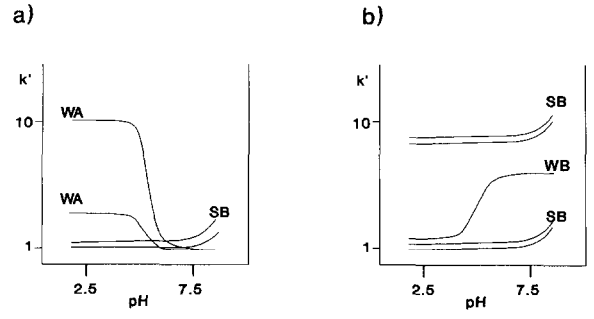

Fig. 9. Schematic k' *vs.* pH behavior of solute mixtures which contain either (a) hydrophilic strong bases (SB) and both hydrophilic and hydrophobic weak acids (WA), or (b) hydrophilic weak base (WB) and both hydrophilic and hydrophobic strong bases (SB).

decide when and which of these parameters need to be selected for optimization. Such rules are part of a knowledge-base currently under development in our laboratory. This knowledge-base can be used to implement the sample composition-based parameter selection method in an ion-pair chromatographic expert system. The expert system will select the possible combinations of the optimization parameters (pH, negatively charged pairing ion, etc.) and the initial eluent compositions needed for the actual chromatographic experiments. Consequently, it is important to establish the basis of an efficient strategy (which can be used either by the practicing chromatographer or built into an expert system) to find the initial compositions of different organic modifier–aqueous buffer eluents prior to solvent-type optimization in RP-IPC.

Evaluation of the use of solvent strength transfer rules in RP-IPC

In RP-LC, the initial binary eluent compositions used in solvent-type optimization are often selected on the basis of transfer rule equations[21–25]. However, in accordance with other studies[14,15,26,27], we found that the eluent compositions predicted by these transfer rules had to be readjusted experimentally to provide comparable retention conditions for ionic samples chromatographed in the presence of a pairing ion.

To examine this problem in more detail, the eluent compositions predicted by the different RP transfer rules are compared with the eluent compositions which were found experimentally to be isoeluotropic in RP-IPC systems. Five commonly used transfer rule equations are summarized in Table I and examples taken from the literature are shown in Table II. When plotted in Fig. 10, the transfer rules developed for RP-LC agree remarkably with each other, with the only exception that the RP solvent strength parameter[22] predicts higher isoeluotropic compositions for the acetonitrile–water eluents. Apart from this outlying transfer rule, the other RP transfer rule equations predict composition values which are identical within ± 0.05 for $0 \leq \varphi \leq 0.8$.

On the other hand, all experimentally found isoeluotropic eluent compositions for ionic ionizable solute mixtures lie below the plots of the five RP transfer rules.

TABLE I

FIVE TRANSFER RULE EQUATIONS COMMONLY USED TO DETERMINE THE INITIAL ELUENT COMPOSITIONS PRIOR TO SOLVENT-TYPE OPTIMIZATION IN RP-LC

Ref.	*Basis*	*Equation*
21	Solubility parameter	$\varphi_{ACN} = 0.78\varphi_{CH_3OH}$ $\varphi_{THF} = 0.62\varphi_{CH_3OH}$
22	Solvent strength parameter	$\varphi_{ACN} = 0.968\varphi_{CH_3OH}$ $\varphi_{THF} = 0.682\varphi_{CH_3OH}$
23	Empirical	$\varphi_{ACN} = 0.32\varphi^2_{CH_3OH} + 0.57\varphi_{CH_3OH}$ $\varphi_{THF} = 0.66\varphi_{CH_3OH}$
24	Empirical	$\varphi_{ACN} = -0.49\varphi^3_{CH_3OH} + 0.953\varphi^2_{CH_3OH} + 0.447\varphi_{CH_3OH}$ $\varphi_{THF} = -0.42\varphi^3_{CH_3OH} + 0.702\varphi^2_{CH_3OH} + 0.423\varphi_{CH_3OH}$
25	Partition coefficient	$\varphi_{ACN} = 0.081\varphi^2_{CH_3OH} + 0.698\varphi_{CH_3OH}$ $\varphi_{THF} = 0.046\varphi^2_{CH_3OH} + 0.621\varphi_{CH_3OH}$

TABLE II

EXPERIMENTALLY FOUND ORGANIC MODIFIER–AQUEOUS BUFFER ELUENT COMPOSITIONS WHICH PROVED TO BE ISOELUOTROPIC IN DIFFERENT RP-IPC SYSTEMS

