Chapter 11
Non-Aqueous Capillary Electrophoresis

Michał Szumski and Bogusław Buszewski

Abstract Non-aqueous capillary electrophoresis and capillary electrochromatography are special variants of these techniques. Here, organic solvents or their mixtures with or without dissolved electrolytes are used as separation buffer or mobile phase, respectively. The most important features of non-aqueous systems are: better solubility of more hydrophobic ionic substances (many natural products) than in water, much less current and Joule heating allows for using highly concentrated buffers and/or larger capillary internal diameters, polar interactions are enhanced in organic solvents which is often highly advantageous in chiral separation systems. This chapter presents most frequently used solvents, their properties, as well as shows pH* scale which is often used in non-aqueous systems.

In classical CEC and CEC aqueous or hydroorganic solvents are used but it is different in non-aqueous CE (NACE) and CEC (NACEC) which employ a wide range of organic solvents, without any addition of water. Despite non-aqueous approach was described as far as in the 1980s (Walbroehl and Jorgenson) [1, 2], a systematic increase in number of published papers has been dated since 1994 [3, 4]. There are several reasons for using NACE:

- Improvement in selectivity.
- Electrophoresis in large bore capillaries—"preparative" CE.
- Electrophoresis of compounds insoluble or slightly soluble in water.
- Employing during some separations (for example chiral compounds) polar interactions which are not present in water because of its "leveling effect".
- In some cases an increase of fluorescence intensity may be observed when switching to NACE, which positively affects LOD.

M. Szumski (✉) · B. Buszewski
Faculty of Chemistry, Nicolaus Copernicus University, Toruń, Poland
e-mail: michu@chem.umk.pl

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11.1 Solvents

When compared to aqueous systems, application of a wide range of organic solvents and their mixtures, characterized by different properties opens new possibilities in controlling selectivity. For example, pK_a values in organic solvents may differ significantly from those determined in water, which may allow for separation of analytes impossible to be separated in aqueous conditions. This is so, because the solvents used in NACE can belong to amphiprotic (like methanol) or typical proton acceptors (for example formamide) (Table 11.1).

It may be quite important to pay attention to purity of the solvents used, particularly to possible water content. Sometimes when important data, not only analytical but also thermodynamic (for example dissociation constants), have to be obtained it might be necessary to determine water content, which may be done using Karl-Fischer method [5, 6].

In many pure solvents the electroosmotic flow may be observed during CE or CEC. EOF depends on dielectric constant to viscosity ratio and may vary from one solvent to another—some exemplary data are shown in Fig. 9.6 in Sect. 9.1. For practical reasons the solvents should be characterized by low viscosity (which influences not only EOF but electrophoretic mobility as well), low vapor pressure, and should not be toxic. Absorption of UV–V is radiation should also be taken into account, and if any problems occur, other detection methods need to be considered. Pure solvents or their mixtures as well as solutions of some electrolytes can be used as a running electrolytes. Acids and their ammonium (e.g., ammonium acetate, ammonium oxalate) or sodium salts are most popular as well as some organic buffers like Tris. Their cations and anions may have significant influence

Table 11.1 Classification of organic solvents in terms of their acid–base properties (Brønsted theory) according to Kolthoff [7, 8]

Properties		Relative acidity	Relative basicity	Example
Amphiprotic	Neutral	+	+	Methanol, glycerol, phenol, tert-butyl alcohol
	Protogenic	+	−	Sulfonic acid, formic acid, acetic acid
	Protophilic	−	+	Liquid ammonia, formamide, N-methylacetamide, NMF
Aprotic	Dipolar protophilic	−	+	DMSO, DMF, THF, 1,4-dioxane, pyridine
	Dipolar protophobic	−	−	Acetonitrile, acetone, nitrobenzene, sulfolan, propylene carbonate
	Neutral	−	−	Aliphatic hydrocarbons, benzene, 1,2-dichloroethane, carbon tetrachloride

+ or − indicate whether given type of solvent is stronger or weaker acid or base than water

Table 11.2 pH* values of methanolic electrolytes, according to [10]

Electrolyte composition	pH*
2 mol/L TFA, 20 mmol/L NH_4OAc	2.90
1 mol/L TFA, 20 mmol/L NH_4OAc	3.15
10 mmol/L oxalic acid, 10 mmol/L ammonium hydrogen oxalate	5.79
10 mmol/L succinic acid, 10 mmol/L lithium hydrogen succinate	8.75
20 mmol/L NH_4OAc	10.51
50 mmol/L $NaOCH_3$, 20 mmol/L NH_4OAc	13.50
0,1 mol/L $NaOCH_3$, 20 mmol/L NH_4OAc	15.24

on resolution in NACE due to not impaired (compared to water conditions) electrostatic interactions with analytes [3, 4, 9].

