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Effect of additives on chiral selectivities by β-cyclodextrin in capillary electrophoresis based on the phenomenon of solvatochromism

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Abstract

To explore the relationship between solvatochromic parameters and chiral selectivity, we measured the effect of organic solvents as buffer additives on the chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol by β-cyclodextrin in capillary electrophoresis. Negative relationships between the additive's log L^{16} and chiral selectivities were observed for the systems added C_1–C_6 alcohols, C_1–C_5 amines, and C_1–C_5 acids. The chiral selectivities also decreased significantly with the additive's increasing hydrogen bond basicity. The decreased selectivities of additives on dansyl-phenylalanine is much greater than on dansyl-leucine. The chiral selectivities of propranolol remained unchanged with all the additives in the buffer. Although most additives studied decreased chiral selectivities, the data presented in this work provided some initial information on chiral selectivities relating to solvatochromic parameters.

Keywords: Enantiomer separation; Solvatochromism; Buffer composition; Propranolol; Amino acids

1. Introduction

The chiral resolution in capillary electrophoresis (CE) can be described in the following equation:

$$Rs = \frac{\sqrt{N}}{4} \cdot \left(\frac{\Delta\mu}{\mu_{av} + \mu_{eo}} \right)$$

where N is the number of theoretical plates, $\Delta\mu$ is the difference in electrophoretic mobility of two enantiomers, μ_{av} is the average electrophoretic mobility of two enantiomers, and μ_{eo} is the mobility of electroosmotic flow.

Wren and Rowe [1,2] described a chiral separation model in CE assuming that a pair of free enantiomers have identical electrophoretic mobilities. The electrophoretic mobility of an enantiomer is then expressed in terms of concentration of cyclodextrin, [C], and binding constant K between an enantiomer and cyclodextrin as in the following equation:

$$\mu = \frac{\mu_f + \mu_c K_1[C]}{1 + K_1[C]}$$

The difference in electrophoretic mobility between two enantiomers is then given as:

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$$\Delta\mu \frac{[C](\mu_f - u_c)(K_2 - K_1)}{1 + [C](K_1 + K_2) + K_1K_2[C]^2}$$

where μ_f and μ_c are the electrophoretic mobilities of the free and complexed enantiomers. K_1 and K_2 are the binding constants between cyclodextrin and enantiomers 1 and 2, respectively. The theoretical expression given above predict that the chiral resolution in cyclodextrin-based separations can be affected by (1) the concentration of cyclodextrin, (2) the difference of the binding constants of the two enantiomer–cyclodextrin complexes, (3) the effective electrophoretic mobility of the free enantiomers, (4) the electroosmotic flow (EOF).

Chiral resolution will be zero if the cyclodextrin concentration is zero and decreases towards zero at extremely high concentration. Therefore an optimal cyclodextrin concentration should exist to give the maximum resolution in a particular system. The resolution also decreases as the difference between the binding constants of the two enantiomer–cyclodextrin complexes decreases. The binding constants change with the type of chiral selector as well as the buffer composition, e.g. percentage of organic solvent. It has been shown that most organic solvents in buffer decrease the host–guest binding constants significantly [3,4]. Organic solvent in buffer also changes selectivities by changing the EOF.

A majority of CE research on chiral separation has been focused on how to improve chiral separation by varying the type of chiral selector, concentration of chiral selector, pH, ionic strength of the buffer, temperature, etc. [5–7]. However there is a lack of models to predict chiral selectivity based on bonding mechanism between chiral selector and the analytes.

In order to obtain direct separation with cyclodextrin in CE, it is necessary for three different interactions to take place between the enantiomers and cyclodextrin, namely, hydrogen bonding, hydrophobic partition or inclusion into the cavity of cyclodextrin, and specific steric interactions. [8,9]. Besides the parameters can be changed in Wren and Rowe's model, e.g. concentration of chiral selector, chiral resolution in CE may also be achieved or enhanced with organic additives in buffer to increase the hydrogen bonding and/or hydrophobic partition between analytes and cyclodextrin. These factors may be well related to solvatochromic parameters of organic additives, e.g., hydrogen bond acidity α, hydrogen bond basicity β, and hydrophobicity term $\log L^{16}$.

