# 10
# Nonaqueous Capillary Electrophoresis

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## 10.1
## Introduction

The versatility of capillary electrophoresis (CE) is due to its numerous modes of operation. When analytes possessing the same charge-to-size ratio ($q/r$) cannot be separated by conventional aqueous capillary zone electrophoresis (CZE), alternative modes can be used, such as (i) the addition of complexing agents in the background electrolyte (BGE) (e.g., chiral separation), (ii) the use of a pseudostationary phase (e.g., micellar or microemulsion electrokinetic chromatography), or (iii) the use of a stationary phase (e.g., capillary electrokinetic chromatography). Nonaqueous capillary electrophoresis (NACE) using pure organic solvents or mixtures thereof can also be used to modify the selectivity. Because the separation principle is based on differences in zone velocity, NACE should be considered a subclass of the CZE mode and may also be termed nonaqueous CZE.

The theoretical fundamentals underlying CZE separations in aqueous and nonaqueous systems are essentially the same and are based on the physicochemical properties of the analytes and the separation system, which are crucial to ion migration and zone dispersion. The substitution of water by an organic solvent may have an impact on (i) the analyte mobility due to changes in the size of the solvated ion and its p$K_a$, (ii) a modification in electrolyte p$K_a$ and pH, and/or (iii) interactions between the analyte and the electrolyte constituents (e.g., homo- and heteroconjugation, ion pairing, etc.).

The level of differential migration between analytes defines the selectivity achievable, while band dispersion occurs as the analytes migrate through the CE system. In this chapter, both aspects in the context of NACE will be considered, with one section dedicated to selectivity aspects and another devoted to efficiency. The operational conditions will also be discussed, including the composition of the BGE and the sample solvents that should be used for best performance in NACE. A dedicated section will be addressed to the coupling of NACE with mass spectrometry (MS), which is now considered the gold standard detection method for numerous applications.

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## 10.2 Features of NACE

In addition to their effects on selectivity and efficiency, organic solvents may contribute to improved solubility and/or stability of the compounds, particularly for highly lipophilic molecules, including not only the analytes but also the matrix constituents, thus enabling the direct injection of crude mixtures in the organic solvent. Additionally, NACE may also be preferred because complex matrices (e.g., biological fluids) often require a pretreatment or preconcentration step such as liquid–liquid extraction or solid-phase extraction. At the end of the extraction process, the compound of interest is commonly eluted in an organic solvent. Evaporation, sample dilution in water, and analysis by aqueous CZE may then be carried out. These steps are not required for NACE, thus improving the overall workflow in terms of analysis throughput and variability.

NACE electrolytes may generate low electric current and low Joule heating, enabling the use of (i) short capillaries (rapid analyses), (ii) wide-bore capillaries with internal diameters (ID) >50 µm (sensitivity enhancement), (iii) high voltages (rapid analyses and efficiency improvement), and (iv) MS detection (sensitivity and selectivity enhancement). However the contrary can be found and will be discussed thereafter.

## 10.3 Theoretical Aspects

### 10.3.1 pH and p$K_a$

pH measurements are generally carried out using a glass electrode containing an aqueous solution, and pH calibration is achieved with aqueous buffers as well. Therefore, the concept of pH is strictly defined only for pure aqueous solutions and is theoretically not applicable to organic solvents. In the organic solvents, the liquid junction potentials, which cancel each other in pure aqueous solutions, influence the pH measurement. This results in the so-called apparent pH (pH*), which can be determined in nonaqueous media using the pH$^*$ scale introduced by De Ligny and Rehbach [1].

Similar to the measurement of pH, the p$K_a$ values of both electrolytes and analytes are significantly influenced by the nature of the organic solvent, and the apparent p$K_a$ values (p$K_a^*$) should be used. For acidic compounds, this value can be considerably increased due to the poor solvation ability of organic solvents for anions, whereas it is increased to a lesser extent for basic compounds. The appropriate p$K_a$ values for the analytes and electrolytes can be estimated on the basis of their p$K_a$ values in water by taking into account the shift that results in organic solvents [2]. For instance, the p$K_a$ value for acetic acid increases from 4.8 in water to 9.7 and 22.3 in MeOH and ACN, respectively. However, the value for

**Table 10.1** Effect of organic solvent on the p$K_a$ of electrolytes and analytes [7].

| | In water | In MeOH | In ACN |
|---|---|---|---|
| Electrolyte | | | |
| Acetic acid | 4.8 | 9.7 | 22.3 |
| Ammonium ion | 9.2 | 10.8 | 16.5 |
| Analyte | | | |
| Benzoic acid | 4.2 | 9.4 | 20.7 |
| Cocaine | 8.4 | 9.9 | 17.3 |

the ammonium ion only increases from 9.2 to 10.8 and 16.5, respectively. The same trend is observed for the analytes shown in Table 10.1 for acidic and basic compounds. For instance, the p$K_a$ values for benzoic acid are 4.2, 9.4, and 20.7 in water, MeOH, and ACN, respectively, corresponding to p$K_a$ shifts of 5–16 units. Cocaine, on the other hand, has p$K_a$ values of 8.4, 9.9, and 17.3 in water, MeOH, and ACN, respectively, which correspond to p$K_a$ shifts of 1–9. In other words, acidic compounds are less acidic in organic solvents, while basic compounds are more basic, but the extent of the change is smaller. Of special importance in the context of CE and the effects on electroosmotic flow (EOF), the p$K_a$ values for silanols also become less acidic in the presence of organic solvents (see Section 10.5.1.2).

For ionizable compounds, the possibility of coexistence of the protonated and unprotonated forms must also be considered, and the relative concentration of each species will depend on the pH of the BGE. Protonated and unprotonated forms of a given compound may show different solubilities. Generally, the solubility of an organic weak acid in water increases with increasing pH, while the opposite trend is often observed in organic solvents. If the ionized form of a basic organic compound is less soluble in the organic solvent, the solubility might be improved with increasing pH as a result of transformation into the free base. These principles not only apply to the analytes but are also valid for the BGE constituents, for which the concentrations are generally higher than those of the analytes.

### 10.3.2 Interactions

NACE offers the potential for separation mechanisms based on interactions that cannot take place or that are too weak in aqueous BGEs.

#### 10.3.2.1 Conjugation

In contrast to water, many organic solvents have poor solvation ability for ions, especially when they are unable to act as H-bond donors. In such solvents, small

ions and ions with localized charge may be stabilized by H-bonding with other molecules considered H-bond donors.

Heteroconjugation (or heteroassociation) is defined as the association between a base and the conjugate acid of a different base through an H-bond. An uncharged H-bond donor (HR) and a small anion (A−) can be in equilibrium (RH···A−, where the dotted line represents an H-bond). In CE, heteroconjugation mechanisms can be used either to influence the mobility of the ions or to impose electrophoretic mobility on an uncharged molecule. For this purpose, the BGE solvents must be poor H-bond donors, and ACN is the solvent of choice to amplify heteroconjugation between neutral analytes and electrolytes. In contrast to ion pairing, the net charge of the ion is conserved, but its mobility is reduced. Because the concentration of HR in the BGE is reduced upon heteroconjugation, the pH of the solution is also influenced according to the Henderson–Hasselbalch equation.

Homoconjugation (or homoassociation) follows the same principle except that the association takes place between the charged and the uncharged forms of the same species: for the anion A− originating from dissociation of the acid HA, a stabilized homoconjugate, AH···A−, can be formed.

#### 10.3.2.2 Ion Pairing

Ions, coions, and counterions are present together within the BGE and allow ion–ion interactions to occur. Electrostatic principles cause the counterions to be attracted to the ion to be analyzed, whereas the coions are repelled. The counterions are thus found in close proximity to the ion with higher probability than the coions and form an oppositely charged ion cloud around the central ion. In some cases, ion pairing can occur with direct contact of the ions (Figure 10.1a) or association through solvent molecules (Figure 10.1b and c). Especially in solvents with a low relative permittivity, the formation of ion pairs is likely to occur. These aggregates have a reduced, or even zero, net charge, and the degree of mobility reduction depends on the extent of their formation. This ion-pairing effect can be used deliberately to modify the mobilities and improve the separation selectivity. This effect is considered nonexistent in water or *N*-methylformamide (NMF) but is likely to occur in ACN or MeOH.

