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Review

Non-aqueous capillary electrophoresis

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Abstract

The benefits of non-aqueous capillary electrophoresis have been described in a number of recent publications. The wide selection of organic solvents, with their very different physicochemical properties, broadens our scope to manipulate separation selectivity. The lower currents present in non-aqueous solvents allow the use of high electric field strengths and wide bore capillaries, the latter in turn allowing larger sample load. In many cases detection sensitivity can also be enhanced. The potential of non-aqueous capillary electrophoresis is discussed throughout the paper, and the feasibility of capillary electrophoresis under non-aqueous media is demonstrated with reference to several applications.

Keywords: Reviews; Nonaqueous capillary electrophoresis; Enantiomer separation; Detection, electrophoresis; Buffer composition

Contents

1. Introduction

Conventional electrophoresis with low electric fields is a well-established separation method for proteins, used especially by biochemists. The two-

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dimensional approaches that are available nowadays have further increased the resolving power of the method. As early as the 1950s, electrophoresis was also applied to more hydrophobic compounds such as carbon black particles, cholesterol, higher fatty acids and steroid hormones with the aid of non-aqueous and mixed solvents as separation media. The need for better separation efficiencies, automation and reliable quantitation led to the development in the early 1980s of techniques allowing electrophoresis to be carried out in capillaries under high electric field [1].

At the moment the electromigration techniques form a versatile separation family covering the application area from small ions to macromolecules and single cells. Even though for many years organic solvents have provided an alternative to aqueous media in conventional electrophoresis and isotachophoresis, and although they have commonly been employed as modifiers in capillary electrophoresis (CE), the potential of applying them in CE as background electrolyte solutions has only recently begun to attract close attention. The low currents present in non-aqueous capillary electrophoresis not only allow the use of higher electrolyte salt concentrations and higher electric field strengths, but also the sample load can be scaled-up by employing capillaries with wider inside diameter. CE under non-aqueous media holds great promise, especially for easy manipulation of separation selectivity by changing the solvent and for semipreparative applications. In addition, an effective sample introduction to the mass spectrometer, in terms of volatility, surface tension, flow-rate and ionization, can be expected to further extend the use of non-aqueous CE. This paper reviews current research in the field through a look at the major approaches and achievements.

2. Physicochemical properties of organic solvents

Non-aqueous CE exploits the vastly different physicochemical properties (Table 1) of organic solvents to control electroosmotic flow (EOF) and analyte migration. The ability of organic solvents to accept protons from the silanol groups of the capillary wall appears to play a crucial role in the development of EOF. Although EOF may not be required or even may be completely undesired in a few electromigration capillary techniques, it plays a significant role in separations in free solution CE. We have recently shown that EOF appears in a number of solvents even without addition of electrolyte [4]. We would note, however, that the purity of the solvent may affect the EOF and zeta potential. Even high purity solvents may contain foreign ionic

Table 1
Properties of solvents at 25°C [2,3][a]

Solvent	t_{boil} (°C)	η (mPa s)	ε	pK_{auto}	γ (10^{-2} N m^{-1})
Methanol (MeOH)	64.7	0.545	32.70	17.20	2.212
Ethanol (EtOH)	78.3	1.078	24.55	18.88	2.190
1-Propanol (1-PrOH)	97.2	1.956	20.33	19.43	2.330
2-Propanol (2-PrOH)	82.3	2.073	19.92	20.80	2.124
1-Butanol (1-BuOH)	117.7	2.593	17.51	21.56	2.416
Acetonitrile (ACN)	81.6	0.341	37.5[b]	≥33.3	2.760
Propylene carbonate (PC)	242	2.513	66.1	–	4.14
Formamide (FA)	210.5	3.30	111.0	16.8[b]	5.791
N-Methylformamide (NMF)	~180	1.65	182.4	10.74	3.87
N,*N*-Dimethylformamide (DMF)	153.0	0.802	36.71	29.4	3.52
Dimethyl sulphoxide (DMSO)	189.0	1.996	46.68	33.3	4.286
Tetrahydrofuran (THF)	66.0	0.460	7.58	–	2.64
Water	100.0	0.890	78.39	14.00	7.181

[a] t_{boil}, boiling point; η, coefficient of viscosity; ε, dielectric constant (relative permittivity); pK_{auto}, autoprotolysis constant; and γ, coefficient of surface tension.

[b] At 20°C.

species, and solvents such as *N*-methylformamide, *N*,*N*-dimethylformamide and *N*,*N*-dimethylacetamide hydrolyse easily. The velocity of electroosmotic flow is higher in pure solvents than in electrolyte solutions, which may be beneficial when fast liquid transport is desired. Salt-free solvents may also be advantageous in mass spectrometric detection.

Organic solvents differ widely in their autoprotolytic behaviour and the right choice of solvent may yield separations that are not possible in aqueous systems. Because viscosity has an inverse effect on electroosmotic and electrophoretic mobilities, it should be low, to allow the separations in a reasonable time frame. Stable solvents with low vapour pressures and present as liquids at room temperature are the most convenient for practical purposes. Toxic solvents should be avoided. Unlike water, organic solvents may exhibit strong absorbance of ultraviolet light, so that indirect ultraviolet (UV) or other alternative detection methods are required. These may, in fact, provide better sensitivity than is achievable in water; for example, the sensitivity in fluorescence detection can be enhanced [5], and the low heat of vaporisation and low surface tension of organic solvents can be exploited in coupling of CE with electrospray ionization mass spectrometry [6].

3. Selectivity

One of the most attractive features of organic solvents is that their physical and chemical properties differ widely, both from each other and from water (Table 1). Accordingly, selectivity manipulation in non-aqueous CE can be achieved simply by changing the organic solvent or varying the proportions of two solvents [7]. pK_a values in organic solvents can be significantly different from those in water allowing separations which are difficult to achieve in aqueous media. It has been shown that all solvents in which a measurable zeta potential is developed around the uncoated fused-silica capillary wall are either amphiprotic or aprotic solvents [4]. In addition to self-dissociation, amphiprotic solvents act as proton donors or acceptors if there are other proton donors or acceptors in the separation system. However, there are differences in their proton donor and acceptor capabilities. Methanol, like water, has an equal tendency to donate and accept protons, but basic amide-type solvents are worse proton donors than proton acceptors. Aprotic solvents, such as acetonitrile, which have very weak autoprotolysis constant, can only accept protons. Inert solvents are capable of neither autoprotolysis nor donation–acceptance of protons to a considerable extent, which makes them less suitable for non-aqueous CE.

