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Review

Influence of organic solvents on the separation selectivity in capillary electrophoresis

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Abstract

A review is presented dealing with the investigation of the effect of organic solvents, either pure, mixed with other organic co-solvents, or as aqueous–organic mixtures used as separation medium for capillary electrophoresis (CE). The review deals with those capillary electrophoretic techniques that are based on the different effective mobilities of the separands, namely capillary zone electrophoresis (CZE) and capillary isotachophoresis (ITP) (displacement electrophoresis). Methods like micellar electrokinetic chromatography (MEKC), or CZE with inclusion complexes, that apply organic solvents for adjusting hydrophobic interactions, are not included. This review focuses on the discussion of fundamental physico-chemical aspects of the effects of organic solvents on the separation potential of CE and emphasizes the application of organic solvents on the separation selectivity for the following classes of compounds: inorganic ions, small organic ions, pharmaceuticals, amino acids, peptides and proteins. © 1997 Elsevier Science B.V.

Keywords: Reviews; Selectivity; Buffer composition; Organic solvents

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1. Introduction

Organic solvents, either pure or as a mixture with water, are used in capillary electrophoresis (CE) for a number of reasons. They may increase the solubility of analytes, making CE analysis available for those substances with slight solubility in pure aqueous buffer solutions. Organic solvents are favorably applied to enhance the separation selectivity of CE by influencing the effective mobility of the separands and the mobility of the electroosmotic flow, the EOF. The selectivity can be expressed for a certain pair of separands, i and j, by the selectivity coefficient, r_{ji}, given by

$$r_{ji} = \frac{\mu_i^{\text{tot}}}{\mu_j^{\text{tot}}}$$

where μ_i^{tot} is the total (or apparent) mobility of separand, i; it is the sum of the effective mobility, μ_i^{eff}, and the mobility of the EOF, μ^{eo}: $\mu_i^{\text{tot}} = \mu_i^{\text{eff}} + \mu^{\text{eo}}$. Note that the mobilities are signed quantities, in contrast to the usual theory of conductance. The sign is positive for cation separands, and negative for anions. The mobility of the EOF has positive sign when it is directed towards the cathode.

Organic solvents may affect the electrophoretic properties of the separands two-fold: on the one hand by changing the actual mobility (that of the fully charged ion, which depends obviously on the viscosity of the solution); on the other hand by influencing the pK_a value of weak electrolytes specifically. Both effects are the result of solvation processes that take place in different solvents (cf., e.g., [1–6]).

Before capillary zone electrophoresis (CZE) became commercially available, organic solvents were applied in the other analytical capillary electrophoretic technique, also basing on the effective mobility as the same physico-chemical separation parameter: isotachophoresis (ITP) [5,7–19].

The present review deals with the use of organic solvents in CE. It is, however, limited to CE in free solution, without considering the methods where separation is based either on the partitioning of the analytes between the buffer and micelles (micellar electrokinetic chromatography, MEKC), or on interaction with complex forming agents like those leading to inclusion complexes (e.g., cyclodextrins). In these two areas organic solvents are used in most cases, and the papers dealing with these techniques will not be referred here.

The following abbreviations will be used for the organic solvents: methanol, MeOH; ethanol, EtOH; propanol, PrOH; acetonitrile, ACN; tetrahydrofuran, THF; formamide, FA; N-methylformamide, NMF; N,N-dimethylformamide, DMF; N,N-dimethylacetamide, DMA; dimethylsulfoxide, DMSO; acetone, ACET.

The concentrations of the mixed solvents are given in % (v/v), if not stated otherwise.

2. Effect of organic solvents

2.1. Fundamental aspects

Most of the papers reviewed here deal with the investigation of the separation selectivity concerning a special analytical problem. A smaller number of papers describe more fundamental aspects of organic solvents and its influence on physico-chemical parameters decisive for selectivity in CE: the actual mobility and the pK_a value of the separands, and, most important, the mobility of the EOF, determined by the zeta potential near the surface of the capillary, and the viscosity and dielectric constant of the background electrolyte (BGE) solution close to the surface.

An investigation of the effect of organic solvents (mixed aqueous–organic solutions of MeOH and DMSO) on the pK_a values of substituted benzoic acids and aliphatic amino bases shows that in all cases the pK_a values of organic acids increase; those of bases are influenced to a minor extent [17–19]. This behavior was explained by the concept of the transfer activity coefficient or the ‘medium effect’. A detailed discussion of the topic is given in Ref. [6]. The concept of the medium effect enables to explain changes in the selectivity e.g., of small organic acids in pure methanolic buffer [20].

