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Chiral separation of dansyl-amino acids in a nonaqueous medium by capillary electrophoresis

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Abstract

In this study the feasibility of using pure organic solvents as separation media in capillary electrophoresis was tested. The chiral separation of individual dansyl-amino acids was compared in aqueous phosphate–borate buffer and in N-methylformamide. In both cases β-cyclodextrin was used as a chiral selector. The low solubility of β-cyclodextrin in water may have hindered the separation of some dansyl-amino acid enantiomers when the aqueous buffer was used. By contrast, N-methylformamide proved to be an excellent solvent for β-cyclodextrin. The chiral separation of ten dansyl-amino acids is demonstrated for β-cyclodextrin in N-methylformamide. The results indicate the potential of organic solvents for chiral separations by capillary electrophoresis.

Keywords: Enantiomer separation; Buffer composition; Amino acids, Dns derivatives; N-Methylformamide

1. Introduction

Capillary electrophoresis (CE) is now a widely employed separation technique which can be a useful counterpart of the conventional chromatographic methods. In general, only aqueous buffers are considered suitable for CE. Indeed, the overwhelming majority of separations reported so far, have been achieved in aqueous background electrolytes. However, organic solvents, most often alcohols (e.g. methanol, ethanol, isopropanol) or acetonitrile, have been applied as buffer additives at concentrations typically not higher than 40% in order to improve the resolution and alter the selectivity [1–3]. In this concentration range the addition of alcohols to the background electrolyte results in significantly reduced electroosmotic flow and low electrophoretic mobility [3].

On the other hand, there are numerous examples of nonaqueous media being used in classical electrophoretic separations. As long ago as 1951 Hayek investigated the mobility of carbon black particles in kerosene [4]. Classical electrophoresis in nonaqueous media has been reviewed by Korchemnaya et al. [5]. To date however, there have been only a few reports on the use of nonaqueous media in CE [6–16]. Methanol [6–12,16], acetonitrile [13,16], formamide [14,16], dimethylformamide [12,16] N-methylformamide [15,16], dimethylacetamide [16], and dimethyl sulfoxide [16] have been used and it is highly likely that several other organic solvents could be applied either alone or in combination in CE. As the physical and chemical characteristics of organic solvents vary in a wide range, their electrophoretic

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properties and overall applicability as electrophoretic media in CE are expected to differ considerably from each other and from the conventional aqueous buffers.

In selecting the proper electrophoretic medium for CE, several characteristics of the solvents should be considered including viscosity, dielectric constant, electrical and thermal conductivity, self-dissociation constant, polarity and boiling point. The solubility of the analytes, salts and different modifiers added to the background electrolyte may also determine which is the most suitable solvent for a particular application. Detection is immensely affected by the solvent. Since most organic solvents absorb more strongly in the UV region than water, the use of alternative detection methods such as electrochemical [12], indirect UV [12], fluorescent or mass spectrometric detection [8–10] may sometimes be preferred. The use of organic solvents should also be carefully considered in terms of their general safety and toxicity.

The separation of optical isomers is a rapidly growing area in CE. Several approaches have turned out to be useful [17–25]. Usually the chiral selector is dissolved in the separation buffer but gel electrophoresis [17] and capillary electrochromatography [18] can also be successfully used for enantioseparation. The separation in the reported studies have been based on ligand exchange complex formation [19]; micellar electrokinetic chromatography (MEKC) with chiral surfactants [20]; the application of an oil-in-water microemulsion containing a lipophilic chiral selector [21]; host–guest complex formation with cyclodextrins [22], chiral crown ethers [23] or macrocyclic antibiotics [24]; affinity-type interactions with proteins [25] or oligosaccharides [26]. The chiral separation of dansyl amino-acids (Dns-AAs) has been reported with several chiral selectors and different electrophoretic techniques [17,19,34–54]. A comprehensive review of chiral separations in capillary electrophoresis has been published by Ward [27]. All the separations reported so far have been achieved in aqueous buffers. Cyclodextrins (CD) have proved to be the most successful chiral selectors. However, the poor aqueous solubility of β-CD considerably restricts its use because the resolution is a function of the chiral selector concentration. According to Wren and Rowe [28] the mobility difference of the enantiomers first increases with the increasing concentration of the chiral selector and then, after a maximum, it decreases. The chiral selector concentration that gives the best resolution (c_{opt}) is determined by the complex stability constants of the enantiomers (K_1 and K_2) according to the following equation [28]

