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Analysis of catecholamines by capillary electrophoresis and capillary electrophoresis–nanospray mass spectrometry Use of aqueous and non-aqueous solutions compared with physical parameters

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Abstract

Catecholamines were analysed in aqueous and alcoholic non-aqueous solutions by capillary electrophoresis and capillary electrophoresis–mass spectrometry using sheathless nanospray coupling. Decreases in the electrophoretic mobilities of the catecholamines and in the electroosmotic mobilities were observed from water to 1-propanol. Separations were more efficient in all non-aqueous media than in water. The diffusion coefficients of the catecholamines in the different media were determined. The solvent had little effect on the sensitivity of the UV or MS detection. Both methods were successfully applied to the analysis of urine samples.

Keywords: Non-aqueous capillary electrophoresis; Background electrolyte composition; Catecholamines

1. Introduction

Capillary electrophoresis (CE) is increasingly being applied in non-aqueous separations [1]. Important analytical parameters, including efficiency and selectivity, can be controlled when aqueous analysis is replaced by non-aqueous [2–4]. Many physical properties need to be considered when selecting the solvent for a non-aqueous separation [5,6]. Among the most important of these are the dielectric constant and viscosity. Dielectric constant affects the autoprotolysis constant of the solvent but also the pK_a values of the analytes and thus their dissociation [7]. The comparatively large dielectric constants of water and small alcohols like methanol and ethanol make them suitable media for ionizable analytes. In addition to the dielectric constant, also the hydrogen bonding capability of the solvent stabilizes the charges. Viscosity has a direct effect on the electrophoretic mobility of the analytes [8]. Organic solvents widely used in non-aqueous separations include alcohols, acetonitrile and amides and mixtures of these [9,10]. Urine samples have been analysed in non-aqueous systems [5,11,12].

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CE has been interfaced to electrospray ionization mass spectrometry (ESI-MS) in various ways, including the sheath liquid and sheathless nanospray methods [13]. The highly robust sheath liquid method is the routine coupling [14–16]. However, the flow-rate from the CE capillary is several orders of magnitude lower than flow-rate of the sheath liquid, which means that the sample is heavily diluted and sensitivity is poor. In the sheathless nanospray method [17–19], the CE capillary is tapered at the tip to a few micrometers. A gold coating at the tip provides the electrical contact. In comparison with methods relying on sheath liquid, sheathless coupling allows a greater fraction of the analyte to be converted to gas phase ions so that sensitivity is increased [20]. CE with non-aqueous electrolyte system has been coupled to MS with the sheath liquid approach [21–25], but, to our knowledge, not yet by the sheathless nanospray method. In the sheath liquid coupling the ionization is affected by the sheath liquid, while in nanospray coupling it is determined by the CE electrolyte solution. Non-aqueous electrolytes thus play an important role in sheathless nanospray with CE.

The aim of this research was to study the properties of eight structurally related catecholamines by determining electrophoretic mobility, selectivity, efficiency and sensitivity in aqueous and non-aqueous media by CE. Water, methanol, ethanol and 1-propanol were selected as solvents to study the effect of increasing carbonchain length on the separation of the catecholamines. In addition, study was made of the effect of the composition of the separation medium in analyses with CE–MS with sheathless nanospray coupling. Separation of catecholamines in urine was tested under optimized non-aqueous conditions.

2. Experimental

2.1. Materials

The catecholamines (Fig. 1), 3,4-dihydroxybenzylamine hydrobromide (DHBA), 4-hydroxy-3-methoxybenzylamine hydrochloride (HMBA), dopamine (3-hydroxytyramine hydrochloride, DA), 3-methoxytyramine (3-methoxy-4-hydroxyphenethylamine hydrochloride, 3MT), [±]-noradrenaline-[+]-hydrogentartrate (NA), DL-normetanephrine (3-methoxybenzenemethanol hydrochloride, NMN), adrenaline (A) and DL-metanephrine (DL-*m*-*O*-methylepinephrine hydrochloride, MN), (98%), were obtained from Sigma–Aldrich (Steinheim, Germany).

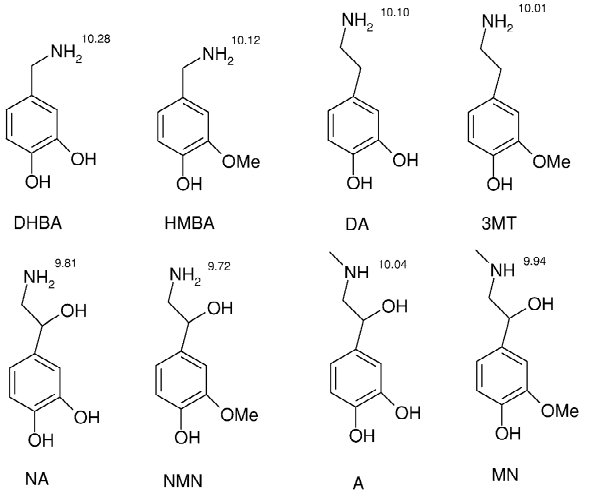

Fig. 1. Structures of catecholamines with pK_a values.

