Journal of Chromatography, 499 (1990) 423–434
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 790

BASIS OF THE RATIONAL SELECTION OF THE HYDROPHOBICITY AND CONCENTRATION OF THE ION-PAIRING REAGENT IN REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

AKOS BARTHA[a] and GYULA VIGH*
Chemistry Department, Texas A&M University, College Station, TX 77843-3255 (U.S.A.)
and
ZITA VARGA-PUCHONY
Institute for Analytical Chemistry, University of Chemical Engineering, Veszprem (Hungary)

SUMMARY

The retention of ionic solutes in ion-pair chromatographic separations can be controlled efficiently only when the surface concentration of the pairing ion, and the resulting surface potential, vary over a reasonably broad range. To achieve a broad operating range, the hydrophobicity and the mobile phase concentration of the pairing ion must be matched to the organic modifier concentration of the eluent. Preferred combinations of the eluent methanol concentrations and the commonly used alkylsulphonate and tetraalkylammonium pairing ions are reported, allowing for the rational selection of these parameters.

INTRODUCTION

The effects of the chain length and the concentration of ion-pairing reagents on the retention of ionic solutes have been studied since the early applications of ion-pair chromatography. Horváth *et al.*[1], Deelder and co-workers[2,3] and Knox and Hartwick[4], among many others[5–11], pioneered this subject. In addition to pH, organic modifier and pairing ion concentrations of the eluent, the hydrophobicity (length of the alkyl chain) of the pairing ion is one of the main parameters in the optimization of retention[12]. Generally, the retention of oppositely charged ionic solutes increases with increasing hydrophobicity of the pairing ions when used at identical mobile phase concentrations.

An important step in the progress of ion-pair chromatography was the recognition that solute retention depends primarily on the surface concentration of the adsorbed ion-pairing reagent[2–5]. Alkylsulphonate pairing ions of different chain length at similar surface concentrations were shown to result in identical solute retention[4,10]. This implies that the hydrophobicity of ion-pairing reagents affects solute retention only through their hydrophobicity-controlled adsorption[4,10,11].

[a] On leave from the University of Chemical Engineering, Veszprem, Hungary.

0021-9673/90/$03.50

Goldberg *et al.*[13] also suggested that, in principle, a single pairing ion can be used for the optimization of separation selectivity, provided that its adsorption covers a sufficiently wide range. On the other hand, studies by Bartha and co-workers[10,14] have shown that the organic modifier concentration of the eluent greatly influences the adsorption of both positively and negatively charged ion-pairing reagents. No single, commercially available pairing ion could ensure sufficiently high surface concentrations in eluents with widely differing organic modifier concentrations.

Pairing ion selection is still largely a trial-and-error procedure in most optimization schemes and previous chromatographic experience plays a large role. A rule of thumb calls for the replacement of the pairing ion with a more hydrophobic ion when sufficient retention shifts are not obtained.

Unfortunately, most optimization strategies[13–16], and even some expert systems[17], continue to adopt this very simple approach. They rely on the (convenient) use of a single (positively or negatively charged) pairing ion, irrespective of the organic modifier concentration in the eluent. Arguments used to support pairing ion selection (such as solubility and column equilibration time[13] or "reasonable effect on solute retention"[17]) apply only over a limited range of organic modifier concentrations. Popular choices tend to favour the less hydrophobic reagents.

Low *et al.*[18] suggested a rational approach for the selection of the types and ranges of the mobile phase variables to be used in the optimization of ion-pair chromatographic separations. The primary parameters include the charge type of the pairing ion, the pH and/or the methanol (or other organic modifier) concentration of the eluent. Essentially, these parameters are determined by the nature (charge type and relative hydrophobicity) of the sample solutes[18].

This approach can be extended to the selection of the hydrophobicity (chain length) and mobile phase concentration of the pairing ion. Until now, however, the complexity of this problem (*i.e.*, the interrelationship of the mutually dependent parameters ionic strength, chain length and concentration of the pairing ion and organic modifier concentration) prevented the rational selection of these parameters. Recently, two important developments in the electrostatic theory of ion-pair chromatography[19–21] contributed to an improved understanding of the simultaneous effects of these parameters. The basic assumption of this theory is that the adsorbed amphiphilic ions and the counter ions form an electrical double layer at the surface and create a surface potential. This surface potential will influence both the adsorption isotherm of the pairing ion and the retention of ionic solutes. The magnitude of the surface potential depends primarily on three parameters: the surface concentration of the pairing ion, the dielectric constant and the ionic strength of the mobile phase[19].