Changes in relative mobilities (so-called RE values, used in ITP) in methanolic buffer solutions with less than 0.5% (w/w) water content were shown for 55 organic acids [10]. Based on these data, cluster analysis enabled to demonstrate that methanolic

buffer solutions can lead indeed to selectivity changes compared with purely aqueous buffers [21].

The influence of pure NMF and aqueous mixtures with NMF on the zeta potential and the EOF in fused-silica capillaries was investigated [22], and substantial EOF was observed even in pure NMF.

The change of the EOF in a series of mixed aqueous–organic solvents consisting of up to 80% MeOH, EtOH, PrOH, ACN or DMF, was systematically investigated in fused-silica capillaries [23–25] and narrow tubes made from synthetic organic polymers like polyfluorocarbon, polyethylene and poly(vinyl chloride) [26]. It was found that organic solvents reduce the mobility of the EOF in nearly all cases, even under conditions of full dissociation of the chargeable groups of the wall, namely at high pH (see Fig. 1). In addition, the pK_a values of the dissociable groups on the capillary surface are found to be shifted to higher values, which is in accordance to expectations based on the concept of the transfer activity coefficient.

The observed effect of water concentrations up to 10% in organic solvents (NMF, DMSO, ACN, MeOH) on the EOF mobility was not very pronounced. The measurements were carried out in a BGE consisting of 25 mmol/l ammonium acetate and 1 mol/l acetic acid [27].

2.2. Inorganic ions

The change in selectivity for the separation of inorganic anions was investigated in non-aqueous and mixed aqueous–organic solvents with MeOH, ACN or DMF as constituents. Furthermore, the addition of organic solvents had not only a major influence on the EOF in comparison to a pure aqueous solution, but also a significant increase on the separation selectivity was observed, with many ions showing reversed separation order [24,28]. Changes in selectivity (see Fig. 2) were explained on the basis of the relative hydration enthalpies [29], therefore evaluating thermodynamical aspects affecting the separation selectivity. Inorganic mono-, di- and trivalent cations (ammonium, alkali, alkali earth, transition metal ions) were separated in non-aqueous and mixed solvents by adding MeOH or ACN to the BGE [30,31].

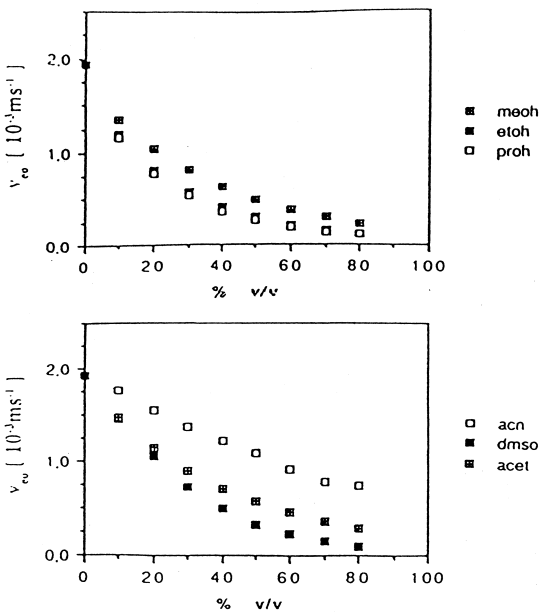

Fig. 1. Change of the velocity, v_{eo}, of the EOF in fused-silica capillaries in dependence on the percentage of the organic modifier in aqueous–organic buffer solutions (field strength 208 V/cm, temperature 25°C, ionic strength about 10 mmol/l, apparent pH between 9 and 12). Taken with permission from Ref. [23].

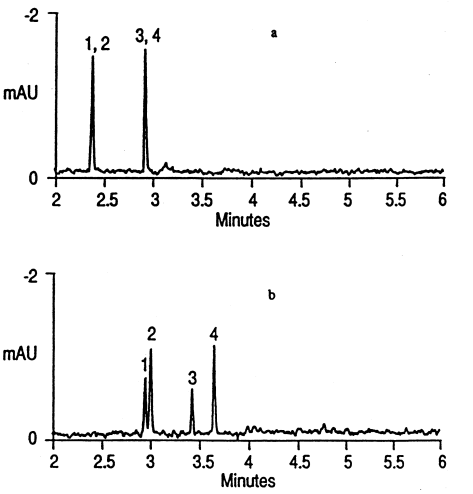

Fig. 2. Effect of methanol on the separation selectivity of four inorganic anions. Electrolyte 2.5 mmol/l pyromellitic acid, 6.5 mmol/l NaOH, 0.75 mmol/l hexamethonium hydroxide; pH 7.7. Detection: indirect UV absorbance at 250 nm. (a) Pure aqueous; (b) 15% (v/v) MeOH. 1, Iodide; 2, chloride; 3, perchlorate; 4, azide. Taken with permission from Ref. [29].

