Chapter 7

Nonaqueous Capillary Electrophoresis Mass Spectrometry

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Abstract

The term nonaqueous capillary electrophoresis (NACE) commonly refers to capillary electrophoresis with purely nonaqueous background electrolytes (BGE). Main advantages of NACE are the possibility to analyze substances with very low solubility in aqueous media as well as separation selectivity that can be quite different in organic solvents (compared to water)—a property that can be employed for manipulation of separation selectivities. Mass spectrometry (MS) has become more and more popular as a detector in CE a fact that applies also for NACE. In the present chapter, the development of NACE–MS since 2004 is reviewed. Relevant parameters like composition of BGE and its influence on separation and detection in NACE as well as sheath liquid for NACE–MS are discussed. Finally, an overview of the papers published in the field of NACE–MS between 2004 and 2014 is given. Applications are grouped according to the field (analysis of natural products, biomedical analysis, food analysis, analysis of industrial products, and fundamental investigations).

Key words Nonaqueous capillary electrophoresis, Mass spectrometric detection

1 Introduction

Only 3 years after the introduction of capillary electrophoresis (CE) by Jorgenson and Lukacs [1], the first paper on CE employing a nonaqueous electrolyte (tetraethyl ammonium perchlorate/hydrochloric acid in acetonitrile) was published [2]. From that time on nonaqueous capillary electrophoresis (NACE) was distinguished from aqueous CE by the use of background electrolytes (BGE) based on purely organic solvents. This definition will also be followed in the present review. Some of the most convincing reasons for favoring NACE over CE with aqueous BGEs are [3, 4] as follows:

- Improved solubility of large number of analytes.
- Improved separation selectivity.
- Lower electric current allowing the use of higher separation voltages.
- Higher plate numbers.

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Due to these features NACE has faced increasing interest over the last decade with more than 200 publications since 2004 (found in SciFinder). This is also reflected in several review papers discussing theoretical aspects [3–6] as well as listing applications of NACE [7–10]. Thereby, similar as in aqueous CE in most cases spectrophotometric detection is employed and only a fraction (less than one quarter) of NACE applications describe the use of mass spectrometric (MS) detection. There is only one review article so far, specifically dedicated to NACE–MS which was published by Scriba in 2007 [11]. Nevertheless, most review articles focusing on CE–MS in general also include sections dealing with NACE–MS [12–17].

1.1 BGE Systems for NACE

BGEs for NACE commonly consist of an electrolyte (either a salt and acid/base or mixtures thereof), additives, and a solvent or solvent mixture. In the subsequent sections, these BGE constituents will be discussed focusing on their role in NACE–MS with respect to both, separation and detection.

1.2 Solvents for NACE and NACE–MS

One main asset of NACE is the possibility to select from a large range of different solvents. Table 1 gives an overview of physicochemical properties of solvents frequently used in NACE in comparison with water. As can be seen from these data, relevant physicochemical properties vary substantially between solvents and it seems obvious that the choice of solvent can be a valuable tool for manipulating separations. Separation of analytes in electrophoresis is governed by differences in their electrophoretic mobility μ_{ep}, i.e., their ability to migrate according to their ionic radius/charge ratio.

Table 1
Properties of solvents at 25 °C [9]

Solvent[a]	ε	η (mPa s)	ε/η (mPa^{-1} s^{-1})	T_{boil} (°C)	γ (N m^{-1})	pK_{auto}
Water	78.4	0.89	88.1	100.0	0.0718	14.0
Methanol	32.7	0.55	59.5	64.5	0.0223	16.9
Ethanol	24.6	1.08	22.8	78.2	0.0219	19.1
1-Propanol	20.5	1.94	10.6	97.1	0.0231	19.4
2-Propanol	19.9	2.04	9.8	82.2	0.0212	21.1
Acetonitrile	35.9	0.34	105.6	81.6	0.0283	32.2
Formamide	109.5	3.30	33.2	210.5	0.0582	16.8
N-Methylformamide	182.4	1.65	110.5	199.5	0.0395	10.7
N,*N*-Dimethylformamide	36.7	0.80	45.9	153.0	0.0364	23.1
Dimethylsulfoxide	46.5	1.99	23.4	189.0	0.0430	31.8

[a] ε, relative permittivity; η, viscosity coefficient; T_{boil}, boiling point; γ, surface tension; pK_{auto}, autoprotolysis constant

These parameters (ionic radius and charge) are both influenced substantially by the type of solvent employed. Different solvents lead to changes in the size of the solvated ion thereby influencing its ionic radius; dielectric constants ε and acid base properties of the solvent affect the degree of protonation/deprotonation and with it the charge of the analyte. In addition to that, also the viscosity (η) of the solvent determines migration velocities and subsequently the speed of separation. Actually the ratio of ε/η (given in Table 1) can be seen as good parameter for comparing solvents or solvent mixtures with respect to ion mobilities, whereby lower ε/η values imply slower migration of the ions [11]. An in-depth discussion of solvent effects in NACE would be beyond the scope of this book chapter, but more comprehensive information is available from several review articles [3, 4, 18, 19].

