

Aqueous and non-aqueous capillary electrophoresis of polar drugs

Heli Sirén,^{*ab} Tarja Hiissa^{ab} and Yuan Min^b

^a VTT Chemical Technology, P.O.Box 1401, FIN-02044 VTT, Finland.

E-mail: heli.siren@vtt.fi

^b Laboratory of Analytical Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, FIN-00014 Finland

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Aqueous and non-aqueous capillary electrophoresis (CE) systems were employed for the separation of basic and acidic polar drugs using capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MECC) techniques. This work was performed to determine the differences in selectivity, after chemical adjustment of the media, of the separation of closely related drugs (by measurement of separation efficiency, resolution and detection) in aqueous and non-aqueous solvents. Various electrolytes were tested in aqueous and organic solvent media. The best separation in aqueous CZE was obtained in 25 mM phosphate solution at pH 7.3. In aqueous MECC, the best performance was obtained in pH 10.5 buffer containing glycine and dodecyl sulfate. Non-aqueous CE separations were performed in electrolyte mixtures containing methanol–acetonitrile, ethanol–acetonitrile and propan-2-ol–acetonitrile (50:49, v/v) modified by ammonium acetate and water-free glacial acetic acid. Acetone was also used instead of acetonitrile in the separation media. In non-aqueous CE systems, the effect of water in the non-aqueous sample and as part of the electrolyte solution played a significant role in the separation of polar anionic drugs.

1. Introduction

Capillary electrophoresis (CE) has been applied to various analyses of pharmaceuticals and their mixtures.¹ The flexible adjustment of the many chemical, physical and instrumental parameters allows stable performance to be achieved in aqueous CE, which has been used in most published studies. The pH of the aqueous electrolyte solution is mainly chosen to influence the dissociation of the analytes. In organic solvents, it is possible to separate the analytes as in aqueous solutions. Most analytes dissociate in non-aqueous solutions as in water, and water as the background electrolyte (BGE) can be completely replaced by organic solvents.² However, the solubility and mobility of analytes in different BGE vary, depending on the dissociation of the analytes and the ionic strength of sample matrices which significantly influences migration.

In capillary zone electrophoresis (CZE), the separation power in aqueous solutions can be affected by various factors, *e.g.*, pH, nature of the buffering ion or organic modifiers in the electrolyte.³ In micellar electrokinetic chromatography (MECC), the surfactants and other additives in the BGE provide more separation power in the electrophoresis medium. In addition, there are many chemical methods available to improve peak resolution and component identification.³ Increased selectivity may be achieved by the addition of an extra reagent to the electrolyte, such as tetraborate, leading to complexation to the analyte or analyte-to-additive reactions. Considerable improvements in separation selectivity, in particular of cationic compounds with similar structures, have been obtained by the use of tensides or cyclodextrins as additives in electrolyte solvents.^{4,5}

When separating closely structurally related polar compounds, as in this study, the effects of chemical parameters on selectivity are restricted, because the pK_a values of similar analytes will be of a similar magnitude.³ Therefore, changing the pH to retain full protonation of the analytes may have a unilateral effect for all the analytes involved. The CZE

separation can be optimized by choosing a pH value of the electrolyte solution that lies between the pK_a values of the analytes. However, the pH should not be too low. When low pH values are used, the electrophoretic mobilities of amine-containing pharmaceuticals, in this study stimulants and morphine analogues, are slow. On the other hand, at high pH, they migrate with high speed, but with poor resolution. Compounds containing sulfonic and carboxylic acid groups, in this study diuretics and weak acids, will separate in a strong polar medium with polar interactions between the analytes and the solvent molecules. The use of the MECC technique is another way to affect selectivity, being dependent on the interactions of the analytes with micelles and the BGE.⁶ In this case, the polarity of the separation medium does not play as significant a role as in CZE.

Hydrophobic interactions are weak in organic solvents. Therefore, separation in a non-aqueous medium (non-aqueous CE) involves interactions between the organic solvent molecules and the analytes caused by electrostatic ion–dipole forces.⁷ Methanol and acetonitrile, in particular have been commonly used in electrolyte solutions.^{8–10} They are mainly favoured for their low absorption cut-off level against a UV detector, but also because they dissolve both cationic and anionic pharmaceuticals. Chemical differences, which affect the viscosity and relative permittivity of organic modifiers, influence the electrophoretic behaviour of drugs. Possibly the most attractive feature of organic solvents is that their physical and chemical properties are very different from each other and from water, allowing the important characteristics of separation to be controlled on a wider scale than with water alone.²

The migration time and peak resolution will increase as the viscosity of the organic modifier increases and the relative permittivity decreases. However, acetonitrile and acetone have a minor influence on electro-osmosis, such that anionic analytes will migrate rapidly. Usually, propan-2-ol increases the electrophoretic mobility, but unfortunately without an increase in peak

resolution. The addition of methanol may sometimes be inadvisable, since it increases the noise.^{8–11}

This study describes a comparison of aqueous and non-aqueous CE in the simultaneous separation of some closely related morphine analogues, stimulants and diuretics. The aqueous BGE was modified with buffering reagents or selective reagents. Non-aqueous systems were controlled with chemical reagent modifications to obtain an electric field in the capillary. Modification of alcoholic electrolyte solution mixtures was made with ammonium acetate and glacial acetic acid. The choice of BGE was made on the basis of the pK_a values of the drugs in order to retain full dissociation for all analytes, except hydrochlorothiazide.