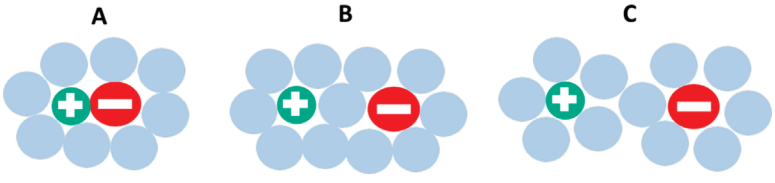


**Figure 10.1** Types of ion pairs produced in organic solvents: (a) "tight ion pair," (b) "solvent-shared ion pair," and (c) "solvent-separated ion pair."

#### 10.3.2.3 Dimerization

A dimerization process can also occur in organic solvents. For instance, two molecules of acetic acid (AcOH) can associate to produce a dimer $(AcOH)_2$, which can produce a $(AcOH)_2^+$ cation by autoprotolysis. This cation can in turn participate in the formation of ion pairs with acetate ions or other anions present in the BGE.

## 10.4 Practical Considerations

### 10.4.1 Composition of the Background Electrolyte

#### 10.4.1.1 Solvents

In CZE, several properties of the various possible solvents should be considered: (i) physical and chemical stability, (ii) compatibility with the capillaries and detectors, (iii) solubilization of both the analytes and the electrolytes, (iv) volatility, (v) availability at acceptable quality, and (vi) cost. Water is a favorable solvent to dissolve ionic compounds due to its high relative permittivity ($\varepsilon$) and its excellent solvation ability for both anions and cations. However, it is less suitable to dissolve nonpolar and/or neutral compounds. This property limits its applicability for lipophilic analytes and therefore alternative organic solvents have to be considered. The most frequently employed organic solvents for NACE are MeOH, ACN, and mixtures of the two. The strong preference for these solvents has several motivations, one certainly being that many analysts are familiar with these solvents from their use in liquid chromatography. Other features include (i) low optical cutoff required for the commonly used UV/VIS detection methods, (ii) excellent compatibility with MS detection, and (iii) commercial availability at high purity at a relatively cost-effective price, especially for alcohols. The use of mixed solvent systems such as ACN–MeOH is also widespread and may permit the possibility of optimizing the solubility of neutral compounds and ionic species, which are better soluble in ACN and MeOH, respectively. Other less commonly used organic solvents with interesting properties include other alcohols (ethanol, EtOH; 1-propanol, 1-PrOH; 2-propanol, 2-PrOH), amides (formamide, FA; *N*-methylformamide, NMF; *N*,*N*-dimethylformamide, DMF; *N*,*N*-dimethylacetamide, DMA), propylene carbonate (PC), dimethylsulphoxide (DMSO), and nitromethane (NM).

Some physicochemical properties of the most commonly employed solvents are reported in Table 10.2, including their relative permittivity ($\varepsilon$), viscosity ($\eta$), autoprotolysis constant ($pK_{auto}$), surface tension ($\gamma$), and UV cutoff [3]. The solvents with higher relative permittivity values are generally more polar and dissociate electrolytes more efficiently than solvents with lower $\varepsilon$ values; for NACE, the range of solvents is restricted to those having $\varepsilon$ values approximately 30 and larger, to be able to (i) dissolve electrolytes and establish a certain pH and (ii)

**Table 10.2** Physicochemical properties of organic solvents [3].

| Solvent | Abbreviation | Relative permittivity, $\varepsilon$ | Viscosity, $\eta$ (cP) | p$K_{auto}$ | Surface tension, $\gamma$ (mN·m$^{-1}$) | UV cutoff (nm) |
|---|---|---|---|---|---|---|
| Water | $H_2O$ | 78.4 | 0.89 | 14.0 | 71.8 | 190 |
| Alcohols | | | | | | |
| Methanol | MeOH | 32.7 | 0.55 | 16.9 | 22.3 | 205 |
| Ethanol | EtOH | 24.6 | 1.08 | 19.1 | 21.9 | 205 |
| 1-Propanol | 1-PrOH | 20.5 | 1.94 | 19.4 | 23.1 | 210 |
| 2-Propanol | 2-PrOH | 19.9 | 2.04 | 21.1 | 21.2 | 210 |
| Amides | | | | | | |
| Formamide | FA | 109.5 | 3.30 | 16.8 | 58.2 | 245 |
| *N*-methylformamide | NMF | 182.4 | 1.65 | 10.7 | 39.5 | 245 |
| *N*,*N*-dimethylformamide | DMF | 36.7 | 0.80 | 23.1 | 36.4 | 268 |
| *N*,*N*-dimethylacetamide | DMA | 37.8 | 0.78 | 24.0 | 36.7 | 268 |
| Other | | | | | | |
| Acetonitrile | ACN | 35.9 | 0.34 | 32.2 | 28.3 | 195 |
| Propylene carbonate | PC | 64.9 | 2.53 | — | 41.4 | 280 |
| Dimethylsulphoxide | DMSO | 46.5 | 1.99 | 31.8 | 43.0 | 265 |
| Nitromethane | NM | 35.9 | 0.63 | 23.7 | 36.8 | 380 |

carry the electric current upon voltage application. Viscosity corresponds to the resistance of the solvent to laminar flow; ion mobility is higher in low $\eta$ solvents such as ACN. The p$K_{auto}$ value is a measure of the auto-dissociation (or self-ionization) of the solvent on a logarithmic scale: lower p$K_{auto}$ values imply more self-ionization and thus, more ions in solution. The p$K_{auto}$ value can be used to evaluate the pH scale shift that occurs in organic solvents; these pH shift ranges from moderate for MeOH to extreme for ACN. Surface tension is caused by attraction among liquid molecules; solvents with high $\gamma$ such as water and FA form droplets of a larger size and are thus generally more difficult to evaporate than solvents with lower $\gamma$ (e.g., alcohols and ACN). The UV cutoff value indicates the maximum extent of a solvent's UV-visible window; UV detection with DMF, DMA, and DMSO, for instance, must be conducted above 270 nm.

The choice of an organic solvent or a solvent mixture for a given separation is mainly driven by its impact on the separation selectivity. While the details on the physicochemical and thermodynamic properties of neat solvents may be found in the literature, data on solvent mixtures are scarce (e.g., p$K_a$ values and mobilities; viscosity on the other hand can be experimentally measured). Consequently, systematic studies of how to choose solvents and electrolytes to control the selectivity are limited, and thus the choice of the separation media is still a matter of trial-and-error, involving the screening of multiple solvents and binary solvent mixtures.

In practice, it is important to ensure that all forms of the analyte present at equilibrium are sufficiently soluble in the pure organic solvent or solvent mixture; otherwise, the different forms may precipitate on reaching the solubility limits. Approaching the solubility limits may lead to a variety of effects, such as (i) formation of oversaturated solutions, (ii) precipitation followed by rapid redissolution after being spatially separated from the moving ionic form, which can lead to additional peak distortion, and (iii) irreversible adsorption to the capillary wall.

One question that is often raised in connection with practical work using NACE concerns the presence of water in the electrophoresis medium. The presence of water in NACE is often introduced unintentionally from the humidity of the laboratory atmosphere, especially when hygroscopic solvents are employed. Introduction of water into the organic solvent may also occur through dissolution of chemicals that contain crystalline water or as an impurity, or if the compounds cannot be obtained in a water-free form (e.g., perchloric acid). Additionally, water may also be formed as a reaction product upon neutralization of an acid and a base during the production of the BGE. Because commercially available conventional CE instruments do not protect against such an uptake, water may be present in many organic solvents employed for NACE. However, because the selection of solvents is based mainly on the selectivity obtained, the presence of undesired water is acceptable if repeatable buffer preparation and experimental conditions can be maintained. It is generally accepted that a difference in water content in the BGE up to 1% does not influence the separation efficiency and selectivity significantly, and the potential presence of residual water in solvents can only induce some discrepancies from the theoretical values found in Table 10.2.

#### 10.4.1.2 **Electrolytes**

Similar to aqueous CZE, the presence of electrolytes is needed to perform efficient and repeatable separations with NACE, because proper buffering is essential to control the effective and apparent mobilities of the tested compounds. The physicochemical properties of the BGE may, therefore, differ from those reported for pure solvents in Table 10.2.