Most organic solvents are capable of dissolving electrolytes, at least to some extent, and acids and their ammonium salts are the most commonly used electrolytes. Electrolyte cation or anion also has a clear effect on the capillary electrophoretic selectivity in non-aqueous media [8]. Since the association constants of host–guest complexes in organic solvents differ from those detected in aqueous solutions, non-aqueous media can be exploited in further optimisation of the selectivity of chiral separations [9,10]. Unfortunately, micelles cannot be used to improve the selectivity: micelle formation is scant in non-aqueous solvents because the weakness of hydrophobic interactions prevents surfactants from aggregating. Other limitations are high temperatures (Krafft temperature) and the high micellar concentrations needed for micellation.

4. Non-aqueous wide bore capillary electrophoresis

With aqueous electrolyte solutions, very small internal diameter (I.D.) capillaries have to be used to ensure effective dissipation of Joule heat. Less heat is produced in non-aqueous CE because of the low electrical conductivities of the non-aqueous electrolyte solutions. Organic electrolyte solutions have somewhat lower thermal conductivities than water, which slightly increases the resistance to heat transfer but has only a minor effect on the performance. Low heat production leads to enhanced separation efficiency owing to lowered longitudinal diffusion and more uniform temperature distribution inside the capillary. We have observed negative temperature dependence of electrical conductivity for some electrolyte solutions, which may also prove to be advantageous for the separation efficiency [11]. With non-aqueous CE the capillary diameter can be increased without excessive rise in the electrolyte solution

temperature. The heating power can be further reduced by appropriate choice of CE solvent, for example, by using longer chain alcohols (Fig. 1) [12]. Although some solvents frequently used in non-aqueous CE have fairly low boiling points, also much higher boiling solvents (e.g. formamides) can be utilised. With low boiling electrolyte solutions the maximum applicable running current may be decreased and also the evaporation from the vials may be significant.

The maximum sample load in conventional CE usually is limited by the length of the sample plug.

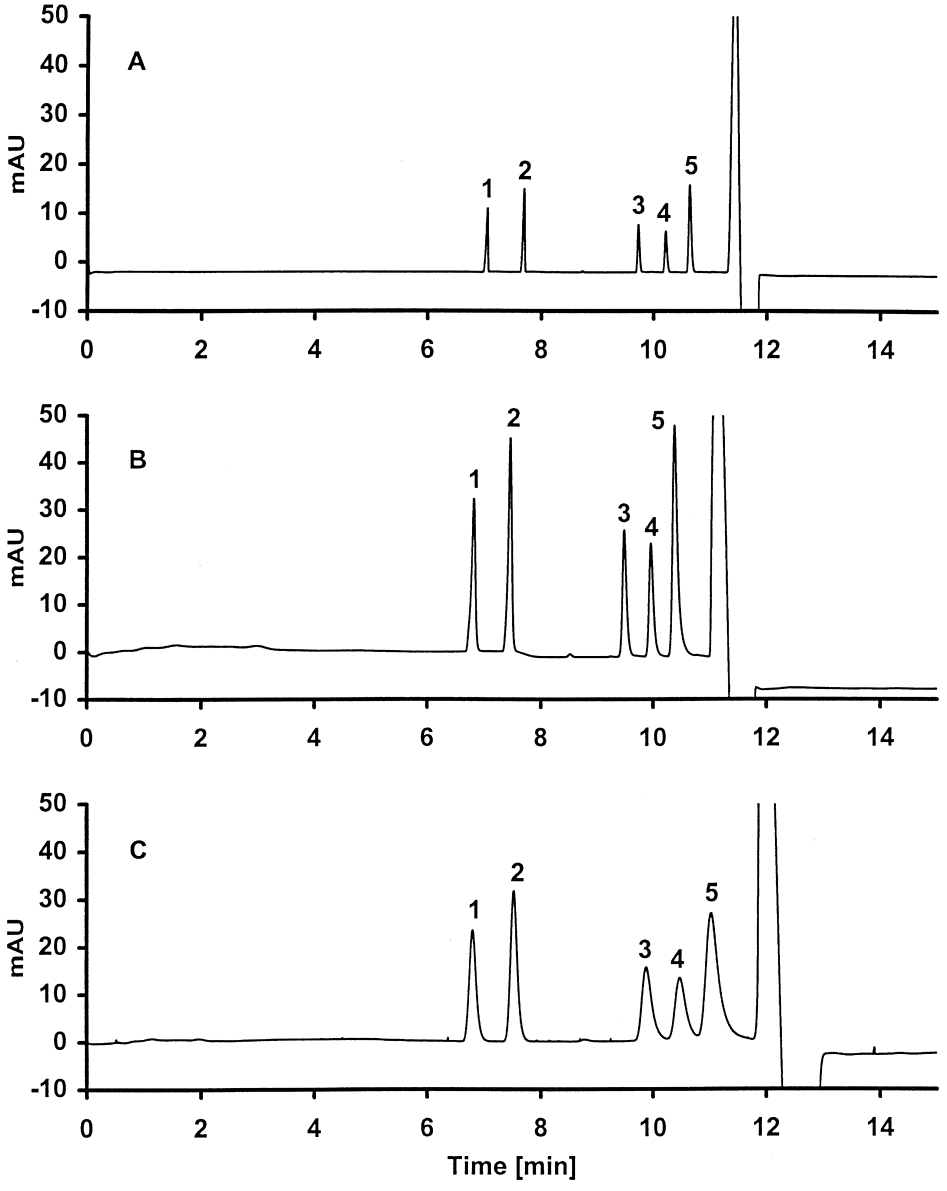

Fig. 1. Electropherogram of dipipanone (1), methadone (2), pentazocine (3), levorphanol (4) and dihydrocodeine (5) using (A) 50, (B) 200 and (C) 320 μm I.D. capillary. Electrolyte solution: 20 m*M* NH_4Ac in 1-BuOH-ACN-HAc (50:49:1, v/v); capillary length: 58.5 cm (50 cm to the detector); separation voltage: 20 kV; detection: UV 214 nm [12].