2. Experimental

2.1. Materials

The chemicals used were methadone hydrochloride, dextropropoxyphene, levorphanol, hydrocodone bitartrate, pentazocine, dipipanone hydrochloride, codeine hydrochloride, ethylmorphine hydrochloride, amphetamine hydrochloride, morphine hydrochloride, dextromoramide bitartrate and dihydrocodeine bitartrate anhydride, which were donated to our laboratory by the Crime Laboratory of the Finnish National Bureau of Investigation (Vantaa, Finland). Ethacrynic acid, D,L-ephedrine, hydrochlorothiazide, bumetanide, bendroflumethiazide, clopamide, trichlormethiazide, chlorothiazide, chlorthalidone, dichlorphenamide, benzoic acid, 1,2-phenylenediacetic acid, acetazolamide, 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS), *N*-2-hydroxyethylpiperazine-*N'*-2-methanesulfonic acid (HEPES) and 3-(*N*-morpholino)propanoic acid (MOPS) were obtained from Sigma (St. Louis, MO, USA). *meso*-2,3-Diphenylsuccinic acid was obtained from TCI Ltd. (Tokyo, Japan). Glycine, sodium hydroxide, potassium hydroxide, phthalic acid, acetonitrile (HPLC grade), disodium hydrogen phosphate, sodium dihydrogen phosphate, ammonium acetate, acetic acid (water free), propan-2-ol (HPLC grade) and methanol (HPLC grade) were purchased from Merck Ltd. (Darmstadt, Germany), and sodium dodecyl sulfate (SDS) from BDH Chemicals Ltd. (Poole, Dorset, UK). Ethanol (AAS grade) was purchased from Primalco Ltd. (Helsinki, Finland). All reagents were of analytical grade and were used as received. Water was distilled, deionized (conductivity, $0.05 \mu\text{S cm}^{-1}$) and filtered ($0.45 \mu\text{m}$ pore size of the membrane) before use.

2.2. Instrumentation

Three commercial CE instruments were used. For the Beckman P/ACE 2050 (Beckman, Bellefonte, PA, USA), UV detection was at 220 and 214 nm in aqueous and non-aqueous separations, respectively. The capillaries ($50 \mu\text{m}$ id) were from 23 to 87 cm (Composite Metal Services, Hallow, Worcestershire, UK), with separation lengths of 16 to 80 cm, respectively. The separation voltages in aqueous and organic solvent media were +22 kV and +30 kV, respectively. The samples were injected by application of $3.447 \times 10^3 \text{ N m}^{-2}$ of pressure for 5 s. The temperature was kept at +22 °C with a liquid coolant system. The capillaries were rinsed with buffer for 3 min before each run.

The HP 3D CE system (Hewlett-Packard, Avondale, PA, USA) contained a diode-array detector (DAD) and an air cooling system for the capillary. UV detection was at 214 nm with a range of 10 nm. A reference wavelength was not used in detection. The separation capillaries were 32 cm and 50 cm with $50 \mu\text{m}$ id (separation lengths of 23.5 cm and 58.5 cm, respectively) in non-aqueous CE. The samples were injected using $3 \times 10^3 \text{ N m}^{-2}$ of pressure for 5 s. MECC separations

were performed with the HP 3D CE system with 68.5 cm capillaries (separation length, 60 cm).

When screening the analytes in non-aqueous media and estimating the most convenient injection method for the studies, a Quanta 4000 CE system (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) with a 214 nm UV filter was used. All experiments were carried out at ambient temperature. Samples were injected hydrostatically for 10 s by lifting the inlet end of the capillary to 10 cm height compared to the outlet end.

The absolute viscosity of the electrolyte solutions at +22 °C was calculated from the experimental viscosity and density values. The equipment used for the measurements included a 0.003 S.I.L. type viscometer (Gallenkamp, London, UK) and a bubble-shaped pycnometer (laboratory made; volume, 10.7 mL). The conductivity of the solutions at +22 °C was measured with Radiometer Model CDM 3 apparatus with a 1 cm wide conductivity cell (Radiometer CDC 304). The pH values of the aqueous electrolyte solutions were measured with a PHM220 Lab pH meter and a glass electrode (Radiometer, Copenhagen, Denmark) at +22 °C.

2.3. Aqueous electrolyte solutions

The buffers were chosen depending on their interactions with the analytes, their pK_a values and their buffer capacity. Therefore, five buffers were used to optimize the CZE separations: 30 mM CAPS (pH 10.6), 30 mM HEPES (pH 7.0), 30 mM MOPS (pH 6.5), 20–40 mM sodium dihydrogen phosphate–disodium hydrogen phosphate (pH 7.0) and 30 mM disodium hydrogen phosphate (pH 6.5–8.0). In the CZE technique, after laboratory optimization, the final BGE solution was chosen to be 25 mM sodium dihydrogen phosphate with the pH adjusted to 7.3 using 25 mM disodium hydrogen phosphate. The prepared buffers were filtered through $0.45 \mu\text{m}$ filters (Millipore, Molsheim, France) degassed by an ultrasonicator before use. All the above-mentioned organic buffers were tested in the hope of obtaining better selectivity, since they gave lower currents than the phosphate buffer. Low currents are preferred to minimize the heat expansion generated by the high voltage used during the separation. Unfortunately, in our studies, they did not give better resolution due to their different buffer capacity ranges, which influenced the dissociation of the polar drugs. The physicochemical values of the BGE solutions are listed in Table 1.

