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Influence of electrolyte nature on the separation selectivity of amphetamines in nonaqueous capillary electrophoresis: Protonation degree *versus* ion pairing effects

The simultaneous analysis of Ecstasy and its derivatives in an acetonitrile-methanol (80:20 v/v) mixture was previously shown to be strongly dependent on the nature of the electrolyte (acetate *versus* formate). To elucidate the phenomena involved, systematic experiments were conducted in this solvent medium. Conductivity measurements allowed to evaluate the ion-pairing rate in the background electrolyte (BGE) and thereby distinguish between electrolyte concentration and ionic strength. The influence of electrolyte concentration on analyte effective mobilities (μ_{eff}) was studied by capillary electrophoresis (CE). As μ_{eff} extrapolated to infinite dilution proved to be independent of the nature of the electrolyte, selectivity changes could not be attributed to a modification in the protonation degree of amphetamines. Experimental mobility data were then confronted to existing theoretical mobility models to discriminate between ion pairing or simple ionic strength effect. Ion-pair formation in a BGE containing acetate was highlighted with an ion-pairing model and ion-pair formation constants between each amphetamine and acetate ion were calculated.

Keywords: Amphetamines / Background electrolyte composition / Ion-pairing / Nonaqueous capillary electrophoresis / Solvation effects DOI 10.1002/elps.200305372

1 Introduction

1.1 General aspects

During the last decade, there has been growing interest for nonaqueous buffers in capillary electrophoresis (CE), and a number of reviews and applications have been published [1–3] on this subject. Organic solvents are useful in several situations, especially in the analysis of hydrophobic compounds and substances which are difficult to separate in aqueous buffers [4, 5]. Due to their different physicochemical properties, organic solvents may improve or modify selectivity. The phenomena involved are shifts in acido-basic [6–9] or complexation equilibria [10], occurrence of new noncovalent interactions (*e.g.*, ion-pairing [11, 12], charge transfer [13, 14], heteroconjugation [7, 15]), modification of the dielectric friction [16, 17] or of solvation shell [18–20]. Some of these phenomena can be cross-correlated. Recent work has focused on understanding these phenomena [12, 21, 22]. Nevertheless, the role played by each phenomenon in selectivity changes remains of particular interest.

Amphetamine and other related derivatives are powerful stimulants of the central nervous system and their analysis is of great importance in the clinical and forensic fields [23]. Several aqueous CE methods have been developed for the determination of amphetamine and its derivatives in seized tablets [24] or biofluids [25, 26] by investigating parameters, such as buffer pH and concentration, the presence of organic modifiers, the applied voltage, and nature of the capillary. Because of the relatively low sensitivity afforded by the short optical path length through the capillary, the direct assay of these drugs in biological matrices by CE coupled with UV absorbance detection remains of limited practical utility. Among the different strategies contemplated to enhance sensitivity in CE, the on-line coupling of CE with electrospray ionization-mass spectrometry (ESI-MS) was recently applied to determine amphetamine derivatives and, in order to make this coupling easier, nonaqueous background electrolytes (BGEs) were set up [27]. The best compromise in terms of selec-

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Abbreviations: A, amphetamine; **DHO**, Debye-Hückel-Onsager; **FP**, Falkenhagen-Pitts; **MA**, metamphetamine; **MDA**, 3,4-methylenedioxyamphetamine; **MDEA**, 3,4-methylenedioxyethylamphetamine; **MDMA**, 3,4-methylenedioxymethylamphetamine; **MDPA**, 3,4-methylenedioxypropylamphetamine; **MeCN**, acetonitrile; **NACE**, nonaqueous capillary electrophoresis

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tivity, efficiency, and analysis time was obtained with an acetonitrile-methanol 80:20, v/v mixture containing 25 mM ammonium formate and 1 M formic acid. Nevertheless, in the course of this study, differences in separation selectivity and migration orders appeared upon substitution of acetate for formate electrolyte, keeping the salt/acid molar ratio constant. Such phenomena might be attributed to several factors, including differences in the protonation degree of amphetamines, ion-pair formation between amphetamines and electrolyte anions, or even possible solvation alterations.

The aim of the present work was to evaluate the influence of the above-mentioned physicochemical parameters on the separation selectivity of some selected amphetamines, according to the electrolyte nature. For this purpose, both acetate and formate concentrations in the BGE were varied, keeping the salt/acid molar ratio and the composition of the organic solvent mixture constant. It is noteworthy that formate and acetate are by far the most common electrolytes in nonaqueous capillary electrophoresis (NACE) applications. Moreover, these electrolytes are volatile and thus are suitable for NACE-MS coupling. Therefore, understanding the physicochemical phenomena involved with these electrolytes in nonaqueous media is of utmost interest. First, conductivity measurements were performed to evaluate the rate of ion pairing and determine the free ion concentration in the BGE. Thus, the actual ionic strength was expressed as a function of the total salt concentration in the electrolyte. Afterwards, NACE measurements were carried out to study the effect of electrolyte concentration on effective mobilities (μ_{eff}). Finally these variations were confronted to existing theoretical mobility models, in an attempt to discriminate between simple ionic strength, protonation degree, ion pairing or solvation effects.