Ammonium acetate was obtained from Sigma–Aldrich; glacial acetic acid from Rathburn (Walkerburn, UK); methanol, ammonium hydroxide and sodium hydroxide solutions from J.T. Baker (Deventer, Netherlands); 1-propanol from Riedel-de Haën (Seelze, Germany) and ethanol (96% v/v) from Primalco (Rajamäki, Finland). Ammonium acetate was dried overnight in a desiccator over silica before use. All reagents were of analytical purity unless otherwise stated and used without further purification. The deionized water was purified with a Milli-Q Plus system (Millipore, Bedford, MA, USA).

2.2. Instruments

2.2.1. CE instruments

The CE equipment used in CE–UV analyses was a P/ACE MDQ instrument (Beckman-Coulter Instruments, Fullerton, CA, USA). The temperatures of the capillary and sample trays were maintained at +25 °C and +15 °C, respectively. Since the temperature of the buffer trays in the instrument is not adjustable, large buffer vials of 30 ml volume were

used to minimize the effect of the change in the electrolyte composition due to vaporization. Hydrodynamic injections were adjusted for the different solvents, to 6 nl, by using the viscosities for the pure solvents reported in the literature (Table 1) in the Hagen–Poiseuille equation:

$$t_{inj} = 128 L_{tot} V\eta / \Delta P\, d^4 \pi \quad (1)$$

where L_{tot} is the total length of the capillary, V injection volume, η viscosity of the solvent, ΔP pressure difference between the capillary ends and d the capillary I.D. [26]. The sample introduction was 3.1–8.9 s with pressures of 0.4–0.5 p.s.i. (1 p.s.i. = 6894.76 Pa). The voltage applied in separation was +20 kV corresponding to an electric field of 500 V/cm. The capillaries, which were 40 cm (effective length 10 cm)×50 μm I.D.×375 μm O.D., were obtained from Composite Metal Services (The Chase, Hallow, Worcestershire, UK). UV detection was performed at 214 nm.

The urine samples were analysed under optimized conditions in ethanol-based electrolyte solution with a P/ACE 2200 series CE instrument (Beckman-Coulter Instruments). The capillary was 37 cm (effective length 30 cm)×50 μm I.D.×375 μm O.D. Temperature was kept at +25 °C. Injection was performed hydrodynamically at 0.5 p.s.i. pressure for 12 s, separation voltage was +30 kV corresponding to a field strength of 810 V/cm and UV detection was at 214 nm.

The CE instrument used in the CE–MS analyses was from Prince Technologies (Emmen, Netherlands). Capillaries were 83 cm×(50 μm I.D.×360 μm O.D.) with a multi-layer conductive coating (US Patent 5788166) on the tip (8±1 μm I.D.) (New Objective, Woburn, MA, USA). To obtain equal sampling volumes with the different solvents, pressure injections were made at 35 mbar (1 bar = 10^5 Pa) for 3.0–10.8 s, varying with the solvent and corresponding to a volume of 3.6 nl (Eq. (1)). Separation of the catecholamines was performed with +30 kV aided with 21 mbar pressure from the capillary inlet. The sample tray and capillary were kept at +15 and +25 °C, respectively.

2.2.2. Mass spectrometer

The MS was an API 300 triple quadrupole instrument (Perkin-Elmer Sciex Instruments, Foster City, CA, USA) with a nanospray ion source (Protana, Odense, Denmark). The capillary voltage was 1900, 1700, 1300 and 2000 V with water, methanol, ethanol and 1-propanol, respectively. The orifice and ring electrode voltages were 20 and 200 V, respectively. The curtain gas was N_2 with flow-rate of 1.08 l/min. Nebulizing gas was not used. The catecholamines were detected by multiple ion monitoring with ions m/z 123, 137, 152, 154, 168, 180 and 184 amu for DHBA, HMBA, NA, DA, 3MT, MN, NMN and A, respectively. The dwell and pause times were 12.7 and 2.0 ms, respectively. The instrument was tuned with m/z 137 using 50 μM of HMBA in the electrolyte solution with +30 kV and 21 mbar pressure.