2.3. Small organic ions

It is obvious that amino acids or most of the pharmaceuticals belong to this class of compound. The influence of organic solvents on the separation of these analytes is, however, not discussed in this section.

2.3.1. Anions

D-Gluconic acid produced during fermentation was determined in a mixed aqueous–organic buffer solvent consisting of 15% ACN. Fast separation of saturated and unsaturated mono- and dicarboxylic acids by co-electroosmotic CZE, achieved through cetyltrimethylammonium bromide (CTAB) and hexadimethrine bromide (HDB) as EOF modifiers, with an electrolyte containing 30% EtOH and using either direct or indirect detection was obtained [32]. Although small changes in selectivity were obtained for the separation of small organic fermenting acids in silage using methanol as solvent compared to water, aqueous buffers were preferred [33].

Positional isomers of substituted benzoic acids were fully separated in aqueous–organic solvents by mixing up to 60% of MeOH or ACN with the BGE. ACN was favored above other organic solvents due to short analysis and high separation efficiency [34] (see Fig. 3). The isomers of monohydroxy benzoic acid were separated in a phosphate buffer at pH 10.3, consisting of 0.20 mmol/l CTAB as EOF modifier and 1-PrOH added at 10% [35]. The effect of organic modifiers on the separation of a number of closely related (mono-, di- and tri-) hydroxybenzoic acids in buffer solutions containing MeOH, EtOH, ACN, DMSO, THF or ethylacetate in proportions of up to 30% was studied [36].

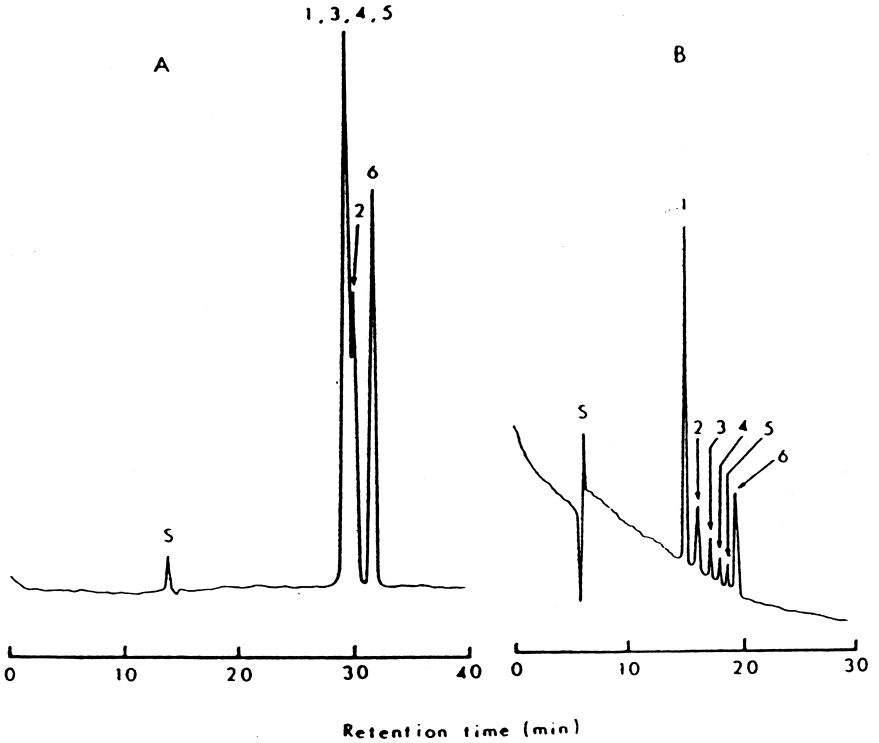

Fig. 3. Improvement of the separation selectivity for positional isomers of amino- and methylbenzoic acids by addition of ACN to the buffer (0.02 mol/l phosphate, pH 7.0). (A) Pure aqueous system; (B) 50% (v/v) ACN. Benzoic acids: 1, *p*-amino-; 2, *m*-amino-; 3, *p*-methyl-; 4, *m*-methyl-; 5, *o*-methyl-; 6, *o*-amino-. Taken with permission from Ref. [34].