Due to the low solubility of many electrolytes in organic solvents, it can be difficult to find a suitable electrolyte. The more polar solvents, such as MeOH, DMSO, FA, NMF, and DMF, possess a good solvating power toward the electrolytes commonly used in NACE, while aprotic solvents such as ACN may exhibit low solvating ability. For instance, the molar solubility of sodium acetate is 5.53 in water versus 0.0014 in ACN, that is, the salt is about 4000-fold more soluble in water than in the aprotic organic solvent, due to the rather poor solvating properties of this organic solvent for ionic compounds [4]. Significantly improved solubility can be achieved by switching from the alkali to an ammonium or alkylammonium ion as counterion or by combining ACN with another organic solvent. For this reason, ammonium acetate is used in combination with acetic acid, which is miscible in nearly all solvents, in many

applications that use the most common solvent ACN, and in mixtures of MeOH and ACN.

The electrolytes used for the analysis of basic compounds combine a high concentration of formic or acetic acid (generally between 0.1 and 2 M), with a low concentration (25–50 mM) of ammonium formate or acetate. These conditions ensure full protonation of the compound during analysis. On the other hand, ammonium acetate (5–20 mM) is conventionally used to prepare basic BGEs for the analysis of acidic compounds. A certain amount of acid or base can also be added to adjust the apparent $pH^*$ to a selected value. This approach may cause differences in the BGE preparation within a single laboratory and, to a greater extent, between laboratories, because the $pH^*$ measurements in nonaqueous solvents are subject to deviations. The specification of the exact amount of each electrolyte included in the BGE is more relevant and for most applications, NACE analyses can be carried out without the knowledge of the exact BGE $pH^*$ values and analyte $pK_a^*$ values.

#### 10.4.1.3 Additives

As in aqueous CZE, additives such as ionic liquids, chiral selectors, or surfactants can be included in the BGEs used for NACE. Room-temperature ionic liquids have melting points below or only slightly above the ambient temperature. They are composed of inorganic or organic cations and anions. Their use as additives in CE improves the separation performance or modifies the electroosmotic flow. Because several ionic liquids (e.g., BMIm NTf2) are poorly soluble in water, NACE may greatly extend the range of their applications in CE. Chiral separations with neutral cyclodextrins (CD) require hydrophobic interactions for enantiomer separation. ACN is thus generally avoided due to its strongly aprotic nature, and MeOH is the most commonly used solvent for chiral separation by NACE. The use of highly charged CD can also be successful in NACE [5]. Finally, a number of polyalcohols and surfactants (e.g., Tween) can be added to the BGE to decrease the electroosmotic flow.

### 10.4.2 Composition of the Sample

The compatibility of organic BGEs with the solvent or the matrix in which the analytes are dissolved is a key parameter in NACE. A compatibility mismatch in terms of solvent or ionic strength between sample and BGE zones can greatly change the homogeneity of the local electric field and reduce the separation performance.

It is recommended to avoid water in the sample dissolution solvent and to prepare the sample in a solution without electrolytes and either with the same composition as the BGE or with a composition that improves sample stacking. For the analysis of basic compounds at an acidic pH, the sample zone can be prepared in a solvent (or a mixture of solvents) that enables higher mobilities than the BGE, while for the analysis of acidic compounds at a basic pH, a solvent

(or mixture of solvents) that allows for equal or lower mobilities than the BGE should be used. This is illustrated by the analysis of nonsteroidal anti-inflammatory drugs (NSAIDs) by NACE, using a BGE consisting of 5 mM ammonium acetate in ACN–MeOH 80 : 20 (v/v) [6]. Several sample zones were tested and good results were achieved with a sample dissolution solvent composed of maximum 80% of ACN (Figure 10.2a–d), in order to keep the analyte mobility in the zone at a level equivalent or lower than that in the BGE. This solvent mixture provided an efficiency of >170 000 compared to 100% ACN as a dissolution solvent ($N < 100\,000$, Figure 10.2e).

## 10.5 Separation Performance

NACE is recognized as an alternative to aqueous CZE to solve selectivity and solubility issues because organic solvents can have a great influence on the analyte mobility and the electroosmotic flow [7]. Concerning the overall separation performance in NACE, the following benefits are frequently claimed: (i) higher separation efficiency can be obtained and (ii) lower electric current and lower Joule heating are produced, allowing for higher voltage and a shorter analysis time. Although these benefits are not universally true (and were critically questioned, for example, in Ref. [8]), these improvements can be achieved using the appropriate organic solvents. Furthermore, because many variables (e.g., solvent nature and proportion, electrolyte type and concentration, voltage, and capillary dimensions) may affect the selectivity and the separation efficiency [9], and because the latter is poorly predictable and the parameters may interact, chemometric tools are recommended for rational method optimization.

### 10.5.1 Mobility and Selectivity

NACE is frequently used to improve selectivity. However, the use of NACE does not automatically provide better selectivity than aqueous CZE and may even decrease separation. Therefore, CZE with nonaqueous solvents produces *different* selectivity relative to water and given the number of available solvents, there is a good chance of finding one solvent or a mixture of solvents that enable suitable separation conditions. For instance, rarely used solvents, such as FA and NMF offer acceptable alternatives to the more commonly used MeOH, ACN or water for the analysis of basic compounds (Figure 10.3) [10]. In this case, the selectivity among these drugs is not always improved with the organic solvents compared to aqueous CZE, but the selectivity is clearly different. On the other hand, NACE can be particularly valuable for the separation of closely related compounds, including structurally related compounds, positional isomers, diastereoisomers, and Z/E isomers [11]. As shown in Figure 10.4, three drugs commercialized as mixtures of Z/E isomers were analyzed with different separation

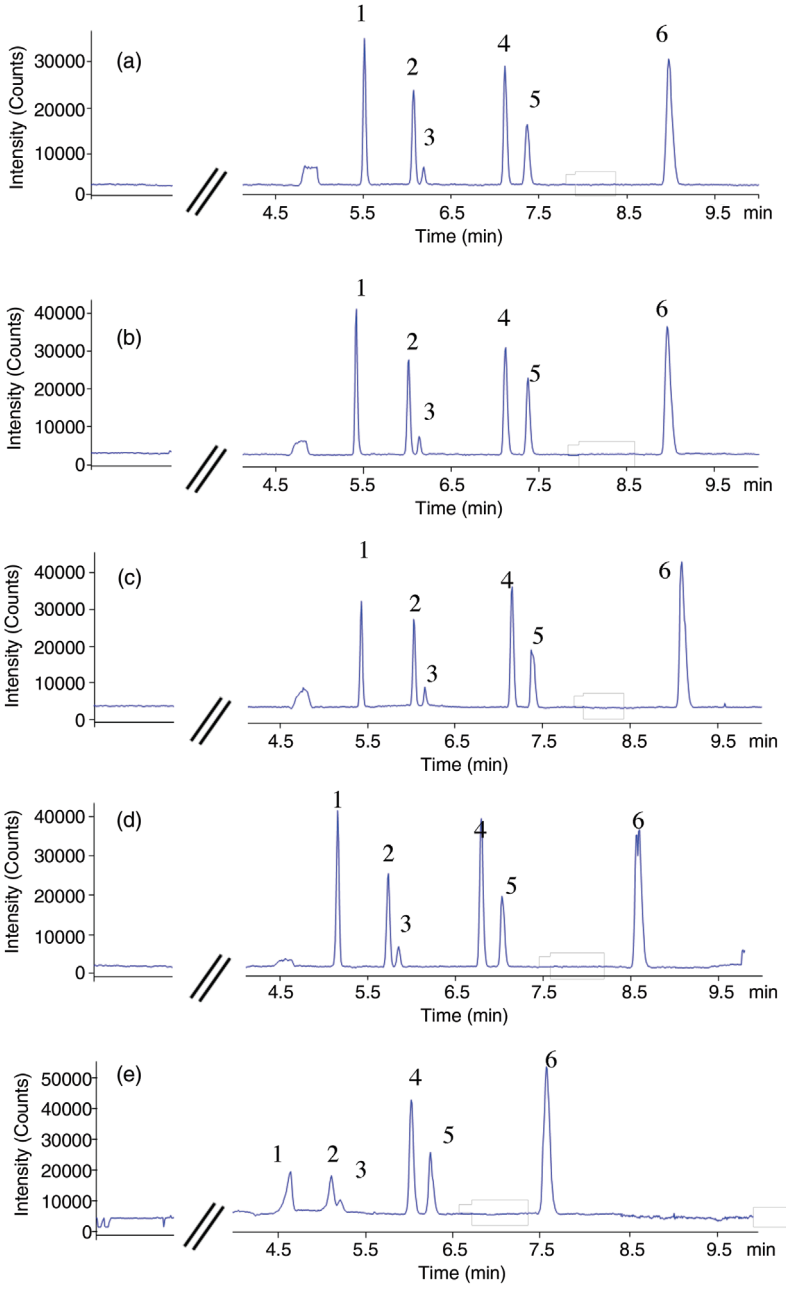


**Figure 10.2** Effect of the sample dissolution solvent. Analysis of NSAIDs at 1 μg/ml dissolved in (a) MeOH 100%, (b) ACN–MeOH 30:70 (v/v), (c) ACN–MeOH 60:40 (v/v), (d) ACN–MeOH 80:20 (v/v), (e) ACN 100%. BGE: ammonium acetate 5 mM in ACN–MeOH 80:20 (v/v). Sample: (1) ibuprofen, (2) indomethacin, (3) suprofen, (4) mefenamic acid, (5) diclofenac, and (6) flufenamic acid. (Reproduced with permission from Ref. [6].)