However, since the sample loadability increases with the cross-sectional area of the capillary, even semi-preparative fractionations can be performed with a wide bore capillary. Special arrangements (such as restrictors) may be needed to minimise siphoning during the vial change in semi-preparative work. Fairly effective separations by wide bore CE have already been achieved in non-aqueous media (Figs. 1 and 2) [11–13]. Owing to the larger sample load, wide bore capillaries also serve to increase the overall detection sensitivity. According to Beer's law the sensitivity of photometric detection is enhanced by the longer light path length, and the greater illuminated volume is beneficial to fluorescence detection.

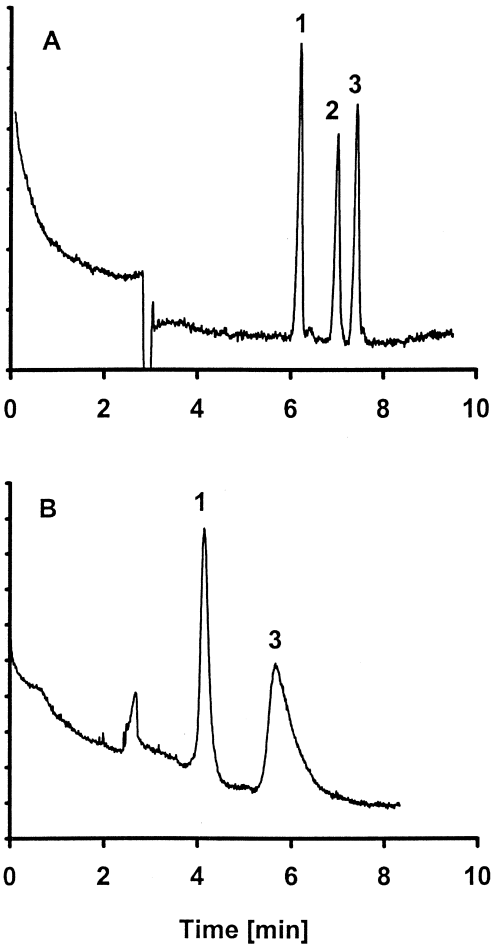

Fig. 2. Separation of diuretics using (A) 320 [11] and (B) 530 μm [13] I.D. capillary. Peaks: bumetanide (1), furosemide (2) and ethacrynic acid (3); electrolyte solution: (A) 1 m*M* KAc in EtOH–ACN (50:50, v/v); (B) 2.5 m*M* NH_4Ac in EtOH–ACN (50:50, v/v); lift rate: (A) 0.72 mm/min; (B) 2.3 mm/min; capillary length: 59 cm (46 cm to the detector); separation voltage: 30 kV; detection: UV 214 nm.

Wide bore capillaries provide much less friction to suppress Poiseuille flow induced by hydrostatic pressure (i.e. siphoning flow) than do conventional 50-μm capillaries. Usually siphoning can be avoided by exact levelling of the CE electrolyte vials, but with wide bore capillaries the volume of solution carried by electroosmosis from inlet vial to outlet vial during the run is great enough to change the liquid levels in the vials and establish siphoning flow. Valkó et al. [14] have shown how, with 200-μm capillaries, siphoning can ruin the separation with a difference in level of just 2 or 3 mm. They filled buffer vials so that initial liquid levels differed by a few millimetres. With just a 3-mm difference in initial level the peaks were about five times as broad and the migration time of the last migrating peak was almost double that in a run with balanced solution levels. Their results also showed that forward directed flow may have a less detrimental effect on the separation efficiency than backward flow, probably because the migration accelerates with increasing forward driving flow.

Yin et al. [15] have described two effective ways to suppress siphoning: by adding small I.D. restrictors to the ends of the separation capillary and by increasing the cross-sectional area of the vials. Alteration of the vial height during the run can also be used to equalise the liquid levels [11]. The inlet vial can be lifted with a special computer controlled instrument, and if the lifting speed is carefully optimised the siphoning will be completely eliminated. The approximate lift rate can be calculated [11], but the final optimisation must be carried out experimentally. With this technique, 320- or even 530-μm I.D. capillaries can produce acceptable separation efficiency (Fig. 2).

5. Detection in non-aqueous CE

5.1. UV detection

UV detection, the commonly used method in aqueous CE, is usually applicable to non-aqueous CE

as well. Organic solvents exhibiting strong UV absorbance at the detection wavelength cannot be exploited. However, many common solvents such as methanol and acetonitrile can be utilised even at 200 nm. In some cases the solvent effects on the analyte spectra must also be taken into account.

5.2. Fluorescence detection

Tjørnelund and Hansen [5] have investigated fluorescent metal complexes of tetracyclines in milk and plasma using non-aqueous CE relying on laser-induced fluorescence detection (LIF). They obtained more intense fluorescence in organic solvents than in aqueous conditions, especially in *N*,*N*-dimethylformamide, which provided a 34 times stronger signal for the oxytetracycline Mg^{2+} complex than did water. *N*-Methylformamide provided 6-fold intensity enhancement for 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS), whereas the order of intensity of the fluorescence signal for the other solvents studied was *N*-methylformamide, dimethyl sulphoxide, formamide, methanol, acetonitrile, water [16]. The enhanced fluorescence was attributed to lowered quenching due to the molecular oxygen present in organic solvents.

Indirect fluorescence detection (IFD) has been utilised in the study of free fatty acids where fluorescein and potassium hydroxide were added to the background electrolyte (methanol–acetonitrile mixture) [17]. The amount of additive needed to produce the fluorescent background can be smaller in IFD than in indirect absorbance detection. This allows lower detection limits, while still affording a good background signal for detection over a wide dynamic range.

5.3. Electrochemical detection

Electrochemical detection systems usually involve highly sensitive devices, which are prone to interference from external electrical fields. The problem is especially acute in CE where high voltages are inherently present. Interference can be expected to be less in non-aqueous solvents because of the high resistance of the electrolyte solution. Non-aqueous media also extend the potential window available for detection, enabling detection of compounds that are difficult to oxidise or reduce under aqueous conditions [18]. Acetonitrile has proved especially suitable for electrochemical measurements [19].

Very low detection limits (10^{-9} to 10^{-8} mol/l) have been found for some inorganic ions (SCN^-, N_3^-, I^- and NO_2^-) when electrochemical amperometric detection was used in non-aqueous CE with *N*,*N*-dimethylformamide-acetonitrile based electrolyte medium [20]. The detection limits were much lower than those obtained for electrochemical detection in aqueous CE. Electrochemical detection has recently been explored by Matysik [21,22], who studied dyes and some pharmaceutically relevant compounds with acetonitrile (1 *M* acetic acid, 10 m*M* sodium acetate) as electrolyte solution. A detection limit of 13 ng/ml and good linearity within the range 0.1–10 μg/ml was observed for oxidative detection of nicotine [23]. Even though their platinum microelectrode showed low coulometric efficiency, it provided comparable detection limits and even better separation efficiency in CE than the coulometrically more effective macroelectrode [24].