Positional isomers of amino benzoic acids were investigated in mixed solvents with up to 25% MeOH or 12.5% i-PrOH [37] The migration behavior of a variety of polycarboxylic acids in ACN at high pH with cyclodextrin as lipophilic additive was investigated [38].

Changes in selectivity resulting in separation of aromatic and aliphatic acids in non-aqueous MeOH at different apparent pH values were demonstrated [20]. This was mainly because of changes in the size of the solvated particles and their acid–base features, expressed by the variation of the pK_a values of the analytes, which was related to the transfer activity coefficient.

The successful separation of substituted phenols [39] and methylphenols [40] in mixed aqueous solvents consisting of MeOH, EtOH, 1-PrOH, 2-PrOH and ACN in the presence of cationic additives (CTAB; HDB) to reverse the EOF through dynamically coating and thus shortening analysis time was demonstrated. The impact of the chain length of the alcohols used as organic additives on the separation behavior of alkyl phenols was also noted.

Rapid separation of 2-pyridinecarboxylate and salicylic acid in pure NMF was obtained without electrolyte added to the solvent [22]. This result was achieved due to the advantageous features of the organic solvent, which besides the high solubilizing power, are two-fold: the high dielectric constant and the low conductivity. Unfortunately, the use of NMF as a solvent for CE with UV absorption detection is limited by its high extinction coefficient below 250 nm.

C_2 to C_{18} linear saturated fatty acid separation was carried out by ITP [11] in methanol as a solvent and an electrical conductivity detector, and by CZE containing up to 60% MeOH, THF, ACN and substituted cyclodextrins (trimethyl-β-CD) with indirect UV absorbance detection [41].

Alkanesulfonates (C_2–C_{16}), alkylsulfates (C_8–C_{18}) and linear alkyl benzenesulfonates were separated in aqueous and non-aqueous methanolic solutions and in ACN–MeOH mixtures. Recording was performed either by direct or by indirect detection, respectively [42]. Undesirable sorption effects of the surfactants onto the capillary wall were almost fully reduced by working with a non-aqueous medium.

To increase the solubility of the sample [2-(2,4-dichlorophenoxy)-propionic acid in the respective isooctylester] 70% MeOH in water was used for the leading electrolyte solution in ITP [43].

2.3.2. Cations

Quinoline type compounds were separated in pure ACN at an early stage of the development of CZE [44]. Polar organic solvents such as ACN (30%), THF (40%) and ACET (50%) were added to a methanolic buffer solution (>60%) to disrupt micelles formed by alkylbenzyl- and alkylphenyl quaternary ammonium compounds into individual surfactant ions, necessary for effective separation. [45,46]. Quaternary ammonium herbicides were determined in an aqueous buffer containing 10% of MeOH as modifier by CE–MS [47]. Optimization by orthogonal array design and overlapping resolution mapping to separate heterocyclic amines was carried out by variation of the organic modifier content (MeOH, 31%) [48]. The result of the optimization led to the use of 31% MeOH as constituent of the aqueous–organic buffer (see Fig. 4).

2.4. Pharmaceuticals, drugs

A number of publications dealing with the use of organic solvents in CE focus on this category of substances. The lower alcohols, ACN, DMSO, NMF, FA, DMA, DMF are applied either pure, in binary mixtures with organic co-solvents or in aqueous–organic mixtures. Hansen et al. [49] investigated pharmaceuticals and drug metabolites in non-aqueous media consisting either of pure or mixed organic solvents (NMF, DMF, DMA, DMSO). Advantage of the high separation selectivity to test drug purity and study drug metabolism was taken. The same group developed a method for the quantitative determination of opium alkaloids in crude opium, drug preparations and other alkaloids based on non-aqueous CE with ACN, DMF, DMA, NMF, DMSO and MeOH as organic solvents [49,50]. For the characterization of cationic drug substances, differing only in the amine functionality or even having the same charge-to-mass ratio, non-aqueous media with solvents similar to those used in Refs. [49,50] and varying ratios of solvent mixtures were applied. Tetracyclines were separated in an aqueous–methanolic solvent of 40% MeOH [51]. In ternary mix-