1.2 Theoretical background

1.2.1 Concentration effects on electrolyte conductivity

According to the simple Debye-Hückel-Onsager model (DHO model), for a strong 1:1 electrolyte the equivalent conductivity Λ decreases linearly with the square-root of its concentration c [28–30]:

$$\Lambda = \Lambda^{\infty} - (A\Lambda^{\infty} + B)\, c^{1/2} \quad (1)$$

Λ^{∞} is the equivalent conductivity of the electrolyte at infinite dilution, while A and B are parameters accounting for the relaxation and electrophoretic effects, respectively. The latter parameters are directly related to the electrolyte temperature T, dielectric constant ε, and viscosity η:

$$A = 0.818\ 10^6/(\varepsilon T)^{3/2} \quad (2)$$

$$B = 82/\eta(\varepsilon T)^{1/2} \quad (2')$$

For consistency with Eqs. (1, 2, 2'), η, c and Λ^{∞} are given in 10^{-1} Pa·s (Poise), mol·L^{-1} and S·cm^{2}eq^{-1}, respectively. It should be remembered that the DHO model is only valid for very diluted electrolyte solutions and ions are considered as point charges, *i.e.*, ionic radii are ignored.

In the case of ion-pairing between electrolyte ions, the variation of the equivalent conductivity upon concentration can be described by the more sophisticated Fuoss equation [31]:

$$\Lambda = \Lambda^{\infty} - S\, c^{1/2}\, \alpha^{1/2} + E\, c\, \alpha \ln(c\, \alpha) + J\, c\, \alpha \quad (3)$$

where S is the slope of the linear variation of Λ as a function of the square-root of the concentration in the DHO model ($S = A\Lambda^{\infty} + B$), E is a constant defined by the same variables as S, and J a parameter depending on the ion size. α is the ion-pair dissociation coefficient, related to the ion-pair formation constant K_{ip} by:

$$1 - \alpha = K_{ip}\, c\, \alpha^2\, f^2 \quad (4)$$

f being the activity coefficient of the electrolyte ions. In view of determining K_{ip}, Eq. (3) can be rearranged under the form [32]:

$$F(z)/\Lambda = 1/\Lambda^{\infty} + c\, \Lambda\, f^2\, K_{ip}/\Lambda_{\infty}^2\, F(z) \quad (5)$$

$$\text{with } z = ((A\Lambda^{\infty} + B)/\Lambda^{\infty 3/2})(c\, \Lambda)^{1/2} \quad (6)$$

$$\text{and } F(z) = 4/3 \cos^2 [1/3 \cos^{-1}(-3^{3/2} z/2)] \quad (7)$$

Experimental conductivity data as a function of electrolyte concentration can then be linearized by plotting $F(z)/\Lambda$ in terms of $c\, \Lambda\, f^2\, /\, F(z)$ [33, 34]. Y-intercept and slope of the regression straight line give access to Λ^{∞} and K_{ip} values.

1.2.2 Ionic strength effects on ion mobility

The DHO model, described for equivalent electrolyte conductivity, applies also in a similar form to absolute ionic mobility. However, to take into account the influence of ion sizes, the more refined treatments by Falkenhagen *et al.* [35] and Pitts *et al.* [36] are considered below. For an uni-univalent electrolyte, the actual mobility μ_i^0 of ion i can then be expressed as a function of ionic strength I according to:

$$\mu_i^0 = \mu_i^{\infty} - \left[\left(8.20 \times 10^5 \mu_i^{\infty}/(\varepsilon T)^{3/2}\right) + 42.75/\eta(\varepsilon T)^{1/2}\right] \times \left[\sqrt{I}/\left(1 + 50.29 a (\varepsilon T)^{-1/2}\sqrt{I}\right)\right] \quad (8)$$

where a is the distance of the closest approach between ion i and its counterion and μ_i^{∞} is the absolute mobility of ion i. For unit consistency, η is reported in 10^{-1} Pa·s, a in

Å, I in mol·L^{-1}, and μ^{∞} in 10^{-5} cm^{2}V^{-1}s^{-1}. The terms $8.20 \times 10^5\ \mu_i^{\infty}/(\varepsilon T)^{3/2}$ and $42.75/\eta\ (\varepsilon T)^{1/2}$ describe relaxation and electrophoretic effects, respectively. In Eq. (8), the denominator of the last term between brackets is the unit if ions are considered as point charges (a = 0), and mobility decreases linearly with $\sqrt{I}$, with the so-called Onsager limiting slope.

In order to analyze experimental mobility data by means of Falkenhagen and Pitts model (FP model), parameter a is estimated as the sum of Stokes radii of analyte ion and electrolyte counterion [37], Stokes radius $R_{St,i}$ of ion i being defined by:

$$R_{St,i} = z_i e/6\pi\mu_i^{\infty}\eta \quad (9)$$

where z_i is the charge number of ion i and e is the elementary charge. It is noteworthy that the Stokes radius accounts for solvation effects.

1.2.3 Ion-pairing effects on ion mobility

In the case of the formation of a neutral ion-pair of 1:1 stoichiometry between an analyte ion i and the electrolyte counterion C, the effective mobility of analyte i, μ_i^{eff}, can be expressed as a function of the ion-pair formation constant K_{ip} and the free counterion concentration [C] [11, 38] according to:

$$\mu_i^{eff} = \frac{\mu_i^0}{1 + K_{ip}[C]} \quad (10)$$

which can be rearranged as:

$$\mu_i^0/\mu_i^{eff} - 1 = K_{IP}[C] \quad (11)$$

The plot of the left-hand term of Eq. (11), calculated from experimental values of μ_i^{eff} in terms of the free counterion concentration, should lead to a straight line, the slope of which yields the ion-pair formation constant K_{ip}. It is noteworthy that in Eqs. (10) and (11) as before, μ_i^0, represents the actual mobility of ion i which should be calculated from the absolute mobility, μ_i^{∞}, from Eq. (8), to discriminate ion-pairing from ionic strength effects. As for the free counterion concentration, it should be calculated by considering ion-pairing between electrolyte ions.

