Comparison of separation selectivity in aqueous and non-aqueous capillary electrophoresis[☆]

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Abstract

The use of non-aqueous capillary electrophoresis in free solution in uncoated fused-silica capillaries was investigated and compared with capillary electrophoresis using aqueous systems. Cationic drug substances differing only in the amine functionallity or even having the same charge-to-mass ratio were used as test solutes. Some of these test substances are difficult to separate in plain aqueous buffers and even when using micellar electrokinetic chromatography or by adding cyclodextrins to the electrophoresis buffer it is difficult to obtain baseline separations. Very high separation selectivity may be obtained in non-aqueous capillary electrophoresis systems without adding surfactants or complexing agents to the electrophoresis medium. Major selectivity changes may be obtained using different organic solvents (formamide, N-methylformamide, N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, methanol and acetonitrile) for the electrophoresis medium, e.g., the relative electrophoretic mobilities of a primary, a secondary and a tertiary amine with otherwise identical structures are reversed on replacing acetonitrile with methanol as solvent.

1. Introduction

Improvement of separation selectivity is of fundamental importance and one of the main challenges in separation science. In capillary electrophoresis (CE), significant improvements in separation selectivity were obtained with the introduction of micellar electrokinetic chromatography (MEKC) [1,2], where the electrophoretic rate processes are combined with partition-ing processes. Also, different kinds of complexation may be used in order to improve selectivity and this has found widespread application in the separation of mixtures of enantiomers [3].

Recently, considerable improvements in separation selectivity between cationic compounds with very similar structures and even with same charge-to-mass ratio were obtained [4,5] using either surfactants or cyclodextrins as additives to the electrophoresis buffer. However, these techniques suffer from a number of drawbacks: some of the additives may be fairly expensive, and the additives are often non-volatile and the separation systems are therefore not suitable for on-line coupling to a mass spectrometer.

A number of papers on the use of non-aqueous electrophoresis have been published, and in 1976, Korchemnaya et al. [6] reviewed the research that had been performed in that field until then. Since this review appeared, only a single investigation on the use of the non-aqueous mode has been reported [7]. However, recently the topic has gained renewed interest [8–12].

In this paper, it is demonstrated that high separation selectivity of very similar compounds may easily be obtained by using non-aqueous systems and a volatile salt as the electrolyte. In non-aqueous systems, changes in separation selectivity may be obtained that would be very difficult to achieve in capillary electrophoresis systems based on aqueous buffers.

2. Experimental

2.1. Capillary electrophoresis system

An HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode-array detector was used. A detection wavelength of 214 nm was used for all samples unless stated otherwise. The separation was performed in a fused-silica capillary (64 cm × 50 μm I.D.; 55.5 cm to the detector; for impurity testing a 64 cm × 100 μm I.D., 55.5 cm to the detector, capillary was used) (Polymicro Technologies, Phoenix, AZ, USA). The capillary was thermostated at 25°C with air. Samples were kept at ambient temperature in the autosampler and injected by applying a pressure of 5 kPa (50 mbar) for 3 s. A voltage of 25 kV was applied during analysis.

A Quanta 4000 capillary electrophoresis system (Waters, Milford, MA, USA) was used for studies with the surfactants in 0.05 M 6-aminocaproic acid buffer (pH 4.0). Detection was performed by measuring UV absorption at 214 nm. The separation was performed in a fused-silica capillary (60 cm × 75 μm I.D.; 56 cm to the detector) (Polymicro Technologies). Sample injection was accomplished by hydrostatic injection for 15 s. All analyses were performed using an applied voltage of 20 kV. Data collection was performed using Turbochrom version 3.3 software (PE Nelson, Cupertino, CA, USA).

Prior to use, the capillaries were rinsed with 1 M sodium hydroxide for 60 min, 0.1 M sodium hydroxide for 20 min, distilled water for 20 min and the final electrophoresis medium for 10 min. Between analyses, the capillaries were flushed with electrophoresis medium for 2 min.