The phenomenon of solvatochromism refers to the effect of solvent on the solute's properties and can be applied to intermolecular interactions in a chromatographic system. In fact the partition or retention in a gas and liquid chromatographic system has been studied extensively by applying the phenomenon of solvatochromism [10]. For example, based on the linear solvation energy relationship, it has been observed that the capacity factor in reversed-phase LC correlated very well with the solute's $\log L^{16}$ and β values [11]. However the relationship between solvatochromic parameters and selectivity has not been explored before, especially relating to chiral selectivity.

We chose organic solvents which have solvatochromic parameters available as additives in CE. Since only small amounts of these additives (50–100 m*M*) are added to the aqueous run buffer, the solvatochromic parameters used in this study and shown in Tables 1 and 2 are their solute values. The objective of this work is to initially investigate the effect of these additives on the chiral selectivities by β-cyclodextrin in terms of the solvatochromic pa-

Table 1
Values of dipolarity–polarizability π^*, hydrogen-bond acidity α, and hydrogen-bond basicity β of selected buffer additives

	π^*	α	β	$\log L^{16}$
Water	0.45	0.82	0.35	0.260
Methanol	0.44	0.43	0.47	0.970
Ethanol	0.42	0.37	0.48	1.485
1-Propanol	0.42	0.37	0.48	2.031
1-Butanol	0.42	0.37	0.48	2.601
1-Pentanol	0.42	0.37	0.48	3.106
1-Hexanol	0.42	0.37	0.48	3.610
Formic acid	0.60	0.75	0.38	
Acetic acid	0.65	0.61	0.44	1.750
Propanoic acid	0.65	0.60	0.45	2.290
Butanic acid	0.62	0.60	0.45	2.830
Pentanoic acid	0.60	0.60	0.45	3.380
Hexanoic acid	0.60	0.60	0.45	3.920
Methylamine	0.35	0.16	0.58	1.300
Ethylamine	0.35	0.16	0.61	1.677
n-Propylamine	0.35	0.16	0.61	2.141
n-Butylamine	0.35	0.16	0.61	2.618
n-Pentylamine	0.35	0.16	0.61	3.139
n-Hexylamine	0.35	0.16	0.61	3.655

Table 2
Values of dipolarity–polarizability π^*, hydrogen-bond acidity α, and hydrogen-bond basicity β of selected buffer additives

	π^*	α	β	Log L^{16}
Trimethylphosphate	1.10	0	1.0	Not available
N,*N*-Dimethylformamide	1.31	0	0.75	3.173
Dioxane	0.75	0	0.64	2.892
Tetrahydrofuran	0.52	0	0.48	2.636
Ethylacetate	0.62	0	0.45	2.314
Acetamide	1.30	0.54	0.68	Not available
Trifluoroethanol	0.60	0.57	0.25	1.224
Chloroacetic acid	1.08	0.74	0.36	Not available
Dichloroacetic acid	1.20	0.90	0.27	Not available

rameters, mainly the additive's hydrophobicity, log L^{16}, and hydrogen bond basicity, β. It is not the intention of this work to achieve the maximum chiral separation. Dansyl-phenylalanine, dansyl-leucine, and propranolol were chosen as the analytes because they showed various chiral selectivities in the run buffer without any additives.

2. Experimental

2.1. Apparatus

All separations were carried out using a HP^{3D} capillary electrophoresis system (Hewlett–Packard, Avondale, PA, USA). Barefused-silica capillaries of 80.5 cm (effective length to the detector window 72 cm)×50 μm I.D. were used. The system was operated at constant temperature (25°C) and constant voltage (25 kV) using the normal polarity mode, with detection towards the cathodic end of the capillary. Samples were injected by applying a pressure of 50 mbar for 3 s. The separation was monitored at 210 nm with a diode-array detector.

2.2. Chemicals

β-Cyclodextrin and (*R*,*S*)-propranolol hydrochloride were from Fluka (Buchs, Switzerland). (D,L)-Dansyl-phenylalanine and (D,L)-dansyl-leucine were from Sigma (St. Louis, MO, USA). Sodium phosphate monobasic monohydrate, phosphoric acid, methanol, 1-propanol, 1-butanol, 1-pentanol, *N*,*N*-dimethylformamide, and tetrahydrofuran were from EM Science (Darmstadt, Germany). 1-Hexanol, formic acid, propanoic acid, butanic acid, methylamine, 1-propylamine, 1-butylamine, 1-pentylamine, trimethylphosphate, dioxane, acetamide, 2,2,2-trifluoroethanol, chloroacetic acid, and dichloroacetic acid were from Aldrich (Milwaukee, WI, USA). Acetic acid (glacial), pentanoic acid, ethylamine, and ethylacetate were from J.T. Baker (Philipsburg, NJ, USA). Ethanol was from Quantum Chemical (Anaheim, CA, USA).