Stahlberg and Hagglund[22] have shown that the type and concentration of the electrolyte influences both solute retention and pairing ion adsorption through the surface potential, confirming the notion that eluent pH and ionic strength can be considered independent parameters.

Stahlberg and Bartha[23] demonstrated that the hydrophobicity of the pairing ion and the concentration of the organic modifier affect the surface potential by influencing the adsorption of the pairing ions.

In this paper, we discuss the interrelationship of the organic modifier and the hydrophobicity and concentration of the pairing ion, and provide a rational basis for their selection. We show that the use of a single pairing ion irrespective of the concen-

tration of the organic modifier may be convenient, but it is not efficient. Based on an extensive compilation of adsorption isotherms and retention data, recommendations are made for matching the hydrophobicity and mobile phase concentration of typical positively charged (tetraalkylammonium) and negatively charged (alkylsulphonate) ion-pairing reagents to the organic modifier concentration in the eluent. Pairing ions used in special applications, such as indirect UV detection and enantiomer or peptide separations, will not be discussed.

EXPERIMENTAL

All chemicals were of analytical-reagent grade. Drugs and ion-pairing reagents were obtained from Janssen (Beerse, Belgium), Fluka (Buchs, Switzerland) and Merck (Darmstadt, F.R.G.). Distilled, ion-exchanged water was used for the preparation of buffer solutions and eluents. An LC 5000 liquid chromatograph, equipped with UV (254 nm) and RI detectors (all from Varian Aerograph, Walnut Creek, CA, U.S.A.) and two Model 7010 six-port injection valves (Rheodyne, Cotati, CA, U.S.A.) were used. Columns were thermostated at 25°C. The chromatographic system allowed the determination of both the breakthrough curves of the pairing ions (surface concentrations) and the retention data (capacity factors) of the solutes, as described previously[24]. The analytical columns (120 × 4.6 mm I.D.) were slurry packed with 5-μm ODS-Hypersil (Shandon, London, U.K.), Nucleosil C_{18} (Macherey-Nagel, Bad Dürkheim, F.R.G.), Supelco S C_{18} (Supelco, Bellefonte, PA, U.S.A.) and 5-μm LiChrosorb RP-8 and RP-18 (Merck). Three non-commercial stationary phases, received as gifts, were also used: DiMeODS (10-μm dimethyloctadecylsilica) (Prof. E. Sz. Kovats, Ecole Polytechnique, Lausanne, Switzerland), BST C_{18} (5-μm octylsilica) (Bio-Separation Technologies, Budapest, Hungary) and HTS RP6 (10-μm dimethylhexylsilica) (Prof. Th. Welsch, Karl-Marx University, Leipzig, G.D.R.). Details of the stationary phase studies will be published elsewhere[25].

The eluents were prepared by weighing as described previously[24]. They contained 25 m*M* phosphoric acid, 25 m*M* sodium dihydrogenphosphate and different concentrations of ion-pairing reagents and sodium bromide (to maintain the ionic strength constant). Methanol was used as an organic modifier.

RESULTS AND DISCUSSION

Role of the surface concentration of the pairing ion in the optimization of separations

A nine-component mixture of strong bases and weak acids (catecholamines and some of their acidic metabolites) was taken as an example to demonstrate the importance of pairing-ion selection in the retention control of ionic solutes. All solutes are considered to be of interest and must be separated from each other.

In Fig. 1 the solute capacity factors are shown as a function of pH using a methanol–aqueous buffer (10:90, v/v) eluent. The curves represent the idealized behaviour of weak acids (solutes 1–4) and strong bases (solutes 5–9) between pH 2.5 and 7.5. This separation problem can be solved fairly simply: the retention of the early eluting solutes must be increased. One alternative is to decrease the methanol concentration at high pH in order to increase the retention of all ionized solutes. However, their retention remains very low even in pure aqueous buffer. The same strategy at