When electrolytes solutions in organic solvents are employed as background electrolytes their pH* values are sometimes used to characterize them. The values presented in Table 11.2 have been obtained with a pH-meter with glass electrode calibrated with the following methanolic buffers: oxalic acid/ammonium hydrogen oxalate and succinic acid/Lithium hydrogen succinate of pH* 5.79 and 8.75, respectively [10]. Exemplary plots of dependency of EOF on pH and pH* are presented in Fig. 11.1 [10].

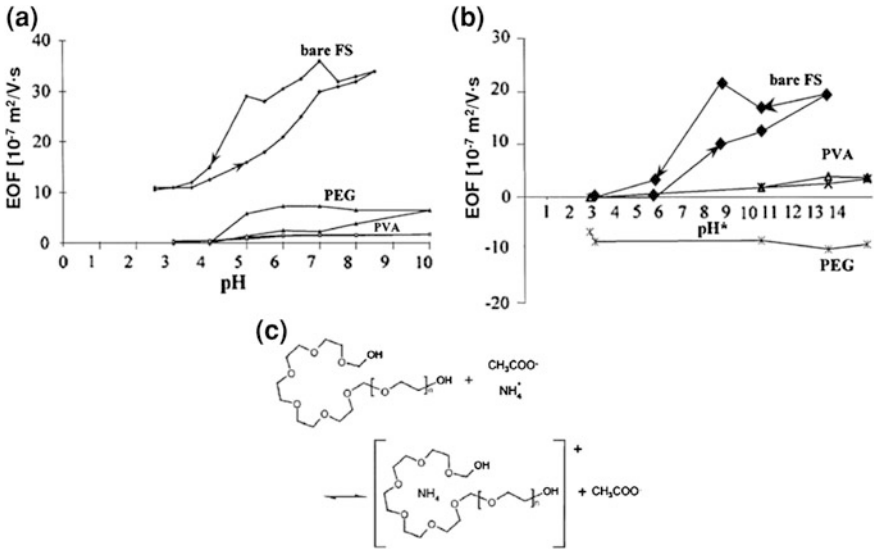

Fig. 11.1 Comparison of EOF in capillaries (coated with hydrophilic polymers) in aqueous and non-aqueous conditions and different pH and pH*. Bottom picture explains the reversed EOF: ammonium ions are complexed by PEG in non-aqueous conditions, which results in positive net charge on the inner capillary wall. Reprinted with permission from Belder et al. [10]. Copyright 2000 Elsevier

11.2 Separations in Wide Bore Capillaries

In classical CE the capillaries are characterized by rather small internal diameters (25–75 μm) as the Joule heat needs to be efficiently dissipated. Joule heating is much lower in NACE due to much less conductivity of electrolytes in organic solvents, which allows to use high electrolyte concentrations, wide bore capillaries (150 or even 530 μm), and higher voltages [11, 12]. Contrary to separations performed in aqueous solutions, lower Joule heat generated in organic solvents give linear plot I = f(V) in a broad range of voltages and capillary diameters (Fig. 11.2).

Larger capillary diameter may in turn allow for larger volumes to be injected which make "preparative" but with high resolution CE separations possible. However, when using large bore capillaries a siphoning (i.e., flowing of the liquid from the vial of higher liquid level to another vial of lower liquid level) effect may be quite significant. Such phenomenon may adversely influence band width and resolution. It can be minimized by using capillaries tapered at both ends (to restrict hydrodynamic flow) or by compensating the change of level of liquid by controlled rise of the vial in which the level becomes lower due to the EOF—Fig. 11.3 [12, 13].