Focusing on the situation in NACE–MS, when choosing an appropriate solvent not only factors related to separation have to be observed, but also the effect of the chosen solvent on the performance of the MS detector. When using a triaxial sheath flow interface (as done in the majority of NACE–MS applications published so far) the effluent from the separation capillary is substantially diluted by the sheath liquid (which will be discussed later), so the sprayed solution should mainly consist of the sheath liquid and conditions were thus supposed to be dominated by its composition. Nevertheless, the solvent used for NACE still influences the efficiency of the electrospray process and thereby important parameters like signal-to-noise (S/N) ratio and limit of detection (LOD). This has been discussed in more detail in two interesting papers, focusing on solvent properties and their role in detection in NACE–MS [19, 20]. Studies employing an organic/aqueous (isopropanol:water = 4:1) sheath liquid and BGEs based on several solvents (methanol, acetonitrile, dimethylsulfoxide, formamide, *N*-methylformamide, and *N,N*-dimethylformamide) revealed substantial differences in the LODs obtained. Only methanol and acetonitrile provided similar results for the tested analytes (2-aminobenzimidazole, procaine, propranolol, and quinine) as observed with aqueous electrolytes; for the other solvents less favorable LODs were recorded. In the case of 2-aminobenzimidazole, the LOD with formamide and *N*-methylformamide in the BGE was more than 300-fold higher than the one obtained with methanol, acetonitrile, or the aqueous BGE [19]. A consequence of these findings may be the fact that when browsing the applications listed in Table 2 the majority of NACE–MS applications is performed using BGEs containing methanol, acetonitrile, or mixtures of these two solvents.

1.3 Electrolyte Systems for NACE–MS

Focusing on electrolyte systems for NACE, the impact of the solvent employed has to be considered first. One major prerequisite is the solubility of the selected electrolyte system in nonaqueous solvent systems. Some of the most popular buffer systems in aqueous

Table 2
Applications of nonaqueous capillary electrophoresis–mass spectrometry (since 2004)

	Method details	Ref.
Plants and natural products		
Crude root bark extracts from an African Ancistrocladus species	BGE: 100 mM ammonium acetate in methanol/acetic acid 60/40 v/v Ion trap MS with ESI in positive ion mode Sheath liquid: 2-propanol/water 1/1 v/v *Remark:* comparison with HPLC	[21]
β-Carboline alkaloids extracted from dried leaves	BGE: 40 mM $(NH_4)_2CO_3$ in methanol/glacial acetic acid 80/20 v/v Ion trap MS with ESI in positive ion mode Sheath liquid: 2-propanol/water 1/1 v/v *Remark:* combined detection system laser-induced fluorescence and MS; comparison with aqueous CE	[32]
Isoquinoline alkaloids in Fumaria officinalis	BGE: 60 mM ammonium acetate and 2.2 M acetic acid in acetonitrile/methanol 9/1 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: water/2-propanol 1/1 v/v	[33]
Nicotine-related alkaloids in chewing gums, beverages, and tobaccos	BGE: 50 mM ammonium formate in acetonitrile/methanol 50/50 v/v, apparent pH 4.0 Ion Trap MS with ESI in positive ion mode Sheath liquid: isopropyl alcohol/water 80/20 v/v	[36]
Alkaloids in Central European Corydalis species	BGE: 50 mM ammonium acetate and 1 M acetic acid in methanol/acetonitrile 1/9 v/v	[34]
Quinolizidine alkaloids in the roots of Sophora flavescens Ait. and S. tonkinensis Gagnep	BGE: 50 mM ammonium acetate and 0.5% acetic acid in methanol/acetonitrile 7/3 v/v Quadrupole MS with ESI in positive ion mode Sheath liquid: 0.5% acetic acid in methanol/water 80/20 v/v *Remark:* Field-amplified sample stacking with electromigration injection	[37]
Pyrrolo- and pyrido[1,2-a] azepine alkaloids in Stemona	BGE: 50 mM ammonium acetate, 1 M acetic acid, and 10% methanol in acetonitrile Ion Trap MS with ESI in positive ion mode Sheath liquid: water/2-propanol 1/1 v/v	[35]
Cinchona alkaloids in cinchona bark	BGE: 80 mM formic acid, 20 mM acetic acid, and 30 mM ammonium formate in methanol/ethanol/acetonitrile 50:35:15 v/v/v QTOF MS with ESI in positive ion mode Sheath liquid: 0.1% formic acid in 2-propanol/water 8/2 v/v	[38]
Alkaloids from psychoactive plant extracts	BGE: 58 mM ammonium formate and 1 M acetic acid in acetonitrile QTOF and Ion Trap MS with ESI in positive ion mode	[39]

(continued)

Table 2
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	Method details	Ref.
Alkaloids from a plant extract of Mitragyna speciosa	BGE: 60 mM ammonium formate and in acetonitrile/acetic acid 1000/35 v/v QTOF MS with ESI in positive ion mode Sheath liquid: 5% acetic acid in 2-propanol/water 66/34 v/v *Remark:* design of experiments to study the influence of the background electrolyte on separation and detection in NACE–MS	[20]
Mesembrine alkaloids in Sceletium tortuosum	BGE: 75 mM ammonium acetate in acetonitrile/glacial acetic acid 9/1 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: 5% acetic acid in 2-propanol/water 66/34 v/v	[40]
Matrine and oxymatrine in Sophora flavescens	BGE: 30 mM ammonium acetate and 1% acetic acid in methanol/acetonitrile 85/15 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: 2-propanol/water 2/1 v/v	[59]
Alkaloids isolated from Amaryllidaceae plants	BGE: 40 mM ammonium acetate and 0.5% acetic acid in methanol/acetonitrile 2/1 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: water/2-propanol 1/1 v/v	[60]
Biomedical analysis		
Lidocaine and its metabolites in human plasma	BGE: 70 mM ammonium formate and 2 M formic acid in acetonitrile/methanol 6/4 v/v Quadrupole MS with ESI in positive ion mode Sheath liquid: 2% formic acid in water/2-propanol 1/1 v/v	[41]
Peptaibol alamethicin F30 isolated from the culture broth of Trichoderma viride	BGE: 12.5 mM ammonium formate in methanol Ion trap and TOF MS with ESI in positive ion mode Sheath liquid: 1% formic acid in 2-propanol/water 1/1 v/v *Remark:* comparison with aqueous CE	[42]
Determination of salbutamol enantiomers in urine	BGE: 0.75 M formic acid, 10 mM ammonium formate, and 15 mM Heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-cyclodextrin in methanol Ion Trap MS with ESI in positive ion mode Sheath liquid: 0.1% formic acid in 2-acetonitrile/water 3/1 v/v	[28]
Amino acid sequences of alamethicins F30	BGE: 12.5 mM ammonium formate in methanol Ion trap and TOF MS with ESI in positive ion mode Sheath liquid: 1% formic acid in 2-propanol/water 1/1 v/v	[43]
Phospholipids extracted from rat peritoneal surface	BGE: 20 mM ammonium acetate and 0.5% acetic acid in acetonitrile/methanol 60/40 v/v Ion Trap MS with ESI in negative ion mode Sheath liquid: 50 mM ammonium acetate in acetonitrile/methanol 60/40 v/v	[44]