2.2. Chemicals and reagents

Imipramine hydrochloride (IMP), desmethylimipramine (DMI), methylimipramine iodide (IMP-CH$_3$) and imipramine N-oxide hydrochloride (DINO) were obtained from Dumex (Copenhagen, Denmark), didesmethylimipramine hydrochloride (DDMI) from Ciba-Geigy (Basle, Switzerland), maprotiline hydrochloride (MAP), litracene hydrochloride (LIT), amitriptyline hydrochloride (AMI), nortriptylene hydrochloride (NOR) and protriptyline hydrochloride (PRO) from H. Lundbeck (Valby, Denmark), 6-aminocaproic acid (6-ACA) and polyoxyethylenesorbitan monolaurate (Tween 20) from Sigma (St. Louis, MO, USA), 3 - (N,N - dimethylmyristylammonium)propanesulphonate (MAPS) and N,N-dimethylacetamide (DMA) from Fluka (Buchs, Switzerland), acetic acid and N,N-dimethylformamide (DMF) were obtained from Riedel-de Häen (Seelze, Germany). Dimethyl sulphoxide (DMSO), HPLC grade acetonitrile and sodium acetate were obtained from Merck (Darmstadt, Germany). Ammonium acetate, formamide and N-methyl formamide (NMF) were obtained from Aldrich (Steinheim, Germany). The used methanol was of HPLC-grade and all chemicals were used without further purification.

2.3. Sample preparation

Mixed test sample I consisted of IMP, DMI, DDMI, IMP-CH$_3$ and DINO in methanol at concentrations of 0.06, 0.04, 0.02, 0.04 and 0.04 mg/ml, respectively, or at a concentration of 0.04 mg/ml each.

Mixed test sample II consisted of: AMI, NOR, PRO, MAP and LIT in methanol at a concentration of 0.04 mg/ml each.

When investigating formamide, NMF, DMF, DMA and DMSO, the mixed test sample was prepared in the respective organic solvent.

For testing the impurities in DINO, 5.1 mg/ml of this substance was dissolved in methanol.

Standard addition was performed by adding 10 μl of each of 0.05 mg/ml methanolic solutions of IMP-CH$_3$, IMP, DMI and DDMI to 300 μl of the DINO test solution.

3. Results and discussion

CE is normally performed using either aqueous buffers or buffers with the addition of only smaller amounts of organic solvents to regulate the electroosmotic flow and the selectivity. As many larger biomolecules are only compatible with aqueous buffers, the aqueous electrophoresis mode will also be the dominant technique in the future. However, using CE, especially smaller molecules such as pharmaceuticals may be separated in non-aqueous systems with increased separation selectivity. In this study, cationic drug substances differing only in the amine functionallity or even having the same charge-to-mass ratio were used as test substances (Fig. 1).

The separations of cationic drug substances using non-aqueous systems were compared with
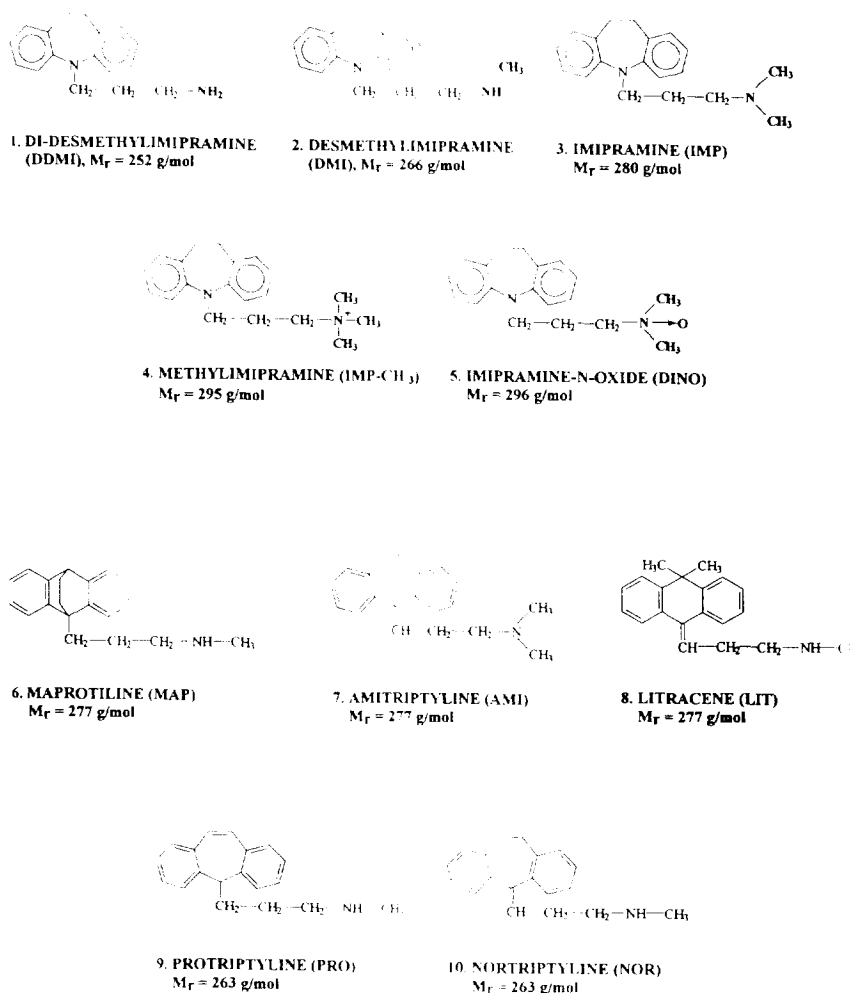