Ref.	*Solutes*	*Pairing ion*[a]	φ_{CH_3OH}	φ_{ACN}	φ_{THF}
6	Doxycyclines	1 m*M* TBA	0.4	0.21	0.1
13	Bile acids	5 m*M* TBA	0.73	0.5	–
14	Tricyclic antidepressants	20–70 m*M* HSA	0.66	0.52	0.34
15	Sympathomimetic amines	5 m*M* HSA	0.5	0.24	0.18
16	Water-soluble vitamins	5 m*M* HSA	0.15	0.07	0.03
26	Pyrroloquinoline quinone	15 m*M* TEA	0.44	0.3	0.15
27	Local anaesthetics	5 m*M* OSA	0.5	0.3	0.18
[b]	Catecholamines	2 m*M* OSA	0.1	0.06	0.025
[b]	Aromatic sulfonic acids	5 m*M* TBA	0.42	0.28	0.14

[a] TBA = Tetrabutylammonium bromide; TEA = triethylamine; HSA = sodium hexanesulfonate; OSA = sodium octanesulfonate.

[b] This study.

A consistently large deviation (about -0.15 in φ_{THF}) is found for the tetrahydrofuran–aqueous buffer eluents. For acetonitrile some of the eluent compositions are close to, or coincide with, the predictions made by the RP transfer rules.

This behavior strongly suggests that the RP transfer rules which translate the eluent methanol concentration into equivalent acetonitrile or tetrahydrofuran concentrations provide only a conservative overestimate of the isoeluotropic eluent

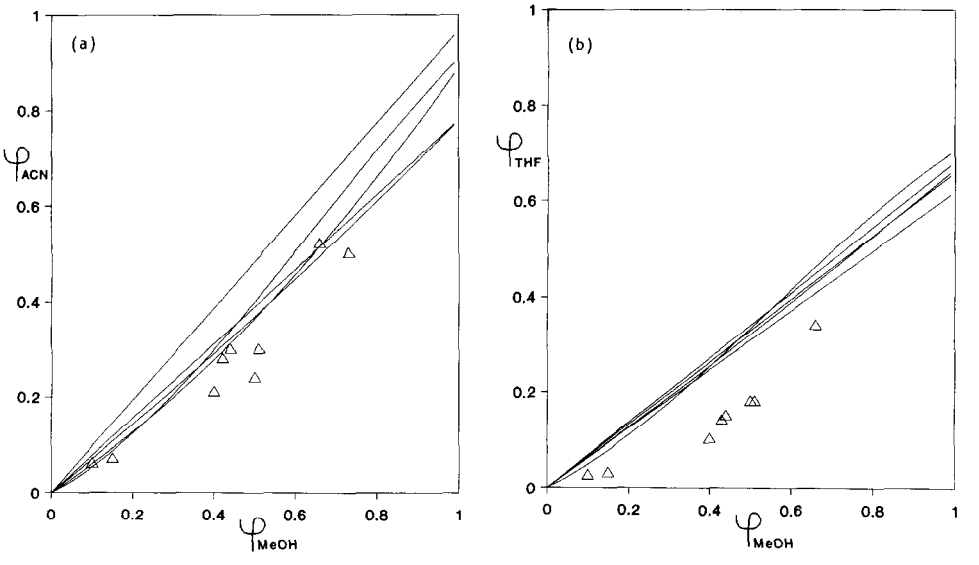

Fig. 10. Comparison of the five commonly used reversed-phase transfer rule equations listed in Table I (solid lines) with the experimentally found ion-pair chromatographic isoeluotropic mobile phase compositions listed in Table II (triangles) for (a) acetonitrile and (b) tetrahydrofuran *vs.* methanol–aqueous buffer eluents. MeOH = Methanol.

compositions in RP-IPC systems. In other words, the solvent strength of the eluent defined by any of the RP transfer rules will be sufficiently high to elute the sample components with retention times comparable to or lower than those in the RP mode, irrespective of the charge type of the components in the sample mixture. When ionized solutes and oppositely charged pairing ions are present, one may need to decrease the predicted volume fraction concentration of the organic modifier (tetrahydrofuran or acetonitrile) by as much as 0.15–0.2 in order to obtain comparable retentions.

The main reasons why transfer rule equations fail for RP-IPC systems seem to include (i) the different $\ln k'$ *vs.* φ behaviors of the ionic and the uncharged solutes in the regular RP mode and (ii) the diverging adsorption behavior of the pairing ion(s) in the three common organic modifier (methanol, acetonitrile, tetrahydrofuran)–aqueous buffer eluent systems. Both effects can be qualitatively understood by invoking the electrostatic retention theory of RP-IPC.