(continued)

Table 2
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	Method details	Ref.
Three anesthetic drugs in human plasma	BGE: 2 M formic acid and 70 mM ammonium acetate in acetonitrile/methanol 60/40 v/v Quadrupole MS with ESI in positive ion mode Sheath liquid: 2% formic acid in methanol/water 8/2 v/v *Remark:* microextraction by packed sorbent in combination with CE	[45]
20 Antidepressants in plasma samples	BGE: 60 mM ammonium acetate and 1 M acetic acid in acetonitrile/water/methanol 100/1/0.5 v/v/v TOF MS with ESI in positive ion mode Sheath liquid: methanol/water 9/1 v/v	[47]
Amphetamine and related compounds in equine plasma	BGE: 25 mM ammonium formate and 1 M formic acid in acetonitrile/methanol 2/8 v/v Ion Trap MS with ESI in positive ion mode sheath liquid: 0.5% formic acid in water/2-propanol 1/1 v/v	[48]
Identification of fentanyl derivatives	BGE: 200 mM ammonium acetate in glacial acetic acid/acetonitrile 1/9 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: 2-propanol/water 1/1 v/v	[49]
Five fluoroquinolones in Urine	BGE: 20 mM ammonium acetate in acetonitrile/methanol 50/50 v/v adjusted to pH 4 with formic acid Quadrupole MS with ESI in positive ion mode Sheath liquid: 2% formic acid in acetonitrile/methanol 50/50 v/v *Remark:* microextraction by packed sorbent in combination with CE	[46]
Pregabalin in human urine	BGE: 10 mM ammonium formate and 0.05% acetic acid in methanol QTOF MS with ESI in positive ion mode Sheath liquid: 10 mM ammonium formate and 0.05% acetic acid in methanol	[61]
Nonsteroidal anti-inflammatory drugs (NSAIDs) and glucuronides in urine samples	BGE: 5 mM ammonium acetate in acetonitrile/methanol 80/20 v/v Quadrupole MS with ESI in negative ion mode Sheath liquid: 2-Propanol/water/NH_4OH 49.5/49.5/1 v/v/v *Remark:* comparison of a sheath liquid and sheathless interface	[30]
Food		
Phenolic compounds from olive oil	BGE: 25 mM ammonium acetate in methanol/acetonitrile 1/1 v/v, apparent pH adjusted to 5.0 with acetic acid TOF MS with ESI in negative ion mode Sheath liquid: 5 mM sodium hydroxide and 0.2% formic acid in water/2-propanol 1/1 v/v	[31]

(continued)

Table 2
(continued)

	Method details	Ref.
Analyses of clenbuterol, salbutamol, and terbutaline in pork	BGE: 18 mM ammonium acetate in methanol/acetonitrile/glacial acetic acid 66/33/1 v/v/v TOF MS with ESI in positive ion mode Sheath liquid: 5 mM ammonium acetate in methanol/water 80/20 v/v	[51]
Glycerophospholipids in olive fruit and oil	BGE: 100 mM ammonium acetate and 0.5% acetic acid in methanol/acetonitrile 60/40 (v/v) Ion Trap MS with ESI in positive ion mode Sheath liquid: 0.5% acetic acid in methanol/water 8/2 v/v	[50]
Environmental and industrial		
Detection of hexamethonium–perchlorate association complexes	BGE: 2-propanol/acetone 2/1 v/v Ion trap MS with ESI in positive ion mode Sheath liquid: methanol	[52]
Separation and characterization of ionizable organic polymers nonsoluble in water	BGE: 1 M acetic acid, 20 mM ammonium acetate in methanol/acetonitrile 87.5/12.5 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: methanol/acetonitrile 87.5/12.5 v/v	[53]
Degradation products of the herbicide oxasulfuron	BGE: 50 mM ammonium acetate and 1.2 M acetic acid in acetonitrile/methanol 9/1 v/v Ion Trap MS with ESI in positive ion mode Sheath liquid: 1% acetic acid in water/methanol 1/1 v/v	[54]
Characterization of nonderivatized Brij 58 oligomers	BGE: 20 mM NH4I in methanol Ion Trap MS with ESI in positive ion mode Sheath liquid: 25 mM ammonium acetate in methanol/water 95/5 v/v *Remark:* EOF reversal with hexadimethrine bromide	[27]
Six pharmaceutical compounds and their respective process-related impurities	BGE: 10 mM ammonium acetate and 100 mM acetic acid in methanol/acetonitrile with varying ratios Ion trap MS with ESI Sheath liquid: 0.1% formic acid in methanol/water 50/50 v/v	[55]
Separation of basic drugs, including their enantiomers	BGE: 10 mM HP-β-CD or 10 mM HDMS-β-CD in methanol containing 10 mM ammonium and 0.75 M formic acid Ion trap MS with ESI in positive ion mode Sheath liquid: 0.1% formic acid in acetonitrile/water 75/25 v/v *Remark:* Nonaqueous electrokinetic chromatography using anionic cyclodextrins;	[56]

(continued)