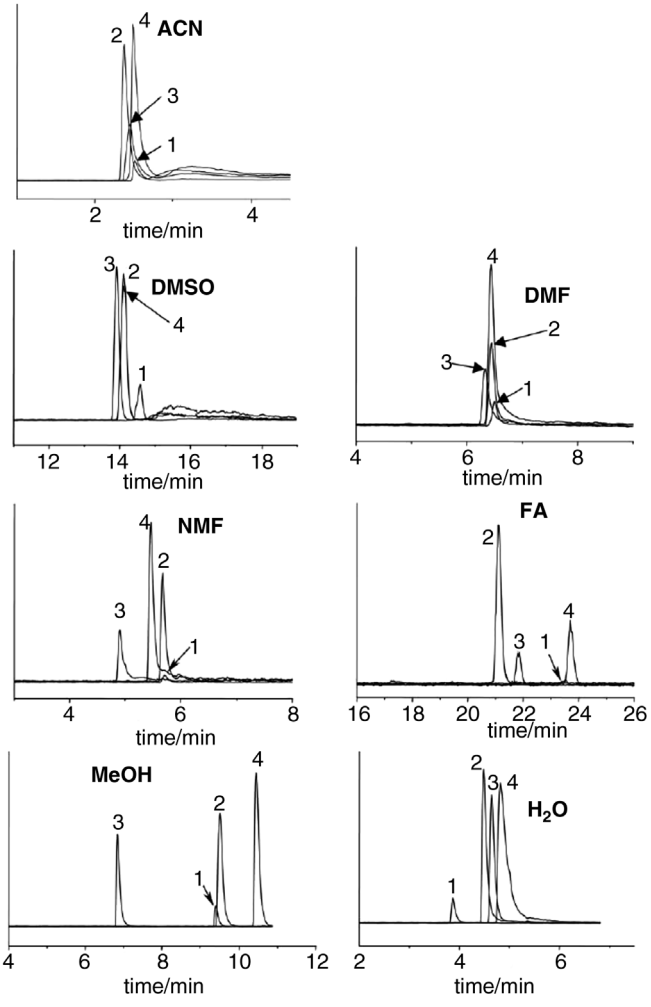


**Figure 10.3** Effect of the BGE solvent. Analysis of basic compounds in (a) ammonium acetate 10 mMin ACN, (b) DMSO, (c) DMF, (d) NMF, (e) FA, (f) MeOH, and (g) water. Sample: (1) 2-aminobenzimidazole, (2) procaine, (3) propranolol, and (4) quinine. (Reproduced with permission from Ref. [10].)

techniques. While UHPLC, UHPSFC, and CZE could not resolve the isomers, a baseline separation for each of the drugs was achieved using NACE.

In addition to the solvent, the modification of the electrolyte (nature and/or concentration) can also modify selectivity. While this is true in aqueous CZE, the effect can be further amplified in nonaqueous solvents, due to the conjugation and ion-pairing effects that occur as a result of the relatively low $\varepsilon$ value of the organic systems (except for FA and NMF, which possess higher $\varepsilon$ than

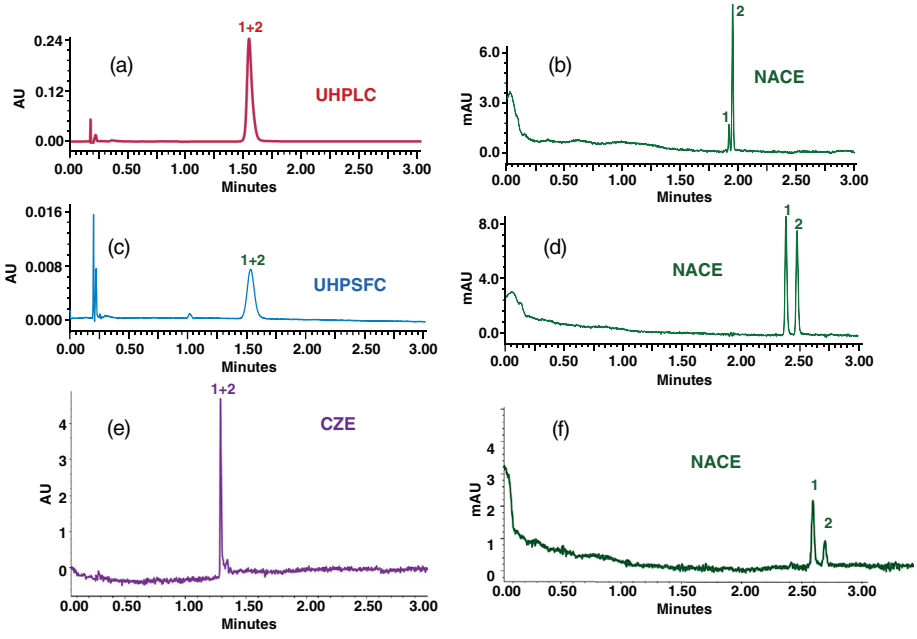


**Figure 10.4** Comparison of separation techniques for the analysis of Z/E isomers. Analysis of doxepin (a–b), endoxifen (c–d), zuclopentixol (e–f) by (a) UHPLC with ammonium formate 20 mM pH 9 buffer–ACN (60 : 40, v/v), (c) UHPSFC with $CO_2$–MeOH + ammonium hydroxide 20 mM, gradient from 20 to 27% MeOH in 3 min, (e) aqueous CZE with tris-phosphate 50 mM pH 2.5, (b–d–f) NACE with ammonium formate 35 mM + acetic acid 590 mM in ACN. (Reproduced with permission from Ref. [11].)

water). In order to investigate the effect of the electrolytes on the separation of alkaloid mixtures, a design of experiments (DOE) was employed to obtain best separation between the structurally related compounds, including diastereoisomers (Figure 10.5) [12]. In this example, the separation power increased with increasing ammonium formate concentration due to an effect of (i) increased ionic strength, reducing the EOF, pronouncing the electrophoretic separation, and leading to a superior resolution with extended analysis time (Figure 10.5a and b); (ii) higher heteroconjugation by the presence of more ammonium ions; (iii) higher apparent pH* of the BGE, introducing some separation selectivity due to differences in the apparent p*K*a* values of the analytes, as mentioned in the Section 10.3.1. Interestingly, the increase in ACN content broadened the separation window toward a lower migration time range without increasing the analysis time (Figure 10.5c and d). Overall, the DOE approach emphasized best separation with a combination of a high concentration of ACN and formate (Figure 10.5d).