2.3. Procedures

Racemic (*R*,*S*)-propranolol was used in this study. For dansyl-(DL)-phenylalanine and dansyl-(DL)-leucine, about 25% of pure L-isomers were added to the racemic mixture to identify the migration order of isomers. The individual analyte was dissolved in deionized water at 0.5 mg/ml. The sample solution was ultrasonicated and filtered through a 0.45-μm acrodisc membrane filter. The buffer of 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate was prepared with sodium phosphate monobasic monohydrate and adjusted to pH 2.5 with phosphoric acid. The individual buffer with additive was prepared by adding the required amount of each additives to the above buffer except for the C_1–C_5 amines and acids. These systems were prepared by adding the required amount of β-cyclodextrin, sodium phosphate, and additives then adjusting the pH to 2.5 with phosphoric acid or sodium hydroxide. The buffer was ultrsonicated for 10 min and filtered through a 0.45-m acrodisc membrane filter before use. The capillary was flushed for 3 min with water, 3 min with 0.1 *M* sodium hydroxide, 3 min with water, and 5 min with buffer before run. It was flushed for 5 min with buffer between the runs.

3. Results and discussions

3.1. Effect of the additive's log L^{16}

Abraham published the theory on construction of hydrogen bond scales as well as values of dipolarity–polarizability π^*, α, β, and log L^{16} for more than 300 commonly used solutes/solvents [12]. We chose C_1–C_6 alcohols, C_1–C_5 amines, and C_1–C_5 acids as

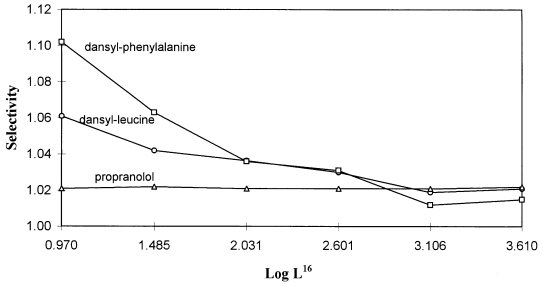

Fig. 1. Effect of 100 m*M* of C_1–C_6 alcohol additives (plotted as $\log L^{16}$) on chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol. Buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate at pH 2.5. Capillary: 72 cm×50 μm, voltage: 25 kV, temperature: 25°C.

buffer additives to investigate the effect of the $\log L^{16}$ on chiral selectivities since they all have nearly the same π^*, α, and β values but sequentially increasing $\log L^{16}$ (Table 1). Therefore the results observed with the above additives could be counted as the contribution of $\log L^{16}$ only.

The effects of C_1–C_6 alcohols, C_1–C_5 amines, and C_1–C_5 acids on chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol are shown in Figs. 1–3. The selectivities in this study were actually migration separation factors which were calculated as follows:

$$\alpha = \frac{t_2 - t_0}{t_1 - t_0}$$

where t_2 is the migration time for the second

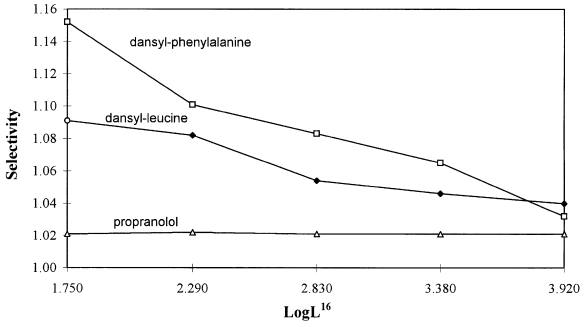

Fig. 2. Effect of 100 m*M* of C_1–C_5 amine additives (plotted as $\log L^{16}$) on chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol. Buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate at pH 2.5. Capillary: 72 cm×50 μm, voltage: 25 kV, temperature: 25°C.