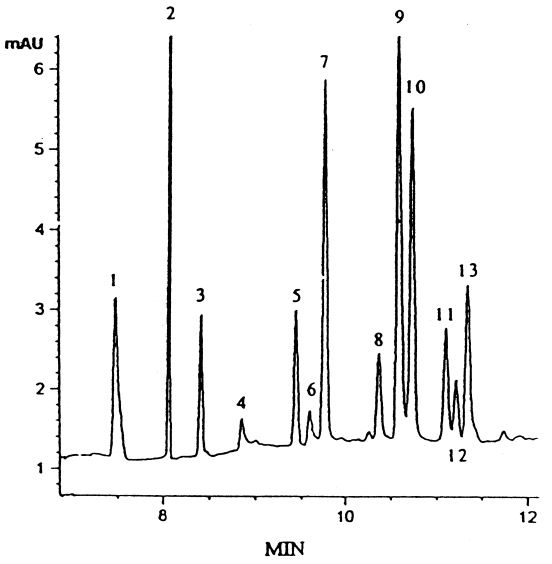

Fig. 4. Optimized.electropherogram of the separation of thirteen HCAs; pH 2.0; 31% (v/v) MeOH, 20 mmol/l Na_2HPO_4. (1) 2-Amino-4-methylimidazo[4,5-*f*]quinoline; (2) 2-amino-3-methylimidazo[4,5-*f*]quinoline; (3) 9H-pyrido[3,4-*b*]indole, β-carboline; (4) 2-amino-dipyrido[1,2-*a*:3′2′-*d*]imidazole; (5) 1-methyl-9H-pyrido[3,4-*b*]indole, β-carboline; (6) 2-amino-6-methyldipyrido[1,2-*a*:3′2′-*d*]imidazole; (7) 3-amino-1-methyl-5H-pyrido[4,3-*b*]indole; (8) 2-amino-9H-pyrido[2,3-*b*]indole; (9) 3-amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole; (10) 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; (11) 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; (12) 2-amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline. Taken with permission from Ref. [48].

tures of MeOH, ACN and a low content of DMF (45:49:6) impurities in tetracyclines were determined quantitatively and the consequences of evaporation of the different organic solvents used were studied [52].

Propanolol and felodipine (Fig. 5) were resolved in pure NMF without addition of electrolyte within 35 s [22].

The successful separation of thirteen sulfonamides in an aqueous–organic solution containing 16.6% of MeOH or 9% of ACN [53] and of three pyrazoloacridines in a buffer consisted of variable amounts of MeOH (10–100%) was demonstrated by CZE [54]. Tamoxifen, a non-steroidal anti-estrogen, and its metabolites were separated in a MeOH–ACN (50:50) mixture [55].

To enhance the solubility of nicotinic acid as analyte and to increase the separation selectivity, a

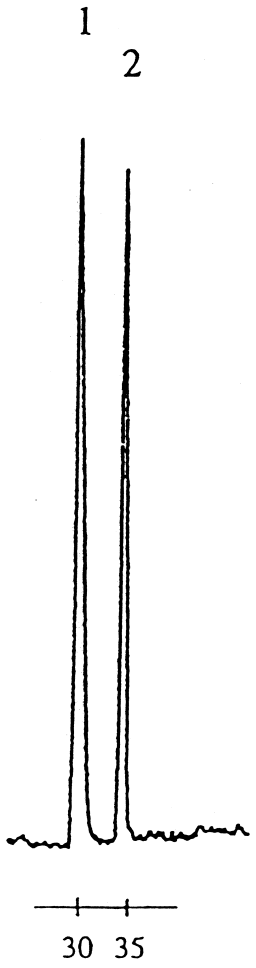

Fig. 5. Rapid separation of (1) propanolol HCl and (2) felodipine in pure NMF. Taken with permission from Ref. [22].

mixed aqueous–methanolic solution (20% MeOH) was used for the ITP of a number of pharmaceutical formulations with different matrices [16].

Pure methanolic buffers, or mixed aqueous–methanolic buffer solutions were applied not only for the separation and determination of drugs like pyrazoloacridine, haloperidol or mifentidine, and their metabolites, but also for on-line capillary electrophoresis–electrospray ionization-mass spectrometry (CE–ESI-MS) [56–59] (see also Ref. [60]). Estrogens and rodenticides were separated with buffers consisting of aqueous mixtures with MeOH (20%) or ACN (20–40%) [61]. Similar conditions were applied to optimize total analysis time and

separation of rodenticides in aqueous electrolytes, such as phosphate buffer modified with MeOH (20–30%) or ACN (5–30%) [62].