The selectivity for the separation of a given pair of analytes results from the difference in their individual migration rates, which in turn is governed by their

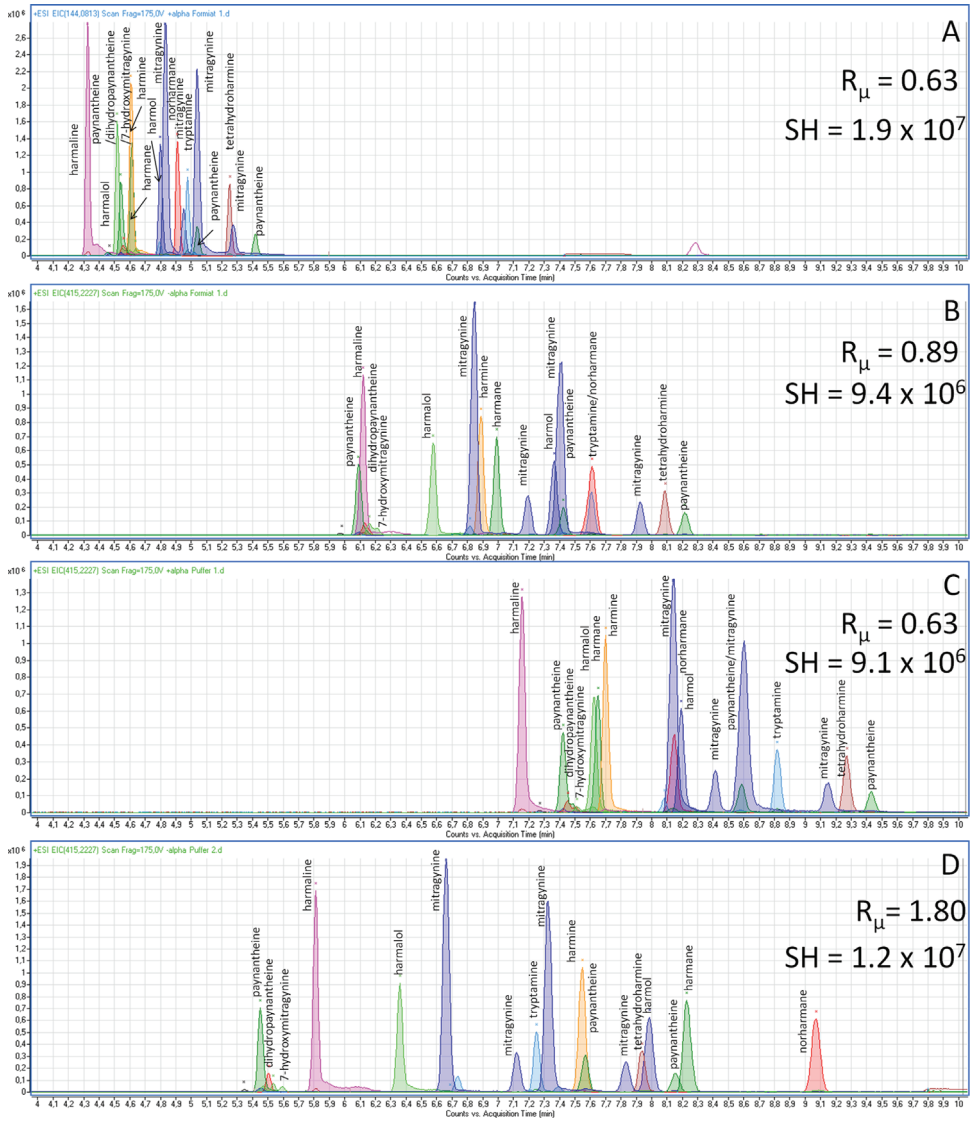


**Figure 10.5** Effect of the BGE electrolyte. Analysis of a mixture of harmala alkaloid standards in (a) ammonium formate 10 mM + acetic acid 2.7 M in ACN, (b) ammonium formate 60 mM + acetic acid 2.7 M in ACN, (c) ammonium formate 35 mM + acetic acid 5.5 M in ACN, and (d) ammonium formate 35 mM + acetic acid 0.7 M in ACN. (Reproduced with permission from Ref. [12].)

effective electrophoretic mobilities ($\mu_{eff}$) and the electroosmotic mobility ($\mu_{EOF}$). The modified selectivity for the separation in organic solvents compared to aqueous systems occurs because both mobilities depend on the physicochemical properties of the BGE and consequently the separation medium.

#### 10.5.1.1 Effective Electrophoretic Mobility

The effective mobility ($\mu_{eff}$) of a compound is a function of the mobility of its fully ionized form at the ionic strength of the separation solution and its degree of ionization. The most common analytes in aqueous CZE are weak acids and bases, which undergo ionization according to protonation and deprotonation equilibria. In NACE, the leveling effect of water is eliminated since acids or bases dissolved in organic solvents show very different protolytic behavior depending on the degree of dissociation.

As described in Section 10.3, in addition to the common acid–base equilibrium, which differs in organic solvents according to their respective permittivity, other processes such as homo- and heteroconjugation and ion pairing impact the electrophoretic mobility of the analytes. The latter interactions produce an ion cloud and reduce the electric charge of the central ion, and thus lead to a decreased electrophoretic mobility. The extent of the mobility decrease depends on the concentration and the charge of the counterions, and most importantly, on the solvent.

The effective mobility, $\mu_{eff}$, also depends on the zeta potential ($\zeta_{ion}$) formed at the electric double layer between the central ion and the solution in contact with it and on the relative permittivity ($\varepsilon$) and the viscosity ($\eta$) of the solvent, according to the following equation:

$$\mu_{\text{eff}} = \frac{2}{3}\frac{\epsilon_0 \epsilon \zeta_{\text{ion}}}{\eta}, \tag{10.1}$$

where $\varepsilon_0$ is the permittivity of vacuum. The effective mobility is thus a function of the solvent $\varepsilon/\eta$ ratio, which differs among organic solvents and mixtures thereof, as reported in Table 10.3 [13].

As previously mentioned, it is difficult to predict the impact of a given solvent on the differential mobility for a specific pair of compounds. However, it is reasonable to assume that strongly solvating solvents have greater effects on the mobilities of ions than poorly solvating media. In the latter case, a given ion exhibits approximately the same hydrodynamic radius, independent of the

**Table 10.3** Effect of selected organic solvents on mobility and efficiency [12].

| Solvent | $\varepsilon/\eta$ ($CP^{-1}$) | $\varepsilon^2/\eta$ ($CP^{-1}$) |
|---|---|---|
| $H_2O$ | 88.0 | 6896 |
| NMF | 110.5 | 20 163 |
| MeOH | 59.8 | 1950 |
| MeOH–ACN (80 : 20) | 77.1 | 2612 |
| MeOH–ACN (55 : 45) | 97.2 | 3393 |
| MeOH–ACN (40 : 60) | 104.8 | 3698 |
| MeOH–ACN (25 : 75) | 108.5 | 3864 |
| ACN | 105.6 | 3801 |

solvent applied, and the change in $pK_a$ is then the main driver for the selectivity changes.

#### 10.5.1.2 Electroosmotic Flow

The EOF is superimposed on the migration of the ions in the solution, and adds a vectorial velocity component to the movement resulting from their specific electrophoretic mobility. The total migration velocity is thus the sum of the velocity contributions to the ions. Depending on the direction of the EOF, the ions may migrate with a higher or lower velocity than predicted by their own electrophoretic mobility, while neutral compounds move with the EOF.

The inner walls of native fused silica capillaries possess silanol groups, which are weak acids with $pK_a$ values of approximately 5–6 in water. As discussed in Section 10.3.1, in organic solvents these $pK_a$ values are shifted to those typical for weak acids, for instance, approximately 11 or 12 in MeOH. In the latter solvent, the EOF mobility is hence much lower than in water, where it is directed toward the cathode at $pH > 3$. At a pH of approximately 1.5 in water, the silica surface is essentially uncharged, because the dissociation of the silanolate groups is completely prevented, and the EOF mobility is nil (the so-called "point-of-zero-charge"). Lowering the pH leads to (i) a protonation of the Si−OH group, (ii) a positively charged surface, (iii) a reversal of the sign of the zeta potential, and (iv) an EOF directed toward the anode. This phenomenon can also be observed with MeOH and according to the $pK_a^*$ of silanol in this solvent, the point-of-zero-charge is shifted to a $pH^*$ of approximately 6, and at a lower $pH^*$, the direction of the EOF is reversed. The effects in other organic solvents follow the same trend according to the $pK_a^*$ of silanol in each solvent.

As for $\mu_{eff}$, the EOF mobility ($\mu_{EOF}$) also depends on the zeta potential ($\zeta_{wall}$) formed at the electric double layer between the charged surface and the solution in contact with it and on the relative permittivity ($\varepsilon$) and the viscosity ($\eta$) of the solvent, according to the Von Smoluchowsky equation:

$$\mu_{EOF} = -\frac{\epsilon_0 \epsilon \zeta_{wall}}{\eta}, \tag{10.2}$$

where $\varepsilon_0$ is the permittivity of vacuum. The EOF mobility is thus a function of the solvent and its $\varepsilon/\eta$ ratio. However, in practice, this dependence is more complex because it also involves the effects of numerous other parameters (e.g., ionic strength, temperature, etc.). It can also be influenced by a surface coating or even irreversibly modified by nondesirable effects (e.g., adsorption of ions on the surface, especially highly charged cations or proteins). All of these factors can alter the EOF, but the magnitude of these changes between the aqueous system and the nonaqueous systems is rather difficult to predict.