2 Materials and methods

2.1 Materials

Standard methanolic solutions of 1 mg·mL^{-1} amphetamines including amphetamine (A), metamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethylamphetamine (MDMA, also known as Ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA) and 3,4-methylenedioxypropylamphetamine (MDPA) (see Fig. 1) were purchased from Alltech (Deerfield, IL, USA). Analytical reagent-grade ammonium formate, formic acid (water content 2% v/v), ammonium acetate, acetic acid (water content 0.2% v/v), benzyl alcohol, sodium hydroxide and potassium chloride were obtained from Fluka (Buchs, Switzerland). HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were supplied by Romil (Kölliken, Switzerland) (water content 0.05% and 0.03% v/v, respectively). Ultrapure water was produced by a Milli-Q unit from Millipore (Bedford, MA, USA).

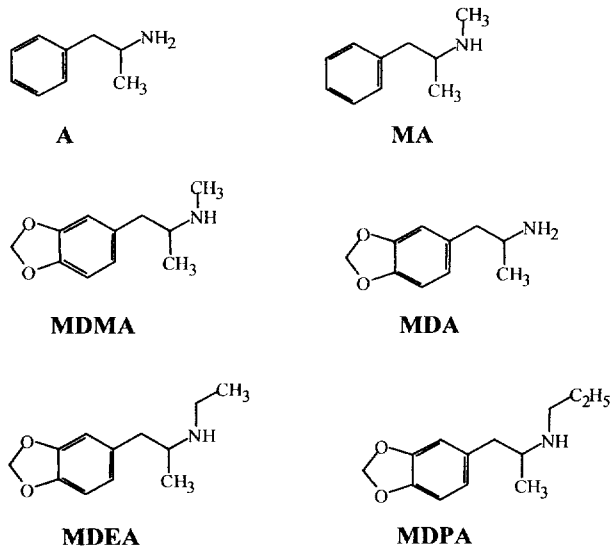

Figure 1. Structures of the investigated amphetamines.

2.2 Capillary electrophoresis

CE experiments were carried out with an HP^{3D} CE apparatus (Agilent, Waldbronn, Germany) equipped with a diode array detector, an autosampler and a power supply able to deliver up to 30 kV. Data were handled by a HP Chemstation software. Bare fused-silica capillaries, 50 µm ID (375 µm OD) × 40 cm in length (31.5 cm effective length), were purchased from Polymicro Technologies (Phoenix, AZ, USA). Samples were injected in hydrodynamic mode (40 mbar, 3 s, approx. 5 nL). Under optimal NACE conditions [27], the separation voltage and temperature were set at 20 kV positive voltage (500 V·cm^{-1} electric field) and 25°C, respectively. UV absorbance detection was carried out at 200 nm with a spectral bandwidth of 20 nm. The acquisition rate was 10 points·s^{-1}.

2.3 Procedures

The nonaqueous BGE were made up by mixing ammonium formate (or acetate) and formic (or acetic) acid in various concentrations but at a constant molar ratio

(1/40) in a MeCN-MeOH (80:20 v/v) mixture. The highest tested acid concentration was 1 M. Benzyl alcohol (0.1% v/v in water) was used as a neutral marker for determining electroosmotic mobility. All BGEs were filtered through 0.2 μm filter units before use. New capillaries were conditioned by successive flushes with 1 M and 0.1 M NaOH, and then with water for 10, 5, and 10 min, respectively. Prior to each sample injection, the capillary was rinsed with BGE for 5 min. When not in use, the capillary was rinsed with water and dried by air. Conductivity of each BGE was directly measured at 25.0 ± 0.5°C using a Radiometer CDM210 conductimeter (Villeurbanne, France). The cell constant was determined by calibration with various concentrations of potassium chloride. BGE viscosity was determined as follows: in a fused-silica capillary filled with the studied BGE, a 50 mbar pressure was applied on a vial containing the neutral marker benzyl alcohol (0.1% v/v in BGE). The detection time corresponding to the absorbance front (t_d, in s) of the neutral marker was used to calculate electrolyte viscosity (in cP) according to the Hagen-Poiseuille equation:

$$\eta = 10^5 (d_c^2 \Delta P / 32 L^2) t_d \quad (12)$$

where d_c is the capillary inner diameter, L is the capillary length (both in m), and ΔP is the applied pressure (in mbar). Throughout the paper, uncertainties given are one standard deviation.

3 Results and discussion

3.1 Nonaqueous separation conditions of amphetamines

In a previous study, the simultaneous separation of six amphetamines was achieved by aqueous CE-UV in less than 7 min, using a 50 mM Tris-phosphate buffer at pH 2.5 [39]. At this pH, all amphetamines were fully protonated and migration took place as expected in the order of their increasing molecular mass, *i.e.*, A<MA<MDA<MDMA <MDEA<MDPA. Later, nonaqueous media were also considered to investigate the potential of NACE for these compounds [27]. A good separation was obtained with a 25 mM ammonium formate, 1 M formic acid in MeCN-MeOH (80:20 v/v) mixture. The choice of the BGE pH was dictated by the fact that the analytes should be protonated and the BGE composition must be compatible with MS detection. This is why the BGE was only slightly buffered. In comparison to the preceding migration order recalled above for aqueous buffer, significant modification in separation selectivity was observed in this nonaqueous medium as illustrated in Fig. 2A. A reversal in A and MA was obtained, while MDA was delayed after MDMA and MDEA. Moreover, by substituting ammonium acetate for