Leung et al. [63] achieved baseline separation of basic drugs such as morpholine analogues, antihistamines, antipsychotics and stimulants by working in a non-aqueous medium of ACN, MeOH and acetic acid mixed in the ratio of (49:50:1). Complete separation of eleven standard heparin and eight standard dermatan sulfate disaccharides, which belong to the family of glycosaminoglycans, with both the addition of triethylamine and ACN and mixtures of themselves to the buffer solution was possible [64].

2.5. Amino acids, peptides, proteins

In two publications the enhanced detection sensitivity of amino acids by derivatization prior to CE separation in mixed aqueous–organic solvents was described. In one paper the solvent normally used for the separation by HPLC [water, 15% MeOH, 0.05 mol/l sodium dodecyl sulfate (SDS), 1% THF] is applied for the determination of amino acids after pre-column derivatization with *o*-phthalaldehyde [65]. The other deals with the use of tricarbocyanine dyes as labeling agents for amino acids resulting in high sensitivity of near-IR fluorescence detection after separation in a mixed aqueous–methanolic buffer solution [66].

Thermooptical absorbance detection after separation in aqueous–organic medium consisting of water and MeOH (50:50) allowed determination of amino acids in the attomole range [67].

Peptides were separated in aqueous mixtures of 2,2,2-trifluoroethanol (TFE) in a capillary coated with a monolayer of acrylamide [68]. TFE was used due to its structure-inducing properties. Furthermore, separation of peptides was achieved in FA [69], emphasizing the increase in efficiency and the reduction of analysis time when working with pure FA and in aqueous mixtures of ACN, MeOH, EtOH and i-PrOH having a concentration of the organic constituent of up to 30% [70]. CZE of dipeptides was carried out in aqueous–organic solutions of the lower alcohols, THF and ACN at concentrations of the organic modifier between 0 and 40% [69,71].

Aqueous PrOH (up to 20%) and ACN (up to 25%) solutions were used for the separation of hydrophobic polypeptides, such as fatty acid-acylated peptides, in untreated and 3-aminopropyltrimethoxysilane-derivatized fused-silica capillaries prior to their structural characterization by ^{252}Cf plasma desorption and ESI-MS [72]. Weinmann et al. [73] achieved separation of hydrophobic lipoproteins in buffer solutions containing up to 70% of 2-PrOH prior to desorption ESI-MS.

Microheterogeneities in the charged carbohydrate moieties of recombinant human erythropoietin with a molecular mass range of 34 000 to 38 000 were investigated using a variety of concentrations of ACN (5–40%), MeOH (5–30%), ethylene glycol (5–30%), PrOH and BuOH as organic modifiers [74]. In addition, diol compounds were used as additives and showed a higher separation efficiency than alcohols in increasing the viscosity or decreasing the EOF of the running buffer, respectively.

2.6. Miscellaneous

CZE in pure MeOH was applied to determine complex constants of neutral polyethers (crown ethers and dinitrobenzoyl polyoxyethylene) with different ammonium ions in the background electrolyte [75]. Solvophobic association of tetraheptyammonium as additive to the buffer (with 80% ACN as organic cosolvent to water) led to different migration of non-ionic aromatic compounds, enabling their separation [76].

References

[1] R.G. Bates, in J.F. Coetzee and C.D. Ritchie (Editors), Solute–Solvent Interactions, Marcel Dekker, New York, 1969, pp. 45–96.
[2] E.J. King, in A.K. Covington and T. Dickinson (Editors), Physical Chemistry of Organic Solvent Systems, Plenum Press, London, 1973, pp. 331–403.
[3] I.M. Kolthoff, M.K. Chantooni, in I.M. Kolthoff and P.J. Elving (Editors), Treatise on Analytical Chemistry, Wiley, New York, 1979, pp. 239–301.
[4] M. Spiro, in A.K. Covington and T. Dickinson (Editors), Physical Chemistry of Organic Solvent Systems, Plenum Press, London, 1973, p. 635.
[5] F.M. Everaerts, J.L. Beckers and T.P.E.M. Verheggen, Isotachophoresis—Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.