Table 2
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	Method details	Ref.
Separation of 10 acidic drugs, including their enantiomers	BGE: 20 mM PA-β-CD in methanol containing 20 mM ammonium acetate or 5 mM IPA-β-CD in methanol containing 40 mM ammonium acetate Ion trap MS with ESI in negative ion mode Sheath liquid: 5 mM ammonium acetate in acetonitrile/water 75/25 v/v *Remark:* Nonaqueous electrokinetic chromatography using cationic cyclodextrins; polyacrylamide and polyvinylamide coated capillaries were used	[26]
Organotin compounds in water samples	BGE: 50 mM ammonium acetate and 1 M acetic acid in acetonitrile/methanol 80/20 v/v QTOF MS with ESI in positive ion mode Sheath liquid: 0.2% formic acid in 2-propanol/water 1/1 v/v *Remark:* speciation of organotin compounds	[57]
Fundamental investigations		
2-Aminobenzimidazole, procaine, propranolol, and quinine	BGE: 10 mM ammonium acetate in 7 different solvents Ion Trap MS with ESI in positive ion mode Sheath liquid: 0.1% formic acid in 2-propanol/water 4/1 v/v *Remark:* assessment of the influence of the solvent on selectivity, separation speed, and peak efficiency for a given set of model compounds	[19]
2,4-Dinitrophenol, pentachlorophenol, 3,4-dichlorocinnamic acid, 2-methyl-4,6-dinitrophenol, 2,3,4,5-tetrachlorophenol	BGE: 25 mM ammonium formate in methanol, apparent pH adjusted to 8.0 with 25 mM NH_4OH Triple quadrupole MS with ESI in negative ion mode Sheath liquid: 2 mM ammonium acetate in 2-propanol/water 80/20 v/v *Remark:* Large volume stacking using an EOF pump	[58]
Separation of carvedilol enantiomers	BGE: 0.75 M formic acid, 10 mM ammonium acetate and 10 mM sulfated β-CD in methanol Ion Trap MS with ESI in positive ion mode Sheath liquid: 0.1% formic acid in 2-propanol/water 3/1 v/v *Remark:* addition of different single-isomer sulfated β-CD derivatives	[29]

CE such as phosphate and borate are hardly soluble in nonaqueous media. Second, the critical parameter giving an idea about the dissociation of an electrolyte is the relative permittivity ε of the solvent. As can be seen from Table 1, for most solvents employed in NACE, ε is lower than in the case of water. As a consequence of this fact, comparing a given concentration of an electrolyte substance in water and in a nonaqueous solvent (such as methanol or acetonitrile) its conductivity will be lower in the nonaqueous

medium. For this reason, relatively high concentration of electrolyte substances can be employed in NACE without reaching the limiting electrical current. This can clearly be seen from the applications listed in Table 2, where electrolyte systems containing 100 mM ammonium acetate and 40% of acetic acid could be used without any problems with excessive current [21].

Comparing NACE with spectrophotometric detection and NACE–MS, it can be observed that using MS as detector has a clear impact on the selection of components that can be included in the BGE, whereby no significant difference exists between aqueous and nonaqueous electrolyte systems. The MS detector is far more prone to interferences caused by physicochemical properties of electrolyte ingredients than a photometric one. Unfortunately, the need to ensure compatibility with MS reduces the number of selectable electrolyte ingredients substantially. Focusing on MS with the most commonly used ionization technique, namely, electrospray ionization (ESI) [22] the use of volatile BGE components is mandatory. This prerequisite excludes a series of substances which are of very popular in CE, such as buffers based on phosphate or borate (although these two are not employed in NACE due to solubility issues in the commonly employed organic solvents), capillary coatings based on alkylammonium salts or sulfonate/sulfate additives. BGEs mostly used in CE–MS are based on formic acid, acetic acid (and their ammonium salts), carbonate, and solutions of ammonia or alkylamines if strongly alkaline conditions are needed. Here the situation in NACE–MS is quite similar. Nevertheless, there are some reports on the use of other BGE systems containing nonvolatile ingredients and their impact on ionization in ESI–MS exist [23]. In-depth information about requirements for MS-compatible CE electrolytes, in general, can be found in a comprehensive review article published by Pantuckova et al. [24].

1.4 pH in Nonaqueous Systems

A crucial parameter in optimizing the BGE for CE in general is the selection of the appropriate pH value, as this factor influences the direction and magnitude of the electro endoosmotic flow as well as the degree of protonation/deprotonation of the analytes and with it the direction and magnitude of their electrophoretic mobilities. Whereas measuring and adjusting the pH is a more or less simple task in aqueous systems, the concept of pH measurement and adjustment cannot be transferred easily to nonaqueous conditions. Several approaches to overcome this problem have been suggested so far [11]. One option is to measure pH in nonaqueous solvents employing the same procedure as in aqueous media. This leads to the so-called apparent pH a value that is quite often used to describe nonaqueous buffer systems. Nevertheless, it has to be taken into account, that this “apparent pH” only allows comparison between BGEs based on the same solvent. An alternative is pH calculation using the Henderson–Hasselbalch equation. This approach can only be used if the pK values of the employed acid or

base in the respective solvent are available. A comprehensive discussion on the issue of pH measurement and adjustment in nonaqueous solvents has been published by Porras and Kenndler [6].

1.5 Capillary Coatings and Additives

Capillary coatings are quite popular in CE. They are used, for example, to reduce analyte/capillary wall interactions or to suppress or even reverse the electro endoosmotic flow, just to name a few reasons for their application [25]. Dynamic coating (i.e., the coating substance is added to the BGE) and static coating (the coating is attached to the capillary wall either by covalent bonds or by strong electrostatic interaction) can be distinguished. When moving from aqueous conditions to NACE it has to be ensured that the coating is still stable even when purely organic BGEs are employed. Furthermore, most substances used for dynamic coating are not compatible with MS. So either capillaries with covalently bonded coatings are employed in NACE–MS [26] or substances like hexadimethrine bromide that show strong interactions with the capillary wall even under completely nonaqueous conditions [27].