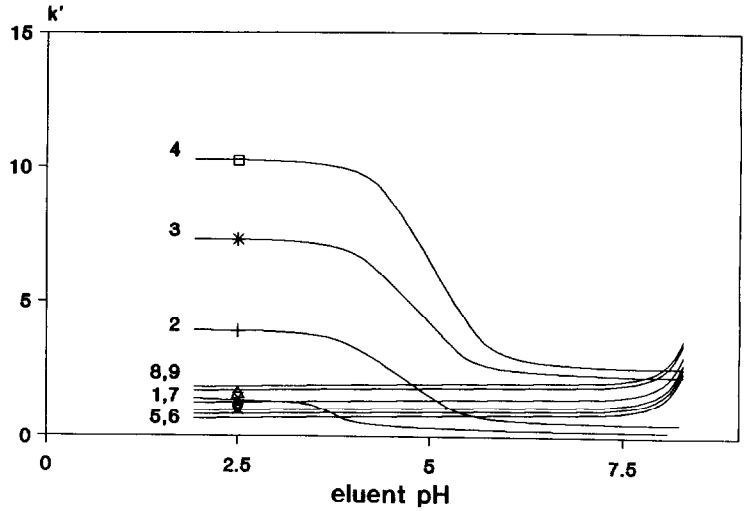

Fig. 1. Variation of the capacity factors (k') of a mixture of weak acids and strong bases as a function of the eluent pH. Column, 5-μm ODS-Hypersil; eluent, 10% (v/v) methanol in 50 mM aqueous phosphate buffer, constant (175 mM) ionic strength adjusted with sodium bromide. Solutes: 1 = homovanylmandelic acid; 2 = 3,4-dihydroxyphenylacetic acid; 3 = 3,4-dihydroxymandelic acid; 4 = 5-hydroxyindole-3-acetic acid; 5 = noradrenaline; 6 = adrenaline; 7 = octopamine; 8 = dopamine; 9 = 3,4-dihydroxyphenylalanine.

low pH results in excessively retained weak acids. The only good alternative is to increase the retention of the strong bases by adding a negatively charged pairing ion to the eluent. However, this must be done at low pH, because at high pH, owing to the ionic repulsion of the ion-pairing reagent, all acids elute close to the solvent front. These considerations result in a simple optimization vector space: a single line [pH 2.5, 10% (v/v) methanol and varying concentration of a negatively charged pairing ion], shown in a three-dimensional representation in Fig. 2. Next, the concentration limits and the hydrophobicity of the pairing ion to be used along this single line must

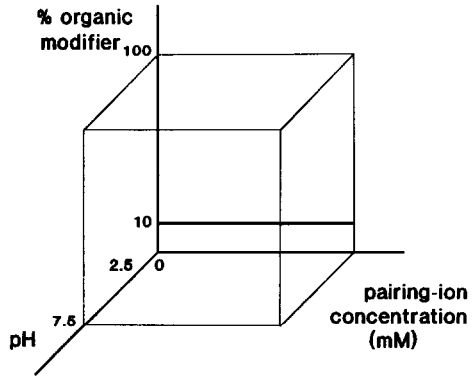

Fig. 2. Three-dimensional representation of the combination of eluent optimization parameters: eluent pH, methanol concentration and ion-pairing reagent concentration. The bold horizontal line at 10% (v/v) methanol represents the selected optimization parameter space.

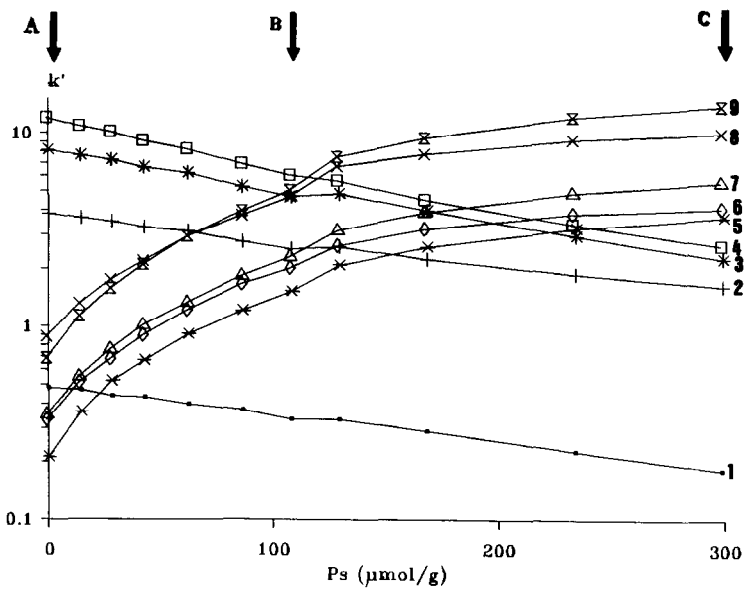

Fig. 3. Capacity factors (k') of solutes 1–9 in Fig. 1 as a function of the surface concentration (Ps) of hexyl- and octylsulphonate pairing ions. Mobile phases between arrows A and B contained 0 to 70 mM hexylsulphonate and those between B and C contained 0.5 to 10 mM decylsulphonate pairing ion. Other conditions as in Fig. 1.

be selected. First, we shall analyse the possible consequences of an improper choice, and in the last part of the paper we show a practical solution to this problem.