According to this theory[20], the retention of an ionic solute B ($\ln k'_{cB}$) is influenced by the concentration of the organic modifier (φ) in at least two ways:

$$\ln k'_{cB}(\varphi) = \ln k'_{0B}(\varphi) - (z_B F/RT)\,\psi_0(\varphi) \tag{1}$$

where k'_{cB} is the capacity factor of ionic solute B, k'_{0B} is the capacity factor of solute B in the absence of a pairing ion, z_B is the charge of B, ψ_0 is the electrostatic surface potential between the surface of the stationary phase and the mobile phase, F is the Faraday constant, R is the gas constant and T is the absolute temperature. When an organic modifier is added to the eluent, both k'_{0B} [related to problem (i) above] and ψ_0 [related to problem (ii) above] will change.

(i) In the regular reversed-phase mode (in the absence of a pairing ion), solute retention and separation selectivity depend only on $k'_{0B}[\Delta G^0_B(\varphi)]$, where ΔG^0_B is the free energy of adsorption of solute B. Extensive $\ln k'$ *vs.* φ data sets (related to ΔG^0_B *vs.* φ) are available for uncharged solutes (or acids and/or bases in their non-ionized form), but not for ionized solutes. Analysis of the retention data of limited sets of ionic and non-ionic solutes[28,29] indicates that the $\ln k'$ *vs.* φ (*i.e.*, ΔG^0_B *vs.* φ) behavior of ionic solutes differs significantly from that of the uncharged solutes, the slope being steeper for the ionic solutes.

(ii) In RP-IPC, the surface potential depends on the organic modifiers via the free energy of adsorption of the pairing ion A [$\Delta G^0_A(\varphi)$]. Were the surface potentials identical in the different eluents, ionic interactions between the pairing ion and the solute ions would contribute to solute retention equally. Assuming that the ionic strength and the pairing ion concentration are constant, and that the changes in the dielectric constant of the eluent have a negligible effect, identical surface concentrations of the pairing ion in the different organic modifier–aqueous buffer eluents will lead to identical surface potentials[20].

Previously, we reported[20] the adsorption isotherms of octylsulfonate pairing ion as a function of the type and concentration of the organic modifier in aqueous buffer–organic solvent eluents. These data were used to define the isopotential eluent compositions by fitting empirical equations to the adsorption term $\ln (n_0 K_{As})$ *vs.* φ data, where n_0 is the monolayer capacity and K_{As} is the adsorption constant of pairing ion A. Each equation was forced to pass a common point at 0 organic modifier volume fraction concentration. Pairs of these empirical expressions were used to derive the relationships sought:

$$\varphi_{ACN} = 0.604\varphi_{CH_3OH}^{1.25} \quad (2)$$

$$\varphi_{THF} = 0.782\varphi_{CH_3OH}^{1.875} \quad (3)$$

where φ is the volume fraction of the given organic modifier. It must be noted that the equations are limited to the $0 < \varphi_{CH_3OH} < 0.4$ concentration range, and have an error margin of at least ± 0.05.

In these isopotential eluents the effects of varying pairing ion adsorption are supposedly eliminated and comparable overall retentions should result for ionic solutes in the different organic modifier–aqueous buffer eluents. The calculated isopotential eluent compositions (eqns. 2 and 3), the experimentally determined isoeluotropic eluent compositions from Table II and the eluent compositions calculated by the other two transfer rules defined on the basis of retention data of ionized solutes are compared in Fig. 11. The dashed lines show transfer rules defined by Sekulic *et al.*[15] and the dotted lines show data taken from a nomogram presented by Tomlinson and Riley[17].

The good agreement between predictions from eqns. 2 and 3 and all the other data supports our hypothesis: the retention of ionic solutes depends significantly on the electrostatic interactions in the presence of a pairing ion (*cf.*, second term in eqn. 2), and the deviation between the RP transfer rules and the experimentally determined isoeluotropic eluent compositions (*cf.*, Fig. 10) are caused primarily by the adsorption (and surface potential) differences of the various pairing ions in the different organic modifier–aqueous buffer eluents.