2.2.3. Capillary conditioning

A fresh fused-silica capillary was used with each change in solvent. For aqueous analyses in CE–UV studies, the capillaries were conditioned by flushing at 20 p.s.i. pressure sequentially with 0.1 M sodium

Table 1
Physical and chemical properties of the solvents (from Refs. [1,7]) together with measured pH/pH*s and currents in the electrolyte solutions used in CE–UV

Solvent	ε	η (mPas)	ε/η	γ (10^{-2} N/m)	pK_{auto}	pK_a(AcH)	pH/pH*	Current[b] (μA)
Water	78.3	0.890	87.9	7.181	14.0	4.7	3.74	23.8
Methanol	32.7	0.545	60.1	2.212	17.2	9.7	5.94	18.3
Ethanol	24.6	1.089	22.6	2.190	18.9	10.3	6.28	3.7
1-Propanol	20.3	1.956	10.4	2.330	19.4	11.3[a]	6.35	0.5

ε, dielectric constant; η, viscosity; γ, surface tension; AcH, acetic acid.
[a] Value in 2-propanol.
[b] At 500 V/cm.

hydroxide and water for 15 min each and with the electrolyte solution for 60 min. For non-aqueous separations the flushing was performed with alcohol for 90 min and with the electrolyte solution for 60 min. Between analyses the capillary was flushed with the electrolyte solution for 2 min.

For aqueous analyses in CE–MS, capillaries were conditioned by flushing at 1400 mbar pressure with 0.1 *M* ammonium hydroxide and water for 15 min each and with the electrolyte solution for 30 min. In non-aqueous separations, capillaries were flused for 60 min with alcohol and with the electrolyte solution for 30 min. Between analyses the capillaries were flushed with the electrolyte solution for 1 min.

2.3. Electrolyte solutions

The electrolyte solutions used in CE–UV analyses were 20 m*M* ammonium acetate in water–acetic acid (99:1, v/v) or in alcohol–acetic acid (99:1, v/v). Alcohol was methanol, ethanol or 1-propanol. The optimized electrolyte solution in urine analyses was 15 m*M* ammonium acetate in ethanol–acetic acid (98.5:1.5, v/v). The electrolyte solutions were filtered through 0.45 μm nylon membranes. The pH measured for organic solutions with a glass electrode using aqueous solutions and aqueous calibration buffers is called apparent pH and is marked as pH*. The pH and pH* values of the electrolyte solutions reported in Table 1 were measured with an inoLab pH meter and a combination electrode (WTW, Weilheim, Germany), which was calibrated with commercial aqueous buffers of pH 4 and 7 (Merck, Darmstadt, Germany).

In CE–MS studies the electrolyte solutions were 10 m*M* ammonium acetate in water–methanol–acetic acid (59.5:40:0.5, v/v/v), 10 m*M* ammonium acetate in ROH–acetic acid (99.5:0.5, v/v) (ROH was methanol or ethanol) and 5 m*M* ammonium acetate in 1-propanol–methanol–acetic acid (89.5:10:0.5, v/v/v). All electrolyte solutions were filtered through 0.2 μm nylon membranes.

2.4. Standard solutions

The 5 m*M* stock solutions of DHBA, HMBA, DA, 3MT, NA, NMN, A and MN were prepared in 0.5% (v/v) acetic acid in water. For aqueous separations the stock solutions were diluted with water to the concentrations desired. For non-aqueous analyses the stock solutions were diluted with methanol to 500 μ*M* concentrations. The final 5 μ*M* mixtures of standards were prepared in the alcohol under investigation. The non-aqueous samples contained 0.1–0.8% water, depending on the mixture. For determination of diffusion coefficients, the standard mixtures were prepared in the electrolyte solutions used in analyses to avoid electromigration dispersion [27,28]. The CE–MS analyses were performed with a mixture of catecholamines at 50 μ*M* concentration each in pure solvent.

2.5. Urine samples

Urine samples from a healthy volunteer were purified by the solid-phase extraction (SPE) method reported earlier [29]. Phosphate buffer (0.5 *M*, pH 7.0) was added to the sample, after which the sample was introduced to a SPE sorbent (Oasis HLB, Waters, Taunton, MA, USA) conditioned with methanol and phosphate buffer. The sorbent was washed with water and the analytes were eluted with methanol. Differing from the reported method, the final dissolution after evaporation was made in ethanol. The urine samples were also analysed after spiking with 5 μ*M* of the catecholamine mixture excluding DHBA.