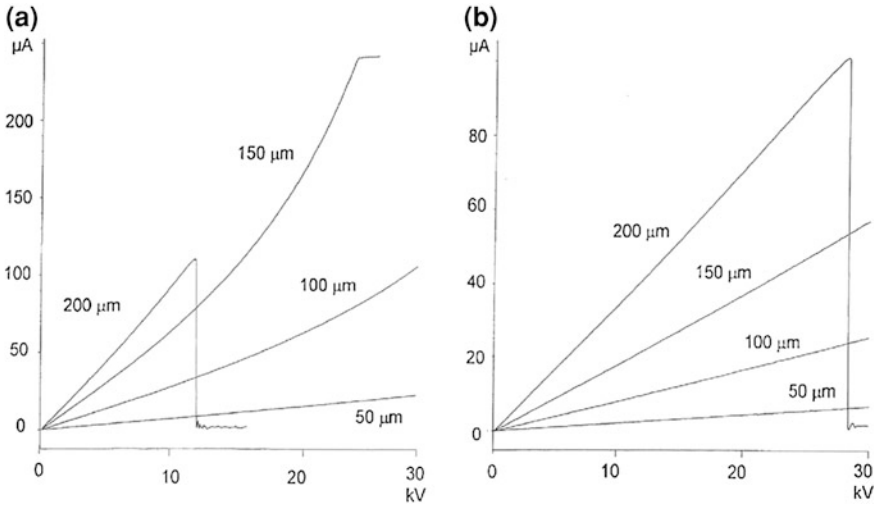

Fig. 11.2 Dependency of current on the applied voltage for capillaries of L_{tot} = 58.5 cm and different internal diameters. Electrolytes: **a** 20 mmol/L ammonium acetate in water/acetic acid (99/1, v/v), **b** 20 mmol/L ammonium acetate in ethanol/acetonitrile/acetic acid (50/49/1, v/v). Temperature: 20 °C. Breakdown on the curves indicate the conditions when the medium starts to boil which breaks the electric circuit. Reprinted with permission from Valkó et al. [20]. Copyright 1998 Elsevier