The use of two alkylsulphonate pairing ions of different hydrophobicity was evaluated experimentally. The mobile phase concentration was varied from 0 to 70 mM for sodium hexylsulphonate and from 0 to 10 mM for sodium decylsulphonate. In Fig. 3 the solute capacity factors are plotted against the surface concentration (Ps) of the alkylsulphonates. All strong bases become more retained as the pairing ion is added, while the retention of the four weak acids (1–4) gradually decreases. Although there is a small break in the retention curves as hexylsulphonate is replaced with decylsulphonate, the difference is negligible, in accordance with earlier findings[10,11].

The chromatograms shown in Fig. 4 were obtained as follows: (A) without any ion pairing reagent, (B) with 70 mM sodium hexylsulphonate and (C) with 10 mM decylsulphonate. Although sodium hexylsulphonate enhanced the retention of the positively charged solutes, certain solute pairs (*e.g.*, 2–7 and 3–8) could not be separated. The highest surface concentration of this pairing ion is 110 μmol/g. When sodium decylsulphonate is used, higher surface concentrations (and potentials) are obtained; the positively charged basic solutes become more retained than the weak acids. High surface concentrations of decylsulphonate result not only in increased retention, but also in improved separation selectivity as indicated by the complete separation of the components in chromatogram C in Fig. 4. (Incidentally, a number of local optima can be found in this high surface concentration range.)

Thus, the main question is how one can find the eluent composition limits that probably contain the global selectivity optimum. According to our experience, the

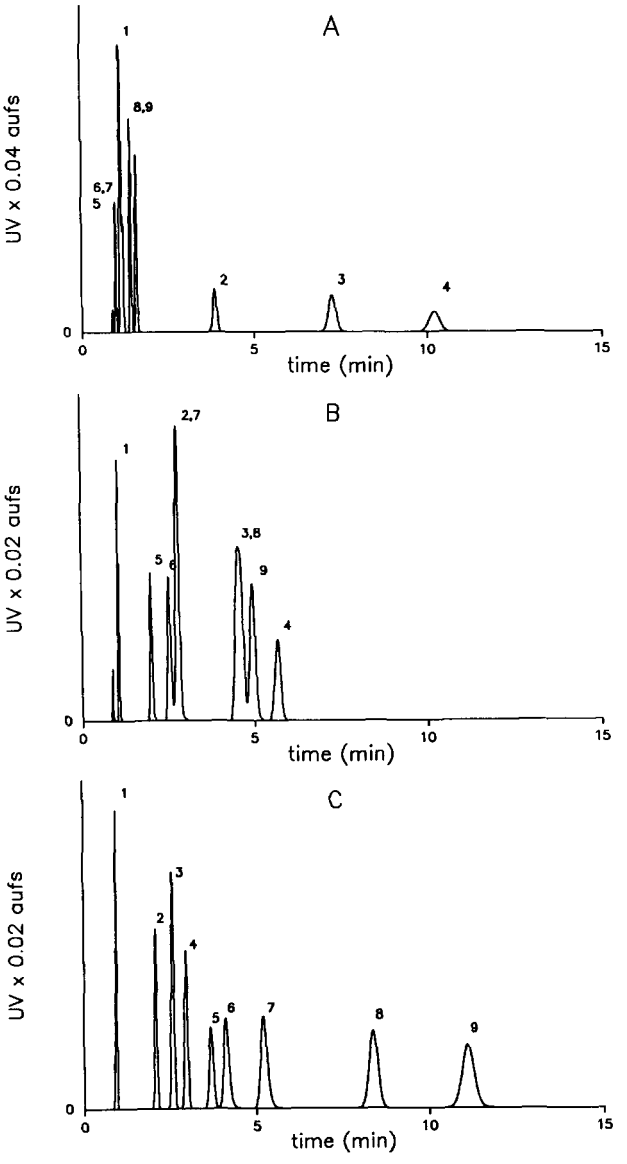

Fig. 4. Chromatograms of the catecholamine mixture shown in Fig. 1. (A) No pairing ion added; (B) eluent containing 70 m*M* sodium hexylsulphonate; (C) eluent containing 10 m*M* sodium decylsulphonate. Other conditions as in Fig. 1.

surface concentration of the ion-pairing reagents should reach at least 100–200 μmol/g if the solute retention is to be affected significantly (see, *e.g.*, Fig. 3). One possibility is to increase the concentration of hexylsulphonate even further and another is to use a more hydrophobic pairing ion.