5.4. CE–electrospray ionisation mass spectrometry

Replacement of water with organic solvents in CE–electrospray ionisation mass spectrometry (ESI–MS) meets practically all the criteria required to obtain stable spray and high sensitivity. The formation of the spray is favoured by the lower surface tension and heat of vaporisation of organic solvents. Furthermore, the risk of electrical breakthrough is less due to lower spray onset voltage. With the low currents typical of non-aqueous CE, the grounding of the capillary outlet at the ESI interface is not critical, since a large portion of the current is carried by the spray itself. Easy evaporation of the solvent allows the use of lower drying gas flow-rate, thus increasing the yield of ions to the MS. To date, very few solvents have been utilised in on-line coupled non-aqueous CE–MS, and almost all the studies have been performed using positive ion mode of ESI. A comprehensive review of non-aqueous CE–MS was recently published by Yang et al. [6].

The first experiments with on-line coupled non-aqueous CE were published in 1994 by Tomlinson et al. [25] who separated mifentidine and its metabo-

Table 2
Conditions used in coupling non-aqueous CE to MS for various types of analytes[a]

Analyte	CE-voltage (kV)	ESI-voltage (kV)	CE-solvent	Electrolyte/Additive	Sheath liquid	Ref.
Mifentidine, pyrazoloacridine, haloperidol metabolites	+20–30	3.2–3.5	MeOH	20 m*M* NH_4Ac, 1% HAc; or 5 m*M* NH_4Ac, 100 m*M* HAc	2-PrOH–water–HAc (60:40:1), 2 μl/min	[25–30]
Tamoxifen metabolites	+25	2.48	MeOH	0–30 m*M* SDS, 2.5–5 m*M* NH_4Ac, 50–100 m*M* HAc	MeOH, 1% HAc, 2.5 μl/min	[31]
Tricyclic antidepressants	+25	4.6	ACN-MeOH (varying ratios)	5-50 m*M* NH_4Ac	MeOH, 2.0 μl/min	[32]
Phospholipids	-/+30	4.5	ACN–2-PrOH–*n*-hexane (57:38:5)	20 m*M* NH_4Ac, 1% HAc	MeOH–water (80:20), 0.5 μl/min (pos.), 5 μl/min (neg.)	[33]
Narcotics, amines	+30	4.0	ACN–MeOH (84:16 and 25:75)	25 m*M* NH_4Ac, 1 *M* HAc	ACN–MeOH (84:16), 1 *M* HAc	[34]

[a] The solvent compositions are expressed as volumetric ratios.

lites with methanol as a solvent in CE electrolyte and 2-propanol–water as sheath liquid for the coaxial electrospray interface (Table 2). Later they extended their studies to the metabolism of haloperidol and pyrazoloacridine [27] and utilised CE–MS–MS runs for the structural elucidation of mifentidine metabolites [29,30]. The CE–MS conditions were virtually the same in all these studies (Table 2). Enhanced spray stability and detection sensitivity were observed when the CE and ESI currents were similar. Because of the lower conductivities of non-aqueous media relative to aqueous solutions, close matching of the ESI and CE currents was easier in non-aqueous CE.

In studies of the metabolism of tamoxifen by aqueous and non-aqueous CE–MS, Lu et al. [31] found better sensitivity, more stable electrospray and better CE separation of metabolites under non-aqueous conditions. The enhancement of the ESI–MS performance was attributed to lower surface tension and faster solvent evaporation when methanolic electrolyte solution and methanolic sheath liquid were used. They also added surfactants to improve the separation of hydroxytamoxifen isomers. Genapol+C-100 and Mega-10 were reported to cause insignificant analyte ion suppression in non-aqueous CE–MS, but they did not provide satisfactory isomer separation. Even though about 66% suppression of peak intensity in MS was observed when 7 m*M* of sodium dodecyl sulphate (SDS) was used in the electrolyte solution, the analytes could be separated by CE and detected by MS. The system was operational for at least 1 working day without cleanup, despite the fouling of the ion source by the surfactant.

Liu et al. [32] found methanol, acetonitrile and their mixtures to show good spray characteristics and to give high signals for tricyclic antidepressant metabolites when these were directly infused into ESI–MS. Highest signals and best baseline stability were obtained when methanol was used as a sheath liquid. Too high sheath flow-rate weakened the MS signals, while too low flow increased the baseline noise. The sheath gas flow was found to affect migration times and resolution in CE.

Acetonitrile-2-propanol-*n*-hexane has been used as CE electrolyte in the CE–MS study of phospholipids [33]. Since a deactivated capillary with suppressed EOF was used, low pressure had to be applied to the capillary inlet to provide enough flow for ESI. Separations with both positive and negative polarity were carried out with methanol–water (80:20, v/v) sheath flow. Methanol–acetonitrile was also employed by Bjørnsdottir et al. [34] to analyse 14

amines in a single non-aqueous run. Although not all the compounds were resolved by CE the peaks could be detected selectively by MS.

We have constructed a new type of sheathless interface, where a very thin (ca. 10 μm O.D.) glass capillary inserted into the 50-μm I.D. CE capillary is used as a spray needle [35]. The needle provides stable spray and good sensitivity with low ESI voltages and drying gas flow.

6. Separation of uncharged analytes

The most widely used capillary electrophoretic technique for the separation of uncharged analytes is micellar electrokinetic capillary chromatography (MECC), first introduced by Terabe et al. in 1984 [36]. However, as discussed above, MECC does not lend itself to non-aqueous solvents and other approaches for the separation of uncharged compounds are preferred. Organic solvents offer the potential for separation mechanisms based on interactions that cannot take place or are too weak to be measured in aqueous media. Furthermore, the solubilities of many promising additives for analyte–additive interactions are substantially greater in some organic solvents than in aqueous solutions. Selected examples of the separation of uncharged analytes by non-aqueous CE are presented in Table 3 [37–45].