$$c_{opt} = \frac{1}{\sqrt{K_1 K_2}} \quad (1)$$

In practice the optimum concentration for chiral separation is sometimes higher than the aqueous solubility of the β-CD. The solubility of β-CD can be enhanced by dissolving urea in the background electrolyte [29] or by using water–cosolvent mixtures [30] or more soluble β-CD derivatives [31]. However, the selectivity of the derivatized β-CDs differs from that of the native β-CD. A drawback to using derivatized CDs is that some of them (e.g. hydroxypropyl- and hydroxyethyl-β-CD) are not pure entities but mixtures of analogues having different degrees and patterns of substitution [32]. An alternative approach is to find a better solvent for β-CD which at the same time is a suitable medium for electrophoresis. In this work we tested the applicability of N-methylformamide (NMF) as a medium for chiral separations of Dns-AAs by CE.

Some characteristic properties of NMF and water are listed for comparison in Table 1. The electrophoretic mobility (μ_{ep}) is a function of the dielectric

Table 1
Comparison of characteristic properties of water and N-methylformamide (NMF)

	Water	NMF
Viscosity (cP)	0.89025[a]	1.65[a]
Dielectric constant	78.54[b]	182.4[a]
Boiling point (°C)	100[a]	180–185[a]
Density (g/cm^3)	0.9970474[a]	0.9988[a]
Dipole moment (D)	1.85[b]	3.83[b]
Specific conductance (ohm^{-1} cm^{-1})	$5.49 \cdot 10^{-8}$ [a]	$8 \cdot 10^{-7}$ [a]

[a] Data from Ref. [59].
[b] Data from Ref. [60].

constant (ε), the viscosity (η) of the solvent and the zeta potential (ζ_{ep}) around the analyte ions according to the Hückel equation [33]

$$\mu_{ep} = \frac{\varepsilon \zeta_{ep}}{6\pi\eta} \quad (2)$$

The high dielectric constant of NMF reduces the Coulombic interaction between the oppositely charged ionic species, therefore the shielding effect of counterions in the shear plane near the analyte ions and the capillary wall is weak. This results in higher effective charge and zeta potential leading to the enhanced electroosmotic and electrophoretic mobilities. The higher viscosity of NMF compared to water has the opposite effect on the mobility.

2. Experimental

2.1. Materials

The reagents used in this study were of analytical grade. Asparagine, aspartic acid, proline, serine, threonine and valine were obtained from Hoffmann la Roche (Basel, Switzerland), lysine and methionine from BDH (Poole, UK), norvaline and alanine from Fluka (Buchs, Switzerland), dansyl chloride from Aldrich (Steinheim, Germany), β-cyclodextrin and the Dns-AA standards from Sigma (St. Louis, MO, USA), N-methylformamide from EGA-Chemie (Steinheim, Germany), acetonitrile, $NaH_2PO_4 \cdot 2H_2O$ and $Na_2B_4O_7 \cdot 10H_2O$ from Merck (Darmstadt, Germany). Distilled water was further purified with a Water-I system (Gelman Science, Ann Arbor, MI, USA). The Dns-AAs were prepared by the method of Okafo and Camilleri [39] as follows. The individual racemic amino acids were dissolved in 100 m*M* borate buffer (pH 9.5) in a concentration of 1 m*M*; then 50 μl of the amino acid stock solution was diluted with 100 μl of the same borate buffer and treated with 100 μl dansyl chloride (8 m*M* solution in acetonitrile). The reaction mixtures were allowed to stand at room temperature for one hour. The Dns-AA solutions were kept in dark at 5°C. The final concentration of the Dns-AAs was 0.2 m*M* and the solution also contained the by-products of the reaction. All the samples were filtered through 0.45 μm Acrodisc PTFE membrane filters (Gelman Sciences) before electrophoresis.