[6] E. Kenndler, in N.A. Guzman (Editor), Capillary Electrophoresis Technology; Marcel Dekker, New York, Basel, 1993, pp. 161–186.
[7] J.L. Beckers, F.M. Everaerts, J. Chromatogr. 51 (1970) 339.
[8] J.L. Beckers, F.M. Everaerts, J. Chromatogr. 68 (1972) 207.
[9] Y. Hirama, H. Yoshida, J. Chromatogr. 322 (1985) 139–148.
[10] T. Hirokawa, T. Tsuyoshi, Y. Kiso, J. Chromatogr. 408 (1987) 27.
[11] M. Koval, D. Kaniansky, M. Hutta, R. Lacko, J. Chromatogr. 325 (1985) 151–160.
[12] J.C. Reijenga, V.A. Aben, T.P.E.M. Verheggen, F.M. Everaerts, J. Chromatogr. 260 (1983) 241–254.
[13] J.C. Reijenga, H.J.L.A. Slaats, F.M. Everaerts, J. Chromatogr. 267 (1983) 85–89.
[14] S. Tanaka, T. Kaneta, H. Yoshida, J. Chromatogr. 472 (1989) 303–307.
[15] H. Yoshida, Y. Hirama, J. Chromatogr. 298 (1984) 243–251.
[16] E. Kenndler, R. Katzenberger, Microchim. Acta (1986) 415–428.
[17] E. Kenndler, P. Jenner, J. Chromatogr. 390 (1987) 185–197.
[18] E. Kenndler, P. Jenner, J. Chromatogr. 390 (1987) 169–184.
[19] E. Kenndler, C. Schwer, P. Jenner, J. Chromatogr. 470 (1989) 57–68.
[20] M. Chiari, E. Kenndler, J. Chromatogr. A 716 (1995) 303–309.
[21] E. Kenndler, B. Gassner, Anal. Chem. 62 (1990) 431–436.
[22] M. Jansson, J. Roeraade, Chromatographia 40 (1995) 163–169.
[23] C. Schwer, E. Kenndler, Anal. Chem. 63 (1991) 1801–1807.
[24] H. Salimi-Moosavi, R.M. Cassidy, Anal. Chem. 67 (1995) 1067–1073.
[25] B.B. VanOrman, G.G. Liversidge, G.L. McIntire, T.M. Olefirowicz, A.G. Ewing, J. Microcol. Sep. 2 (1990) 176–180.
[26] W. Schützner, E. Kenndler, Anal. Chem. 64 (1992) 1991–1995.
[27] J. Tjornelund, S.H. Hansen, Chromatographia 44 (1997) 5–9.
[28] W. Buchberger, P.R. Haddad, J. Chromatogr. A 608 (1992) 59–64.
[29] M.P. Harrold, M.J. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. A 640 (1993) 463–471.
[30] H. Salimi-Moosavi, R.M. Cassidy, J. Chromatogr. A 749 (1996) 279–286.
[31] Q. Yang, J. Smeyers-Verbeke, W. Wu, M.S. Khots, D.L. Massart, J. Chromatogr. A 688 (1994) 339–349.
[32] D. Volgger, A.J. Zemann, G.K. Bonn, M.J. Antal, J. Chromatogr. A 758 (1997) 263–276.
[33] W. Buchberger, C.W. Klampfl, J. Chromatogr. A 766 (1997) 197–203.
[34] S. Fujiwara, S. Honda, Anal. Chem. 59 (1987) 487–490.
[35] Z.D. Hu, L. Jia, Z.P. Zhang, Anal. Lett. 29 (1996) 2573–2586.
[36] Y.J. Lee, W.E. Price, M.M. Sheil, Analyst 120 (1995) 2689–2694.
[37] M.W.F. Nielen, J. Chromatogr. 542 (1991) 173–183.
[38] W. Buchberger, K. Winna, J. Chromatogr. A 739 (1996) 389–397.
[39] S.M. Masselter, A.J. Zemann, Anal. Chem. 67 (1995) 1047–1053.
[40] A.J. Zemann, J. Cap. Electrophoresis 2 (1995) 131–136.
[41] R. Roldan-Assad, P. Gareil, J. Chromatogr. A 708 (1995) 339–350.
[42] H. Salimi-Moosavi, R.M. Cassidy, Anal. Chem. 68 (1996) 293–299.
[43] E. Kenndler, J.F.K. Huber, Mikrochim. Acta (1989) 271–282.
[44] Y. Wahlbroehl, J.W. Jorgenson, J. Chromatogr. 315 (1984) 135.
[45] C.E. Lin, W. Chiou, W. Lin, J. Chromatogr. A 723 (1996) 189–195.
[46] C.E. Lin, W. Chiou, W. Lin, J. Chromatogr. A 722 (1996) 345–352.
[47] E. Moyano, D.E. Games, M.T. Galceran, Rapid Commun. Mass. Spectrom. 10 (1996) 1379–1385.
[48] J. Wu, M.K. Wong, S.F.Y. Li, H.K. Lee, C.N. Ong, J. Chromatogr. A 709 (1995) 351–359.
[49] S.H. Hansen, J. Tjornelund, I. Bjornsdottir, Trends Anal. Chem. 15 (1996) 175–180.
[50] I. Bjornsdottir, S.H. Hansen, J. Pharm. Biomed. Anal. 13 (1995) 1473–1481.
[51] J.J. Pesek, M.T. Matyska, J. Chromatogr. A 736 (1996) 313–320.
[52] J. Tjornelund, S.H. Hansen, J. Chromatogr. A 737 (1996) 291–300.
[53] C.E. Lin, W.C. Lin, W.C. Chiou, E.C. Lin, C.C. Chang, J. Chromatogr. A 755 (1996) 261–269.
[54] L.M. Benson, A.J. Tomlinson, J.M. Reid, D.L. Walker, M.M. Ames, S. Naylor, J. High Resolut. Chromatogr. 16 (1993) 324–326.
[55] C.L. Ng, H.K. Lee, S.F.Y. Li, J. Liq. Chromatogr. 17 (1994) 3847–3857.
[56] A.J. Tomlinson, L.M. Benson, S. Naylor, J. High Resolut. Chromatogr. 17 (1994) 175–177.
[57] A.J. Tomlinson, L.M. Benson, J.W. Gorrod, S. Naylor, J. Chromatogr. B 657 (1994) 373–381.
[58] A.J. Tomlinson, L.M. Benson, S. Naylor, J. Cap. Electrophoresis 1 (1994) 127–135.
[59] A.J. Tomlinson, L.M. Benson, K.L. Johnson, S. Naylor, Electrophoresis 15 (1994) 62–71.
[60] S. Naylor, L.M. Benson, A.J. Tomlinson, J. Cap. Electrophoresis 1 (1994) 181–189.
[61] K.J. Potter, R.J.B. Allington, J. Algaier, J. Chromatogr. A 652 (1993) 427–429.
[62] C. Stathakis, P.L. Warburton, R.M. Cassidy, J. Chromatogr. A 659 (1994) 443–447.
[63] G.N.W. Leung, H.P.O. Tang, T.S.C. Tso, T.S.M. Wan, J. Chromatogr. A 738 (1996) 141–154.
[64] L. Scapol, E. Marchi, G.C. Viscomi, J. Chromatogr. A 735 (1996) 367–374.
[65] J.P. Liu, K.A. Cobb, M. Novotny, J. Chromatogr. 468 (1989) 55–65.
[66] J.H. Flanagan, B.L. Legendre, R.P. Hammer, S.A. Soper, Anal. Chem. 67 (1995) 341–347.
[67] M. Yu, N.N. Dovichi, Anal. Chem. 61 (1989) 37–40.