In the MECC technique, the best separation efficiency was obtained with an electrolyte solution containing 50 mM glycine and 50 mM SDS. In addition, CAPS, HEPES and MOPS modified by SDS were tested. The pH of the electrolyte solution (Table 1) of the final tests in MECC was adjusted to 10.5 with 0.1 M NaOH, after which the BGE was pretreated as the CZE solution.

2.4. Non-aqueous electrolyte solutions

The organic BGE solutions were chosen by first varying methanol–acetonitrile contents from 60:39 (v/v) to 40:59 (v/v). All solutions contained 1% acetic acid. In the solutions, the ammonium acetate concentration was between 5 and 20 mM. Finally, 20 mM ammonium acetate and 1 M acetic acid in acetonitrile–alcohol mixtures (50:49, v/v) were chosen as the non-aqueous media giving the best separations for the analytes. The alcohols used in the studies were methanol, ethanol and propan-2-ol. Due to the lack of reactivity of the solutions containing acetonitrile or acetone, the BGE solvents were freshly used. The effects of salt concentration and the amount of water in the sample were tested in non-aqueous CE. The conductance, density and viscosity of the solutions were

measured at +25 °C according to the procedures described elsewhere.⁹

2.5. Conditioning of the capillaries

Commercial, silica capillaries were washed before use with 0.1 M NaOH, rinsed with purified water and cleaned with the aqueous electrolyte solution for 20 min, 10 min and 20 min, respectively. One blank run (no injection) was needed before the first analysis. When separations in organic solvents were made, the capillary was conditioned with methanol for 30 min at high pressure. After rinsing, the voltage of +30 kV was switched on and maintained for 30 min.

2.6. Sample mixtures

The standard compounds were diluted with HPLC grade methanol to obtain 1000 mg L⁻¹ stock solutions of each. The final sample mixtures for capillary electrophoretic studies were prepared from the stock solutions at the concentrations needed. For aqueous separations, the samples contained HPLC grade methanol (10%, v/v), 0.1 M NaOH (10%, v/v) and water. For non-aqueous separations, the samples were only diluted with the alcohol needed in the studies. Tests to estimate the effects of water content in the sample were made with samples containing 1, 2, 5, 7, 10, 15, 20, 25, 30, 35 or 40% (v/v) of purified water.

2.7. Calculations

Resolution was defined as the extent of separation between two compounds and was calculated as

$$R = 2(t_2 - t_1)/(w_1 + w_2) \quad (1)$$

where t_1 and t_2 are the migration times of the drugs and w_1 and w_2 their peak widths. Plate numbers were calculated using the formula

$$N = 5.545(\mu_{1/2}/w_{1/2})^2 \quad (2)$$

where $\mu_{1/2}$ is the theoretical electrophoretic mobility of the analyte (amphetamine) at the time of peak maximum corrected with the electro-osmotic flow and $w_{1/2}$ is the peak width at half height of the peak seen in the electropherogram.

3. Results and discussion

The compounds investigated in the studies were used in different mixtures depending on the separation performance of the system. The analytes formed two drug groups: cationic and anionic. The wide polar range and the differences between the dissociation of dissimilar groups made it possible to manipulate

the separation by selective pH adjustment and to optimize it by modification of the BGE. Chemical modification of the electrolyte solutions provided rapid analyses and reliable identification of the drugs. Twelve drugs could be simultaneously separated by MECC in a single run under the same micellar conditions. The other techniques were used for both separation and fractionation of the drugs on the basis of their charges.

3.1. Separation of analytes

Various aqueous and non-aqueous solvent systems were tested (Figs. 1–4). The electropherograms showed that the selectivity of the cationic analytes was almost identical in aqueous CZE and non-aqueous CE (alcohol–acetonitrile based BGE mixtures). However, the cationic drugs migrated in a different order when aqueous CZE rather than non-aqueous CE was used. This was probably based on the molar masses of the drugs and their pK_a values. Anionic drugs migrated according to their size per charge ratios in aqueous solvent, but in non-aqueous solvents their velocities were totally influenced by the conductance, the viscosity and autoprotolysis of the organic alcohol solutions.¹² The physical parameters had a particular effect on the selectivity of probenecid (pK_a 3.4), ethacrynic acid (pK_a 3.5) and chlorothiazide (pK_a 6.7 and 9.5). The studies showed that, in methanol–acetonitrile, the migration order of the anionic drug was chlorothiazide < probenecid < ethacrynic acid; in ethanol–acetonitrile, probenecid < chlorothiazide < ethacrynic acid; and, in 2-propanol–acetonitrile, probenecid < ethacrynic acid < chlorothiazide. The migration order was the same in the presence of acetone in methanolic BGE solution. However, acetone as a part of the other alcohol mixtures changed the selectivity: in ethanolic solution, the order was chlorothiazide < probenecid < ethacrynic acid and, in propanolic solution, probenecid < ethacrynic acid < chlorothiazide. The reason for this behaviour has been suggested to be solvation, which in methanol is favoured due to the formation of hydrogen bonds. The strength of intermolecular hydrogen bonding between two methanol molecules can be decreased by the addition of an organic solvent modifier to the mixture.^{3,12} Acetonitrile has a dipolar non-hydroxylic or non-hydrogen bond donor group,^{12,13} which is why it has been shown to be a good choice as a modifier in non-aqueous solvents. However, it has been suggested that a cyano group is converted to a carboxylic group at pH values above 5,¹⁴ which obviously changes the chemical behaviour of acetonitrile-containing separation medium. Under basic conditions, it can be dissociated and its functionality will influence the electrophoretic mobility of the analytes.