2.6. Procedures

The analytical parameters discussed below were determined by CE–UV in five replicate analyses, and the results given are mean values. The neutral electroosmotic flow (EOF) marker used in methanol electrolyte was ethanol, and methanol was used with all the other solutions. In determination of the apparent diffusion coefficients of the catecholamines by the stopped migration method, two sequential electrophoresis runs were made under identical experimental conditions. In the first run, the total variance of peak width was determined. In the second run, the electric field was turned off for 2 h and the sample was allowed to diffuse freely in the capillary. The separation voltage was turned on again and the catecholamines passed the detection window. Analyses for the diffusion coefficients were repeated

three times. The pK_a values for catecholamines in aqueous conditions were predicted using the Pallas 1.2 program (CompuDrug Chemistry, Budapest, Hungary).

3. Results and discussion

3.1. Electrophoretic mobility, resolution and selectivity

The electrophoretic mobility of an ion in an electric field correlates with the molecular size and the degree of ionization. The degree of ionization, α, of a conjugated acid of a weak base correlates with the pK_a value of the ion as well as the pH (pH*) of the electrolyte solution according to Ref. [7]:

$$\alpha = 1/\left(1 + 10^{(\mathrm{pH} - \mathrm{p}K_a)}\right) \quad (2)$$

The electroosmotic mobility, μ_{eo}, and the electrophoretic mobility of an analyte, μ_{ep}, can be written as:

$$\mu_{eo} = \varepsilon_0 \varepsilon \zeta / 4\pi\eta \text{ (the Smoluchowski equation):} \quad (3)$$

and

$$\mu_{ep} = 2\varepsilon_0 \varepsilon \zeta_{ion} / 3\eta \quad (4)$$

where ε_0 is the permittivity of free space, ε the dielectric constant, ζ the zeta potential, ζ_{ion} the zeta potential of the ion and η the viscosity of the solvent [8,30]. If a constant zeta potential is assumed, the formation of the electroosmotic flow in the different solvents can be compared in terms of the ε/η ratios of solvents (Table 1).

The electrophoretic mobilities of the catecholamines decrease from water to 1-propanol (Table 2). The decrease is affected by the decrease in dielectric constant and the increase in viscosity of the solvents. In addition, the dissociation of the catecholamines is decreased as the pH (pH*) is increased from water to 1-propanol (Table 1). Our results show that the changes in the electrophoretic mobilities of the catecholamines coincide well with the changes in the ε/η ratio of the solvents (Table 1), and that both the dielectric constants and the viscosities of the solvents have a major effect on the mobility of catecholamines.

The change from water to alcohol decreases the electroosmotic mobility, as also has been noticed earlier [31]. The measured electroosmotic mobilities were 1.60, 0.51, 0.17 and $0.13 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} in water, methanol, ethanol and 1-propanol, respectively. However, the change in electroosmotic mobility in the solvents correlates with the change in the dielectric constants of the solvents and not with the ε/η ratio (Table 1). Therefore, a constant zeta

Table 2
Electrophoretic mobilities (μ_{ep}), plate numbers (N) and diffusion coefficients (D) for the catecholamines in water, methanol, ethanol and 1-propanol

	DHBA	HMBA	DA	3MT	NA	NMN	A	MN
μ_{ep} ($\cdot 10^{-8}$ m^2 V^{-1} s^{-1})								
Water	2.34	2.30	2.24	2.18	2.11	2.09	2.01	2.00
MeOH	1.79	1.91	1.73	1.88	1.59	1.72	1.58	1.70
EtOH	0.55	0.59	0.52	0.56	0.47	0.49	0.44	0.46
1-PrOH	0.14	0.15	0.13	0.13	0.11	0.12	0.10	0.10
N ($\cdot 10^3$/m)								
Water	62	73	70	80	65	66	75	70
MeOH	154	163	160	158	148	163	167	164
EtOH	277	337	320	342	286	328	271	332
1-PrOH	227	181	172	197	235	174	215	167
D ($\cdot 10^{-6}$ cm^2 s^{-1})								
Water	6.21	4.21	6.33	7.71	5.39	5.81	4.54	7.33
MeOH	8.29	7.34	9.66	9.10	6.95	8.17	7.65	9.14
EtOH	3.19	1.97	2.71	2.83	2.19	3.19	2.61	2.00
1-PrOH	1.43	1.16	1.96	0.95	1.71	1.18	0.73	1.08

potential at the capillary wall cannot be assumed for these solvents. The ionization of the silanol groups is affected by the solvent autoprotolysis constant and the pH* of the electrolyte solution. The decrease in the dielectric constant of the solvent causes an increase in the pK_a of the silanol groups, and thus the electroosmotic mobility is decreased [32].