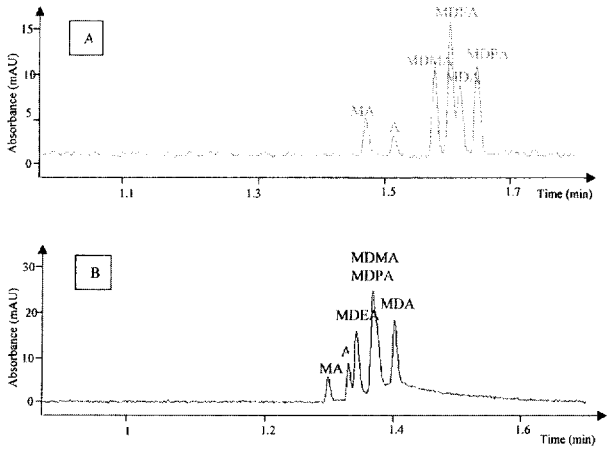

Figure 2. Influence of BGE nature on the NACE separation of amphetamines. Fused-silica capillary, 50 μm ID × 40 cm (detection, 31.5 cm). BGE: (A) 25 mM ammonium formate/1 M formic acid, (B) 25 mM ammonium acetate/1 M acetic acid in MeCN-MeOH (80:20 v/v) mixture. Applied voltage, +20 kV, (A) 30 μA, (B) 20 μA; temperature, 25°C; hydrodynamic injection (40 mbar for 3 s); UV absorbance detection at 200 nm. The sample mixture contained six amphetamines at a concentration of 10 $\mu g \cdot mL^{-1}$ in methanol.

ammonium formate and acetic acid for formic acid (other conditions being unchanged), two additional inversions in migration orders occurred, regarding MDMA/MDEA and MDA/MDPA pairs, while MDMA and MDPA were no longer resolved (Fig. 2B). These alterations are not likely to be simply attributable to differences in water content of formate- and acetate-based BGEs (0.1% v:v and 0.05% v/v, respectively for the more concentrated BGEs). Indeed, Tjörnelund *et al.* [40] have shown that a water content below 0.5% v/v has a minor effect on separation selectivity and efficiency and on electroosmotic flow in solvents such as MeCN and MeOH. In addition, uncontrolled variations of the water content in the time course of the experiments did not impact migration times significantly.

Several physicochemical phenomena can be invoked in these selectivity variations, including acido-basicity, ion-pairing, and solvation. Acido-basic properties are strongly modified on going from water to MeCN-MeOH medium, leading to a stronger decrease in the strength of HA/A^- type acids, compared with BH^+/B type ones [31, 41]. Whether amphetamines remained fully protonated in the studied acetic or formic electrolytes can thus be questioned. Were they not, the migration order of partially protonated amphetamines could be tuned according to their acidity constants. On the other hand, considering the dielectric constant value for nonaqueous media (Table 1), ion-pair formation between analyte and electrolyte could

Table 1. Some relevant physicochemical parameters for water, methanol, acetonitrile and the acetonitrile/methanol (80:20 v/v) binary mixture at 25°C

Solvent	Viscosity η (cP)	Dielectric constant ε
Water	0.89	80
Methanol (MeOH)	0.54	32.7
Acetonitrile (MeCN)	0.34	37.5
MeCN-MeOH (80:20 v/v)	0.34 ± 0.01 ($n = 3$)	35

All values were obtained from [43], except for the viscosity of the MeCN-MeOH mixture, which was measured directly in this work.

occur in these solvents, reducing significantly the electrophoretic mobility of the analytes. Regarding the nature of the counterionic species in the electrolyte, different rates of ion-pair formation could induce alteration of migration orders. Besides, the hydrodynamic radii of the analytes could also be modified by changes in the solvent composition of the electrolyte through solvation effects.

The purpose of this work was to clarify which of these physicochemical phenomena mainly affects migration order and separation selectivity within the studied amphetamine mixture. To this end, the salt concentration was varied for both formate and acetate buffers, keeping the molar ratio of basic to acidic forms (1/40) as well as the organic solvent composition (MeCN-MeOH 80:20 v/v) constant.

3.2 Evaluation of ion-pairing in BGE

In order to assess the rate of ion-pair formation between anions (formate or acetate) and ammonium cation, BGE conductivity was studied as a function of the ammonium salt concentration introduced. As ion pairs behave as electrically neutral dipoles, ion pair formation reduces the apparent equivalent conductivity of BGE. For both acetate and formate BGE, variation of the experimental equivalent conductivity with the square-root of the ammonium salt concentration is shown in Fig. 3. In order to better confront these data with DHO theoretical predictions, the equivalent conductivities at infinite dilution were first estimated by linear extrapolation to zero concentration. As a result, Λ^{∞} values of 125 ± 17 and 159 ± 9 $S \cdot cm^2 eq^{-1}$ were obtained for acetate- and formate-based BGE, respectively. Using these values, theoretical slopes were calculated for the studied medium according to Eqs. (1), 2) and (2') and then represented as broken lines in Fig. 3. It clearly appears that both BGEs