Fig. 1. Structures of the test substances.

respect to the nature of the organic solvent, the observed pH (pH*, see Section 3.2), the temperature and the water content in the electrophoretic medium.

It has been shown previously [4] that adding non-ionic and/or zwitterionic surfactants to the aqueous electrophoresis buffer may provide large increases in separation selectivity between test solutes (Fig. 2). However, instead of using large amounts of surfactants or complexing agents, similar or even better increments in separation may be obtained using "buffers" consisting of methanol, acetonitrile or other organic solvents with electrolytes added.

3.1. Organic solvent

A number of organic solvents were tested (Fig. 3) using mixed test sample I. Rapid and good separations were obtained using methanol and acetonitrile. When using DMF, DMA and DMSO, the analysis time increased owing to the slower electroosmotic flow (EOF). However, in formamide (not shown) no separation of IMP, DMI and DDMI was obtained and in NMF only small separation factors between these three test solutes were obtained. The relative electrophoretic mobilities of the primary, secondary and tertiary amines were reversed on replacing acetonitrile with methanol. The relative order of migration of the test solutes in DMF, DMA and DMSO was similar to that obtained in methanol whereas the relative order of migration in NMF resembled that in acetonitrile.

A more detailed study of the use of methanol and acetonitrile was performed. The gradual replacement of acetonitrile with methanol was studied (Fig. 4). On changing the solvent from 100% acetonitrile through 25% methanol in acetonitrile and 75% methanol in acetonitrile to 100% methanol, the mixed test sample I with imipramine and its derivatives exhibited major changes in selectivity. At 50% methanol in acetonitrile (not shown) no separation of the primary, secondary and tertiary amines could be observed. With the mixed test sample II it was primarily the migration of the tertiary amine (AMI) that changed relative to the four sec-

ondary amines. Smaller changes in selectivity were seen between the four secondary amines. These changes in selectivity may be ascribed to the "basicities" of the amines in the various solvents and solvent mixtures and thus to the solvation of the test solutes. It is known that there are differences in the relative basicities of primary, secondary and tertiary alkylamines when determining them in water or in the gas phase [13].

It is also interesting to observe the change in the EOF on changing the organic solvent. However, in methanol, acetonitrile and mixtures thereof the electrophoretic migration ($\mu_{ep} = \mu_{ap} - \mu_{eof}$) remained virtually constant (about $2 \cdot 10^{-4}$ cm^2/V·s), although the electroosmotic migration increased by a factor of ca. 10 on changing from methanol to acetonitrile and was highest at acetonitrile–methanol (75:25, v/v). These changes in EOF are probably due primarily to changes in viscosity. The viscosity of methanol is decreased with the addition of acetonitrile owing to formation of monomeric methanol [14]. The viscosity reaches a minimum at about 80% acetonitrile in methanol. However, viscosity is not the only explanation, as DMSO has a higher EOF than methanol although DMSO has a relatively high viscosity [15]. Therefore, changes in dielectric constants of the solvents and the zeta potential at the inner capillary wall may also play a role.

Detection can be problematic in non-aqueous capillary electrophoresis. A significant limit of detection is obtained at lower wavelengths when using formamide, NMF, DMF, DMA and DMSO as the noise level increases owing to the high UV absorbance of the organic solvent at lower wavelengths. When using methanol or acetonitrile, a detection wavelength at 214 nm may be used without problems.