With the short capillaries used in this study, baseline separation of all the catecholamines was not obtained in any solvent tested. However, the resolution in ethanolic separation was promising, and a minor optimization of the separation conditions resulted in baseline separation of seven of the eight catecholamines (Fig. 2). Since resolution between DHBA and 3MT could not be achieved, and since DHBA had been successfully replaced by HMBA as internal standard in earlier studies [29], DHBA was removed from the standard mixture for urine sample analysis. With minor modifications, the separation of all the catecholamines was achieved in aqueous conditions (Fig. 2).

Selectivity is proportional to the differences in the mobilities of the analytes expressed relative to the average mobility [8]. The analysis can be optimized for selectivity by choosing a solvent in which analytes are ionized in an optimally different way [33]. Ionization of the analytes in a solvent is affected by the ability of the solvent to accept or donate protons to the analytes and to solvate the separated species. Both the dielectric constant and the hydrogen bonding ability of the solvent are relevant [34]. Because the pK_a values of the catecholamines do not differ significantly from one another (Fig. 1), differences in their electrophoretic mobilities are not great in any solvent. Since the electroosmotic flow changes the average mobility of the separation system, it affects the selectivity in the different solvents.

The differences in selectivity of the separation in different solvents are observed as changes in the migration order of the analytes. The migration order of the catecholamines was determined in all the electrolytes (Table 3). In all cases, the four catecholamines with no benzylic hydroxy group (DHBA, HMBA, DA, 3MT) migrate first, then the four catecholamines with a benzylic hydroxy group (NMN, NA, MN, A) (Fig. 1), which increases the molecular size and decreases the mobility. In addition, the benzylic hydroxyl group decreases the pK_a value of the amine group by internal hydrogen bonding resulting in decrease in ionization and mobility. The migration order of the catecholamines

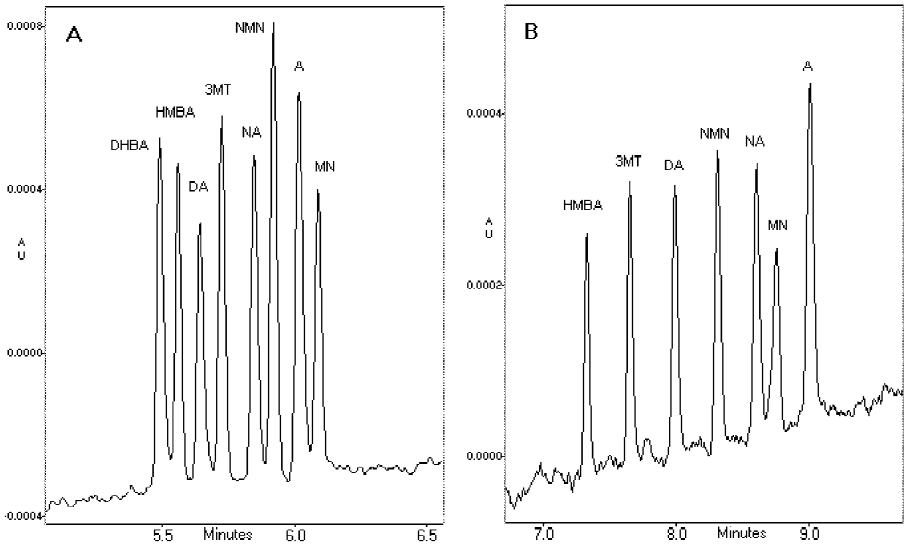

Fig. 2. Catecholamines in optimized aqueous (A) and ethanolic (B) separations. (A) 20 m*M* ammonium acetate in water–acetic acid (99:1, v/v), capillary 50/57 cm, separation +20 kV, UV detection at 214 nm. (B) 15 m*M* ammonium acetate in ethanol–acetic acid (98.5:1.5, v/v), capillary 30/37 cm, separation +30 kV, UV detection at 214 nm.

Table 3
Migration orders of catecholamines in water and alcohols

Solvent	1	2	3	4	5	6	7	8
Water	DHBA	HMBA	DA	3MT	NA	NMN	A	MN
Methanol	HMBA	3MT	DHBA	DA	NMN	MN	NA	A
Ethanol	HMBA	3MT	DHBA	DA	NMN	NA	MN	A
1-Propanol	HMBA	DHBA	3MT	DA	NMN	NA	MN	A

changes dramatically when the solvent in the electrolyte is changed from water to methanol. In ethanol, the migration order of MN and NA is reversed relative to that in methanol, and in 1-propanol, the migration order of 3MT and DHBA is reversed relative to that in ethanol. The changes in migration order reflect the differences in pK_a values and the solvation of the catecholamines in the solvents.