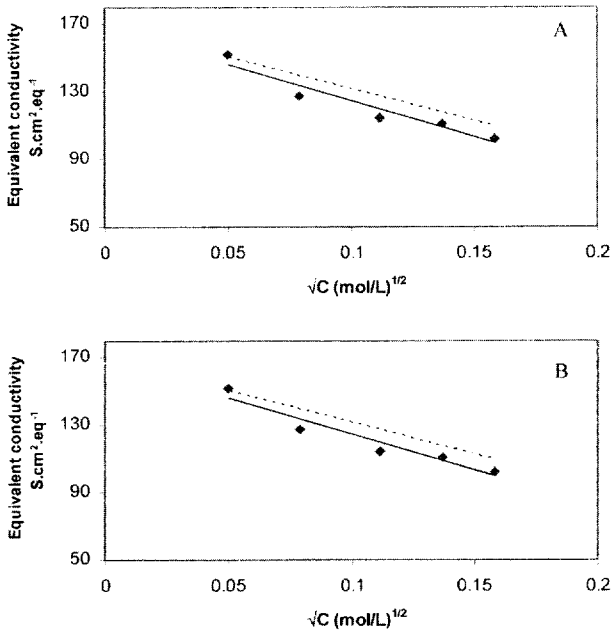

Figure 3. Effect of ammonium salt concentration on equivalent conductivity of (A) ammonium formate/formic acid (molar ratio, 1:40), and (B) ammonium acetate/acetic acid (molar ratio, 1:40) in MeCN-MeOH (80:20 v/v) mixture. Temperature, 25°C. Solid lines: least squares linear regression of the experimental points. Broken lines: theoretical slope of the linear variation for this medium, according to the DHO model (Eq. 1).

did not follow the DHO model. The decrease of the electrolyte equivalent conductivity, stronger than predicted by the model, indicates that ion-pair formation might be involved.

In order to confirm this hypothesis, experimental data were reprocessed according to the Fuoss model (Eq. 3) which takes into account ion association. For the present study, the activity coefficient value was considered as equal to one. Figure 4 shows that a rather good linear correlation was observed using this model equation and Table 2 gives the resulting equivalent conductivities at

Table 2. Ion-pair formation constant K_{ip} and equivalent conductivity at infinite dilution Λ_{∞}

	K_{ip} ($mol^{-1}L$)	Λ^{∞} ($S \cdot cm^2 eq^{-1}$)
Ammonium acetate/acetic acid	18	122
Ammonium formate/formic acid	11.5	156

Medium: ammonium acetate/acetic acid or ammonium formate/formic acid (molar ratio, 1:40) in MeCN-MeOH (80:20 v/v) mixture. Temperature, 25°C. Experimental data processed according to Fuoss model (Eq. 5).

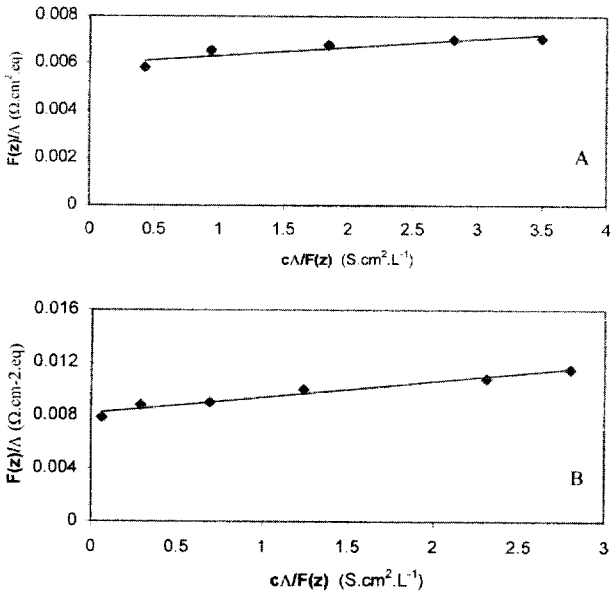

Figure 4. Mathematical treatment of the electrolyte equivalent conductivity as a function of the ammonium salt concentration according to the Fuoss model (Eq. 5). (A) Ammonium formate/formic acid (molar ratio, 1:40) and (B) ammonium acetate/acetic acid (molar ratio, 1:40) in the MeCN-MeOH (80:20 v/v) mixture. The least squares regression straight lines are represented as a solid line. Least squares correlation coefficients are 0.85 and 0.96 for (A) and (B), respectively.

infinite dilution Λ^{∞} and ion-pair formation constant K_{ip} derived from the parameters of the least-squares regression straight lines. K_{ip} values likewise obtained for ammonium association of acetate were higher than with formate. At first sight, this result is not consistent with the concept of contact ion-pairs between acetate (or formate) and ammonium ions, since a smaller crystal radius for formate ion should lead to a stronger association with ammonium counterion. However, these findings can be related to the fact that acetate presents a higher polarizability than formate. The dissociation coefficient (α), which corresponds to the proportion of free electrolyte ions, and the actual BGE ionic strength (αc) were calculated using the K_{ip} values and according to Eq. (4). Results are given in Table 3. Within the considered concentration range (2.5–25 mM), the impact of ion-pairing between the electrolyte ions resulted in up to a 25% decrease in electrolyte conductivity and ionic strength; the corrected BGE ionic strengths were used throughout the study.

3.3 Influence of electrolyte concentration on amphetamine effective mobilities (μ_{eff})

The six amphetamines' μ_{eff} were measured in both acetate- and formate-based electrolytes of varying ammonium salt concentrations (2.5–25 mM) and constant ammonium salt/acid with a molar ratio of 1:40.

3.3.1 Comparison of experimental results with the simple DHO model. Evaluation of analyte protonation degree

Amphetamines μ_{eff} were first analyzed with respect to the simple DHO model (Eq. 8), ignoring the size of the ions (*a* = 0). The variation of these mobilities in terms of the square-root of actual ionic strength, corrected for electrolyte ion association, is presented in Figs. 5A and B for formate- and acetate-based BGE, respectively. As both these plots were considered as fairly linear (see regression coefficients), *Y*-intercept of the least-squares regression straight lines were used to determine analyte μ_{eff} at infinite dilution (μ_{eff}^{∞}). Obtained values are reported in

Table 3. Proportion of free ions and actual ionic strength for ammonium acetate and formate electrolytes as a function of their total concentration

Ammonium salt (total concentration) (mM)	Acetate-based electrolyte		Formate-based electrolyte	
	Free ion (%)	Actual ionic strength (mM)	Free ion (%)	Actual ionic strength (mM)
25	75	18.7	81	20.2
18.7	79	14.8	84	15.7
16.7	80	13.3	86	14.3
12.5	84	10.5	88	11.0
8.3	88	7.3	92	4.7
6.2	91	5.7	93	5.8
2.5	96	2.4	97	2.4

Calculations were performed with Eq. (4), using K_{ip} values given in Table 2.