The group of additives for CE comprises a wide range of quite different substances. These can be micelle-forming agents, ion-pairing reagents, chiral selectors, and so on just to name a few. Also in the case of additives analyte/additive interactions have to be reevaluated when moving from aqueous to nonaqueous media. Similar as in the case of substances employed for capillary coating, most of the additives commonly employed in CE are not compatible with MS detection. A strategy to combine NACE with nonvolatile additives such as cyclodextrins with MS detection is to select conditions where these additives migrate toward the capillary inlet [26, 28, 29]. So no disadvantageous suppression effects are encountered as the cyclodextrins are not reaching the MS ion source.

1.6 Sheath Liquids for Nonaqueous Capillary Electrophoresis–Mass Spectrometry

From the NACE–MS papers listed in Table 2, only in one case a sheathless interface is employed for CE–MS coupling [30]. In this study, a recently developed sheathless interface was compared to a conventional triaxial sheath flow interface with respect to its performance in NACE–MS of acidic compounds. In all other studies, the addition of an appropriate sheath liquid is required for guaranteeing a stable electrospray. The sheath liquid serves as a makeup flow (to reach the minimum flow rates needed for the ESI source), it is needed to close the electric circuit of the CE system (as no second electrolyte vial is present in CE–MS coupling), and its composition should enhance ionization efficiency and probably overcome less favorable characteristics of the BGE (with respect to ionization). When comparing sheath liquid compositions used in aqueous CE–MS and those for NACE–MS not real difference can be detected. In most cases a mixture of water and an alcohol (mainly methanol or 2-propanol) or acetonitrile together with small amounts of a volatile salt (often ammonium acetate or formate) and/or a MS compatible acid or base (formic acid,

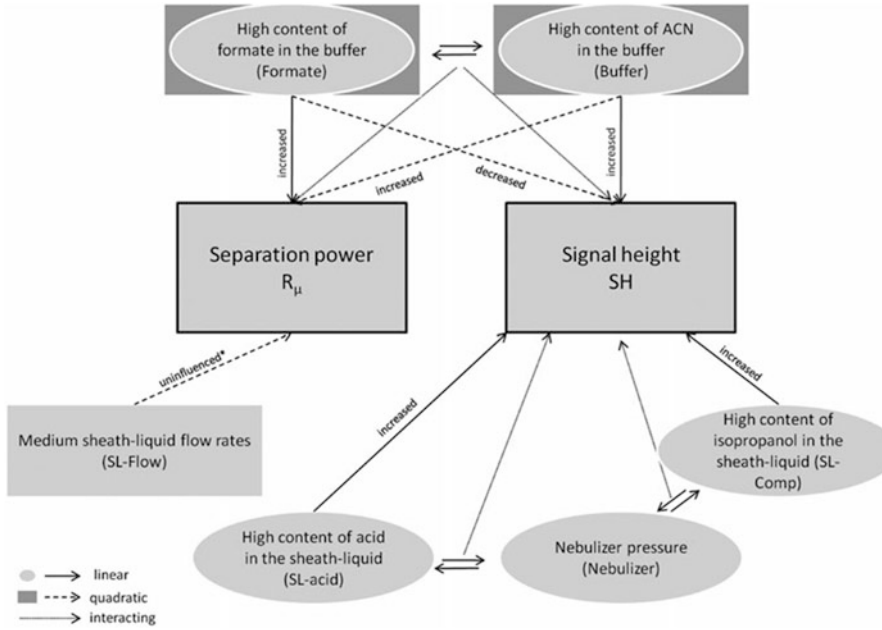

Fig. 1 Visualizationof the interactions and influences of the process parameters on the response variables. CE parameters are shown on top, MS parameters are shown below the response variables. Reproduced from [20] with permission

acetic acid, or ammonia, respectively) are the best choice. An example demonstrating a further functionality of the sheath liquid was presented by Gomez-Caravaca et al. [31]. By adding 2.5 mM of NaOH to sheath liquid, sodium formate clusters were formed that could be directly used for mass calibration of the TOF instrument.

1.7 Optimization of NACE–MS Parameters

When searching for the best operational parameters for NACE–MS experiments, mutual interference between several experimental parameters like BGE composition, composition of the sheath liquid, sheath liquid flow rate, or nebulizer pressure has to be taken into account. Posch et al. have published an in-depth study discussing the possibility to use a design of experiments approach to study the influence of the background electrolyte on separation and detection in NACE–MS [20]. A scheme depicting potential interactions and influences is shown in Fig. 1. In this work it was proven that at high electro endoosmotic flow conditions, separation can be optimized without inferences from the MS detection system.

2 Applications

Table 2 gives an overview of NACE–MS applications published since 2004. Applications are grouped according to the field of application and listed in chronological order. In this table, relevant

information concerning the type of analytes investigated and/or the field of application, BGE and sheath liquid composition, and the type of MS instrument/ionization source used is provided. Additionally, remarks on characteristic features of the work are given.