2.2. Apparatus

Separations were performed using a HP^{3D} CE System (Hewlett-Packard, Avondale, PA, USA) with air cooling. Uncoated fused-silica capillaries with 50 μm I.D. and 360 μm O.D. were used. The total lengths of the capillary in case of separations in NMF and in borate-phosphate buffer were 50 cm and 58.5 cm, respectively. The detection window was made at 8.5 cm from the end of the capillaries. Samples were injected by applying a pressure of 50 mbar for 5 s. The applied voltage was 30 kV. The temperature of the capillary was kept constant at 25°C and the UV absorption was monitored at 254 nm with a diode-array detector.

2.3. Methods

The separations were carried out in NMF containing 10 m*M* NaCl or no salt and various concentrations (25–200 m*M*) of β-CD. To compare the separations in NMF and in an aqueous buffer, samples were also analysed according to the method described by Tanaka et al. in [47] using 10 m*M* β-CD in 50 m*M* NaH_2PO_4–100 m*M* borate buffer (pH 9.0) as background electrolyte. The pH of the phosphate–borate buffer was adjusted with 1 *M* NaOH using a Jenway 3030 pH meter with a Jenway electrode (Jenway, Felsted, UK). The electrode was calibrated with Fixanal solutions (Riedel-de Haën, Seelze, Germany). The electrolyte solutions were de-gassed in an ultrasonic bath before use. The capillary was washed for 20 min with NMF or water then for 20 min with the background electrolyte at the beginning of each day and for 5 min with the background electrolyte between the runs.

Kinematic viscosity of the background electrolytes at different β-CD concentrations was measured with an S.I.L.-type viscometer. Density of the same electrolyte solutions was measured in a pycnometer. Both measurements were carried out at 25±0.5°C.

2.4. Calculations

Resolution was calculated using the following equation [55]

$$R_s = 1.18 \frac{t_2 - t_1}{w_1 + w_2} \quad (3)$$

where t_1 and t_2 are the migration times of the enantiomers and w_1 and w_2 the peak widths measured at half of the peak heights. The term R_s is not suitable for peaks that overlap at half of their peak height. In such cases $R' = 100(h_2/h_1)$ may be used to compare resolutions [48]. In this equation h_1 is the height of the first peak and h_2 is the depth of the valley between the two peaks measured from the apex of the first peak. R' is often used for the comparison of chiral separation of racemic compounds. The advantage of R' is that it allows the calculation of resolutions too poor to be expressed by R_s. The higher the R' value the better the resolution and 100 represents the baseline separation. R' stays constant after baseline resolution. This expression can only be used when the peaks are the same size. The plate number (N) was calculated according to $N = 5.54(t/w)^2$, where t is the migration time and w is the peak width at half of the peak height [56].

3. Results and discussion

The direct comparison of separations in aqueous buffers and organic solvents is difficult because usually the experimental conditions are different. For example, in water higher ionic strength may be necessary for acceptable separation and/or the ionic strength used in water cannot be achieved in the organic solvent. However, to compare the performance of our nonaqueous system with an aqueous system we tested the chiral separation of the same samples in a 50 m*M* phosphate–100 m*M* borate buffer (pH 9.0) containing 10 m*M* β-CD as a chiral selector. This system has been used in four earlier studies [44,47,48,51].

Separation of the enantiomers of the individual Dns-AAs was attempted both in phosphate–borate buffer and in NMF. Since the dansylation reaction mixtures were directly injected to the capillary, the Dns-AAs also had to be separated from dansylic acid which was the main by-product of the reaction [39] and from some small amounts of unidentified impurities. In both the aqueous and nonaqueous systems Dns-AAs were successfully separated from these other compounds in the reaction mixture.

The separation efficiency was good in the phosphate–borate buffer: for Dns-D- and Dns-L-Asp 195 000 and 220 000, respectively. Only the Dns-Asp enantiomers were baseline separated when the phosphate–borate buffer was used (Table 4). We achieved slightly worse resolution for some Dns-AAs with the aqueous system than reported by Tanaka et al. [47] but the overall tendency was the same. The relatively poor resolutions in the phosphate–borate buffer may have been due to the low β-CD concentration that was used. Yoshinaga and Tanaka have shown [51] that the optimum chiral selector concentration is higher than 10 m*M* for Dns-Leu, -Nleu, -Nval, -Phe, -Ser and -Val. In contrast to the poor solubility of β-CD in water (18 m*M*), more than 700 m*M* solution in NMF could be prepared. The current in the phosphate–borate buffer was as high as 115 μA. In NMF the salt concentration was only 10 m*M* and the separation was achieved at significantly lower current (4.5–9 μA).