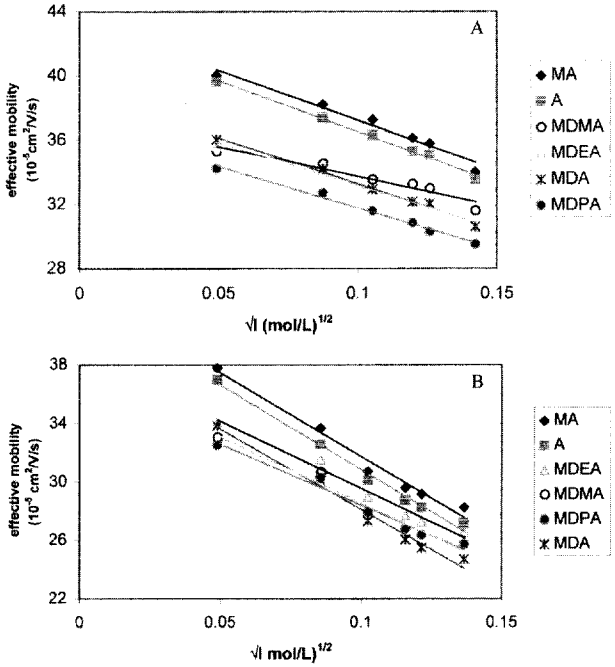

Figure 5. Variation of the amphetamine effective mobilities as a function of the square-root of the actual electrolyte ionic strength for (A) the formate and (B) the acetate-based electrolytes. BGE: (A) ammonium formate/formic acid or (B) ammonium acetate/acetic acid in variable concentrations such that the salt/acid molar ratio was kept constant (1:40), all in a MeCN-MeOH (80:20 v/v) mixture. Temperature, 25°C. Equations of the least-squares regression straight lines: MA: (A) $y = -62.26x + 43.4$ ($R^2 = 0.968$), (B) $y = -114.26x + 43.2$ ($R^2 = 0.979$); A: (A) $y = -63.91x + 42.8$ ($R^2 = 0.993$), (B) $y = -115.5x + 42.4$ ($R^2 = 0.990$); MDA: (A) $y = -57.01x + 39$ ($R^2 = 0.990$), (B) $y = -110.15x + 39.2$ ($R^2 = 0.983$); MDEA: (A) $y = -53.3x + 38.5$ ($R^2 = 0.974$), (B) $y = -91.89x + 38.7$ ($R^2 = 0.976$); MDMA: (A) $y = -36.85x + 37.4$ ($R^2 = 0.920$), (B) $y = -90.22x + 37.6$ ($R^2 = 0.969$); MDPA: (A) $y = -51.48x + 36.9$ ($R^2 = 0.989$), (B) $y = -83.47x + 36.8$ ($R^2 = 0.975$).

Table 4, which revealed that μ_{eff}^{∞} were not significantly dependent on electrolyte nature, confirming that amphetamines were fully protonated in both BGEs. Extrapolated values then correspond to the absolute mobilities of amphetamines in this nonaqueous medium. Undeniably, the selectivity modifications between acetate and formate BGE are not induced by different protonation degrees of analytes.

Furthermore, for all six amphetamines, the slopes of the straight lines represented in Figs. 5A and B were higher for acetate than for formate BGE. As μ_{eff}^{∞} were identical, this difference implied that μ_{eff} of a given analyte was more reduced in acetate than in formate BGE for the same ionic strength. This behavior, as well as the difference observed in the migration orders of amphetamines, could not be explained by the DHO model (Eq. 8, with $a = 0$). Consequently, ion-pair may occur between protonated amphetamines and electrolyte counterions and amphetamines associate more strongly with acetate anions than formate ones.

Table 4. Effective mobilities of amphetamines at zero ionic strength μ_{eff}^{∞} obtained from the *Y*-intercept of the graphs presented in Figs. 5A and B for ammonium formate/formic acid (molar ratio, 1:40) and ammonium acetate/acetic acid (molar ratio, 1:40) in MeCN-MeOH (80:20 v/v) mixture

Amphetamines	μ_{eff}^{∞} (10^{-5} cm$^2 \cdot$V^{-1}s^{-1})	
	Formate-based electrolyte	Acetate-based electrolyte
MA	43.4 ± 1.7	43.2 ± 2.4
A	42.8 ± 0.8	42.4 ± 1.7
MDA	39.0 ± 0.8	39.2 ± 2.1
MDEA	38.5 ± 1.3	38.7 ± 2.1
MDMA	37.4 ± 1.6	37.6 ± 2.3
MDPA	36.9 ± 1.0	36.8 ± 2.0

Temperature, 25°C. As the values do not significantly differ from one BGE to the other, they can be regarded as absolute mobility values.

3.3.2 Comparison of experimental results with the FP model

The analyte mobilities measured at varying salt concentrations in the nonaqueous electrolyte were confronted to the refined FP model, which considers ion dimensions. Indeed, the previous estimation of absolute mobilities for amphetamines using the simple DHO model (see Table 4) enabled to calculate their Stokes radii from Stokes relationship (Eq. 9) and viscosity value for MeCN-MeOH (80:20 v/v) measured in this work. Values ranging from 0.55 nm for MA to 0.68 nm for MDPA were thus obtained (Table 5).