3.2. Resolution

The comparison of the separation efficiencies, *e.g.*, resolution, selectivity and reproducibility, between the aqueous buffers and

Table 1 Physicochemical parameters of the BGE solutions (22 °C)

BGE solution	Conductance $\kappa/\mu\text{S cm}^{-1}$	Density $\rho/\text{g cm}^{-3}$	Kinematic viscosity $\eta_{\text{kin}}/\text{cSt}$	Absolute viscosity $\eta_{\text{abs}}/\text{cP}$
25 mM phosphate, pH 7.3	3200	1.000	0.966	0.966
50 mM glycine–50 mM SDS, pH 10.5	4170	1.050	1.019	1.071
Methanol–acetonitrile–acetic acid ^a	1504	0.789	0.439	0.346
Ethanol–acetonitrile–acetic acid ^a	597	0.786	0.561	0.441
Propan-2-ol–acetonitrile–acetic acid ^a	244	0.782	0.628	0.491
Methanol–acetone–acetic acid ^a	880	0.807	0.496	0.400
Ethanol–acetonitrile–acetic acid ^a	230	0.793	0.577	0.457
Propan-2-ol–acetone–acetic acid ^a	43	0.788	0.645	0.508

^a With 20 mM ammonium acetate; volume ratios 50:49:1 (v/v/v).

water-free organic solvents was tested using identical sample mixtures. However, due to the difficulties of obtaining equal ionic strengths and pH values in the BGE solutions, the chemical comparison of the selectivity in the BGE was performed using absolute mobility values of the individual analytes. There were practical difficulties in the adjustment of the chemical parameters for this comparison, as when the viscosity of the non-aqueous medium was changed, the selectivity was also changed. The chemical parameters (ionic strength, concentration) did not influence the non-aqueous CE separation as much as aqueous CZE and MECC. The resolution was affected by currents in the BGE solutions: in methanol, ethanol and propan-2-ol mixed with acetonitrile these were 18.0 μA , 6.5 μA and 2.2 μA , respectively, but in mixtures with acetone they were 9.0 μA , 2.8 μA and 0.6 μA , respectively. In CZE and MECC, the currents were 34 μA and 53 μA , respectively. The plate numbers (N) of amphetamine in the BGE solutions show the efficiency of the techniques: in CZE and MECC, the values are 890 000 m^{-1} and 1 400 000 m^{-1} , respectively; in non-aqueous CE systems, they are 1 150 000 m^{-1} , 1 730 000 m^{-1} and 1 599 000 m^{-1} in methanol, ethanol

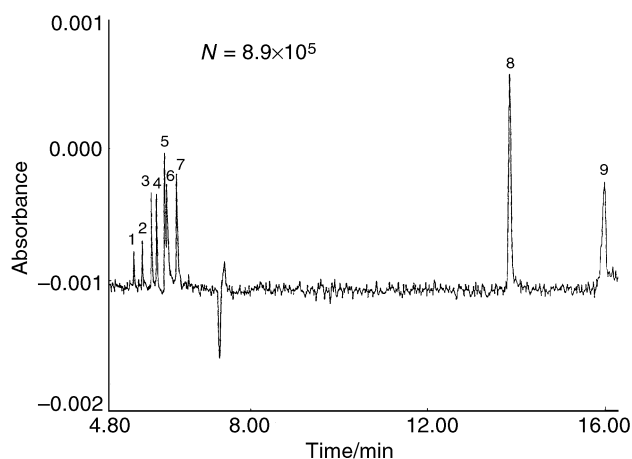


Fig. 1 CZE separation of narcotics and ephedrine with internal standards in 25 mM phosphate buffer at pH 7.3. Compounds in migration order: (1) amphetamine, (2) ephedrine, (3) levorphanol, (4) dextropropoxyphene, (5) hydrocodone, (6) ethylmorphine and (7) dextromoramide with internal marker standards migrating as the two late analytes (8) benzoic acid and (9) 1,2-phenylenediacetic acid. Sample concentrations: 22 $\mu\text{g mL}^{-1}$ of 1–4, 6 and 7, 13 $\mu\text{g mL}^{-1}$ of 5 and 12 $\mu\text{g mL}^{-1}$ of 8 and 9. Instrument: Beckman P/ACE 2050. Capillary: total length, 77 cm (separation length, 70 cm).

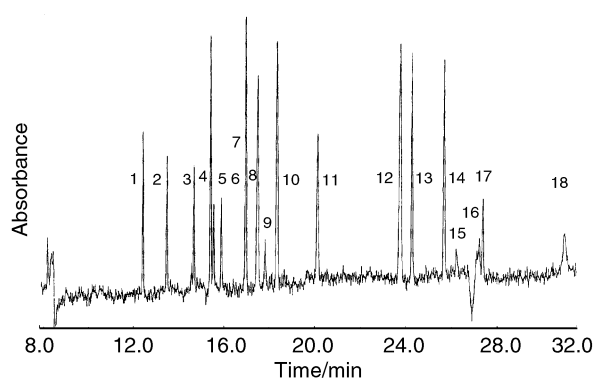


Fig. 2 Separation of diuretics, probenecid, stimulants and morphine analogues with internal marker standards by MECC in 50 mM glycine–50 mM SDS at pH 10.5. Compounds in elution order: (1) clopamide, (2) probenecid, (3) bumetanide, (4) bendroflumethiazide, (5) ethacrynic acid, (6) trichlormethiazide, (7) chlorthalidone, (8) benzoic acid, (9) hydrochlorothiazide, (10) *meso*-2,3-diphenylsuccinic acid, (11) ephedrine, (12) dichlorphenamide, (13) codeine, (14) ethylmorphine, (15) amphetamine, (16) pentazocine, (17) methadone and (18) acetazolamide. Sample amount: 12 $\mu\text{g mL}^{-1}$. Instrument: HP 3D CE system. Capillary: total length, 68.5 cm (separation length, 60 cm).

and propan-2-ol media, respectively; when acetone was used the N -values were decreased, being 1 309 000 m^{-1} , 1 048 000 m^{-1} and 706 000 m^{-1} in methanol, ethanol and propan-2-ol media, respectively. Enolization of acetone changed the chemistry of the BGE.