To conclude, the mobilities are crucial parameters for separation in CZE, regardless of the solvent system. However, they are quite difficult to control and cannot easily be preselected (as compared to the mobile-phase velocity in chromatography for instance). They strongly depend on the specific experimental

conditions and the separation system chosen for a particular type of analysis using organic solvents.

### 10.5.2 Efficiency

The level of resolution that can be achieved between two zones migrating with different velocities is ultimately governed by the extent by which the zones broaden during migration. In CZE, as in chromatographic separations, efficiency is used to describe the extent of the zone broadening of the system. Although it is not perfectly applicable for a nonequilibrium phenomenon, the number of theoretical plates ($N$) for an observed Gaussian peak can be experimentally measured according to the following equation:

$$N = 5.54\left(\frac{t_m}{w_{1/2}}\right)^2, \tag{10.3}$$

where $t_m$ is the migration time and $w_{1/2}$ is the width at half peak height.

In practice, the measured efficiency is usually lower than the calculated one, because several dispersive effects are often present. The processes responsible for zone dispersion include on-capillary and extra-capillary band broadening. Examples of processes involved in the extra-capillary band broadening are the length of the injection zones and the width of the detector aperture. Because these contributions are independent of the nature of the solvent employed, they need no further consideration in the context of NACE. During migration in the separation capillary, on the other hand, the following on-capillary effects can contribute to band broadening: (i) longitudinal diffusion, (ii) thermal broadening due to the radial temperature gradient caused by the generation of Joule heating and heat transport through the capillary wall, (iii) analyte adsorption onto the capillary wall, and (iv) electrodispersion. Additional dispersion effects may be caused by a nonuniform EOF or by hydrodynamic flow caused by different liquid levels in the buffer reservoirs. All contributions to band broadening, except longitudinal diffusion, should be avoidable with the proper tuning of the experimental conditions. Fundamental theories concerning these zone-broadening effects have been established for CE in aqueous solutions, and can be readily applied, after appropriate modification, to the nonaqueous systems.

#### 10.5.2.1 Longitudinal Diffusion

Under ideal conditions (i.e., if other contributions are properly avoided), the main contribution to the analyte band broadening is longitudinal diffusion along the capillary (or axial diffusion), and the efficiency can be expressed according to

$$N = \frac{(\mu_{eff} + \mu_{EOF})U}{2\,D}, \tag{10.4}$$

where $U$ is the applied voltage and $D$ is the diffusion coefficient. In addition to the applied voltage, efficiency is thus only a function of the mobilities and the

diffusion coefficient of the analyte, both of which depend on the properties of the solvent. Combining Equations 10.1, 4.2, and 10.4, a qualitative relationship can be established between efficiency and the significant parameters of the solvents [14], where $N$ is directly proportional to the $\varepsilon^2/\eta$ ratio (Table 10.3). Consequently, solvents with high $\varepsilon^2/\eta$ values permit low longitudinal diffusion and high efficiency, provided that other contributors are negligible. Compared to water, only NMF theoretically enables higher efficiencies, while other commonly used solvents (MeOH, ACN, and mixtures thereof) should exhibit lower efficiencies. In addition, the efficiency for nearly all organic solvents is strongly reduced as a function of the buffer ionic strength, meaning that for a given ionic strength, the plate number in nearly all organic solvents is lower than that in water.

#### 10.5.2.2 Joule Heating

Thermal band broadening caused by Joule heating is associated with the generation of electric power upon the application of an electric field along the separation capillary. This leads to two important effects: (i) the temperature of the electrolyte solution is increased and is therefore different from that selected and (ii) a temperature gradient in a radial direction is formed inside the capillary.

The temperature increase inside the capillary influences mobility, viscosity, $pK_a$ values, complex constants, and so on and depends on the generated power (product of voltage and current), which in turn is determined by the capillary dimensions, applied voltage, and BGE thermal conductivity. Significantly elevated temperatures result when the power generation exceeds the dissipation. The absolute rise in the temperature is generally not detrimental, provided that there is a linear relationship between current and voltage (Ohm's law). On the other hand, a temperature gradient inside the capillary potentially broadens the analyte zone. At the center of the capillary, the temperature, the mobility, and the ion velocity is higher than at a position closer to the wall. It should be noted that the BGE thermal conductivity depends on the concentration of its ionic constituents and on their mobilities. As mentioned in Section 10.5.1.1, the effective mobilities are proportional to the solvent $\varepsilon/\eta$ ratio, and are thus higher in ACN and NMF than in water, while they are lower in MeOH than in water. Consequently, the thermal conductivity of the former BGEs is high compared to that of water, and the temperature gradients in these organic solvents are more pronounced than in water, regardless of the type of cooling. Although it is frequently stated that lower currents occur in NACE than in aqueous CZE, which allows for higher voltages and consequently higher efficiency according to Equation. 10.4 and shorter analysis times, this should be interpreted cautiously because it greatly depends on the type of organic solvent used.

#### 10.5.2.3 Adsorption

The wall adsorption phenomenon involves the transport of an analyte to the capillary surface prior to its interaction and attachment to the surface. The adsorbed compounds can reversibly desorb from the surface (reversible adsorption), thus

retarding their migration time and decreasing the separation efficiency due to adsorption/desorption events. In the particular case of the analysis of proteins, which possess numerous charges and hydrophobic moieties and exhibit a three-dimensional structure, further conformational rearrangements can occur. Under these conditions, the proteins can be definitively adsorbed on the surface (irreversible adsorption), causing the loss of protein within the capillary and an alteration in the EOF velocity [15]. In addition to hydrophilic and hydrophobic interactions, the main driving force for wall adsorption is electrostatic interaction, which depends on the surface and analyte charges, which in turn are driven by the BGE composition (e.g., pH, ionic strength, and electrolyte and solvent nature). The hydrophobic interactions can sometimes be modulated by the presence of an organic solvent in the BGE, although it is generally not possible to reliably predict whether a given solvent will have a favorable effect. Therefore, no absolute ranking of the solvents concerning adsorption-related band broadening can be given.

#### 10.5.2.4 **Electrodispersion**

Electrodispersion is induced by a difference in the effective mobilities between an analyte and a coion in the BGE. Such a mismatch is a function of the specific operational conditions, but not of the solvent nature. Mobility mismatch may occur with equal probability in aqueous and in organic solutions, so neither of these options offers a distinct advantage in this respect.

To conclude, the maximum plate number achievable by NACE greatly depends on the solvent. Efficiency in water is higher than in the most commonly used organic solvents, namely, MeOH and ACN, challenging the broadly held notion that nonaqueous CZE enables higher efficiencies than aqueous CZE.

### 10.5.3 **Analysis Time**

As mentioned in Section 10.5.1, the solvent $\varepsilon/\eta$ ratio affects both $\mu_{eff}$ and $\mu_{EOF}$, which provides a direct estimation of the separation time. Selecting a solvent with a high $\varepsilon/\eta$ ratio, such as NMF, ACN, or mixtures of ACN with MeOH (Table 10.3), can be essential for applications where analysis time is a major issue. Figure 10.3 emphasizes the reduction of the analysis time using ACN under constant conditions of the electrolyte type and concentration. The data indicate that the analysis speed was increased up to 50% [10]. NMF is also interesting, because in addition to a high $\varepsilon/\eta$ ratio, it is the only organic solvent to exhibit a higher $\varepsilon^2/\eta$ ratio than water, thus theoretically providing higher efficiency. However, its use is limited to applications where UV detection is not mandatory because it also possesses a high UV cutoff.