Okada [37] has separated polyethers in methanol by employing a method based on complexation between uncharged polyethers and various cations. The approach is unfavourable in water because cations are too strongly solvated and polyether is not capable of replacing water molecules in the solvation shell of the cation. However, in methanol, which is weaker solvent than water and has moderate dielectric constant (Table 1) to prevent disturbing ion-pair formation, complexation constants between polyethers and various cations could be detected. The study shows the potential of non-aqueous CE to investigate interactions that are too weak to be detected by other methods (conductometry, chromatography).

Miller et al. [38] employed charge-transfer complexation between polycyclic aromatic hydrocarbons (PAHs) and planar organic cations (namely tropylium and 2,4,6-triphenylpyrylium ions) as a separation

Table 3
Separations of uncharged compounds in non-aqueous media[a]

Solvent	Complexing agent	Analytes	Ref.
MeOH	NH_4^+, alkali metal, alkylammonium ions	Polyethers	[37]
ACN	Tropylium, pyrylium ions	PAHs	[38,39]
ACN	Tetrahexylammonium ion	PAHs, mesityl oxide	[40]
Propylene carbonate, ACN	Tetraalkylammonium, alkyltrimethylammonium ions	PAHs, parabens	[41]
MeOH	Tetraheptylammonium ion, heptanesulphonic acid	PAHs	[42]
Mixtures of ACN, chlorobenzene, MeOH and ethylene glycol	Tetraalkylammonium ions	Fullerenes	[43]
MeOH	SDS, STS, SHS	PAHs	[44]
ACN	ClO_4^-, BF_4^- NO_3^-, Br^-, $CH_3SO_3^-$, Cl^-	Undissociated phenols, carboxylic acids, alcohols	[45]

[a] Abbreviations: PAH, polycyclic aromatic hydrocarbon; SDS, sodium dodecyl sulphate; STS, sodium tetradecyl sulphate; SHS, sodium hexadecyl sulphate.

mechanism in acetonitrile. They noted that no reasonable results could be achieved when these organic cations were applied in water or methanol. Their quantitative structure–migration relationship (QSMR) studies showed that the interaction mechanism, which was initially believed to be charge-transfer, was in fact primarily based on weak induced dipole interactions. The strength of the induced dipole interactions between cations and PAHs is directly proportional to the polarisability of PAH molecules. The largest PAHs, having the highest polarisabilities, and therefore strongest binding to the organic cation, migrated first. In more recent work Miller et al. [39] studied the effect of various pyrylium salts on the electrophoretic mobilities of organic cation–PAH complexes.

Tetraalkylammonium ions have traditionally been used in ion-pair chromatography to separate anionic compounds [46]. The ions have low solubilities in water because of entropy losses due to the formation of rigid solvent structures around the bulky cations [47]. The cations are better solvated in dipolar aprotic solvents (e.g. acetonitrile, propylene carbonate), which suggests that they may be useful complexing agents for non-aqueous CE. Walbroehl and Jorgenson [40] have separated PAHs and mesityl oxide in acetonitrile through interactions with tetrahexylammonium ions. The migration order was benzo[*ghi*]perylene, perylene, pyrene, 9-methylanthracene, naphthalene and mesityl oxide. The order was explained by the stronger binding of the large, most hydrophobic PAHs to tetrahexylammonium ion, allowing them to migrate faster. The addition of water to the medium increased the mobilities of larger PAHs more than the mobilities of smaller PAHs, lending further support to the explanation. On the other hand, it seems that the migration order of PAHs follows the polarisabilities of PAH molecules, the first migrating compound, benzo[*ghi*]perylene, having the highest polarisability. This would indicate that the separation, at least in part, was due to induced dipole interactions, which take place when tetrahexylammonium ion induces a dipole on the PAH molecules.

Tjørnelund and Hansen [41] studied the effect of alkyl chain length of tetraalkylammonium and alkyltrimethylammonium ions on the separation of uncharged analytes in propylene carbonate. The alkyl chain length of tetraalkylammonium ion was found to have only a moderate effect on the electrophoretic mobility of phenanthrene, but with alkyltrimethylammonium ions there was a maximum of electrophoretic mobility at about 14 to 16 carbon atoms in the alkyl chain. They also studied the effect of different tetrabutylammonium salts on the electrophoretic mobility of phenanthrene. Both in acetonitrile and propylene carbonate the chloride salt of tetrabutylammonium provided the fastest mobility, with bromide and hydrogen sulphate salts next in order. No reasonable stability was achieved with tetrabutylammonium perchlorate as an additive.

The use of traditional ion-pair reagents as complexing agents in methanol also has been reported [42]. The migration order of uncharged PAHs was reversed when the additive was changed from tetrahexylammonium bromide to heptanesulphonic acid. Wan et al. [43] have shown that non-aqueous medium is suitable for the separation of fullerenes. They separated C_{60}, C_{70} and C_{84} fullerenes with tetra-*n*-decylammonium bromide and tetraethylammonium bromide in a solvent mixture containing acetonitrile, chlorobenzene and methanol. Their results indicated that non-aqueous CE is a useful alternative to high-performance liquid chromatography (HPLC) in fullerene separations.

Recently, anionic surfactants were used as additives to separate PAHs in methanol [44]. No surfactant aggregation was present in the system; rather, the separation was based on interactions between negatively charged surfactant monomers and uncharged analytes, which allowed the surfactant–analyte complex to migrate towards the cathode. With application of negative polarity and use of strongly acidic conditions (phosphoric acid) to suppress EOF, analytes were separated in a reasonable time period. Sodium tetradecyl sulphate (STS) was the preferred surfactant.

Heteroconjugation between Brønsted acids (phenols, carboxylic acids and alcohols) and small inorganic anions has been applied as a separation mechanism in acetonitrile [45]. Phenols, carboxylic acids and alcohols were not dissociated under the separation conditions, but they formed heteroconjugated anions with small inorganic anions, and migrated towards the anode. It was shown that even perchlorate ion, which is usually considered to be

inert, took part in heteroconjugation with phenols and carboxylic acids. Accordingly, non-aqueous CE may also be a useful method in studying the heteroconjugation of Brønsted acids with anions in solution.

7. Separation of enantiomers

The separation of optical isomers has long been at the forefront of interest in analytical chemistry. Along with HPLC and gas chromatography (GC), CE is a common method of choice for chiral separations [48]. Chiral separations indeed appear to be one of the most successful areas in CE. The success may be related to the generally high efficiency and selectivity of CE. Another very useful feature of CE is that chiral separations can be achieved with small volumes of background electrolytes containing a suitable chiral selector.