3.2. pH*

As pH is only defined in diluted aqueous solutions, it has no direct meaning in non-aqueous solvents. However, acidic or basic electrolytes will still exhibit a major influence on the separation selectivity when added to the organic
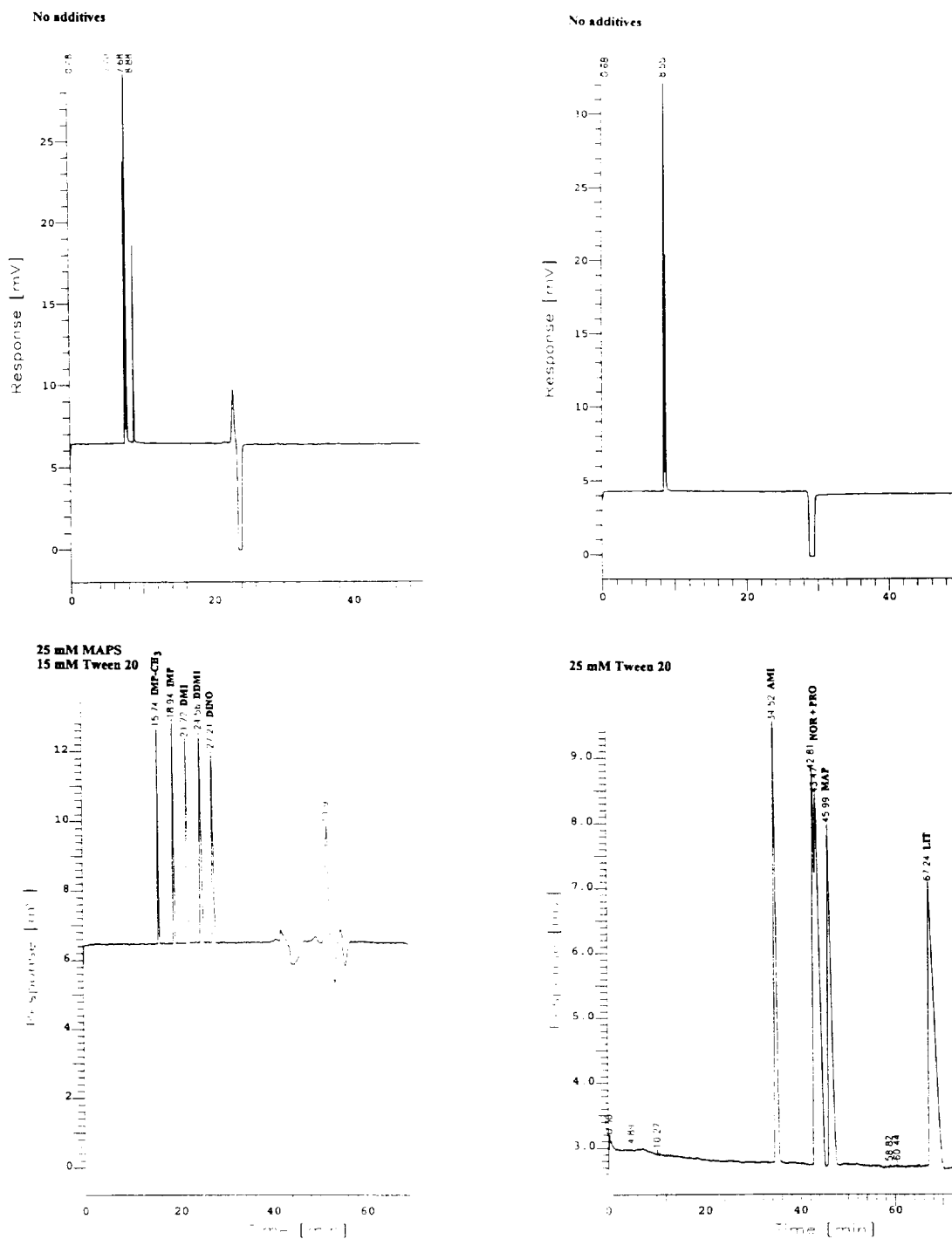
Fig. 2. Electropherograms of mixed test sample I (left) and mixed test sample II (right). Above, no addition of surfactants; below, addition of the surfactants MAPS and Tween 20 to the electrophoresis buffer. Apparatus: Waters Quanta 4000. Conditions: capillary, 56 cm to detector × 75 μm I.D.; temperature, 30°C; detection, 214 nm; buffer, 0.05 M 6-aminocaproic acid (pH 4.0); voltage, 20 kV; current, 62 μA. For peak identification, see Fig. 1.
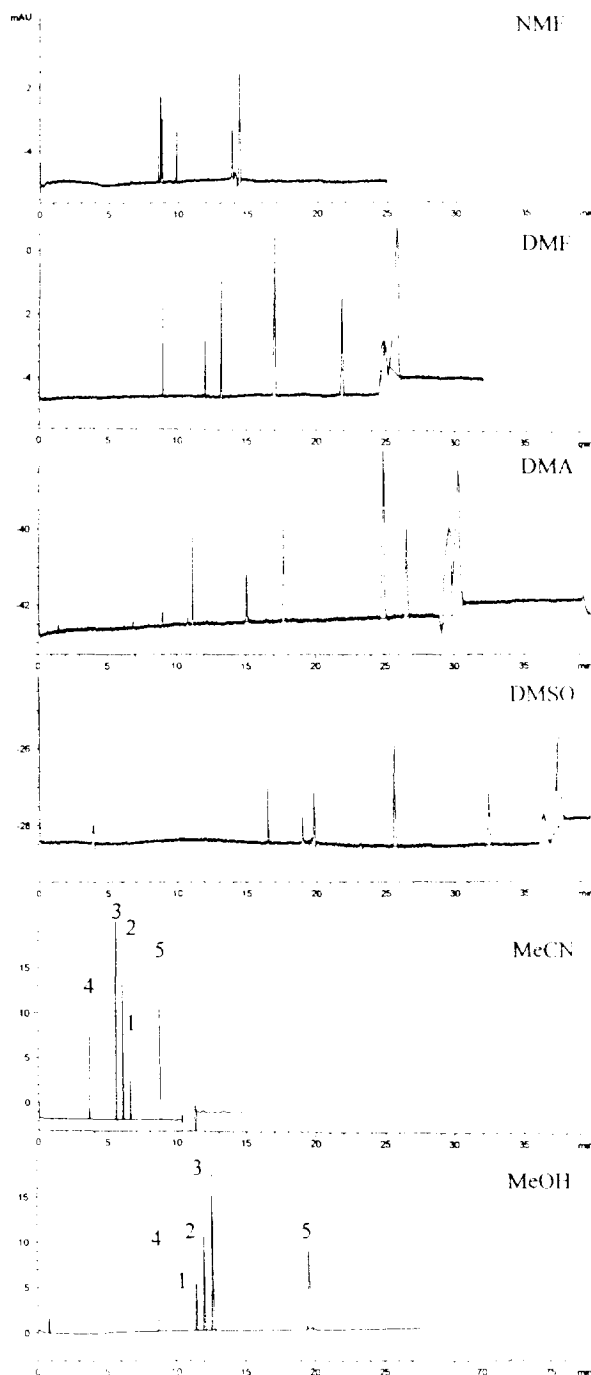
Fig. 3. Non-aqueous CE in NMF, DMF, DMA, DMSO, acetonitrile and methanol. Apparatus: HP[u] CE instrument. Conditions: capillary, 55.5 cm to detector × 50 μm I.D.; temperature, 25°C; detection, 214 nm; electrophoresis medium, 25 mM ammonium acetate–1 M acetic acid in the organic solvent; voltage, 25 kV; current, ca. 7 μA. For peak identification, see Fig. 1