3.2. Separation efficiency and diffusion coefficients

Plate numbers, N, representing the efficiency of the separation can be calculated with the chromatographic equation:

$$N = 5.545(t_m / w_h)^2 \quad (5)$$

where t_m is the migration time and w_h the peak width at half-maximum height. All the factors leading to peak broadening during the migration of the analyte zones decrease the plate numbers and thus the separation efficiency. The total variance of the CE experiment can be taken as the sum of the variances of the several types of dispersion, including longitudinal diffusion, Joule heat, electromigration dispersion, wall adsorption, coiling of the capillary and extracolumn effects (finite plug length of the injection and width of detector aperture) [35]. Of these, the only factor that cannot be avoided is longitudinal dispersion. Several methods have been used to determine the diffusion coefficients, including the ''stopped migration'', the ''low field'', ''graphical'' and ''hydrodynamic velocity'' methods [27,36,37]. In the stopped migration method, peak variance is measured in two separate electrophoretic runs, with and without interruption. To change the variance of the separation from time units to length units, the formula:

$$\sigma_T^2 = 1/8 \ln 2(w_h L_d / t)^2 \quad (6)$$

can be used, where σ_T^2 is the total variance of the peak, w_h the peak-width at half-height, L_d the length of the capillary to the detector and t the migration time in the noninterrupted run [37,38]. The translational diffusion coefficient, D, is then calculated from the Einstein equation:

$$D = \sigma_D^2 / 2\,\Delta T \quad (7)$$

where σ_D^2 is the increase in the variance due to diffusion and ΔT the stopping time.

The plate numbers of catecholamine separation were determined in aqueous and non-aqueous conditions, and found to be higher in non-aqueous conditions (Table 2). In terms of plate numbers, the most efficient separation was achieved in ethanol, which was about six times as efficient as the separation in water. The diffusion coefficients for the catecholamines were determined in the aqueous and non-aqueous separation media by the stopped migration method (Table 2). The diffusion correlates inversely with the viscosity of the solvent (Table 1). Although the diffusion was lower in 1-propanol, the plate numbers were not as high as in ethanol because of the longer migration time. The plate numbers of the methanolic and aqueous separations were considerably lower than those for ethanol and 1-propanol owing to the higher diffusion in the capillary.

3.3. Stokes radius and effective charge

Hydrodynamic (Stokes) radius and effective charge of the analytes can be calculated from diffusion coefficients and electrophoretic mobilities [36,39]. Even though the Stokes equation is valid for spherical molecules larger than cyclohexaamulose in a hydrodynamic continuum of viscosity [40], the

hydrodynamic radius, r, for the catecholamines was calculated using the Stokes–Einstein relation:

$$r = kT/D6\pi\eta \quad (8)$$

where k is the Boltzman constant and T is the absolute temperature. The hydrodynamic radius of the catecholamines were on average 4.2, 4.8, 7.9 and 9.5 Å in water, methanol, ethanol and 1-propanol, respectively. Thus, the hydrodynamic sizes of the catecholamines are increased as the water molecules in the hydration sphere are replaced by the bulky alcohol molecules [41]. This decreases the electrophoretic mobility of the catecholamines (Table 2).

The effective charge, Z, for the catecholamines in different electrolyte solutions was calculated from the formula:

$$Z = \mu_{ep}kT/De \quad (9)$$

where e is the charge of an electron [36]. The effective charges of the catecholamines were on average 0.96, 0.54, 0.52 and 0.26 in water, methanol, ethanol and 1-propanol, respectively. This reflects the decrease in dissociation of the catecholamines, which leads to a decrease in the electrophoretic mobility (Table 2).

3.4. CE–nanospray-MS

The aqueous and non-aqueous separation conditions were applied in CE–MS analysis with sheathless nanospray coupling. The surface tension of water is much greater than that of the alcohols (Table 1). It was found that neither a stable spray nor ionization of the catecholamines could be obtained with 100% aqueous electrolyte solution. An addition of 40% methanol to the aqueous electrolyte solution was needed to decrease surface tension and obtain succesful MS performance (Fig. 3). The stability of the electrospray process was also found to improve with a decrease in the electrolyte concentration to 10 m*M*. Good performance with methanol- and ethanol-based electrolyte solutions could be obtained without any other modifications. However, the viscosity of 1-propanol had to be reduced with 10% methanol to obtain a stable spray. The relatively poor solubility of ammonium acetate in 1-propanol caused a problem in the electrospray process and the electrolyte concentration was further lowered to 5 m*M*.