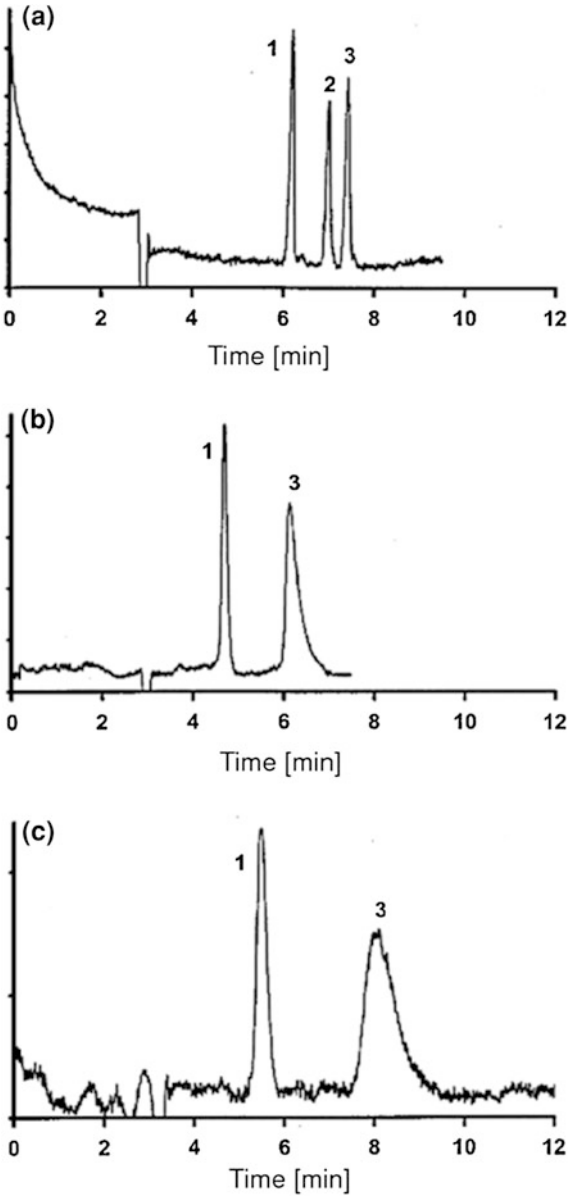

Fig. 11.3 NACE separation of diuretics (1—bumetanide, 2—furosemide, 3—ethacrynic acid) in wide-bore capillaries: 320 μm and total length of 60 cm with controlled vial lifting. Separation medium: ethanol/acetonitrile 50/50 + electrolyte: **a** 1 mmol/L KAc, lifting 0.72 mm/min, no siphoning effect, **b** 5 mmol/L NH_4Ac, lifting 0.84 mm/min, no siphoning effect, **c** 5 mmol/L NH_4Ac, no lifting, significant siphoning effect. Reprinted with permission from Jussila et al. [13]. Copyright 2000 Wiley-VCH

11.3 Separation of Large Molecules Insoluble in Water

Despite their ionic character many molecules of natural of anthropogenic origin are not soluble in water because of their high molecular weight, hydrophobicity or instability in water solutions [14]. Application of NACE may be the only way to

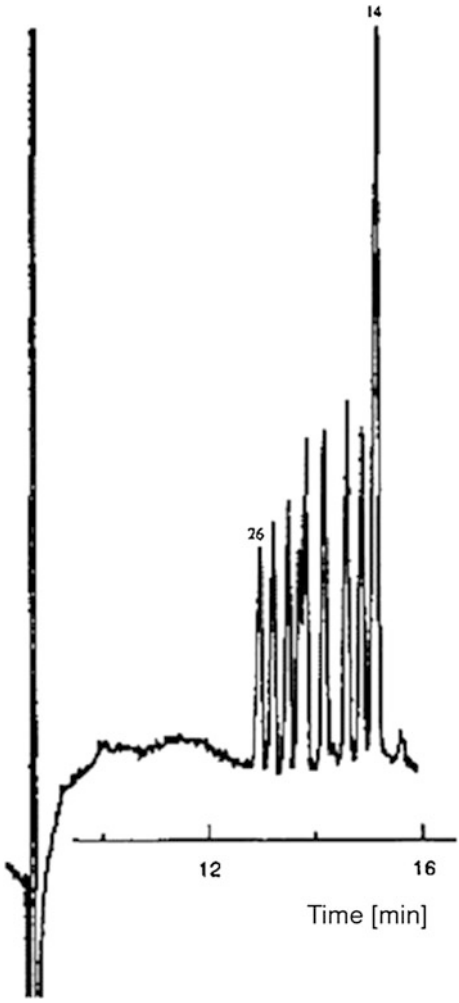

Fig. 11.4 Separation of fatty acids in the following mixtures: 2.5 mmol/L antraquinone-2-carboxylic acid, 40 mmol/L Tris in N-methylformamide/dioxane 3/1 (v/v). Separation conditions: V = 20 kV, L_{tot} = 67 cm, L_{eff} = 46 cm, d_c = 75 μm, indirect detection at λ = 264 nm. The migration order is: n-C_{26}, n-C_{24}, n-C_{22}, n-$C_{22\text{-OH}}$, n-C_{20}, n-C_{18}, n-C_{16}, n-$C_{16\text{-OH}}$, n-C_{14}. Reprinted with permission from Drange and Lundanes [15]. Copyright 1997 Elsevier

separate them provided their solubility in organic solvents. For example, it is possible to separate long chain fatty acids with NACE. Such separations used to be performed with GC, HPLC, or SFC. Drange and Lundanes [15] used 40 mmol/L Tris in NMF/dioxane 3/1 as a separation medium. Due to lack of chromophores in fatty acids, indirect UV detection (λ = 264 nm) was employed in the presence of 2.5 mmol/L antraquinone-2-carboxylic acid (Fig. 11.4).

Xu et al. [16] applied NACE for determination of cholesterol in egg yolk and milk. When compared to classical spectrophotometric analysis, NACE turned out to be much faster and provided good correlation of quantitative data. The authors used 100 mmol/L sodium acetate/acetic acid (99/1) methanolic solution as a background electrolyte. The other exemplary analytes that can be separated with

NACE are phospholipids, polymers, dyes, organomercury compounds, hydrophobic polypeptides, phosphoorganic pesticides, hydrophobic pharmaceuticals, steroids, and alkaloids [9].