solvent used as electrophoresis medium. Fig. 5 shows electropherograms from an investigation where the electrolytes were changed from ammonium acetate–sodium acetate to ammonium acetate–acetic acid at different concentrations. As expected, selectivity changes occurred when acidic or basic salts were added to the electrophoresis medium. This also influences the electroosmotic flow, possible owing to changes in the zeta potential on the inner capillary wall.

3.3. Temperature

Changing the temperature surrounding the capillary is known to have an effect when using aqueous systems [5]. Temperature changes from 10 to 40°C had only a small effect on the mixed test sample I apart from a 25% increase in EOF. With the mixed test sample II an improvement of separation was seen only at 40°C, where all five test solutes were separated. However, a similar investigation on the influence of the temperature on the separation of opium alkaloids (which do not have similar chemical structures) in similar non-aqueous systems showed that major changes in selectivity could be introduced [11].

3.4. Water

On changing the electrophoresis medium from a 100% non-aqueous to a 100% aqueous system, most of the separation is gradually lost. This was to be expected as it is difficult to separate these test solutes (Fig. 2) in aqueous buffers [4].

4. Application

The loadability of the non-aqueous CE system was tested by using the system for drug purity testing. Imipramine-N-oxide (DINO) was investigated for its content of impurities, and a 0.5% solution was loaded to the capillary without any problems (Fig. 6). The limit of detection (defined as a signal three times the baseline noise) is about 1 μg/ml, corresponding to 10 ppm impurity in the drug substance. This is
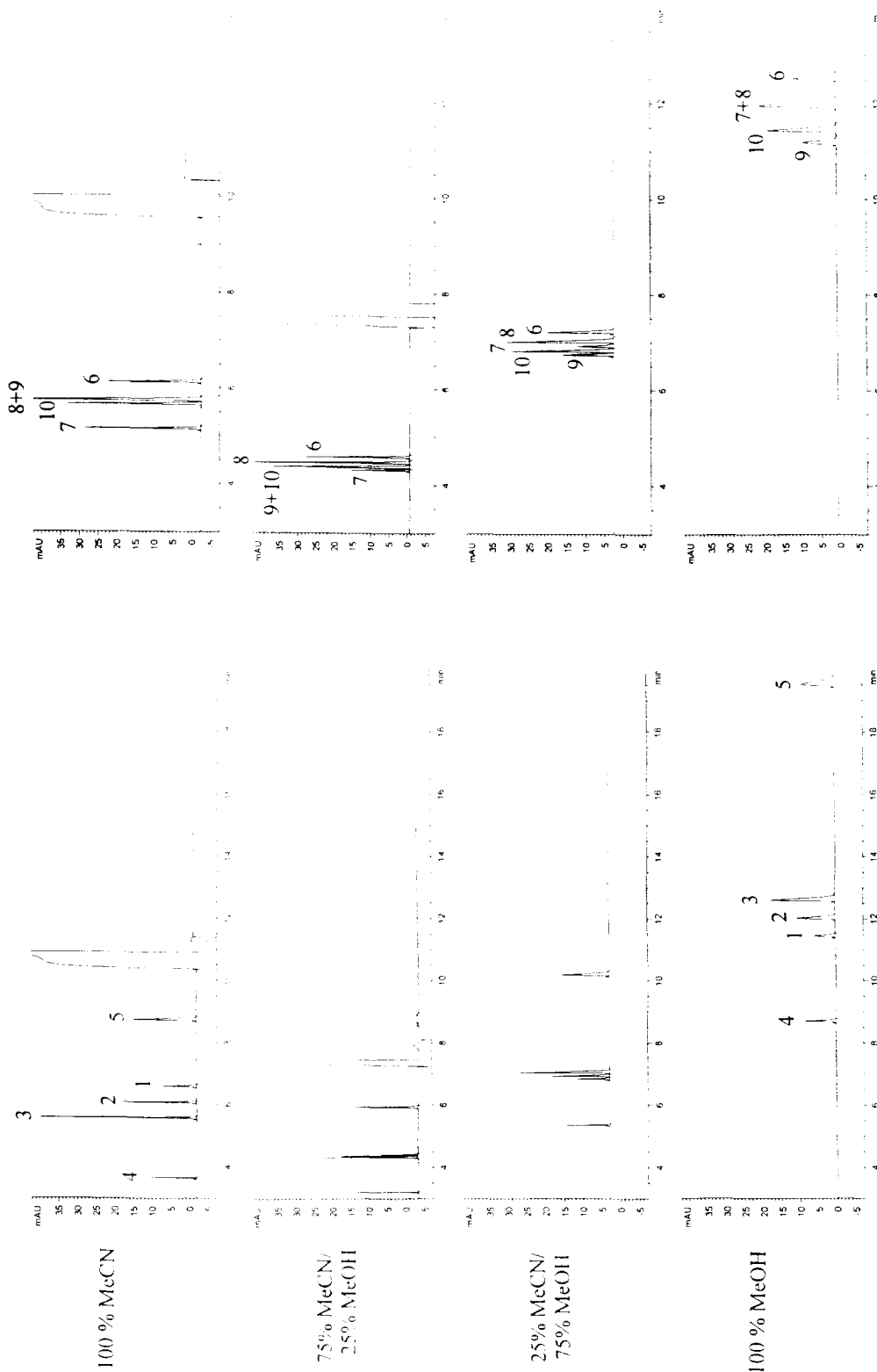
Fig. 4. Non-aqueous CE in acetonitrile, methanol and mixtures thereof. Apparatus and conditions as in Fig. 3. Left row, mixed test sample I; right row, mixed test sample II. For peak identification, see Fig. 1.
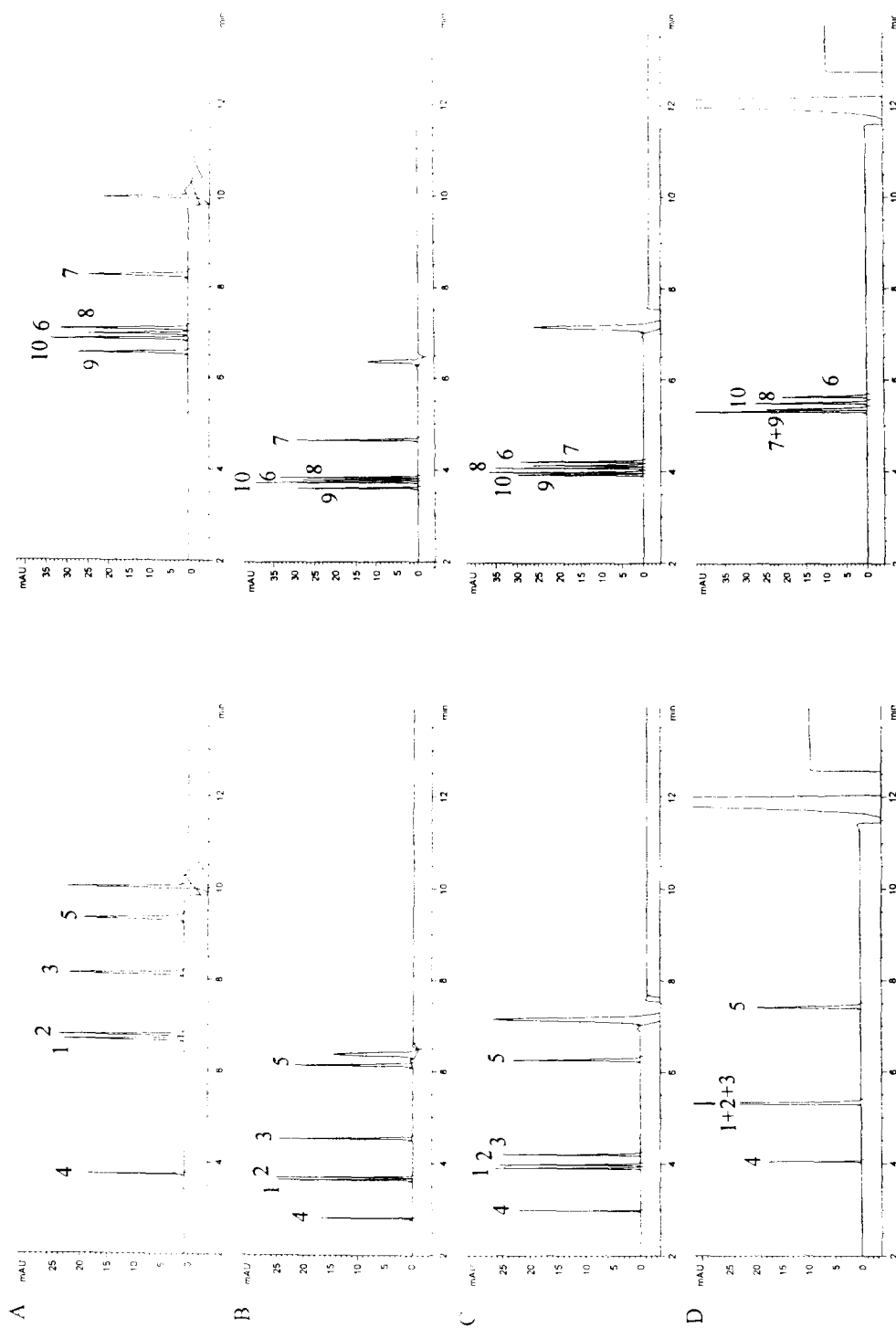
Fig. 5. Non-aqueous CE using basic and acidic electrolytes in acetonitrile–methanol (1:1, v/v). Apparatus, capillary, temperature, detection and voltage as in Fig. 3. Electrophoresis medium [all in acetonitrile–methanol (1:1, v/v)]: (A) 25 mM ammonium acetate–100 mM sodium acetate; (B) 25 mM ammonium acetate; (C) 25 mM ammonium acetate–100 mM acetic acid; (D) 25 mM ammonium acetate–1 M acetic acid. Left row, mixed test sample I; right row, mixed test sample II. For peak identification, see Fig. 1.
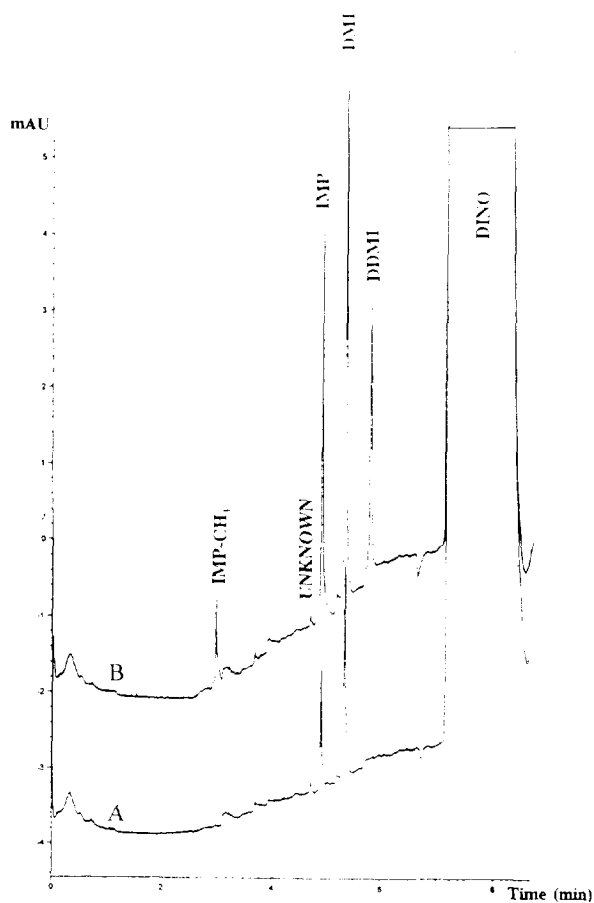
Fig. 6. Drug purity testing of DINO using non-aqueous CE. (A) 5.1 mg/ml sample of DINO in methanol and (B) standard addition of 10 μl of 0.05 mg/ml IMP, DMI, DDMI and IMP-CH₃ dissolved in methanol to 300 μl of DINO sample. Apparatus: HP³ᴰ CE instrument. Conditions: capillary, 55.5 cm to detector × 100 μm I.D.; temperature, 25°C; detection, 214 nm; buffer, 25 mM ammonium acetate–1 M acetic acid in acetonitrile; voltage, 25 kV; current, 30 μA. For peak identification, see Fig. 1.

comparable to what has been obtained previously when using aqueous buffers [4]. A further advantage of the non-aqueous system is that owing to the low current (ca. 30 μA in a 100 μm I.D. capillary using 25 mM ammonium acetate in organic solvent) it is possible to use capillaries with a larger internal diameter, which again may provide higher loadability and thus lower limits of detection.

5. Conclusion

Non-aqueous capillary electrophoresis is a powerful tool for increasing and changing the separation selectivity of smaller molecules, and separation selectivities that would be very difficult to obtain in aqueous buffers may be introduced. Increased possibilities for changing the relative electrophoretic mobility of very similar compounds are obtained. On changing the organic solvent from a solvent with properties for both donating and accepting hydrogen bonds to a solvent which only is an acceptor for hydrogen bonding, major selectivity changes for primary and tertiary amines are observed. The technique is very suitable for drug purity testing but may also be useful for the determination of drugs and metabolites in biological fluids after concentration by solid-phase extraction and elution from the extraction column with organic solvents.

Acknowledgements

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