Although the majority of chiral separations have been carried out in aqueous buffers, non-aqueous media have also proven their usefulness in the past few years. Table 4 gives some examples of chiral separations in non-aqueous media [9,10,49–57].

Organic solvents possess physicochemical properties different from water, which can be exploited in chiral separations. The potential energies of ion–ion ($E_{i\text{-}i}$) and ion–dipole ($E_{i\text{-}d}$) interactions are inversely proportional to the dielectric constant (ε) of the solvent [58]:

$$E_{i\text{-}i} = \frac{z_a z_b e^2}{4\pi\varepsilon_0 \varepsilon r} \quad (1)$$

$$E_{i\text{-}d} = \frac{z_a e \mu \cos\theta}{4\pi\varepsilon_0 \varepsilon r^2} \quad (2)$$

where z_a and z_b are the charges of the ions involved in the interaction; e is the charge of the electron; ε_0 is the permittivity of vacuum; r is the distance between the two interacting ions or ion and dipole; and μ and θ are the dipole moment and the angle of the dipole, respectively. Because of the high dielectric constant, ion–ion and ion–dipole interactions are weak in water. Many organic solvents have dielectric constants significantly lower than water ($\varepsilon = 78.39$), which facilitates ion-pair formation or ion–dipole interactions between the analytes and the chiral selectors. This allows the application of chiral selectors that have not been used in conventional aqueous buffers. For example, Stalcup and Gahm [49] have used quinine for the chiral separation of *N*-3,5-dinitrobenzoyl amino acids in methanol ($\varepsilon = 32.7$). Bjørnsdottir et al. [50] separated the enantiomers of a series of basic pharmaceuticals on the basis of ion-pair formation with optically pure camphorsulphonate ion in acetonitrile ($\varepsilon = 37.5$). Unlike most chiral selectors used in CE, both (−)-*R*- and (+)-*S*-camphorsulphonic acids are commercially available, allowing the analyst to alter the migration order of the enantiomers of the analyte.

Cyclodextrins (CDs) are the most widely used chiral selectors in aqueous media. Some analytes and CDs are not sufficiently soluble in water, which makes organic solvents a useful alternative for the enantioseparations. Vincent and Vigh [51] used the sodium salt heptakis(2,3-diacetyl-6-sulphato)-β-cyclodextrin for the resolution of weak bases in

Table 4
Chiral separations in non-aqueous media[a]

Solvent	Chiral selector	Analytes	Ref.
MeOH	Quinine	*N*-3,5-Dinitrobenzoyl amino acids	[49]
ACN	(+) or (−)-Camphorsulphonate	Basic drugs	[50]
MeOH	Heptakis(2,3-diacetyl-6-sulphato)-β-cyclodextrin	Weak bases	[51]
NMF, FA	β-cyclodextrin	Dansyl-amino acids	[9,52,53]
FA, NMF, (DMF)	Cyclodextrins: β-, γ, Methyl-β-, hydroxypropyl-β-, sulphated-β-	Basic drugs	[10]
FA, NMF, MeOH, DMSO	Quaternary ammonium β-cyclodextrin	Profens, dansyl-amino acids, FMOC-amino acids	[54]
FA	(+)-18-Crown-6 tetracarboxylic acid	Primary amines	[55]
DMF	OD-R (hydrophobic chiral polymer)	Pindolol, atenolol	[56]
FA, NMF	Sulphated β-cyclodextrin	Basic drugs	[57]

[a] Abbreviation: FMOC, g-fluorenylmethoxycarbonyl.

methanol. Unlike many CDs, this cyclodextrin is reasonably soluble in methanol and carries a negative charge, which facilitates the separation of the enantiomers of weak bases. Furthermore, heptakis(2,3-diacetyl-6-sulphato)-β-cyclodextrin is a single isomer CD, which gives it the advantage of having a well-defined structure, unlike the randomly substituted CDs.

Amide-type organic solvents such as formamide and *N*-methylformamide are very good solvents for cyclodextrins. For example, the solubility of β-cyclodextrin in *N*-methylformamide is at least 40 times what it is in water. The chiral separation of dansyl-amino acids with β-CD is hindered by the low aqueous solubility of the chiral selector. In contrast, the chiral separation of dansyl-amino acids was achieved in *N*-methylformamide using 80 m*M* β-CD [52].

Typically, the solvophobic interactions between CDs and the analytes are weaker in amide-type solvents than in water. The association constants of β-cyclodextrins with dansyl-amino acids in *N*-methylformamide lie between 2 and 13 M^{-1} [9]. Wang and Khaledi [10] found the association constants of some basic pharmaceuticals with β-CD in *N*-methylformamide to lie in the 0.1–1 M^{-1} range, and in formamide in the range 1–10 M^{-1}. Despite the low absolute values, the relative difference of the association constants of the enantiomers together with the high β-CD concentrations applied allowed the chiral separations of several substances.

Quaternary ammonium β-cyclodextrin (QA-β-CD) has been used for the chiral separation of profens (nonsteroidal anti-inflammatory drugs), dansyl-amino acids, g-fluorenylmethoxycarbonyl amino acids and 1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate [54]. The selectivity of QA-β-CD varied with the solvent in which it was dissolved, namely, formamide, *N*-methylformamide, methanol and dimethyl sulphoxide. For example, the profens could be separated only in formamide, but the enantioselectivity of QA-β-CD for 1,1′-binaphthyl-2,2′-diyl-hydrogen phosphate in dimethyl sulphoxide was outstanding. The direction of the electroosmotic flow was reversed in the above solvents when QA-β-CD was dissolved in a concentration specific to the particular solvent.