Another aspect is the electric current, and therefore the generated power. These are generally lower in organic solvents than in water, due to their low thermal conductivity. This property permits an increase in the electric field strength, thus decreasing the analysis time without affecting separation efficiency. It should be noted that for BGEs that exhibit high mobilities and

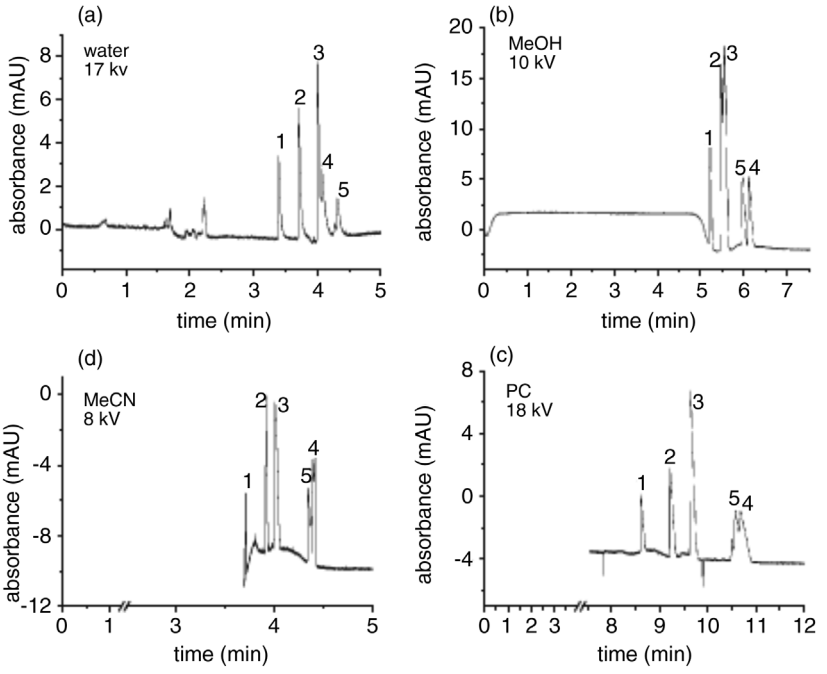


**Figure 10.6** Effect of the BGE on the analysis time. Analysis of sulfonates in tetraethylammonium hydroxyde 5 mM + formic acid 10 mM in (a) water, (b) MeOH, (c) PC, and in tetraethylammonium hydroxyde 5 mM + chloroacetic acid 10 mM in (d) ACN. Sample: (1) benzenesulfonate, (2) 4-toluenesulfonate, (3) 2,5-xylenesulfonate, (4) 8-cyano-1-naphtalenesulfonate, and (5) 4-methylnaphtalene-1-sulfonate. (Reproduced with permission from Ref. [16].)

therefore high electric conductivity (e.g., ACN and NMF, see Section 10.5.2.2), the electric field cannot be increased as much as in water-based BGEs while maintaining equivalent generated power and efficiency, as demonstrated in Figure 10.6 [16]. Taking into account equal generated power and the same temperature increase due to Joule heating, it was shown that organic solvents did not offer an advantage regarding analysis time, even for ACN in which the mobilities are the highest.

To conclude, the high mobilities observed in some solvents such as ACN and NMF enable short analysis times, but they also induce high currents that preclude the use of a high electric field, and consequently the gain in analysis time is not as great as expected compared to aqueous CZE.

### 10.5.4
### Stability

Solvents with a high vapor pressure and thus a high volatility (e.g., MeOH and ACN) may be inconvenient for automated analyses due to the problems

associated with the evaporation of the electrophoresis medium both from the run buffer vials and from the sample vials. To overcome this issue and improve the robustness of the system, changing the vials of BGE every two analyses is recommended. With this procedure, the volume in the vial remains constant, and the modification of the BGE composition due to evaporation is minimized. This practical aspect, in addition to the exclusive use of organic solvents (although in low volumes), prevents the classification of NACE as a "green chemistry" approach.

Quantitative analysis using NACE can be conducted with good performance. With appropriate care to prevent evaporation, the reliability of the NACE methods is comparable to that of aqueous systems [5]. As for other separation techniques, the use of internal standards (IS) is always recommended to compensate for method variability (e.g., sample preparation, injection, and ionization) when a complete quantitative assay is considered.

## 10.6 Coupling NACE with Mass Spectrometry

### 10.6.1 Detection in NACE

In CE, the detection of the analyte is often performed by measuring the UV absorbance at a relatively low wavelength (e.g., at 200 nm) to increase the sensitivity. Solvents such as ACN and MeOH may be used for measurements at wavelengths as low as 205 nm. However, many other organic solvents have higher UV cutoff values (Table 10.2), and higher detection wavelengths are required (>245 nm). Nevertheless, organic solvents often intensify fluorescence detection (FD), compared to the levels observed for the given analytes in aqueous media. NACE-FD can therefore be used to improve the detection limits, for instance, in biological matrices. Because NACE-UV may suffer from a lack of sensitivity due to the strong UV absorbance at low wavelengths, the online coupling of NACE to MS appears particularly attractive. In general, the use of highly volatile organic BGEs with low surface tensions ($\gamma$) improves the formation of easily evaporated droplets, which increases the ionization efficiency while ensuring a stable spray. Moreover, the absence of water reduces the number of electrochemical side reactions, thus stabilizing the ionization current. Finally, the generated electric currents are generally lower in the presence of organic mixtures than in water, allowing for robust CE–MS conditions.

### 10.6.2 MS Detection, Electrospray Ionization, and CE–ESI–MS Interfacing

MS is one of the most universal detection techniques, not only allowing for the detection of virtually any ionized analyte but also providing structural information for unequivocal analyte identification. This is especially true for tandem MS

(MS/MS), which enables the determination and characterization of numerous compounds and their metabolites, including degradation products, in complex biological and environmental samples, as well as in pharmaceutical formulations. Furthermore, in cases of low separation selectivity, the analysis can also be performed using the selected ion monitoring mode (SIM) or the selected reaction mode (SRM), which in turn improve the limit of detection (LOD) of the method. Thus, online coupling of CE with MS improves the identification of unknown compounds and results in versatile and sensitive assays.

Several ionization modes have been described for CE–MS [17], but electrospray ionization (ESI) is used most often, because it is dedicated to polar compounds, which are well-separated by CZE using either aqueous or nonaqueous systems.

Regarding the technical coupling of CE with ESI-MS (i.e., CE–ESI–MS *interfacing*), most of the developed interfaces were initially used for LC hyphenation and have been adapted to the constraints of CE analysis. In establishing online CE–ESI–MS interfacing, several difficulties have to be considered: (i) the presence of nonvolatile constituents of the BGE, such as selectivity modifier additives, may be detrimental to the MS performance, owing to ion source and/or analyzer contamination; (ii) the electrical connection at the interface side of the separation capillary must be achieved with the cathode end of the capillary directly connected to the MS interface; (iii) the typical flow rate in CE capillary, resulting from the EOF, does not exceed 100 nl/min and is generally poorly compatible with conventional LC–MS interfaces. The first issue not only applies to CZE but to any upfront separation technique hyphenated to MS. However, this issue can be avoided in CE with the use of MS-compatible BGEs (e.g., ammonium acetate, ammonium formate, acetic acid, and formic acid). Regarding the electrical connection and flow rate compatibility issues, several approaches have been investigated and two types of CE–ESI–MS interfaces are now conventionally used [18]. The first configuration is the sheath–liquid interface, in which the spray is assisted by a nebulizing gas and an additional sheath liquid. The latter generally consists of an equal mixture of water with alcohol (isopropanol or MeOH) with the addition of formic acid or ammonium ions to support ionization in the positive or negative mode, respectively. This make-up liquid provides electrical contact at the outlet end of the separation capillary, appropriate flow, and proper solvent conditions for ionization, evaporation, and spray stability, independent of the nature of the CE buffer solution. It is delivered at a few µl/min (1–10 µl/min) through a stainless steel needle and is mixed with the CE effluent at the capillary tip. Consequently, the ESI operates in electrospray mode and behaves as a concentration-sensitive detector. The second configuration is the sheathless interface, which functions without pneumatic assistance. In this case, the ESI operates in a pure nanospray mode because the flow rate at the tip is in the range of nl/min. The BGE composition is, therefore, the key parameter that influences both ionization and spray stability. This interface acts as a concentration-sensitive detector at flow rates above 10–15 nl/min and as a mass-sensitive detector below this range. Consequently, the composition and

pH of the BGE determine whether ESI acts as a mass- or concentration-sensitive detector.

When developing a CE–ESI–MS method, one has to keep in mind that not only the CE separation has to be optimized but also the parameters of the ionization source, the interface, and the mass spectrometer must be considered. Because all of these variables may interact, chemometric tools should be applied for rational method optimization.

### 10.6.3 NACE-ESI-MS

In general, the use of highly volatile organic BGEs with low surface tensions ($\gamma$) (all organic solvents in Table 10.2 display a lower $\gamma$ than water) improves the formation of easily evaporable droplets, which increases the ionization efficiency while ensuring a stable spray over a wide range of voltages. Moreover, the absence of water reduces the number of electrochemical side reactions, thus both stabilizing the ESI current and decreasing the background noise. All these properties are particularly well adapted to the negative ionization mode (ESI-), mainly because the deprotonation process can be further improved by using BGEs that are composed of high gas-phase basicity (GB) solvents, such as ACN and MeOH. According to the interface configuration, the separation performance in NACE can differ.