In order to estimate parameter *a* from Eq. (8), Stokes radius of formate and acetate counterions were also calculated from equivalent conductivities of ammonium acetate and ammonium formate at infinite dilution (Table 2). As an approximation, the equivalent conductivity of ammonium ion in pure acetonitrile was considered (97.1 S$\cdot$cm^{2}eq^{-1} [42]). According to Walden's rule this value was also retained for MeCN-MeOH (80:20 v/v) mixtures, since the viscosity remains unchanged (see Table 1). To perform this calculation, the following relationships were used:

Table 5. Stokes radii for the amphetamine analytes, ammonium ion and electrolyte counterions in MeCN-MeOH (80:20 v/v) mixture calculated from absolute mobilities (Table 4) or equivalent conductivities at infinite dilution (Table 2) and Stokes relationship (Eq. 9)

Ions	Amphetamines						Electrolyte counterions	
	MA	A	MDA	MDEA	MDMA	MDPA	Formate	Acetate
Stokes radius (nm)	0.55	0.59	0.64	0.65	0.67	0.68	0.47	0.89

The viscosity value measured in this work was used for this solvent mixture (Table 1).

$$\Lambda^{\infty}_{NH_4^+A^-} = \lambda^{\infty}_{NH_4^+} + \lambda^{\infty}_{A^-} \quad (13)$$

$$\lambda^{\infty}_{A^-} = F\mu^{\infty}_{A^-} \quad (14)$$

where Λ^{∞} and λ^{∞} are electrolytic and ionic equivalent conductivities at infinite dilution, F is the Faraday's constant and A^- stands for formate or acetate ion. Values obtained for Stokes radii of formate and acetate ions are also given in Table 5. Finally, parameter a was calculated from Stokes radii summarized in Table 5. For the investigated amphetamines, estimated a values ranged from 1.02 to 1.15 nm and from 1.44 to 1.57 nm for formate and acetate electrolyte, respectively. Moreover, for each amphetamine and both electrolytes, experimental variation of mobility in terms of actual electrolyte ionic strength was then compared with theoretical predictions derived from the FP model, with ε and η values suitable for a MeCN-MeOH (80:20 v/v) mixture (Table 1). Viscosity value of neat MeCN-MeOH (80:20 v/v) solvent mixture was used in these calculations (Table 1), as viscosity correction for ionic strength did not significantly modify the result (the measured viscosity for the 25 mM ammonium formate (or acetate)/1 M formic acid (or acetic acid) buffer in MeCN-MeOH (80:20 v/v) mixture was 0.37 cP). Equation (8) then becomes:

$$\mu_i^0 = \mu_i^0 - \left[0.77\mu_i^{\infty} + 119.6\right]\sqrt{I}/\left(1 + 0.49a\sqrt{I}\right) \quad (15)$$

As an example, Fig. 6 shows results for metamphetamine. In the case of formate BGE (Fig. 6A), the experimental decrease in mobility was less pronounced than predicted by the FP model with an a value estimated at 1.02 nm. In order to better appreciate the influence of this parameter, which can only be roughly estimated, its value was varied between 1 and 2 nm. Deviation between experimental and theoretical curves remained positive unless an a value higher than 2 nm was considered. Indeed, this positive deviation indicated that calculated parameters a, and thus Stokes radii, were probably underestimated, which may be attributed to an inadequacy of the assumption of spherical shape included in the model used and to uncertainties on absolute mobilities. In any case, ion-pair formation with formate ion could not be highlighted for any tested amphetamines.

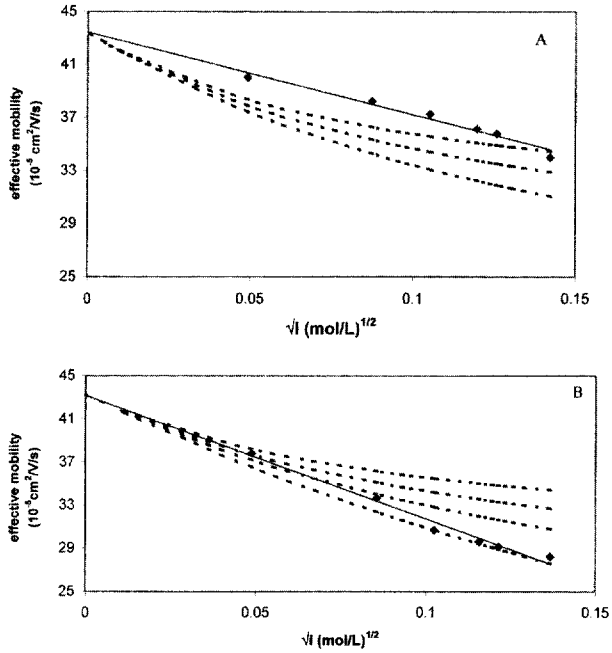

Figure 6. Comparison of the experimental variation for MA mobility in terms of electrolyte ionic strength with theoretical predictions from the FP model. BGE: (A) 25 mM ammonium formate/1 M formic acid or (B) 25 mM ammonium acetate/1 M acetic acid in MeCN-MeOH (80:20 v/v) mixture. Other experimental conditions as stated in Fig. 5. (◆) Experimental points. Solid lines are drawn for a better appreciation of the trend. Dashed lines: theoretical variations (Eq. 15) for various a values. Top to bottom: (A) a = 2, 1.5, 1.02 nm; (B) a = 2, 1.44, 1, 0.5 nm.