11.4 Separation of Uncharged Compounds

Electrophoretic separation in NACE can be completed if analytes are charged in an organic solvent or if non-charged analytes interact with charged additive, introduced to the system for that purpose [17]. Under aqueous conditions neutral and hydrophobic species can be separated with, for example, micellar electrokinetic chromatography (MEKC). In most of the organic solvents (except for formamide and poly (ethylene glycol)) micelles cannot be formed due to very weak hydrophobic (solvophobic) interactions between hydrocarbon chains of the surfactants molecules. Separation of highly hydrophobic substances can be performed using some ion-pairing species as additives. For example, polyaromatic hydrocarbons can be separated employing tetrahexylammonium bromide (THA^+) as an additive to 50 mmol/L ammonium acetate in methanol (Fig. 11.5) [18].

The separation technique used in the above example was called hydrophobic interaction electrokinetic chromatography (HI-EKC). While separating PAHs using HI-EKC with THA^+ the complexes of PAHs molecules with THA^+ are formed, and the number of bound additive molecules depends on hydrophobic interactions and polarizability. Hydrophobic interactions are strong even in methanol, while the influence of polarizability ("electrostatic model") can be explained in such a way that in a medium of low dielectric constant (methanol) delocalized π electrons undergo polarization (induced dipole is formed) in the presence of THA^+ which, in turn, leads to creation of associates of different (depending on hydrophobicity/polarizability of a given PAH) electrophoretic mobility. Both the mentioned mechanisms, with possible domination of the hydrophobic interaction in this particular case, influence the number of THA^+ bound with one PAH molecule (Fig. 11.6) [18].

Other additives that can be used in electrophoretic separation in non-aqueous conditions are: ammonium ions, alkali metal ions, anions ClO_4^-, BF_4^-, NO_3^-, Br^-, Cl^-, $CH_3SO_3^-$, Brij, (+)-18-corona-6-tetraacetic acid, (-)-quinine, 2,4,6-triphenylpyrylium, tetraalkylammonium salts, camphorsulfonic acid, heptanesulfonic acid, tropylium, sodium dodecyl-, tetradecyl-, and hexsadecylsulfates [4, 17]. The mentioned species provide the solvophobic, electrostatic (ion–ion, ion–dipole, dipole–dipole), and donor–acceptor interactions. It is believed that in NACE the interactions of analytes with the additives are of much more complex nature when compared to classical CE performed in aqueous conditions. It should be noted, however, that electrostatic and donor–acceptor interactions rather dominate in NACE (due to low dielectric constant of the medium) while hydrophobic interactions dominate in CE.

11.5 Separation of Optically Active Compounds

Separation of optically active compounds using electromigration techniques is quite popular due to high efficiencies obtained and low chemicals consumption. Despite most chiral separations being performed in aqueous or hydro-organic conditions, application of non-aqueous environment may be profitable, which is the result of different properties of organic solvents and water. The separation of enantiomers usually employs the electrostatic interactions of the analyte with the chiral selector [19]. Potential energy of ion–ion ($E_{i\text{-}i}$) and ion–dipole ($E_{i\text{-}d}$) interactions can be expressed by the following Eqs. [4]:

$$E_{i-i} = \frac{z_a z_b e^2}{4\pi\varepsilon_0 \varepsilon r} \tag{11.1}$$

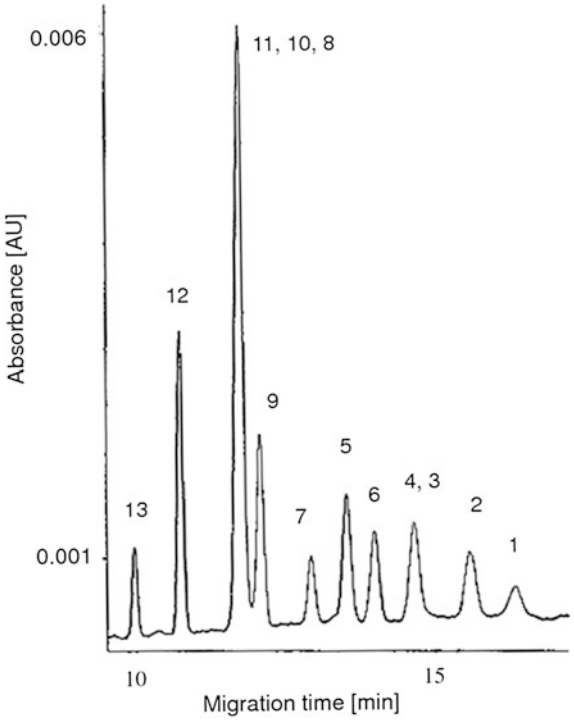

Fig. 11.5 HI-EKC non-aqueous separation of polyaromatic hydrocarbns. Resolved compounds: *13* coronene, *12* benzo[*ghi*]perylene, *11* benzo[*a*]pyrene, *10* benzo[*e*]pyrene, *8* perylene, *9* benzo[*k*]fluoranthrene, *7* chryzene, *5* pyrene, *6* 2,3-benzofluorene, *4* fenanthrene, *3* anthracene, *2* fluorene, *1* naphthalene. Separation medium: 50 mmol/L ammonium acetate–100 mmol/L THA in 100 % methanol. Analyte concentrations ca. 40 μmol/L. Capillary: L_{eff} = 40 cm, E = 532 V/cm, λ = 254 nm. Reprinted with permission from Koch et al. [18]. Copyright 2001 Elsevier

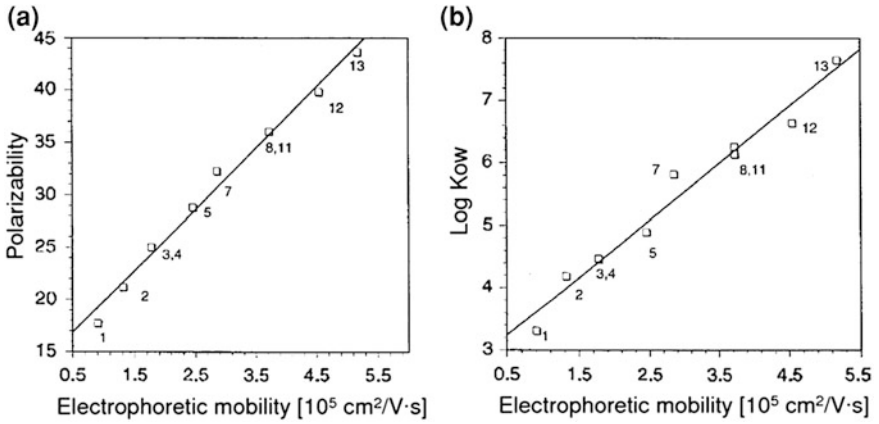

Fig. 11.6 Correlation of electrophoretic mobility of PAHs with polarizability (**a**) and octanol/water partition coefficient (**b**). Analytical conditions the same as in the Fig. 11.4. Reprinted with permission from Koch et al. [18]. Copyright 2001 Elsevier