The sheath liquid setup is robust and yields good results in terms of stability, because the sheath liquid, which is an important driver of the ionization process, is similar between the aqueous and nonaqueous CZE modes. It should be noted that differences in sensitivity between aqueous CZE and NACE are often reported using the same sheath liquid, which supports the fact that even in this configuration the BGE composition has a notable impact on the ionization process, even though its flow rate proportion is relatively low compared to the sheath liquid at the tip. In NACE–MS, the separation efficiency can be decreased by the use of poor viscosity solvents due to the suction effect that is generated by the nebulizing gas and sheath liquid. This issue can be overcome using longer capillaries with small ID. Interestingly, the dilution with the sheath liquid, which results in the loss of sensitivity in aqueous CZE, can be an advantage in NACE. The water in the sheath liquid exhibits higher solvation properties, thus allowing the suppression of covalent and ionic interactions between analytes and ions that can hamper the ionization process, regardless of the ionization polarity. The sensitivity can even be increased in the negative ESI mode for the analysis of acidic compounds at high pH* as the result of two main effects of the organic BGE on the whole electrospray process: (i) the concentration of salts is lower in NACE than in aqueous CZE, which reduces the ion suppression effects; (ii) organic solvents such as ACN and MeOH have higher GB than water, which assists the deprotonation of the acidic groups.

With the second configuration, the sheathless interface, the BGE is the most critical element of the ionization. Hence, the use of organic BGEs appears

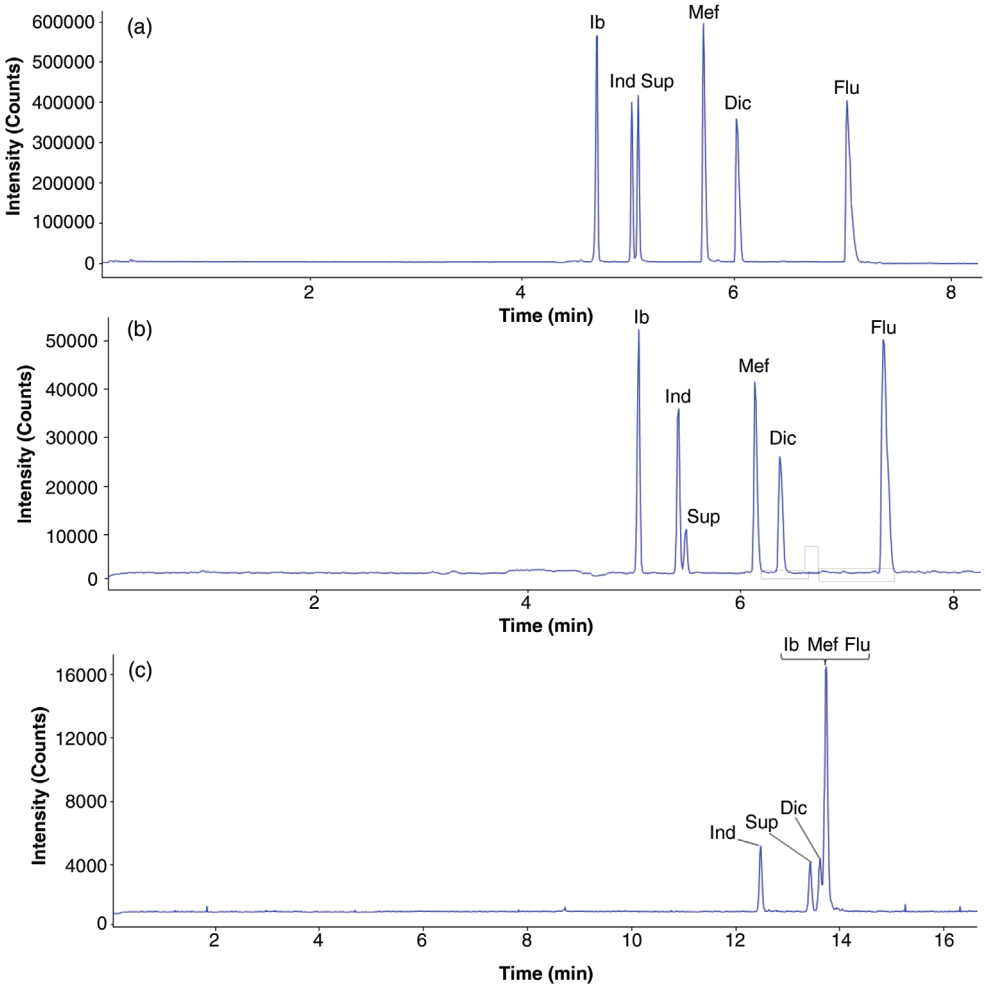


**Figure 10.7** Comparison of CE–ESI–MS interfaces. Analysis of NSAIDs at 1 µg/ml by (a) NACE with the sheathless interface, (b) NACE with the sheath–liquid interface, and (c) aqueous CZE with the sheath–liquid interface. Aqueous BGE: ammonium acetate 50 mM, pH 8.5; nonaqueous BGE: ammonium acetate 5 mM in ACN–MeOH 80 : 20 (v/v). Sample: (1) ibuprofen, (2) indomethacin, (3) suprofen, (4) mefenamic acid, (5) diclofenac, and (6) flufenamic acid. (Reproduced with permission from Ref. [6].)

particularly well adapted to the sheathless interface because an organic solvent with a low surface tension (i) stabilizes the cone-jet mode, (ii) facilitates the desolvation process, and (iii) enhances the deprotonation process (in the case of negative ESI), resulting in a global improvement of the sensitivity of the electrospray process. Moreover, the absence of a suction effect can lead to an improved separation efficiency. As depicted in Figure 10.7, the combination of NACE conditions with the sheathless interface provides separation efficiencies >200 000

(Figure 10.7a) compared to the combination of NACE conditions with the sheath–liquid interface (Figure 10.7b) ($N < 200\,000$) [6]. This excellent performance results from (i) the absence of pneumatic assistance and (ii) the small distance between the ESI tip and the MS entrance in the sheathless configuration (1 mm versus 4 cm in sheath liquid). Regarding selectivity, a slight difference in the migration times between the two interfaces is due to the difference in the capillary ID (30 μm versus 50 μm for sheathless versus sheath liquid, respectively). Regarding sensitivity, a 2–50-fold improvement in the LOD is observed with the sheathless interface, which is attributed to (i) the high charge density of the droplets produced by the nanospray, (ii) a better ionization efficiency provided by the sheathless interface, and (iii) the higher separation efficiency experienced with the sheathless interface as mentioned above. Overall, a sensitivity improvement of between 10- and 100-fold is obtained by replacing the aqueous CZE-ESI-MS method using the sheath–liquid interface (Figure 10.7c) with the NACE-ESI-MS method using the sheathless configuration (Figure 10.7a), which combines the improved detection of the sheathless interface with the higher selectivity obtained with the nonaqueous system.

## 10.7 Conclusions

The use of organic solvents compared to water provides a decisive advantage for the analysis of analytes sparingly soluble in aqueous BGEs, and substantially extends the application range of CZE. Due to their favorable optical properties (low cutoff wavelength) and their wide use in HPLC, the organic solvents most widely used in NACE are MeOH, ACN, and mixtures of these solvents. Only few others are applied, including alcohols (e.g., EtOH, 1-PrOH, and 2-PrOH), amides (e.g., FA, NMF, DMF, and DMA), and more rarely, PC, DMSO, and NM.

Additional advantages of using nonaqueous media for CZE are as follows: (i) the selectivity can easily be modified by changing the nature and proportion of the electrolytes and the organic solvent, or using mixtures of organic solvents, to deliberately modify the protolysis equilibria and induce additional mechanisms (e.g., conjugation or ion pairing); (ii) the separation efficiency may be improved and the analysis times may be shortened (although this is not true in all cases); (iii) the sample preparation is facilitated because the extracts obtained with organic solvents may be injected directly into the nonaqueous system; and (iv) the current generated in organic solvents may be lowered in some cases. These benefits combined with the volatility of the solvents result in optimized conditions for CE-ESI-MS experiments, not only in terms of improved selectivity but also for optimal ESI ionization characteristics, particularly if a sheathless interface is employed.

Although the broadly held notion that nonaqueous CZE is, in principle, superior to aqueous CZE is not always true, the numerous studies in which the use of nonaqueous conditions has enabled solutions to challenging analytical problems demonstrate that the use of nonaqueous CZE has its place in modern separation science.

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