2.1 NACE–MS of Plants and Natural Products

The analysis of plants and natural products is one of the main fields of application of NACE–MS. Unger et al. compared HPLC with CE and NACE for the analysis of crude extracts from Ancistrocladus species [21]. Although the highest number of resolved components was achieved by HPLC, NACE allowed the separation of cis/trans isomers (that could not be resolved using HPLC) whereas conventional CE with an aqueous electrolyte led to comigration of all analytes. Huhn et al. designed a CE system allowing simultaneous laser-induced fluorescence and MS detection [32]. Employing this setup, β-carbolines from an Ayahuasca sample were analyzed, whereby distinctly different migration orders were achieved in NACE compared to CE with an aqueous BGE. The group of Stuppner used NACE–MS with a BGE based on mixed solvents (acetonitrile/methanol=9/1) for the investigation of several plant extracts [33–35]. A NACE–MS method for the analysis of alkaloids in tobacco and chewing gums was developed by Chiu et al. [36]. In their paper they investigated a series of nonaqueous BGEs differing in apparent pH, methanol/acetonitrile ratio, as well as type and concentration of electrolyte employed. The combination of NACE–MS and field amplified sample stacking for the high-sensitivity analysis of quinolizidine alkaloids was presented by Wang et al. [37]. Buchberger et al. achieved the separation of cinchona alkaloids extracted from cinchona bark [38]. Employing a rather complex nonaqueous BGE based on formic acid, acetic acid, and ammonium formate in a mixture of methanol/ethanol and acetonitrile, a series of diastereomeric compounds could be separated. The group of Huhn published a series of papers on the use of NACE–MS for the analysis of forensically interesting alkaloids from several plant species [20, 39, 40]. In one of these papers (as discussed earlier) the use of a "design of experiment" for optimization of NACE–MS parameters is discussed [20].

2.2 NACE–MS for Bioanalytical Applications

A second major field of application of NACE–MS is biomedical analysis. Anderson et al. described a NACE–MS method for the determination of lidocaine and its metabolites in human plasma [41]. Alamethicin peptides from Trichoderma viride were analyzed by Psurek et al. employing NACE–MS [42, 43]. Comparing the results obtained with aqueous and nonaqueous conditions revealed improved separation efficiency for the nonaqueous BGE as well as substantial selectivity changes. The latter might be attributed to changes in the shape of the peptide when switching from aqueous to nonaqueous conditions. Cyclodextrin-mediated NACE–MS was employed for the determination of salbutamol enantiomers in urine by Servais et al. [28]. The developed method allowed the baseline separation of the two enantiomers in less than 12 min. Due to their

low solubility in aqueous media, phospholipids are not easily accessible to CE–MS analysis. Gao et al. investigated the potential of different nonaqueous BGE systems for the NACE–MS analysis of these compounds [44]. Morales-Cid et al. developed sophisticated automated instrumentation, including sample pretreatment steps such as packed sorbent microextraction and microdialysis for the analysis of drugs in body fluids by NACE–MS [45, 46]. Although both, aqueous and nonaqueous electrolytes were studied, the NACE approach was selected due to the increased sensitivity obtained. Employing the right mixture of methanol and acetonitrile allowed to adjust selectivity and to reduce analysis times. A series of antidepressants were separated by NACE and subsequently detected using MS by Sasajima et al. [47]. As can be seen from Fig. 2, great improvement in separation was achieved when moving from an aqueous BGE to a nonaqueous one. Interestingly, the BGE finally selected for this analytical problem can no longer be seen as a purely nonaqueous one, as it contains 1 % of water. Amphetamines in race-horse plasma were analyzed by Li et al. [48]. Fentanyl derivatives were separated using NACE by Rittgen et al. [49]. The analysis of these compounds gains more and more interest as clandestine fentanyl laboratories produce these substances for the illegal drug market. An interesting study comparing not only CE with aqueous

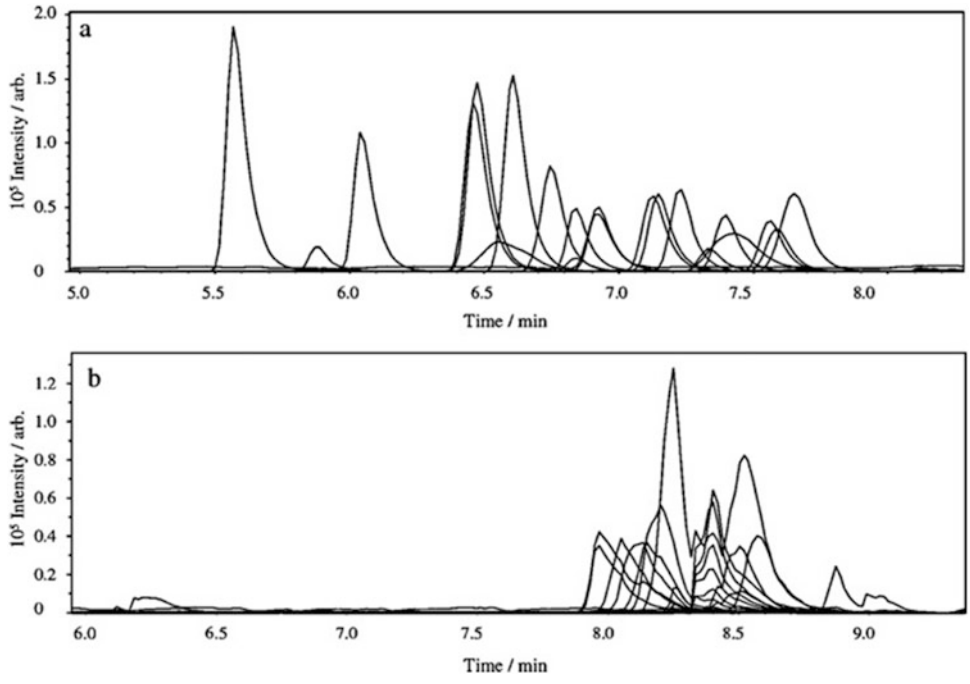

Fig. 2 Separation of 20 antidepressants—comparison between NACE and aqueous CE. BGE, (**a**) 50 mM ammonium acetate and 1 M acetic acid in acetonitrile, (**b**) 1 M formic acid in water; all other parameters are identical. Reproduced from [47] with permission

and nonaqueous conditions, but also two different types of ESI interfaces for CE–MS coupling was published by Bonvin et al. [30]. As can be seen from Fig. 3, switching from an aqueous BGE to NACE substantially improved the resolution of the investigated test substances. In addition, sensitivity obtained with NACE–MS was 5–10 times better than in CE–MS, although also a substantially higher noise level was observed with the nonaqueous BGE.