In this study, suitable ESI voltages varied between 1300 and 2000 V, depending on the solvent and the capillary. Higher voltages were needed for analyses in water and 1-propanol owing to the high surface tension and viscosity, respectively. Methanol- and ethanol-based electrolyte solutions were successfully sprayed with lower voltages. Electrical discharge from the capillary tip easily occurrs with aqueous electrolyte solution, as was observed in the sparks at the tip of the capillary. When the CE separation voltage was on, discharge occurred even with zero ESI voltage. No discharge occurred when the separation voltage was turned off. No electrical discharges were observed with the non-aqueous electrolyte solutions because the current in the capillary was lower. Chang et al. [19], too, note that with aqueous electrolyte solutions, discharge can be avoided by lowering the conductivity of the solution.

3.5. Limits of detection

The limits of detection (LODs) of the catecholamines were determined in aqueous and non-aqueous solvents at signal-to-noise ratio (S/N) of 3. No appreciable differences were observed in the values obtained in aqueous and non-aqueous analyses with UV detection (Table 4). Since ca. 6 nl injection volume was used in the analyses, the limits of detection, from 0.5 to 2.4 μ*M*, in the different solvents correspond to 3–14 fmol.

In CE–MS studies, the sensitivities in the different solvents were compared by an evaluation of signal heights. Only minor differences in sensitivity between the solvents were observed. Despite some dispersion, methanol gave the best sensitivity for most of the catecholamines. This could be explained by the volatility of methanol. LODs of catecholamines in nanospray analyses in ethanol were determined to range from 0.5 to 1.3 μ*M* (Table 4). LODs for the catecholamines obtained in aqueous CE separation with sheath liquid coupling to MS ranged from 1.1 to 4.1 μ*M* [14]. Thus, the sheathless nanospray method is more sensitive, although the difference is not very great.

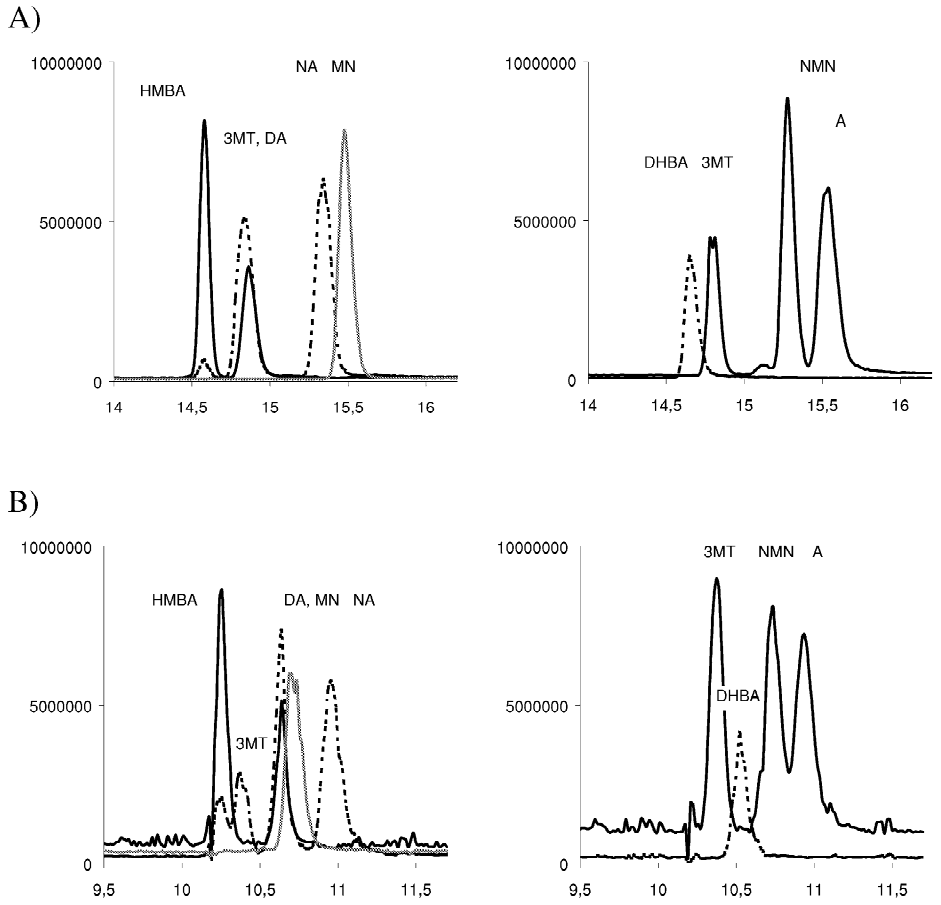

Fig. 3. Separation of catecholamines with capillary electrophoresis–nanospray mass spectrometry in aqueous (A) and methanolic (B) conditions. (A) 10 m*M* ammonium acetate in water–methanol–acetic acid (59.5:40:0.5, v/v/v), capillary 83 cm, separation +30 kV and 21 mbar. (B) 10 m*M* ammonium acetate in methanol–acetic acid (99.5:0.5, v/v), capillary 83 cm, separation +30 kV and 21 mbar.