3.3. Selectivity

In aqueous solutions, the selectivity was easily manipulated by changes in buffer concentrations and other chemical parameters. The effect of instrumental parameters was not so significant, however. The migration times were considerably shorter in CZE than in MECC with acceptable resolution (Figs. 1 and 2). In the MECC technique, the selectivity of the amine-containing drugs could be influenced. This was because, at pH 10.6, the amine-containing drugs were not dissociated or became negatively charged¹⁵ and micelles were needed to

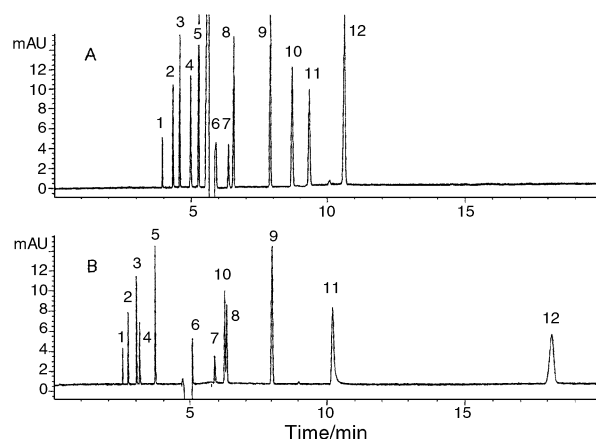


Fig. 3 Separation of diuretics, probenecid, stimulants and morphine analogues with internal marker standards in non-aqueous CE. Compounds in migration order: (1) amphetamine, (2) ephedrine, (3) dextromoramide, (4) levorphanol, (5) morphine, (6) hydrochlorothiazide, (7) benzoic acid, (8) chlorothiazide, (9) *meso*-2,3-diphenylsuccinic acid, (10) 1,2-phenylenediacetic acid, (11) probenecid and (12) ethacrynic acid. Concentrations of the analytes 5 $\mu\text{g mL}^{-1}$. Alcohol–acetonitrile solution (A) ethanol, (B) methanol. Instrument: HP 3D CE system. Capillary: total length, 58.5 cm (separation length, 50 cm).

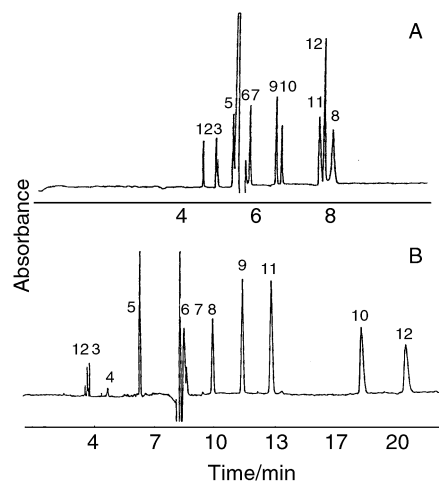


Fig. 4 Effect of water content in the sample on the separation of the analytes. Compounds in migration order: (1) amphetamine, (2) ephedrine, (3) dextromoramide, (4) levorphanol, (5) morphine, (6) hydrochlorothiazide, (7) benzoic acid, (8) chlorothiazide, (9) *meso*-2,3-diphenylsuccinic acid, (10) 1,2-phenylenediacetic acid, (11) probenecid and (12) ethacrynic acid. Concentrations of the analytes 1.5 $\mu\text{g mL}^{-1}$. A, Propan-2-ol–acetonitrile–acetate solution and B, methanol–acetone–acetate solution. Instrument: HP 3D CE system. Capillary: total length, 58.5 cm (separation length, 50 cm).

separate them. The repeatability of the analyses in both methods was excellent, with relative standard deviations for the absolute migration times of 1% (Tables 2 and 3).

In non-aqueous CE separations, the repeatability of the analyses was not as good as in aqueous CZE or MECC. The reason for the poor results was the pH* value (the apparent pH in non-aqueous solution) of the BGE, which was between 6 and 8. It was not easy to further optimize the separation in non-aqueous solution by controlling the time of electro-osmosis, pH or changing the ionic strength in the sample, as can be done in an aqueous medium, because of the lack of indicators to adjust the pH* value. Acetonitrile could not be replaced totally by acetone due to the reactivity of acetone. Acetone in the BGE made the analytes migrate with the mobility of electroosmosis decreasing the peak resolution. The work showed that organic BGE solvents could only be used for separations when the samples did not contain a large amount of water.