On the contrary, with acetate electrolyte (Fig. 6B), the experimental decrease in mobility was markedly more pronounced than predicted by Eq. (15) with previously estimated value of a, 1.44 nm. This parameter a was then varied between 0.5 and 2 nm. The experimental decrease

is closer to the theoretical variation obtained with a = 0.5 nm. This behavior, however, seems unrealistic for the MA-acetate ion-pair, with regard to the preceding estimation. Meanwhile, whatever the exact value of a, experimental mobility values decreased more rapidly than theoretically expected, which strengthens the assumption of ion-pair formation. The same behavior was observed for the six amphetamines.

3.3.3 Comparison of experimental results with the ion-pairing model

In order to validate the assumption of ion-pair formation between protonated amphetamines and acetate ions in BGE, the classical ion-pair model presented in Section 1.2.3 was employed. In this context, μ_i^0 values were calculated for each ionic strength tested from the FP model (Eq. 15), taking for parameter the a values calculated from Stokes radii (Table 5). In addition, values for free acetate ion concentration were taken from Table 3, which accounts for the association of acetate and ammonium ions in BGE. The experimental mobility values of the six amphetamines were then considered as effective mobility data and reprocessed according to the ion-pairing model. Figure 7 represents the linearization of Eq. (11) obtained for all six amphetamines. As reported, the least-squares correlation coefficients varied between 0.85 and 0.97, indicating a rather acceptable linearity. Indeed, linearity problems could be due to the fact that the levels of the free counterion concentration studied (2.4–18.7 mM) were rather low and thus ion pairs were formed in low proportions (see below). The slopes of the regression lines were used to estimate the ion-pair formation constant K_{ip} of each amphetamine with acetate ion. The values obtained

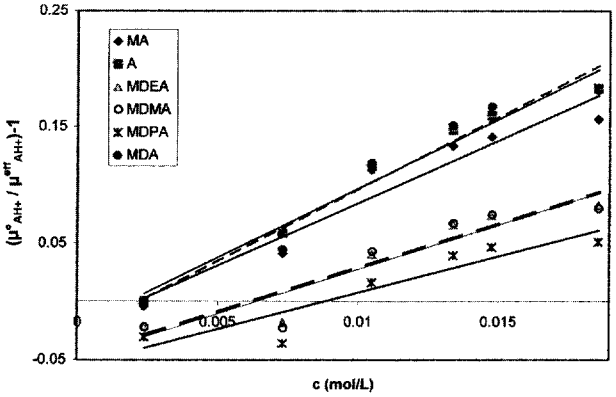

Figure 7. Linearization of the experimental mobility variation of the six amphetamines as a function of the free acetate counterion concentration according to the ion-pair model (Eq. 11). Experimental conditions as for Fig. 5B. Least-squares correlation coefficients vary between 0.85 and 0.97.

(Table 6) seemed acceptable and consistent with those for ammonium ion and electrolyte counterions (Table 2). Association rates derived from these K_{ip} values for each amphetamine under optimized conditions (Fig. 2B) ranged from 1.5 to 18%. Although moderate, these association rates were significant enough to account for changes in the migration order of amphetamines in NACE according to the electrolyte nature. In spite of the rather low precision of these K_{ip} determinations, Table 6 suggests that K_{ip} values decrease from a primary (A, MDA) to a secondary amine (MA, MDMA, MDEA, MDPA) and this, all the more as the secondary amine becomes sterically more hindered. This order is consistent with common sense expectation. Especially, MDA, which presents the highest K_{ip} value, migrates third among ecstasy derivatives in aqueous media (see Section 3.1 and [39]) and last in an acetate electrolyte MeCN-MeOH (80:20 v/v) mixture. This order of K_{ip} values brings a comprehensive clue for most of the migration order and selectivity changes that were observed from aqueous to nonaqueous CE conditions and, in the latter situation, from a formate to an acetate electrolyte.

Table 6. Ion-pair formation constants K_{ip} calculated from the slopes of the least-squares regression straight lines shown in Fig. 7, according to Eq. (11)

Amphetamines	K_{ip} (L/mol)
A	11.8 ± 2.7
MA	10.7 ± 4.0
MDA	12.4 ± 4.0
MDMA	7.6 ± 4.2
MDEA	7.6 ± 3.7
MDPA	6.2 ± 3.6

4 Concluding remarks

This work allowed to elucidate the role of some physicochemical phenomena involved in the selectivity modifications observed in the nonaqueous CE separation of amphetamines according to the separation electrolyte (acetate *versus* formate electrolyte in a MeCN-MeOH (80:20 v/v) mixture). First, amphetamines were confirmed to be fully protonated in both BGEs, allowing to discard the involvement of different effective charges in selectivity modification. Then, the confrontation of the experimental mobility data with theoretical models (simple DHO and refined FP models) highlighted the fact that the ionic strength effect was not the only phenomenon involved in the selectivity changes. An ion-pairing model was next applied to evaluate the influence of ion-pair formation in the case of acetate containing electrolyte. Thereby, ion-

pair formation constants were determined between amphetamines and acetate ions as well as between ammonium and acetate or formate ions in the electrolyte. The values provide a realistic description of the analyte status within the separation electrolyte, which explains the deviation from FP model and most of the changes in migration order observed from aqueous to nonaqueous CE conditions. For the formate electrolyte, ion-pair formation with amphetamines could not be demonstrated, and the positive deviation from FP model may result from solvation effects. Although it cannot be predicted which electrolyte affords the best selectivity, one must remember that the electrolyte nature and concentration may influence the selectivity. Therefore, it is worth considering testing different electrolytes in nonaqueous media.

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