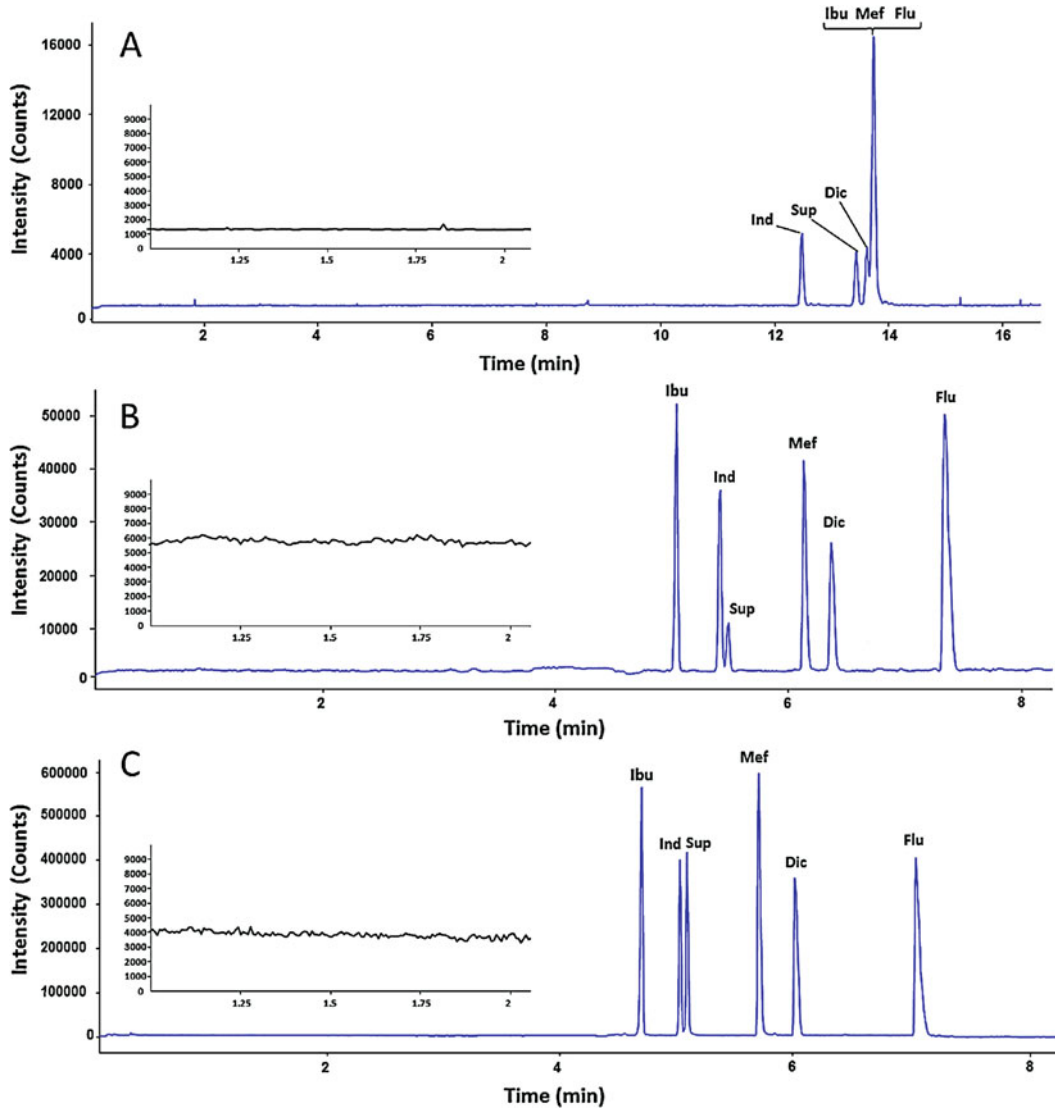

Fig. 3 CE–MS electropherograms of acidic compounds in negative ESI obtained for selected nonsteroidal anti-inflammatory drugs (dissolved at 1 μg/mL in ACN-MeOH 60:40 (v/v)) with the sheath liquid interface in (**A**) aqueous CZE mode; BGE: ammonium acetate 50 mM, pH 8.5 and (**B**) NACE mode; BGE: ammonium acetate 5 mM in ACN-MeOH 80:20 (v/v). (**C**) CE–MS electropherograms with the sheathless interface in NACE mode; BGE: ammonium acetate 5 mM in ACN-MeOH 80:20 (v/v). Peaks: *Ind*: Indomethacin; *Sup* Suprofen; *Dic* Diclofenac, *Ibu* Ibuprofen, *Mef* Mefenamic acid, *Flu* Flufenamic acid. Reproduced from [30] with permission

2.3 NACE–MS in Food Analysis

Nonaqueous conditions are definitely favorable when it comes to the CE analysis of samples with low solubility in water. This fact has been exploited in two studies describing the NACE–MS analysis of olive oils and olive fruit with respect to phenolic compounds [31] and phospholipids [50]. β-Agonists in pork meat were analyzed by NACE–MS and HPLC–MS [51].