In the NMF based background electrolyte, the Dns-AAs were detected after the negative peak of the sample solvent which was used as an electroosmotic flow marker. This indicates that, similarly to the aqueous buffer, the analytes in NMF are negatively charged. Fig. 1 shows the mobility changes as the chiral selector concentration increases. The increasing viscosity of the background electrolyte causes a decrease in the electroosmotic flow and in the electrophoretic mobility of the analytes. NMF has a viscosity about two times higher than water. According to Eq. 2 the mobility is inversely related to the viscosity. However, the exceptionally high dielectric constant of NMF (Table 1) overcompensates the viscosity effect, resulting in relatively low migration times (7–9 min at 25 m*M* β-CD 10 m*M* NaCl concentration). The absolute viscosity (η_{abs}) of the electrolyte increases with β-CD concentration (c) in a power series according to the equation $\eta_{abs} = 58.628c^2 + 6.017c + 1.840$. Even at 200 m*M* β-CD concentration at an absolute viscosity as high as 5.4085 cP the migration times of the analytes ranged

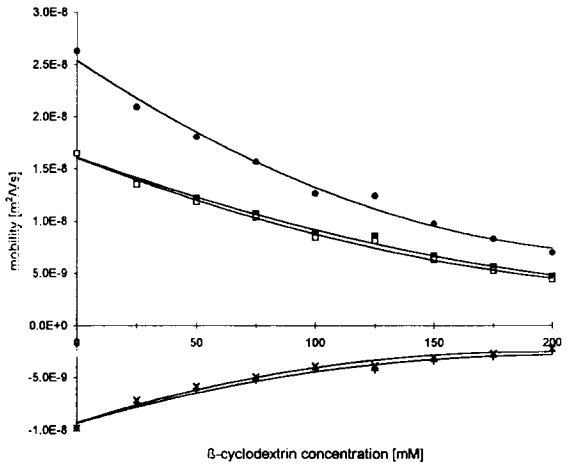

Fig. 1. Mobility as a function of chiral selector concentration in the separation of Dns-Ser enantiomers. Background electrolyte: 10 m*M* NaCl in NMF (containing β-CD as chiral selector); capillary 50 cm×50 μm fused-silica; field strength 600 V/cm; capillary temperature 25°C; detection 254 nm; sample concentration 0.2 m*M*; injection by pressure of 50 mbar for 5 s. Symbols: Electroosmotic mobility (●); apparent mobility of Dns-D-Ser and Dns-L-Ser (■, □) and effective mobility of the Dns-D-Ser and Dns-L-Ser enantiomers (+, ×), respectively.

between 27 and 50 min. The migration times of the Dns-AAs at different chiral selector concentrations are listed in Table 2. The electrophoretic mobilities and the electroosmotic mobility in phosphate–borate buffer and NMF are shown in Table 3. The electrophoretic mobilities were about twice as high in NMF as in the aqueous buffer but difference in the electroosmotic flow was moderate ($\mu_{eo,aq} = 5.4035 \cdot 10^{-8}$, $\mu_{eo,NMF}$ $4.9037 \cdot 10^{-8}$ m^2/Vs).

Table 3
Electrophoretic mobilities of Dns-AAs in 50 m*M* phosphate–100 m*M* borate buffer (pH 9.0) and in NMF containing 10 m*M* NaCl