Table 4
Limits of detection for the catecholamines (μM)

Solvent	DHBA	HMBA	DA	3MT	NA	NMN	A	MN
CE–UV								
Water	0.92	0.86	1.28	0.69	1.46	1.08	0.82	1.92
Methanol	0.76	0.50	1.55	0.92	1.51	0.91	1.15	0.76
Ethanol	0.85	0.76	1.14	1.05	0.86	0.94	0.67	1.43
1-Propanol	1.72	0.89	2.39	1.56	2.15	1.29	1.96	1.83
CE–MS								
Ethanol[a]	0.48	0.53	1.30	0.78	0.83	0.89	1.27	1.05
Water[b]	–	4.12	1.90	1.14	–	3.17	–	3.15

–, not determined.
[a] Sheathless nanospray coupling.
[b] Coaxial sheath liquid coupling [14].

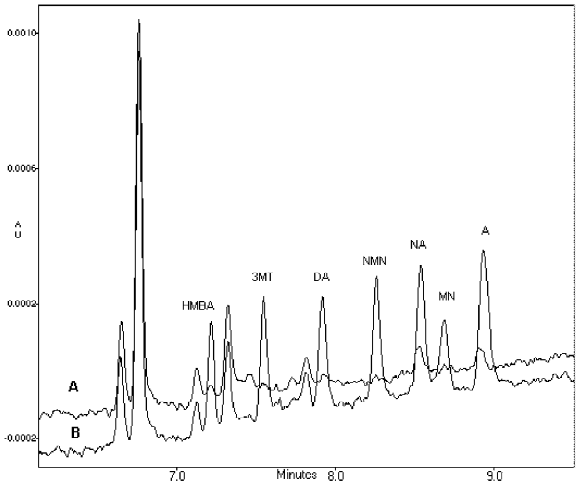

Fig. 4. Analysis of urine sample in ethanolic conditions with CE–UV. Urine sample (A) and spiked sample (B). Conditions as in Fig. 2b.

3.6. Analysis of urine samples by non-aqueous CE and CE–MS

The applicability of non-aqueous CE and CE–MS to the analysis of catecholamines in urine matrix was studied with spiked urine samples under optimized ethanolic conditions. With the SPE technique we have developed [29], a very clean matrix for UV detection was obtained (Fig. 4). However, the number of matrix compounds detected may increase by the inclusion of the hydrolysis step. In aqueous separations, an addition of diisopropylamine to the electrolyte solution was required for adequate resolution between catecholamines and matrix compounds, and this extended the analysis time to 18 min [29]. Our results in ethanol suggest that a faster analysis of catecholamines in urine can be obtained in non-aqueous conditions. The same spiked urine samples were also analysed by CE–MS in ethanolic conditions. The analyses were performed in multiple ion monitoring mode and no interfering peaks in the electropherograms were observed. However, the analysis time was considerably longer in ethanolic than in aqueous CE–MS separation.

4. Conclusions

Comparison was made of aqueous and alcoholic non-aqueous systems for the separation of catecholamines by CE and CE–MS. The electrophoretic mobilities of the catecholamines decreased from water to 1-propanol and correlated with the ratio of dielectric constant to viscosity of the solvent. Also the electroosmotic mobilities decreased from water to 1-propanol, and correlated with the dielectric constants of the solvents. The influence of the solvent on selectivity was evident as changes in the migration order of the catecholamines. With use of identical separation conditions in different solvents, the best resolution was achieved in ethanol. In terms of plate numbers, all the non-aqueous separations were more efficient than the aqueous one. Diffusion constants were determined for the catecholamines, and the largest constants were measured in methanol and the smallest in 1-propanol. This correlated with the viscosities of the solvents. No great differences in sensitivity were observed between the solvents. In study of the ionization efficiency in nanospray MS, the most sensitive analysis was achieved in methanol, even though there was some dispersion between the catecholamines. CE analysis of SPE-purified urine samples using the optimized ethanolic conditions showed good separation of the catecholamines for spiked samples. Also, analyses of urine samples in ethanol with CE–MS were performed, and no interfering matrix peaks were observed. The sensitivity of the non-aqueous nanospray method was only slightly better than that of the aqueous sheath liquid method. In conclusion, the best non-aqueous analysis of the catecholamines was achieved in ethanol.

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