2.4 NACE–MS for the Analysis of Technical Products and Environmental Samples

Groom and Hawari investigated the formation of complexes including hexamethonium perchlorate (substances frequently used as rocket fuel) in both aqueous and polar nonaqueous solvents [52]. The resulting complexes were resolved employing NACE and detected by ESI–MS. A characteristic of this work is the rather unusual BGE based on a mixture of 2-propanol and acetone. Organic polymers are often insoluble in aqueous media. For this reason separation methods working in nonaqueous solution are preferable for the analysis of such samples. Simo et al. demonstrated the suitability of NACE–MS for the analysis of synthetic polymers (poly (*Nε*-trifluoroacetyl-l-lysine)) [53]. Thereby, structures containing up to 38 monomers could be resolved. Scrano et al. developed a NACE–MS method allowing the identification and quantitation of two novel degradation products originating from the photolytic reaction of oxasulfuron [54]. Another polymer-related application of NACE–MS has been published by Morin et al. [27]. The separation of the neutral polyethylene oxide surfactant was based on its complexation with ammonium in methanol as solvent. More than 25 oligomers of this surfactant could be characterized. Aqueous CE, open-tubular capillary electrochromatography and NACE, all coupled to MS, were compared with respect to their potential for the impurity profiling of drugs by Vassort et al. [55]. The results obtained within this study suggest that some of the previously developed CE–MS methods should be replaced by NACE–MS due to improved separation capabilities. Additionally, NACE appears attractive as a large portion of drug candidates are poorly soluble in water. Electrokinetic chromatography with cyclodextrins in nonaqueous media was employed for the analysis of various acidic drugs by Mol et al. [26, 56]. The effect of the cationic cyclodextrins on the ESI process was studied, whereby the separation voltage applied led to migration of these components in direction of the inlet vial, thereby not interfering with the ionization process. A fast NACE–MS method for the speciation of organotin compounds, substances commonly employed as antifouling agents, was presented by Malik et al. [57]. The use of a homemade CE instrument allowed applying separation voltages as high as 35 kV together with the use of short capillaries—thereby reducing analysis times to 2.5 min.

2.5 NACE–MS: Fundamental Investigations

Several papers discussing fundamental issues with respect to the coupling of NACE with MS have been published so far. Steiner and Hassel performed in-depth investigations on the influence of

solvent properties on separation and detection [19]. They compared a series of solvents with respect to analysis times, separation efficiency, as well as performance in combination with ESI–MS detection. Some of the findings from this paper have already been discussed in the previous section on "Solvents for NACE and NACE–MS." The potential of large volume sample stacking in combination with NACE–MS was investigated by Kim et al. whereby a 400-fold enrichment of anionic analytes was achieved [58]. Technical obstacles arising from the long sample matrix plug were solved by supplying a backup run buffer from the outlet vial of the CE system. Cyclodextrins are widely used in electrokinetic chromatography for selectivity manipulations. Unfortunately they can cause adverse effects in ESI–MS detection due to the occurrence of ionization suppression. Nonaqueous electrokinetic chromatography with either anionic [56] or cationic [26] cyclodextrins has successfully been coupled to MS detection. In a further paper, Servais et al. discussed the influence of BGE composition and type of cyclodextrin used on the detector response observed in cyclodextrin-mediated NACE–MS [28].

3 Materials

In this section instrumentation and materials for a typical NACE–MS application are discussed. Some of these points are also valid for normal CE–MS with aqueous BGE.

3.1 BGE for NACE–MS

BGE ingredients have to comply with both, requirements from NACE and requirements from ESI–MS. From the wide range of solvents employed in NACE with spectrophotometric detection only a few are also used in combination with MS: these are alcohols (methanol, ethanol) and acetonitrile. Electrolyte ingredients (salts, acids, or bases) have to be soluble in the selected solvent and have to be compatible with ESI–MS. So in most cases low molecular weight organic acids (formic acid, acetic acid) and/or their ammonium salts are employed.

3.2 Sheath Liquid for NACE–MS

Sheath liquids used in NACE–MS are almost identical to those in aqueous CE–MS. Although purely nonaqueous sheath liquids can be used (methanol, propanol, or acetonitrile/alcohol mixtures) most sheath liquids in NACE contain 20–50% water together with a small amount of acid/base or a volatile salt to enhance ionization.

3.3 CE Instrumentation

"7100 CE System" (Agilent), Beckman PA 800 (SCIEX Separations) or equivalent, equipped with an ultraviolet (UV) absorbance detector, high voltage power supply up to +–30 kV, and autosampler for both hydrodynamic and electrokinetic injection. Also a special capillary cartridge for hyphenation with MS is

needed. Due to the higher volatility of organic solvents (compared to aqueous BGEs) a cooling option for the tray, housing the sample and the electrolyte vials is advisable.

3.4 CE–MS Interface

The majority of CE–MS applications are performed using a triaxial sheath flow interface like the one available from Agilent (G1607A or G1607B). For supplying the sheath liquid ideally an HPLC pump with a 1:100 flow splitter is employed. A second option is the use of a syringe pump (e.g., from Harvard Apparatus, South Natick, MA, USA) whereby an increased baseline noise due to flow rate fluctuations must be taken into account.

3.5 MS Instrument

In most cases, MS instruments that offer commercially available dedicated interfaces for CE–MS coupling are preferable. Apart from that, MS instruments with an ionization source where the sprayer needle is grounded, whereas high voltage is applied to the MS orifice, as is the case in Agilent and Bruker instruments, for example, substantially facilitate CE–MS coupling. As CE is a highly efficient separation technique resulting in narrow peaks a sufficiently fast MS instrument is advantageous. In recent times, TOF and Q/TOF instruments have become the most frequently used instruments in CE–MS coupling.

3.6 Fused-Silica Capillaries

For example, from Polymicro Technologies (Phoenic, AZ) with inner diameter and outer diameter of 50 and 360 μm, respectively, and sufficient length to introduce the capillary into the MS interface. The capillary length can vary significantly due to the different layout of the available CE–MS systems and may be in the range between 60 cm and more than 100 cm. If coated capillaries are employed, stability of the coating in nonaqueous BGEs has to be ensured.

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