Dns-	Mobility (m^2/Vs)	
	Aqueous buffer[a]	NMF[b]
-Pro	$-2.0978 \cdot 10^{-8}$	$-1.0451 \cdot 10^{-8}$
-Ala	$-2.1168 \cdot 10^{-8}$	$-1.0342 \cdot 10^{-8}$
-Ser	$-2.1183 \cdot 10^{-8}$	$-1.0339 \cdot 10^{-8}$
-Lys	$-1.7118 \cdot 10^{-8}$	$-7.5663 \cdot 10^{-9}$
-Asn	$-2.2745 \cdot 10^{-8}$	$-1.0955 \cdot 10^{-8}$
-Leu	$-2.1595 \cdot 10^{-8}$	$-1.0569 \cdot 10^{-8}$
-Nval	$-2.0986 \cdot 10^{-8}$	$-1.0558 \cdot 10^{-8}$
-Asp	$-3.8621 \cdot 10^{-8}$	$-1.5294 \cdot 10^{-8}$
-Met	$-2.1026 \cdot 10^{-8}$	$-9.5683 \cdot 10^{-8}$
-Thr	$-2.0373 \cdot 10^{-8}$	$-9.7387 \cdot 10^{-9}$
-Val	$-2.0008 \cdot 10^{-8}$	$-1.0140 \cdot 10^{-8}$
EOF[c]	$5.4035 \cdot 10^{-8}$	$4.9037 \cdot 10^{-8}$

[a] Experimental conditions as in Table 2.
[b] 50 m*M* phosphate–100 m*M* borate buffer pH 9.0; capillary: 50 cm×50 μm fused-silica; field strength: 513 V/cm. Other experimental conditions as in Table 2.
[c] EOF = electroosmotic flow.

The repeatability of the migration times was remarkably good in NMF. The repeatability was tested in 10 consecutive injections of Dns-Ala using 100 m*M* β-CD and 10 m*M* NaCl in NMF. The relative standard deviations of the migration times for the Dns-D-Ala and Dns-L-Ala were 0.48 and 0.58%, respectively. In the same series of experiment

Table 2
Migration times (min) of Dns-AAs in NMF containing 10 m*M* NaCl and different concentrations of β-CD[a]

Dns-	β-CD concentration (m*M*)									
	25		50		100		150		200	
	D	L	D	L	D	L	D	L	D	L
-Pro	8.82		10.41		12.45	23.74	24.03	35.41	36.14	
-Ala	8.38	8.56	10.57	11.01	12.13	12.90	23.25	25.73	33.90	38.79
-Ser	8.29	8.49	10.43	10.92	12.53	13.36	23.02	26.02	33.25	39.07
-Lys	7.28		8.94	9.04	11.10	11.34	19.24	19.96	27.23	28.51
-Asn	8.01		10.17		12.64		23.63		34.63	
-Leu	7.66	7.73	9.46	9.72	15.00	15.77	20.79	21.92	29.19	31.16
-Nval	7.92	8.08	9.91	10.26	12.23	12.84	22.40	24.27	31.82	35.29
-Asp	9.13	9.29	11.67	12.08	14.66	15.54	30.07	33.59	42.83	50.08
-Met	8.18	8.34	10.18	10.55	12.87	13.56	23.04	25.13	32.70	36.67
-Thr	8.30	8.57	10.31	10.92	12.99	14.17	23.17	26.84	32.93	39.54
-Val	7.91	8.12	9.67	10.01	12.38	13.08	21.68	23.54	30.61	33.76

[a] Capillary temperature 25°C, detection 254 nm, sample concentration 0.2 m*M*, injection by pressure of 50 mbar for 5 s.

the relative standard deviation of the electroosmotic flow was 0.39%. However, the day-to-day repeatability was worse; the difference in the electroosmotic flow sometimes was as high as 10%. Also, the efficiency sometimes was decreased after the use of electrolytes with high β-CD concentrations. The loss of efficiency may be a result of CD binding to the capillary wall which is then difficult to flush away.

The efficiency achieved per unit of time is proportionate to the ε^2/η value of the electrolyte according to the equation [15]

$$\frac{N}{t} \propto \frac{r}{kT\eta} (2\xi_{ion} - 3\xi_{wall})^2 (\varepsilon_0 \varepsilon_r E)^2 \quad (4)$$