3.4. Additives in separation solutions

In aqueous solutions, the dissociation of amphetamine, ephedrine and morphine analogues was strongly dependent on the buffer pH and on the ionic strength of the BGE. Due to its charge in basic solution and therefore its electrophoretic

Table 2 Repeatability of the CZE and MECC analyses in terms of absolute migration times with nine replicates

Drug	CZE/ MECC	Migration time/min	<i>s</i>	RSD (%)
Amphetamine	CZE	5.42	0.009	0.16
	MECC	25.4	0.49	1.92
Morphine	CZE	nd		
	MECC	14.5	0.098	0.67
Ephedrine	CZE	5.61	0.009	0.15
	MECC	25.6	0.173	0.67
Levorphanol	CZE	5.82	0.009	0.15
	MECC	nd		
Dextropropoxyphene	CZE	5.94	0.010	0.17
	MECC	29.7	0.093	0.31
Hydrocodone	CZE	6.12	0.010	0.16
	MECC	23.8	0.26	1.10
Ethylmorphine	CZE	6.18	0.010	0.16
	MECC	25.2	0.41	1.63
Dextromoramide	CZE	6.39	0.010	0.15
	MECC	nd		
Dihydrocodeine	CZE	nd		
	MECC	26.5	0.094	0.36
Dipipanone	CZE	6.53	0.010	0.30
	MECC	29.6	0.104	0.35
Clopamide	CZE	nd		
	MECC	12.24	0.04	0.45
Probenecid	CZE	nd		
	MECC	13.42	0.12	0.96
Bumetanide	CZE	nd		
	MECC	14.34	0.19	1.35
Bendroflumethiazide	CZE	nd		
	MECC	15.27	0.09	0.61
Ethacrynic acid	CZE	nd		
	MECC	15.54	0.08	0.50
Trichlormethiazide	CZE	nd		
	MECC	15.73	0.13	0.82
Chlorthalidone	CZE	nd		
	MECC	17.17	0.13	0.75
Hydrochlorothiazide	CZE	nd		
	MECC	18.85	0.23	1.23
Dichlorphenamide	CZE	nd		
	MECC	23.77	0.30	1.28
Pentazocine	CZE	nd		
	MECC	26.68	0.46	1.72
Methadone	CZE	nd		
	MECC	26.89	0.44	1.62
Acetazolamide	CZE	nd		
	MECC	30.76	0.46	1.51

repulsion, the electrolyte solution did not influence the separation of anionic compounds, which migrated in a similar order in a BGE of equal pH without surfactant.

Surfactants do not form micelles in most non-aqueous solvents because the hydrophobic interactions are too weak³ for surfactant molecules to aggregate. In alcohol-acetonitrile media, the addition of ammonium acetate improved the resolution of the analytes. In the non-aqueous CE system, the conductance of the BGE solution played a role in the separation of the cationic analytes (Figs. 4–6). The higher the conductivity value (Table 1), the better the resolution (Figs. 3–5). However, for the separation of anionic compounds, the selectivity was best in a solution of acetonitrile and alcohol with low conductivity. It has been observed previously that the migration time and peak resolution increase as the viscosity of the organic modifier increases and the relative permittivity decreases.³ However, acetonitrile and acetone have a minor influence on the electro-osmosis such that drugs migrate very rapidly. Propan-2-ol increases the electrophoretic mobility, but unfortunately without an increase in peak resolution. The addition of methanol may sometimes be inadvisable, since it increases the noise.^{9,16}

In all the tests, the reproducibility of the separations was good, as can be seen from the relative standard deviations, which for the absolute migration times were below the 1–2% level. When aqueous buffers were used, the detection limits for the analytes were in the range 1–10 µg mL⁻¹ (S/N > 3) at 220 nm. The detection limit for amine drugs was 0.5 µg mL⁻¹ (S/N > 3) in non-aqueous CE, but was much lower for anionic drugs

Table 3 Repeatability of the non-aqueous analyses in terms of absolute migration times with six replicates. Media modified with ammonium acetate and glacial acetic acid

Compound		Absolute migration time/min	RSD (%)
Amphetamine	A	2.45	0.84
	B	3.46	0.41
	C	4.54	0.59
Ephedrine	A	2.64	0.92
	B	3.78	0.48
	C	4.86	0.81
Levorphanol	A	2.93	0.98
	B	3.98	0.55
	C	nd	—
Dextromoramide	A	3.08	0.61
	B	nd	—
	C	nd	—
Morphine	A	3.59	1.13
	B	4.53	0.76
	C	nd	—
Hydrochlorothiazide	A	4.90	0.41
	B	5.07	1.42
	C	5.54	1.12
Benzoic acid (BA) ^a	A	6.05	0.43
	B	5.48	1.13
	C	5.68	1.12
Chlorothiazide	A	6.30	0.70
	B	7.32	0.66
	C	8.80	2.87
<i>meso</i> -2,3-Diphenylsuccinic acid	A	7.90	0.85
	B	6.46	0.62
	C	6.50	0.90
Probenecid	A	10.1	1.24
	B	6.98	0.96
	C	6.56	0.87
1,2-Phenylenediacetic acid, (PDA) ^a	A	10.4	1.44
	B	8.11	0.43
	C	7.40	2.45
Ethacrynic acid	A	18.9	2.45
	B	8.56	1.74
	C	7.84	1.11

^a Internal standards. A, in methanol-acetonitrile; B, in ethanol-acetonitrile; C, in 2-propanol-acetonitrile.

(10 ng mL⁻¹). The linearity for quantification was tested with 15–120 µg mL⁻¹ sample mixtures, giving correlation values of at least 0.9933. The linearity ranges were not tested in non-aqueous CE.