This example highlights some important points that any optimization strategy must confront. If the hydrophobicity and/or the concentration range of the pairing ion are not selected appropriately, then the surface concentration of the pairing ion (and the retention controlling surface potential) will vary only over a limited range. Higher surface potentials can only be reached by decreasing the ionic strength of the eluent (if possible at all)[19] and/or by increasing the mobile phase concentration and the hydrophobicity of the ion-pairing reagent. Although a given pairing ion can perform well at a certain organic modifier concentration, its effects may not be large enough in a less polar eluent.

The separation selectivity does not necessarily improve as surface concentrations are increased even higher. At very high surface concentrations, the retention of oppositely charged solutes will reach a maximum (see Fig. 3) and a number of secondary effects (which are difficult to handle both theoretically[23] and empirically) might influence the separation selectivity.

Limitations following from the use of a single pairing ion irrespective of the concentration of the organic modifier in the eluent

Ionic solute retention is best controlled through ionic interactions established by sufficiently high surface potentials, *i.e.*, pairing-ion surface concentrations. In this respect, all optimization strategies that rely on the use of a single pairing ion are limited. This limitation becomes obvious when one compares the adsorption isotherms of ion-pairing reagents measured at different organic modifier concentrations.

In Fig. 5 the adsorption isotherms of sodium octylsulphonate are shown for 0, 10, 25 and 40% (v/v) methanol–aqueous phosphate buffer (pH 2.1) eluents. With increasing methanol concentration, the surface concentration of the pairing ion decreases rapidly. This effect is shown in Fig. 6 in a different representation: the corresponding methanol concentrations and pairing-ion adsorption data (for hexyl-, octyl- and decylsulphonates) are plotted at constant mobile phase pairing-ion concentrations. The adsorption decreases almost three-fold when the methanol concentration is increased to 40% (v/v).

Mixture designs used for mobile phase optimization often require the addition of pure methanol to a base eluent with a given pairing-ion concentration[13,15]. This results in even lower surface concentrations, because dilution and weakened adsorption strength act in concert [*i.e.*, the eluent at 40% (v/v) methanol will contain only 3 m*M* octylsulphonate[15]]. Alternatively, one could increase the mobile phase concentration of the selected pairing ion to compensate for the decreased adsorption strength. The limitations of this method are demonstrated by Fig. 6. Compared with octylsulphonate (5 m*M* curve), four-fold higher mobile phase concentrations of sodium hexylsulphonate (20 m*M* curve) still result in much lower (30–50%) surface concentrations.

A more efficient approach adapts the hydrophobicity and concentration of the pairing ion to the organic modifier concentration that is required for the separation. A lower concentration of a more hydrophobic pairing ion can extend the range in which the surface concentration (and potential) can be varied (see data for decylsulphonate in Fig. 6).

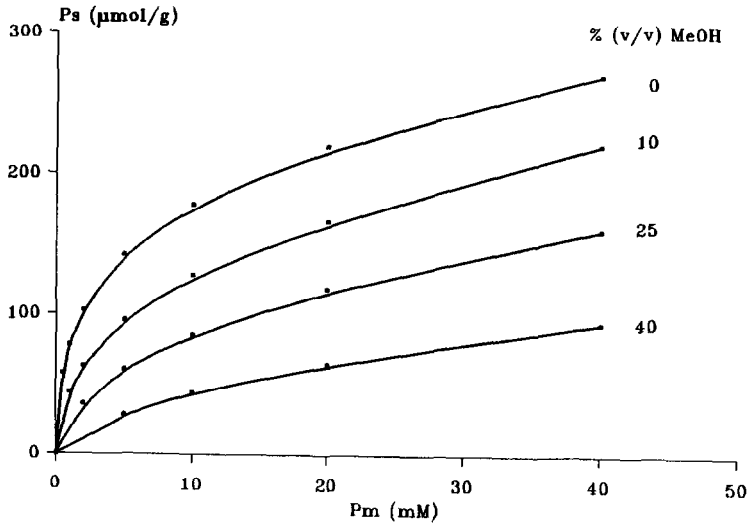

Fig. 5. Adsorption isotherms of sodium octylsulphonate from 0, 10, 25 and 40% (v/v) methanol (MeOH). pH, 2.1; aqueous phosphate buffer eluents on ODS-Hypersil at constant temperature (25°C) and ionic strength (175 m*M*). Ps = surface concentration; Pm = mobile phase concentration.