where N is the plate number, t is the migration time, r is the Stokes radius of the analyte, k is the Boltzmann's constant, T is the temperature, η is the viscosity, ξ_{ion} and ξ_{wall} are the zeta potential of the analyte ion and the capillary wall, respectively, ε_0 is the permittivity in vacuum, ε_r is the relative dielectric constant, and E is the electric field strength. From the dielectric constant and the viscosity values of NMF high efficiency can be predicted (Table 1). However, the efficiency of the separation is not as good as would be expected from the physical constants of the NMF; e.g. the plate numbers for D- and L-Ala are 50 000 and 51 000, respectively. Several factors may be responsible for the relatively low efficiency. Eq. 4 is only valid when diffusion is the sole dynamic factor contributing to band broadening. In our case there were some other factors operating as well. The complex formation between the analytes and the β-CD may have contributed to zone dispersion but more importantly the sample solvent is far not optimal. In CE sample stacking can be achieved by using a sample solvent with lower ionic strength than the background electrolyte [57]. In our case the Dns-AAs were dissolved in the solution used for the dansylation reaction which consisted of a mixture of 100 m*M* borate buffer (pH 9.5) and acetonitrile (3:2, v/v) and this was injected into the low ionic strength background electrolyte. Finally, because of the high β-CD concentration the viscosity of the separation medium was significantly higher than the viscosity of the pure NMF. This according to Eq. 4 results in band broadening.

Table 4 compares the resolution of the optical isomers of the analytes in the aqueous buffer and in NMF containing different concentrations of β-CD. Only Dns-Asn showed no sign of chiral separation, either in the aqueous buffer or in NMF. Eight of the 11 pairs of enantiomers investigated were baseline resolved in NMF. The separation of the Dns-Pro enantiomers was poor even at the highest chiral selector concentration. Fig. 2 shows the resolution (R_s) as a function of β-CD concentration. There is

Table 4
Resolution (R') of Dns-AAs in aqueous and nonaqueous media

Dns-	β-CD concentration (m*M*)								
	In N-methylformamide[a]								In water
	25	50	75	100	125	150	175	200	10
-Pro-	–	–	–	–	–	11.1	25.0	40.0	5.6
-Ala	60.2	87.3	97.3	100	100	100	100	100	5.5
-Ser	61.3	90.4	100	100	100	100	100	100	–
-Lys	–	16.9	39.4	44.8	64.4	75.6	86.1	76.9	–
-Asn	–	–	–	–	–	–	–	–	–
-Leu	16.4	67.5	73.4	100	100	100	100	94.4	13.3
-Nval	47.8	84.3	81.7	91.5	100	100	100	100	7.0
-Asp	27.8	77.4	95.3	100	100	100	100	100	100
-Met	47.6	82.3	93.9	100	100	100	100	100	–
-Thr	74.8	95.8	100	100	100	100	100	100	80.0
-Val	66.1	78.3	100	100	100	100	100	100	48.7

[a] Experimental conditions as in Table 2.

[b] 50 m*M* phosphate–100 m*M* borate buffer pH 9.0; capillary: 50 cm×50 μm fused-silica; field strength: 513 V/cm. Other experimental conditions as in Table 2.

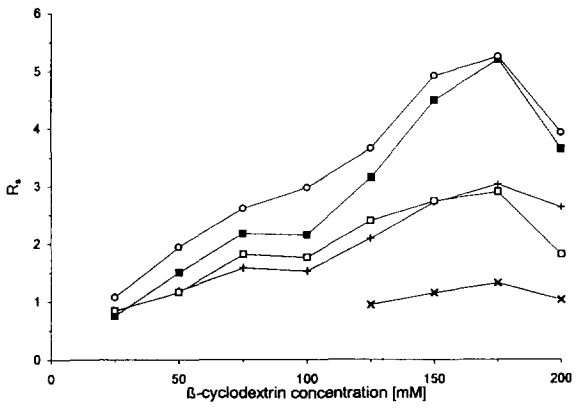

Fig. 2. Effect of the chiral selector concentration on the resolution (R_s) of Dns-amino acid enantiomers. Background electrolyte: NMF containing 10 m*M* NaCl and various concentrations of β-CD. Experimental conditions are the same as in Fig. 1. Symbols: Dns-Thr (○), Dns-Ser (■), Dns-Val (□), Dns-Nval (+), Dns-Lys (×).