Recently, we separated the enantiomers of ephedrine, amphetamine, metoprolol, dipipanone, methadone and propranolol in formamide using carboxymethyl-β-CD (approximate degree of substitution 3) as chiral selector. No chiral separation of these compounds was achieved in formamide using α-CD, β-CD, dimethyl-β-CD, trimethyl-β-CD or hydroxypropyl-β-CD, nor did chiral separation succeed in *N*-methylformamide with up to 50 m*M* carboxymethyl-β-cyclodextrin. Fig. 3 shows the simultaneous enantioseparation of four basic drug

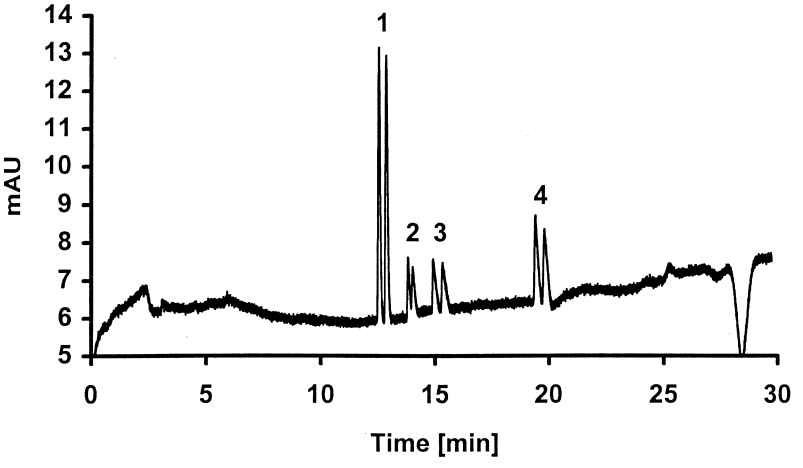

Fig. 3. Chiral separation of methadone (1), ephedrine (2), amphetamine (3) and metoprolol (4) using a 32 cm (24.5 cm to the detector)×50 μm I.D. fused-silica capillary. Electrolyte solution: 75 m*M* carboxymethyl-β-cyclodextrin in FA; injection: 20 kV for 2 s; capillary cassette temperature: 40°C; separation voltage: 15 kV; detection: UV 254 nm [59].

substances with carboxymethyl-β-CD in formamide [59].

(+)-18-Crown-6-tetracarboxylic acid has often been used as chiral selector for the chiral separation of primary amines in aqueous buffers. Mori and co-workers [55] used this selector for eight primary amines in formamide.

A chiral hydrophobic polymer (OD-R) dissolved in *N*,*N*-dimethylformamide was applied by Otsuka et al. [56] for the chiral separation of pindolol and atenolol. The separation was carried out with the partial filling technique, by using a zone of phosphate buffer (water–methanol, 60:40) and a zone of the chiral selector dissolved in *N*,*N*-dimethylformamide.

MECC with chiral surfactants is often used for the separation of enantiomers in water. In most organic solvents, however, the approach is hindered by the lack of micelle formation. A few organic solvents (e.g. formamide, ethylene glycol) can support micelle formation, but the Krafft temperature and the critical micelle concentration (CMC) for the common surfactants are high in these solvents. For example, the Krafft temperatures of SDS and cetyltrimethylammonium bromide (CTAB) in formamide are 55 and 43°C, respectively, with CMC as high as 90 m*M* for SDS and 220 m*M* for CTAB [60]. In addition to the practical problems generated by the high temperatures and strong electric currents in such micellar solutions, the solvophobic interactions between the analytes and the core of the micelles are substantially weaker than in water. As a result, MECC in organic solvents is not likely to have any practical value.

An interesting alternative approach to enantiomer separation could be the use of optically pure chiral solvents. We recently tested *R*-(−)-2-butanol, *S*-(+)-2-butanol and *S*-(−)-2-methyl-1-butanol, (from Fluka, Buchs, Switzerland) for the chiral separation of ephedrine, amphetamine, metoprolol, dipipanone, methadone and propranolol without the addition of chiral selector. These solvents are far from ideal for CE; their viscosity is high ($\eta_{2\text{-butanol}} = 4.21$ mPa s; $\eta_{2\text{-methyl-1-butanol}} = 5.505$ mPa s) and dielectric constant relatively low ($\varepsilon_{2\text{-butanol,}} = 16.56$; $\varepsilon_{2\text{-methyl-1-butanol}} = 14.7$). In addition, the solubility of many common buffer components is poor in these solvents. The solubility of ammonium acetate was nevertheless sufficient to allow the use of 10–20 m*M* ammonium acetate and 100–200 m*M* acetic acid in both 2-butanol and 2-methyl-1-butanol. The analytes were positively charged under these condition, but no chiral separation occurred in any of the optically pure solvents nor in their mixtures with acetonitrile or racemic 2-butanol or 2-methyl-1-butanol. The substantial difference in the size of the analytes and the chiral solvent molecules may be a reason for the lack of enantioseparation.

8. Other applications

As expected, given the potential of non-aqueous media in CE separations, the number of applications of non-aqueous CE is increasing relatively fast. Table 5 [8,12,61–87] lists some of the latest applications in the field. For more comprehensive lists of applications, readers are referred to other contributions [34,88,89]. It is evident from Table 5 that methanol and acetonitrile are still the most commonly used solvents and ammonium acetate and acetic acid the preferred choice for background electrolyte. Note that in some of the applications listed in Table 5, the goal of the work was not to obtain the most efficient separation for the analytes but to use the analytes as model compounds to demonstrate the effect of other separation parameters.

The stability of capillaries coated with polyethylene glycol (PEG) and polyvinyl alcohol (PVA) has been studied in methanol [62]. PVA-coated capillaries proved to be more stable than PEG-coated ones in long series of separations. Esaka et al. [71] have used different PEGs as modifiers to separate benzoic acids in acetonitrile. They report that, in acetonitrile, PEG can work as a hydrogen-bond donor and acceptor, while in water it works predominantly as a hydrogen-bond acceptor.

Fast and efficient separations of cationic *cis–trans* isomers and diastereomers have been reported for non-aqueous media [73]. The separations were achieved without the addition of surfactants, cyclodextrins or other complexing agents. Jussila et al. [78] have separated *cis–trans* and positional isomers of linoleic acid hydroperoxides in a mixture of acetonitrile and methanol containing sodium cholate. When the relative amount of methanol in acetonitrile