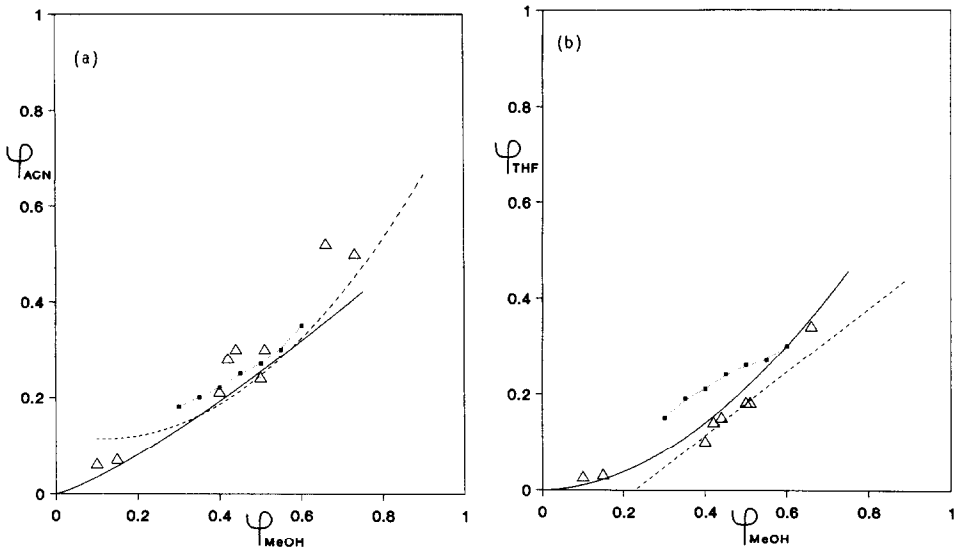

Fig. 11. Comparison of the isopotential eluent compositions defined by eqns. 2 and 3 (solid line), the experimentally determined ion-pair chromatographic isoeluotropic eluent compositions listed in Table II (triangles) and the transfer rules defined for ionic solutes by Sekulic *et al.*[15] (dashed line) and Tomlinson and Riley[17] (dotted line), for (a) acetonitrile and (b) tetrahydrofuran *vs.* methanol–aqueous buffer eluents. MeOH = Methanol.

Unfortunately, the isopotential transfer rules in RP-IPC cannot, in general, be used as the solvent strength transfer rules are in the regular RP mode. They seem to underestimate the acetonitrile (Fig. 11a) and overestimate the tetrahydrofuran (Fig. 11b) isoeluotropic compositions. Tetraalkylammonium pairing ions require acetonitrile concentrations much higher than those predicted by eqn. 7 (*cf.*, Table II). This indicates that the isopotential transfer rules defined by eqns. 2 and 3 are restricted to the alkylsulfonate pairing ions and ionized solutes. Adsorption data, if they were available for pairing ions of different charge type and molecular structure, would probably result in a number of different isopotential transfer rules which would depend on the type of the pairing ion.

A relatively safe strategy for the determination of initial eluent compositions, one which provides satisfactory retention limits prior to solvent-type optimization, involves the use of one of the RP transfer rules as a first estimate, irrespective of the charge type of the solutes in the sample. When the sample contains ionic–ionizable solutes and when an ion-pairing reagent is also present in the eluent, one may need to measure one or two additional isocratic chromatograms at lower solvent strength(s). These conclusions are based on a limited set of experimental data and deviations from this solute behavior are possible, just as in the regular RP mode. However, within these constraints, any of the RP transfer rules considered in this paper will provide a useful first (over)estimate of solvent concentration for ion-pairing systems.

CONCLUSIONS

In RP-IPC, certain solute mixtures which contain ionic and/or uncharged solutes can be separated advantageously by varying the type and/or the concentration of the organic modifier(s) in the aqueous buffer eluent. The simultaneous variation of the type and/or concentration of the organic modifier(s) and the pairing ion leads to complex eluent systems and more parameters to be optimized. In order to rationalize the selection of these parameters, typical sample mixtures which require and benefit from the variation of the type of the organic modifier were discussed. The examples demonstrate the utility of the sample composition-based parameter selection method and the merits of organic modifier optimization in RP-IPC.

A practical approach to defining the initial eluent compositions prior to solvent-type optimization in RP-IPC was also sought. The reasons why transfer rules, so successful in the RP mode, fail were identified. These include the different $\ln k'$ *vs.* organic modifier concentration behaviors of the ionic and the uncharged solutes in the regular RP mode, and the different adsorption behaviors of the pairing ion(s) in the three common organic modifier–aqueous buffer eluents. It was concluded that the RP transfer rules provide a conservative overestimate of the isoeluotropic eluent compositions for RP-IPC systems. When ionized solutes and oppositely charged pairing ions are present, one may need to decrease the predicted concentration of the organic modifier by as much as 0.15–0.2 volume fraction units, and adjust the eluent compositions experimentally.

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