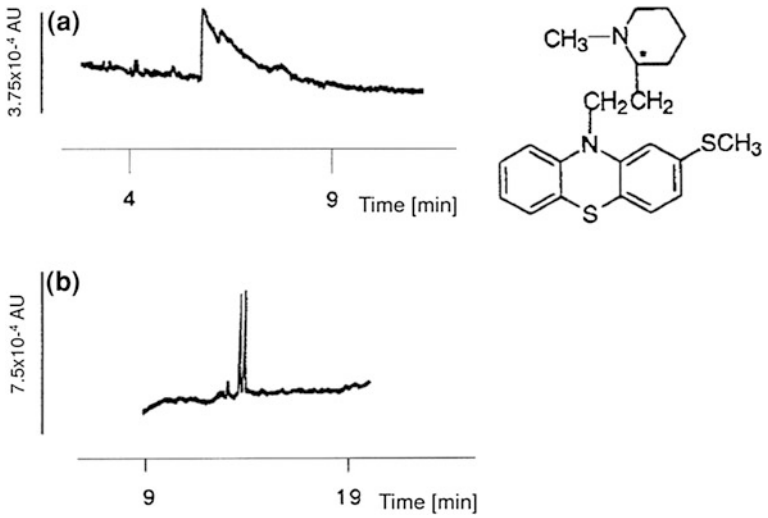

Fig. 11.7 Comparison of separation of thioridazine (antipsychotic drug) enantiomers in aqueous **a** and non-aqueous **b** conditions. Electrolyte: **a** 1.543×10^{-4} w/v % ($\sim$1 μmol/L) β-CD-$(SO_4-)_4$ in aqueous solution of 50 mmol/L Tris-phosphate (pH 2.50); **b** 1.543×10^{-4} w/v % ($\sim$1 μmol/L) β-CD-$(SO_4-)_4$ in 150 mmol/L citric acid–100 mmol/L Tris (pH* = 5.1) in formamide. Electric field: **a** 357 V/cm, **b** 595 V/cm. Reprinted with permission from Wang and Khaledi et al. [23]. Copyright 1999 Elsevier

$$E_{i-d} = \frac{z_a e \mu cos\theta}{4\pi\varepsilon_0 \varepsilon r^2} \tag{11.2}$$

where z is charges of the interacting ions a and b, r is distance between the ions, μ is dipole moment, θ is dipole angle.

Because of lower dielectric constant of the organic solvents, such interactions are stronger than in water ($\varepsilon = 78$). It allows to use such chiral selectors that would not provide the interactions strong enough to obtain separation in aqueous conditions. Moreover, some chiral selectors, like modified cyclodextrins, show much better solubility in organic solvents (formamide or N-methylformamide). For example, separations of dansyl derivatized amino acids enantiomers are difficult to be performed in water because of limited solubility of β-cyclodextrin in water. When NMF is employed as a separation medium such analytes are possible to be separated using 80 mmol/L of β-cyclodextrin as and chiral selector [20]. Other selectors that can be successfully used in chiral separations are: heptakis(2,3-diacetyl-6-sulfato)-[β]-cyclodextrin (separation of weakly basic pharmaceuticals in pure methanol) [21], β-CD-$(SO_4^-)_4$ (Fig. 11.6) and QA-β-CD (QA—*quaternary ammonium*) [22, 23], carboxymethyl-β-CD [4], quinine, (+)-18-corona-6-tetraacetic acid [24] or camphorsulfonic acid. Also, some reports have been published on using pure chiral solvents like R-(−)-2-butanol, S-(+)-2-butanol and S-(−)-2-methyl-1-butanol [4] (Fig. 11.7).

11.6 Summary

Non-aqueous electrophoreis and electrochromatography become more and more important in separation sciences and are a profitable complement to classical aqueous and non-aqueous techniques. The most important advantages are: possibility of separation of the water insoluble species and employing of the electrostatic interactions with the additives that would be too weak in water to give a significant separation effect.

References

1. Y. Walbroehl, J.W. Jorgenson, On-column UV absorption detector for open tubular capillary zone electrophoresis. J. Chromatogr. A **315**, 135–143 (1984)
2. Y. Walbroehl, J.W. Jorgenson, Capillary zone electrophoresis of neutral organic molecules by solvophobic association with tetraalkylammonium ion. Anal. Chem. **58**(2), 479–481 (1986)
3. M.L. Riekkola, Recent advances in nonaqueous capillary electrophoresis. Electrophoresis **23**(22–23), 3865–3883 (2002)
4. M.L. Riekkola, M. Jussila, S.P. Porras, I.E. Valko, Non-aqueous capillary electrophoresis. J. Chromatogr. A **892**(1–2), 155–170 (2000)