Table 5
Some recent applications in non-aqueous CE

Solvent	Electrolyte/additive	Analytes	Ref.
MeOH	Imidazole, HAc, 18-crown-ether-6	Metal ions, nonchromophoric amines, cationic ion-pair reagents, cationic surfactants	[61]
MeOH	NH_4Ac, sodium methanolate	Benzoic acids	[62]
Mixture of MeOH and ACN	NaAc, Tris, trimethylheptyl ammonium bromide, trimethyloctadecyl ammonium bromide	Carboxylic acids	[63]
Mixture of C_1-C_3 alcohol and ACN, mixture of ACN, MeOH and glycerol	NH_4Ac, HAc	Seizure drugs	[64]
MeOH	CAPS, Brij 35	Porphyrins	[65]
ACN, MeOH and their mixtures	Perchloric acid, SDS, sodium dioctyl sulphosuccinate, tetrabutylammonium perchlorate, tetrabutylammonium chloride, $LiClO_4$, LiCl	Triazines	[66]
Mixture of ACN and MeOH	Perchloric acid, SDS	Triazines	[67]
MeOH, ACN and their mixtures	NH_4Ac, HAc, trifluoroacetic acid	Tropane alkaloids and amphetamine derivatives	[68]
Mixture of NMF and dioxane	Anthraquinone-2-carboxylic acid, Tris	Long-chain fatty acids	[69]
Mixture of ACN and MeOH	NH_4Ac, HAc	Cimetidine and related materials	[70]
ACN, MeOH	Tetrabutylammonium perchlorate, tetrabutylammonium hydroxide, tetrapropylammonium perchlorate, tetrapropylammonium hydroxide, polyethylene glycols	Benzoic acids	[71]
MeOH, mixture of MeOH and ACN	NH_4Ac, ammonia	Nonsteroidal anti-inflammatory drugs	[72]
Mixture of MeOH and ACN	NH_4Ac, HAc, formic acid, NH_4Cl, methanesulphonic acid, trifluoroacetic acid	Pharmaceuticals	[73]
Mixture of MeOH and ACN	NH_4Ac, NaAc, hexadimethrine bromide (HDB)	Acetylsalicylic acid and its metabolites	[74]
MeOH	Naphthalene sulphonate, octylbenzene sulphonate, dodecylbenzene sulphonate, *p*-toluenesulphonic acid	Anionic surfactants	[75]
ACN, mixture of ACN and ethylene glycol	HCl, tetrabutylammonium hydroxide	Cationic surfactants	[76]
MeOH, ACN	NaAc, HAc	Tetrahydropalmatine	[77]
MeOH, mixture of ACN and MeOH	Sodium cholate, SDS	Linoleic acid oxidation products	[78]
ACN, mixture of ACN and MeOH	NH_4Ac, HAc, KOH	Phenols	[79]
Mixture of MeOH and ACN	NH_4Ac, HAc, heptakis(2,6-di-*O*-methyl)-β-cyclodextrin	Phytosterols	[80]
MeOH, mixture of MeOH and ACN	Alkalimetal acetates, NH_4Ac, NH_4Cl, NH_4Br	Narcotics, diuretics	[8]
C_1–C_3 alcohols, mixture of C_1–C_5 alcohol and ACN	NH_4Ac, HAc	Narcotics, diuretics	[12]
MeOH, mixture of MeOH and ACN	*p*-Toluenesulphonate, *p*-toluenesulphonic acid, tetramethylammonium hydroxide, HAc, Ca^{2+}, Mg^{2+}, Sr^{2+}	Anionic surfactants	[81]
Mixture of MeOH and ACN	NH_4Ac, HAc	Tamoxifen and its metabolites	[82]
Mixture of MeOH, THF and ACN	Tetraethylammonium hydroxide, tetramethylammonium hydroxide, ammonium hydroxide, HAc	Zinc dialkyl dithiophosphates	[83]
NMF	Tetramethylammonium chloride	Aspartic and glutamic acid	[84]
NMF, mixture of NMF and ACN	NH_4Ac, HCl, HAc, NaAc, Tris, NaOH	Pyridinyl-methyl-sulphinyl-benzimidazoles	[85]
MeOH, mixture of MeOH and ACN	NH_4Ac, HAc, NaAc, HDB	Benzoic acids, organic acids	[86]
ACN	$AgNO_3$	Sulphonamides, N-containing heterocyclics	[87]

was increased, the separation of the *cis–trans* isomers became worse but the positional isomers were better resolved.

Priority pollutant phenols have been studied by non-aqueous CE using field-amplified sample stacking as an on-line enrichment technique [79]. The limits of detection for phenols were substantially lower than with normal hydrodynamic injection. Thibon et al. [83] have analysed zinc dialkyl dithiophosphates in fresh and used lubricant oils by non-aqueous CE. Their results indicate that many problems encountered with other techniques (e.g. HPLC, GC, supercritical fluid chromatography) are overcome by CE. Non-aqueous argentation electrophoresis has been introduced by Wright and Dorsey [87]. They separated sulphonamides and N-containing heterocyclics by complexing with Ag(I) in acetonitrile. The kinetic properties of simultaneous alkylation reactions of secondary amines with the series of benzyl halides has been monitored using non-aqueous CE [90].

A lack of surfactant aggregation and reduced adsorption of surfactants onto the wall of the capillary in non-aqueous media have been exploited in separations of anionic [75,81] and cationic [61,76] surfactants. Cloud point extraction has been reported to be a useful preconcentration step in non-aqueous CE because the adsorption of surfactants in surfactant-rich sample phase onto the capillary wall is avoided [67].

9. Nomenclature

ACN	acetonitrile
ANTS	8-aminonaphthalene-1,3,6-trisulfonic acid
CAPS	3-(cyclohexylamino)-1-propanesulphonic acid
CD	cyclodextrin
CE	capillary electrophoresis
CMC	critical micelle concentration
CTAB	cetyltrimethylammonium bromide
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
EOF	electroosmotic flow
ESI	electrospray ionization
EtOH	ethanol
FA	formamide
FMOC	g-fluorenylmethoxycarbonyl
GC	gas chromatography
HAc	acetic acid
HDB	hexadimethrine bromide
HPLC	high-performance liquid chromatography
I.D.	internal diameter
IFD	indirect fluorescence detection
KAc	potassium acetate
LIF	laser induced fluorescence
MECC	micellar electrokinetic capillary chromatography
NaAc	sodium acetate
MeOH	methanol
MS	mass spectrometry
NMF	*N*-methylformamide
NH_4Ac	ammonium acetate
PAH	polycyclic aromatic hydrocarbon
PC	propylene carbonate
PEG	polyethylene glycol
PVA	polyvinyl alcohol
QSMR	quantitative structure-migration relationship
SDS	sodium dodecyl sulphate
SHS	sodium hexadecyl sulphate
STS	sodium tetradecyl sulphate
THF	tetrahydrofuran
UV	ultraviolet
1-BuOH	1-butanol
1-PrOH	1-propanol
2-PrOH	2-propanol

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