Practical recommendations for pairing-ion selection

Often one must face the problem of pairing-ion selection without a knowledge of the adsorption isotherms of the pairing ions and their dependence on the organic modifier concentrations. Instead of attempting to describe mathematically the effects on the surface potential of the chain length and eluent concentration of the pairing

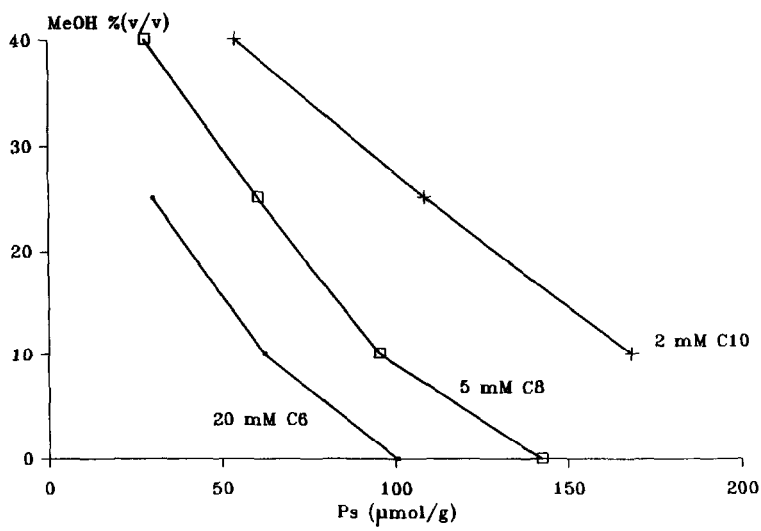

Fig. 6. Variation of the surface concentration (Ps) of hexyl- (C6), octyl- (C8) and decylsulphonate (C10) pairing ions at constant (20, 5 and 2 m*M*) mobile phase concentrations with increasing methanol (MeOH) concentration of the eluent. Other conditions as in Fig. 5.

ion and the organic modifier concentration (which would require a very large and complete dataset for all these variables), we used our extensive solute retention and adsorption isotherm[10,14,23–25] datasets and derived recommended pairing ion–organic modifier combinations which can help the chromatographer to select the appropriate pairing ions. These operating ranges were defined such that the selected pairing ions must (i) be sufficiently soluble to yield at least 100 μmol/g surface concentrations in the organic modifier range assigned, (ii) permit fast column equilibration and regeneration and (iii) not form micelles.

Figs. 7 and 8 summarize our recommendations (which represent a compromise between the above requirements) for the different chain length, ion-pairing reagent and methanol concentration combinations for both alkylsulphonate and tetraalkylammonium ions. These pairing ions are thought to cover most of the common ion-pair chromatographic applications. The bars represent the methanol and highest practical pairing-ion mobile phase concentration combinations that lead to surface concentrations of 100 μmol/g or higher for each reagent, and which can be safely dissolved without micelle formation. The initial electrolyte concentration in these eluents must not exceed 100 mM. Once the methanol concentration of the eluent has been selected, the appropriate pairing ion and its concentration limits (typically between 0 and Pm mM) can be selected from the diagrams. When the bars of several pairing ions overlap, those most centered around the selected methanol concentrations are to be preferred.

Certain other popular pairing ions, such as pentyl- and heptylsulphonates, were not included because sufficient adsorption and retention data were not available. However, their respective limits can be estimated by extrapolation between the neighbouring members of the homologous series. Owing to the possibility of irreversible adsorption on reversed-phase columns, very hydrophobic asymmetric quaternary ammonium salts also were not considered in Fig. 7. However, published adsorption