Hydrochlorothiazide (pK_a 7.9) was thought to be a good internal standard to estimate electro-osmosis and the absolute pH of the non-aqueous BGE solutions used in the study. However, the analyte was not suitable for the purpose due to its

hydrogen-bridged structure at acidic pH. Due to that, it was acting as a neutral molecule and migrating with the electro-osmosis. The effect was tested with chlorothiazide (pK_a 6.9), which has the same structure as hydrochlorothiazide, with exception that one hydrogen molecule is correlated with a double bond in the aromatic ring, averting the formation of the hydrogen-bridged structure. Therefore chlorothiazide was ionized and could have electrophoretic mobility. The pH of the

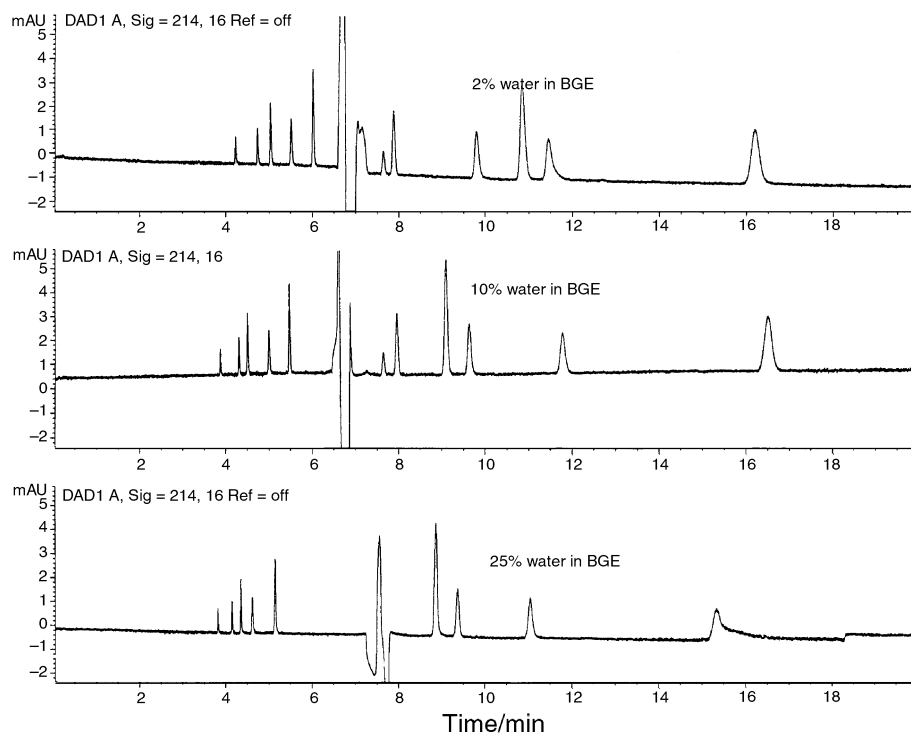


Fig. 5 Effect of water content in the BGE solution on non-aqueous CE separation. Compounds as in Fig. 3. The migration order of the anions has been changed to 7, 10, 9, 8, 11 and 12. Concentration of the analytes: 1.5 µg mL⁻¹. Methanol–acetonitrile solution. Instrument: HP 3D CE system. Capillary: total length, 58.5 cm (separation length, 50 cm).

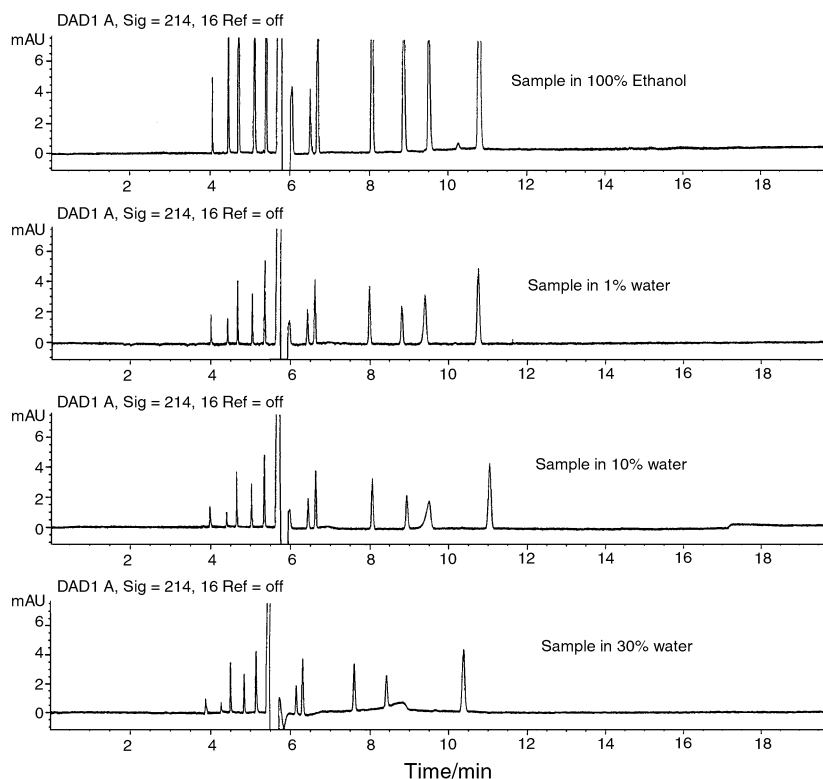


Fig. 6 Effect of water content in the BGE solution on non-aqueous CE separation. Compounds as in Fig. 3. Migration order as in Fig. 5. Concentration of the analytes: 1.5 µg mL⁻¹. Ethanol–acetonitrile solution. Instrument: HP 3D CE system. Capillary: total length, 68.5 cm (separation length, 60 cm).