an increase in the resolution as the concentration of the chiral selector is increased until about 175 m*M* where the resolution of most enantiomers reaches a maximum. In practice, c_{opt} is not the preferred concentration if the baseline resolution can be achieved at lower chiral selector concentration. In this study the best resolutions (Dns-Thr $R_s = 5.24$ and Dns-Ser $R_s = 5.19$) are unnecessarily high. The high optimum of the chiral selector concentration indicates that the complex stability constants were low. According to Eq. 1 the observed c_{opt} corresponds to stability constants of about 5 M^{-1}. The reported complex stability constants of Dns-Glu and Dns-Leu in 200 m*M* phosphate buffer (pH 6.8) containing 20% methanol and different concentrations of β-CD are significantly higher [43]: 220 and 187 M^{-1} for Dns-Glu enantiomers, 170 and 141 M^{-1} for Dns-Leu enantiomers. The effect of the solvent on the complex stability may play an important role in optimisation. Neither too high nor too low complex stability constants are desirable. Complex stability constants higher than 1000 M^{-1} correspond to optimum chiral selector concentrations below 1 m*M* and since the chiral selector should be in excess, the low c_{opt} may require such a low analyte concentration which can cause detection problems [43]. On the other hand, too low complex stability constants may require high concentration of the selector which results in high viscosity, long migration times and the loss of efficiency. The use of organic solvents may provide yet a further possibility to control the stability of the analyte-chiral selector complexes, in terms of both the absolute values of the stability constants and their relative difference.

The D-enantiomers of the Dns-AAs were detected first both in NMF and in the aqueous phosphate–borate buffer. However, the identical migration order may be just a coincidence and does not necessarily indicate a similar molecular mechanism for the chiral separation.

Fig. 3 shows the chiral separation of Dns-Ala at 100 m*M* β-CD concentration with and without 10 m*M* NaCl dissolved in NMF. It was possible to achieve enantioseparation also in salt-free NMF containing the chiral selector because there is strong electroosmotic flow even in pure NMF. This finding is in accordance with the earlier report of Jansson and Roerade [15] who explained the high electroosmotic flow in terms of the presence of autoprotolysis (NMF is an amphiprotic solvent) and hydrolysis products in NMF. Electrolysis at the electrodes also creates ionic components although this seems to have little effect on the electroosmosis as the current is stable during the runs and does not change from run to run. The electroosmotic flow was faster in salt-free NMF than after the addition of NaCl. In aqueous buffers the μ_{eo} is also inversely related to the ionic strength [58] but in pure water there are not enough ionic species to maintain electric current between the electrodes. In salt-free NMF the current was 3.5 μA. However, the resolution of the enantiomers and the stability of the baseline were improved when 10 m*M* NaCl was added to the separation medium. It may be practical to add a known low amount of salt to the NMF in order to minimise the effect of the differences in the impurity profiles of the solvents from various sources.

There are numerous reports on the chiral separation of Dns-AAs by CE some of them describing very good resolution and high efficiency [17,19,34–54]. Although a comprehensive review of the literature is beyond the scope of this paper, a comparison of our results and some from the literature may be useful. Particularly high resolution and good efficiency have been achieved by Karger et al. [17]