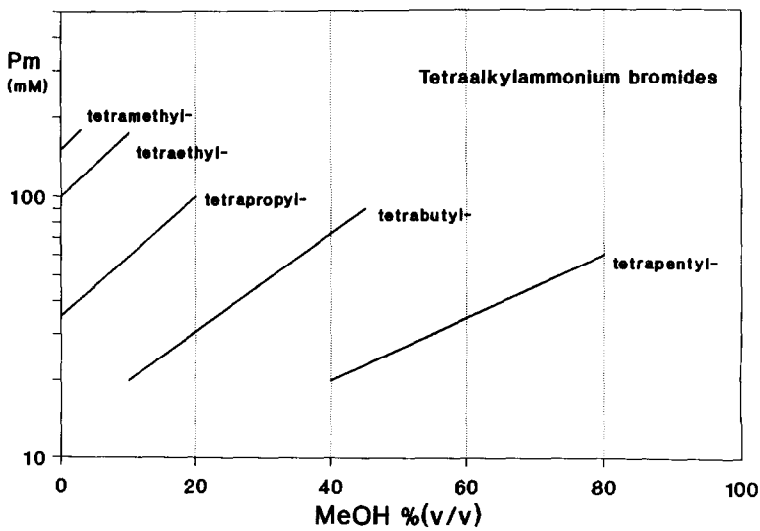

Fig. 7. Recommended maximum mobile phase concentrations (Pm) of tetraalkylammoniumpairing ions and their application ranges as a function of the methanol (MeOH) concentration of the mobile phase.

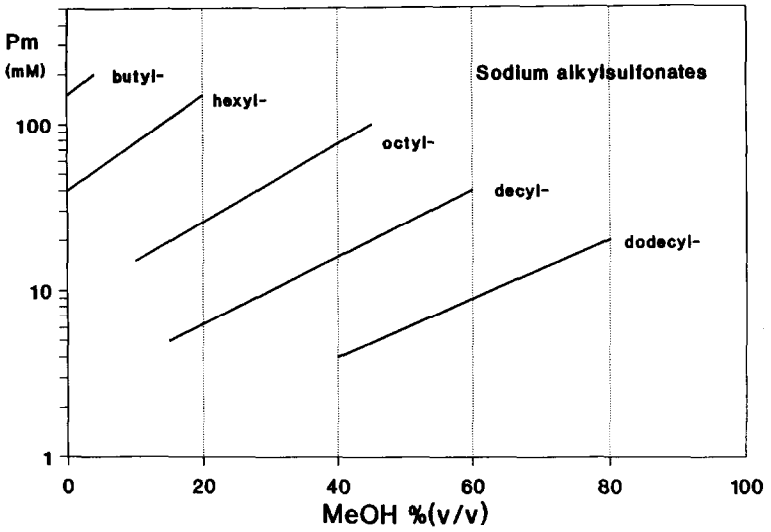

Fig. 8. Recommended maximum mobile phase concentrations (Pm) of alkylsulphonate pairing ions and their application ranges as a function of the methanol (MeOH) concentration of the mobile phase.

data for cetrimide[6,7] indicate that it can be used over a wide range of organic modifier concentrations with characteristics close to the tetrapentylammonium ion.

In Fig. 9 the capacity factors of the positively charged octopamine are plotted against the mobile phase concentration of sodium octylsulphonate for eight different

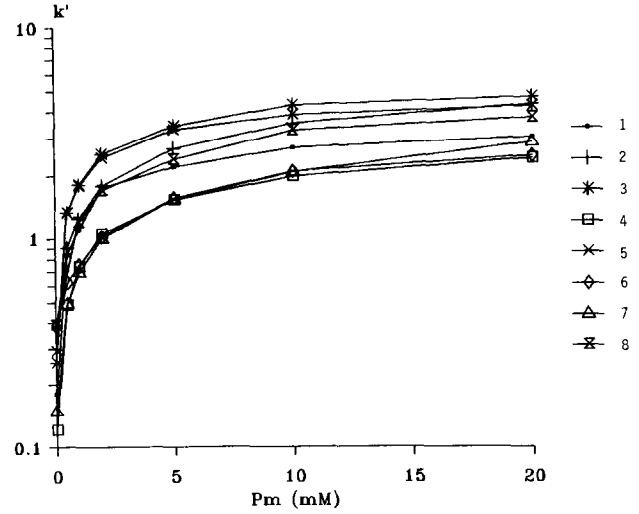

Fig. 9. Capacity factor (k') of positively charged octopamine as a function of the mobile phase concentration (Pm) of sodium octylsulphonate on eight reversed-phase columns. All measurements were made in methanol–aqueous phosphate buffer (pH 2.1) (10:90, v/v) eluents of constant ionic strength (175 mM, adjusted with sodium bromide) at 25°C. 1 = LiChrosorb RP-18; 2 = Nucleosil C_{18}; 3 = DiMeODS; 4 = LiChrosorb RP-8; 5 = Supelco S C_{18}; 6 = BST C_{18}; 7 = HTS RP6; 8 = ODS-Hypersil.

reversed-phase columns. The eluent pH (2.1), organic modifier concentration [10% (v/v) methanol] and ionic strength (175 m*M*, adjusted with sodium bromide) were kept constant throughout these experiments. The most notable feature is that a comparable increase in retention is observed on these diverse packings, indicating that the recommendations in Figs. 7 and 8 are fairly generally applicable. The effects of the stationary phase will be discussed in detail in another paper[25].