5. B. Buszewski, Solvents, In: P.J. Worsfold, A. Townshend, C.F. Poole (eds.), Encyclopedia of analytical Science, 2nd edn. (Elsevier, Amsterdam, 2005)
6. S.P. Porras, M.L. Riekkola, E. Kenndler, Capillary zone electrophoresis of basic analytes in methanol as non-aqueous solvent: Mobility and ionisation constant. J. Chromatogr. A **905**(1–2), 259–268 (2001)
7. I.M. Kolthoff, Acid-base equilibriums in dipolar aprotic solvents. Anal. Chem. **46**(13), 1992–2003 (1974)
8. J. Tjørnelund, S.H. Hansen, Non-aqueous capillary electrophoresis of drugs: Properties and application of selected solvents. J. Biochem. Biophys. Methods **38**(2), 139–153 (1999)
9. H. Teng, B.Q. Yuan, T.Y. You, Recent advances in application of nonaqueous capillary electrophoresis. Chin. J. Anal. Chem. **38**(11), 1670–1677 (2010)
10. D. Belder, K. Elke, H. Husmann, Influence of pH*-value of methanolic electrolytes on electroosmotic flow in hydrophilic coated capillaries. J. Chromatogr. A **868**(1), 63–71 (2000)
11. I.E. Valkó, S.P. Porras, M.L. Riekkola, Capillary electrophoresis with wide-bore capillaries and non-aqueous media. J. Chromatogr. A **813**(1), 179–186 (1998)
12. H. Yin, C. Keely-Templin, D. McManigill, Preparative capillary electrophoresis with wide-bore capillaries. J. Chromatogr. A **744**(1–2), 45–54 (1996)
13. M. Jussila, S. Palonen, S.P. Porras, M.L. Riekkola, Compensation of the siphoning effect in nonaqueous capillary electrophoresis by vial lifting. Electrophoresis **21**(3), 586–592 (2000)
14. H. Cottet, M.P. Struijk, J.L.J. Van Dongen, H.A. Claessens, C.A. Cramers, Non-aqueous capillary electrophoresis using non-dissociating solvents: Application to the separation of highly hydrophobic oligomers. J. Chromatogr. A **915**(1–2), 241–251 (2001)
15. E. Drange, E. Lundanes, Determination of long-chained fatty acids using non-aqueous capillary electrophoresis and indirect UV detection. J. Chromatogr. A **771**(1–2), 301–309 (1997)
16. X.H. Xu, R.K. Li, J. Chen, P. Chen, X.Y. Ling, P.F. Rao, Quantification of cholesterol in foods using non-aqueous capillary electrophoresis. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. **768**(2), 369–373 (2002)
17. M.T. Bowser, A.R. Kranack, D.D.Y. Chen, Analyte-additive interactions in nonaqueous capillary electrophoresis: A critical review. Trends Anal. Chem. **17**(7), 424–434 (1998)
18. J.T. Koch, B. Beam, K.S. Phillips, J.F. Wheeler, Hydrophobic interaction electrokinetic chromatography for the separation of polycyclic aromatic hydrocarbons using non-aqueous matrices. J. Chromatogr. A **914**(1–2), 223–231 (2001)
19. I. Bjornsdottir, S. Honoré Hansen, S. Terabe, Chiral separation in non-aqueous media by capillary electrophoresis using the ion-pair principle. J. Chromatogr. A **745**(1–2), 37–44 (1996)
20. I.E. Valkó, H. Sirén, M.L. Riekkola, Chiral separation of dansyl-amino acids in a nonaqueous medium by capillary electrophoresis. J. Chromatogr. A **737**(2), 263–272 (1996)
21. J.B. Vincent, G. Vigh, Nonaqueous capillary electrophoretic separation of enantiomers using the single-isomer heptakis (2, 3-diacetyl-6-sulfato)-[beta]-cyclodextrin as chiral resolving agent. J. Chromatogr. A **816**(2), 233–241 (1998)
22. F. Wang, M.G. Khaledi, Nonaqueous capillary electrophoresis chiral separations with quaternary ammonium [beta]-cyclodextrin. J. Chromatogr. A **817**(1–2), 121–128 (1998)
23. F. Wang, M.G. Khaledi, Non-aqueous capillary electrophoresis chiral separations with sulfated [beta]-cyclodextrin. J. Chromatogr. B Biomed. Sci. Appl. **731**(2), 187–197 (1999)
24. Y. Mori, K. Ueno, T. Umeda, Enantiomeric separations of primary amino compounds by nonaqueous capillary zone electrophoresis with a chiral crown ether. J. Chromatogr. A **757**(1–2), 328–332 (1997)