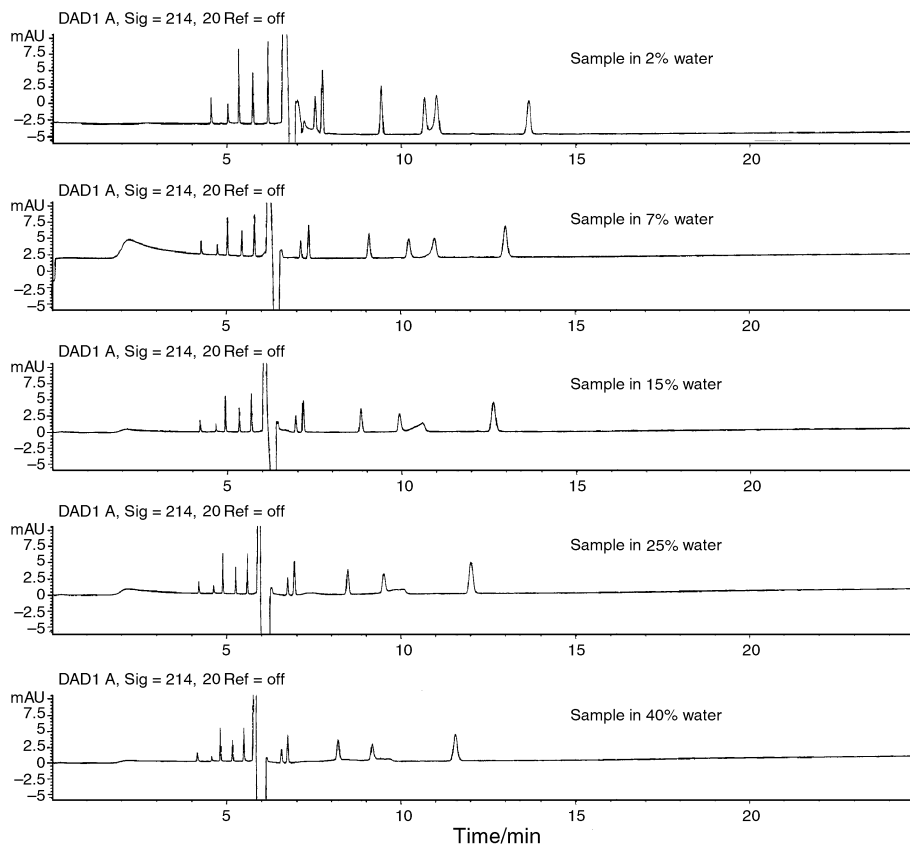


Fig. 7 Effect of water content in the BGE solution on non-aqueous CE separation. Compounds as in Fig. 3. Migration order as in Fig. 3. Concentration of the analytes: $1.5 \mu\text{g mL}^{-1}$. Methanol–acetonitrile solution. Instrument: HP 3D CE system. Capillary: total length, 68.5 cm (separation length, 60 cm).

non-aqueous solutions modified with acetic acid was checked with benzoic acid (pK_a 3.5): in water the pH was 4.2, but with dissociation of benzoic acid the non-aqueous medium was pH 4.0–5.3.

In non-aqueous CE, the best selectivity was obtained by using ethanol as part of the electrolyte solution, although quantitative baseline separations could not be achieved. Propan-2-ol prevented the hydrophobic interactions and the dissociation of the analytes forcing many of them to migrate in electro-osmosis. The results showed that, in non-aqueous medium, 1–5% of water in the sample (Figs. 6 and 7) did not have any effect on the separation profile of the analytes. Figs. 5–7 show that, in methanol–acetonitrile solution, water did not change the separation but influenced the sensitivity of all the peaks. The effect of water on the separation of the analytes was also tested by adding it at 10–25% to the non-aqueous BGE solutions. The dissociation of hydrochlorothiazide was especially sensitive to the water content, since it became uncharged and migrated with electro-osmosis (Fig. 5). The most significant effect of water, was its influence on probenecid: the more water in the sample, the more the peak was fronting. These studies indicate that the sample should contain as small amount of water as possible when non-aqueous CE systems are used.

4. Conclusions

This study shows the applicability of aqueous and non-aqueous media in the separation of basic and acidic pharmaceuticals. The use of phosphate as the buffering ion in the BGE in CZE gave moderately good separation for the amine-containing drugs, but even better resolution for the anionic diuretics. Adjusting the pH with CAPS and using SDS as the micelle in MECC gave the best resolution for all drugs in simultaneous analysis.

Non-aqueous media gave a good resolution for the drugs. The use of the non-aqueous CE system was simple. Dissociation of

both the buffering compounds and the drugs in the organic solvent mixtures was important. The separation of cationic compounds in acetonitrile based media offered a high sensitivity. The detection limits obtained were 10-fold higher than those observed with aqueous buffers. Selectivity changes in chlorothiazide was observed between non-aqueous CE systems, but not in hydrochlorothiazide, although the drug is closely related to chlorothiazide.

Non-aqueous CE is a promising technique for increasing and changing the separation selectivity of smaller molecules, and for separation selectivity that is very difficult to obtain in aqueous buffers. Increased possibilities were obtained for changing the relative electrophoretic mobilities of very similar compounds. Water in the samples was a problem in the separation of analytes containing carboxylic groups. In addition, the narrow sample zone could be enlarged to triangles due to the high water content in the sample.

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