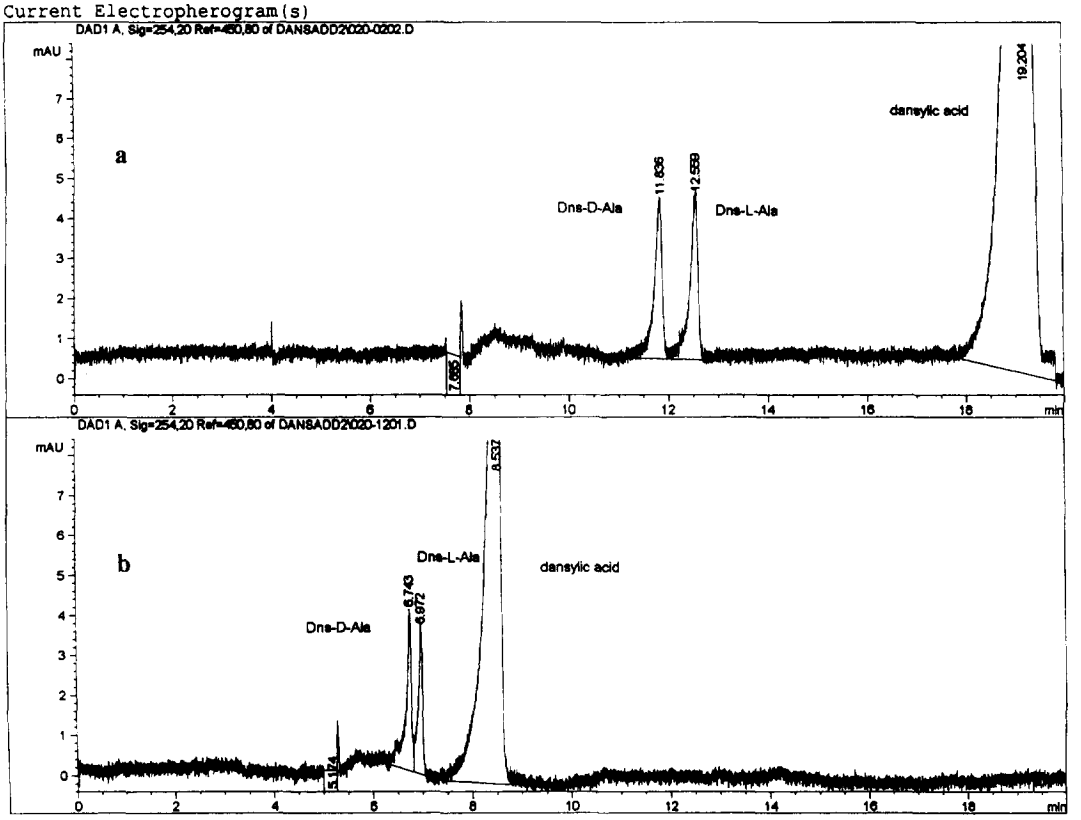

Fig. 3. Separation of Dns-Ala enantiomers with and without NaCl dissolved in the separation medium. Background electrolyte: (a) 100 m*M* β-CD in 10 m*M* NaCl in NMF, (b) 100 m*M* β-CD in NMF. Other experimental conditions are the same as in Fig. 1.

who incorporated 75–100 m*M* β-CD into a polyacrylamide gel. In spite of the good separations achievable by gel electrophoresis the relatively laborious nature of this technique has limited its application. Almost all the reports have shown the chiral separation of racemic Dns-AAs from commercial sources. Only Okafo and Camilleri [39] have reported the separation of in situ derivatized Dns-AAs. Using β-CD in combination with taurodeoxycholate micelles they achieved a good separation for several Dns-AAs. The efficiency in these separations was about the same as in our nonaqueous system. Some results indicate that γ-CD may provide better resolution than β-CD [44,46]. The chiral separation of Dns-Phe within less than 2 min has been reported by Sepaniak et al. [50] using hydroxypropyl-β-CD. Yoshinaga and Tanaka have reported in [51] that addition of urea and urea derivatives to 50 m*M* NaH_2PO_4–100 m*M* borate buffer (pH 9.0) containing 10 m*M* β-CD (the same buffer was used in our study for comparison) can enhance the resolution of Dns-AAs considerably. However, the improved resolutions were achieved at longer migration times and in some cases the efficiency was significantly reduced. The nonaqueous method we describe can provide high resolution even in a relatively complex sample matrix, however the efficiency of the separation is not particularly good.

4. Conclusions

The physical and chemical characteristics of water make it an excellent medium for electrophoresis. Water has always been and most probably will always be the first choice in the development of a CE method. However, the properties of water also determine the limits of its applicability. In some

cases organic solvents will have distinct advantages over water. There are several solvents with very different physical and chemical properties suitable for CE applications. Recently there has been a growing interest in widening the application range of CE by using nonaqueous separation media.

We have demonstrated the chiral separation of Dns-AAs in NMF. NMF is a good solvent for many organic and inorganic compounds. When the solubility of analytes or certain additives such as chiral selectors is low in water, NMF may be an alternative solvent. β-CD is about 40 times more soluble in NMF than in water. The solvent has an immense effect on the stability of the analyte–chiral selector complex. The selectivity and optimum concentration of the selector can be controlled by choosing different nonaqueous systems.

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