A further generalization is possible by extension of the electrostatic theory of ion-pair chromatography to include the effects of the type and concentration of the organic modifier[26]. Using "isopotential" binary methanol–, acetonitrile– and tetrahydrofuran–water eluents, diagrams similar to Figs. 7 and 8 can be obtained.

The recommendations described here are part of a knowledge base used in the development of an ion-pair chromatographic expert system. This expert system is intended to aid users in the selection of the mobile phase optimization parameters by considering the nature (charge type and relative retention) of the solutes in the sample. Once the primary parameters (pH, organic modifier concentration and charge type of the pairing ion) have been selected, the hydrophobicity and concentration of the pairing ion are matched with the organic modifier concentration of the eluent.

CONCLUSIONS

We have shown that one must be able to vary the surface concentration of the pairing ion, *i.e.*, the surface potential, over a reasonably broad range in order to optimize the separation of a complex mixture of differently charged solutes. When the organic modifier concentration is increased, the mobile phase concentration and/or the hydrophobicity of the pairing ion must also be increased to counter the weakened adsorption strength. Based on a comprehensive dataset which contains pairing-ion adsorption isotherms and solute retention data, preferred combinations of typical (both positively and negatively charged) pairing ions (hydrophobicity and concentration) and organic modifier concentrations have been selected. The need for facile column regeneration, solubility and/or the prevention of micelle formation have also been used as constraints.

REFERENCES

1 Cs. Horváth, R. W. Melander and I. Molnár, *Anal. Chem.*, 49 (1977) 142.
2 R. S. Deelder, H. A. J. Linssen, A. P. Konijnendijk and J. L. M. van de Venne, *J. Chromatogr.*, 185 (1979) 241.
3 R. S. Deelder and J. H. M. van den Berg, *J. Chromatogr.*, 218 (1981) 327.
4 J. H. Knox and R. C. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
5 E. Tomlinson, C. M. Riley and T. M. Jefferies, *J. Chromatogr.*, 173 (1979) 89.
6 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 202 (1980) 333.
7 C. T. Hung and R. B. Taylor, *J. Chromatogr.*, 209 (1981) 175.
8 I. S. Lurie and S. M. Demchuk, *J. Liq. Chromatogr.*, 4 (1981) 337.
9 W. R. Melander and Cs. Horváth, in M. T. W. Hearn (Editor), *Ion-Pair Chromatography*, Marcel Dekker, New York, 1985, Ch. 2.
10 A. Bartha, Gy. Vigh, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 303 (1984) 29.
11 A. Bartha and Gy. Vigh, *J. Chromatogr.*, 395 (1987) 503.
12 P. J. Schoenmakers, *Optimization of Chromatographic Selectivity, A Guide for Method Development*, Elsevier, Amsterdam, 1986, Ch. 3.
13 A. P. Goldberg, E. Nowakowska, P. E. Antle and L. R. Snyder, *J. Chromatogr.*, 316 (1984) 241.

14 A. Bartha and Gy. Vigh, *J. Chromatogr.*, 260 (1983) 337.
15 P. M. J. Coenegracht, N. V. Tuyen, H. J. Metting and P. M. J. Coenegracht-Lamers, *J. Chromatogr.*, 389 (1987) 351.
16 H. A. H. Billiet, J. Vuik, J. K. Strasters and L. de Galan, *J. Chromatogr.*, 384 (1987) 153.
17 Y. Hu, A. Peeters, G. Musch and D. L. Massart, *Anal. Chim. Acta*, 223 (1989) 1.
18 G. K.-C. Low, A. Bartha, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 478 (1989) 21.
19 J. Stahlberg, *J. Chromatogr.*, 356 (1986) 231.
20 J. Stahlberg and A. Furangen, *Chromatographia*, 24 (1987) 783.
21 J. Stahlberg, *Chromatographia*, 24 (1987) 820.
22 J. Stahlberg and I. Hagglund, *Anal. Chem.*, 60 (1988) 1958.
23 J. Stahlberg and A. Bartha, *J. Chromatogr.*, 456 (1988) 253.
24 A. Bartha and Gy. Vigh, *J. Chromatogr.*, 265 (1983) 171.
25 Z. Varga-Puchony, A. Bartha and Gy. Vigh, in preparation.
26 A. Bartha, Gy. Vigh and J. Stahlberg, *J. Chromatogr.*, 485 (1989) 403.