[68] M. Castagnola, L. Cassiano, I. Messana, M. Paci, D.V. Rossetti, B. Giardina, J. Chromatogr. A 735 (1996) 271–281.
[69] R.S. Sahota, M.G. Khaledi, Anal. Chem. 66 (1994) 1141–1146.
[70] M. Idei, I. Mezo, Z. Vadasz, A. Horvath, I. Teplan, G. Keri, J. Liq. Chromatogr. 15 (1992) 3181–3192.
[71] G.M. Janini, K.C. Chan, J.A. Barnes, G.M. Muschik, H.J. Issaq, Chromatographia 35 (1993) 497–502.
[72] W. Weinmann, C.E. Parker, K. Baumeister, C. Maier, K.B. Tomer, M. Przybylski, Electrophoresis 15 (1994) 228–233.
[73] W. Weinmann, C. Maier, K. Baumeister, M. Przybylski, C.E. Parker, K.B. Tomer, J. Chromatogr. A 664 (1994) 271–275.
[74] A.D. Tran, T. Blanc, E.J. Leopold, J. Chromatogr. 542 (1991) 459–472.
[75] T. Okada, J. Chromatogr. 695 (1995) 309–317.
[76] Y.C. Shi, J.S. Fritz, J. High Resolut. Chromatogr. 17 (1994) 713–718.