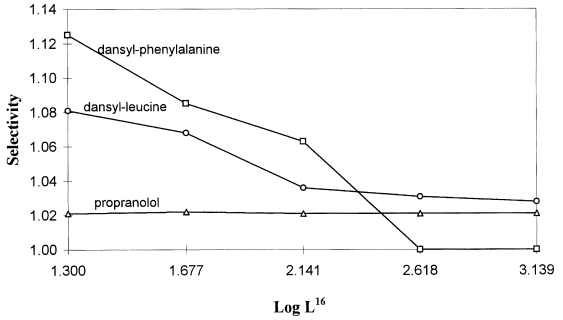

Fig. 3. Effect of 100 m*M* of C_1–C_5 acid additives (plotted as $\log L^{16}$) on chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol. Buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate at pH 2.5. Capillary: 72 cm×50 μm, voltage: 25 kV, temperature: 25°C.

enantiomer, t_1 is the migration time for the first enantiomer, t_0 is the migration time of a neutral marker, which was measured with acetone. Compare to the selectivities obtained from buffer without additives ($\alpha = 1.09$ for dansyl-phenylalanine, $\alpha = 1.05$ for dansyl-leucine), chiral selectivities of dansyl-phenylalanine and dansyl-leucine decreased with most additives except for C_1 systems. Selectivities slightly increased with the addition of methanol, methylamine, and formic acid.

Linear solvation energy relationships (LSERs) have been tested in gas and liquid chromatography systems [10,13]. Positive linear correlations between the capacity factor and solute's $\log L^{16}$ have been observed. The negative correlations between chiral selectivities and additive's $\log L^{16}$ values observed in this work is probably due to the decreases in the host–guest binding constants between cyclodextrin and the analytes or competitive complexation after adding these relatively hydrophobic additives to the aqueous buffer. It is demonstrated by other researchers that the binding constant between cyclodextrin and solute decreased significantly with an increase in buffer hydrophobicity. Others have found that chiral selectivity decreases upon adding extra hydrophobic analytes due to competitive complexations between β-cyclodextrin and solutes [14,15].

The extent of the negative effect of the additive's $\log L^{16}$ on chiral selectivities is in the order of acids > amines > alcohols. The more profound decreases by acid and amine additives are not likely due to the difference in buffer pH values or ionic

strengths compared to those of alcohol systems, since each buffer solution was individually adjusted to the same pH (2.5) values before use. The conductivities were monitored after pH adjustment as the relative measure of buffer ionic strengths. We did not observe significant differences in migration times with different systems; therefore, the viscosity change due to the addition of 100 m*M* additives must be insignificant.

It is worthwhile mentioning that although we used (LSERs) to introduce the results for gas–liquid chromatographic systems, the relationships presented in this study between chiral selectivities and additive's log L^{16} are not really LSERs. The authors just used some solvatochromic descriptors to demonstrate the effect of buffer additives on chiral selectivity. The relationships between selectivities and additive's log L^{16} values are empirical.

It is interesting to note that chiral selectivities of propranolol remained unchanged despite all the different solvents added in the buffer. This indicates that propranolol may have a different chiral recognition mechanism. In fact, it is demonstrated that the pH of the buffer and the type of chiral selector are probably the most predominant factor in controlling chiral resolution of propranolol. Chiral recognition only occurred in a very narrow pH range, 6–8 [16].

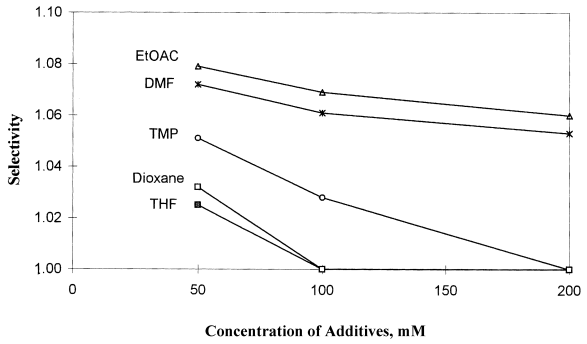

Fig. 5. Effect of 50, 100, and 200 m*M* additives on chiral selectivities of dansyl-phenylalanine. Run buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate, pH 2.5. EtOAC = ethylacetate, DMF = *N*,*N*-dimethylformamide, TMP = trimethylphosphate, THF = tetrahydrofuran.

However, discussion of the chiral recognition mechanism is beyond the scope of this paper.

3.2. Effect of hydrogen bond basicity, β

Due to limited data on hydrogen bond basicity, we did not investigate this parameter at as wide a range as we desired. We chose the additives listed in Table 2 with relative similar log L^{16} and α values. Fig. 4 shows the selectivities vs. concentrations of different

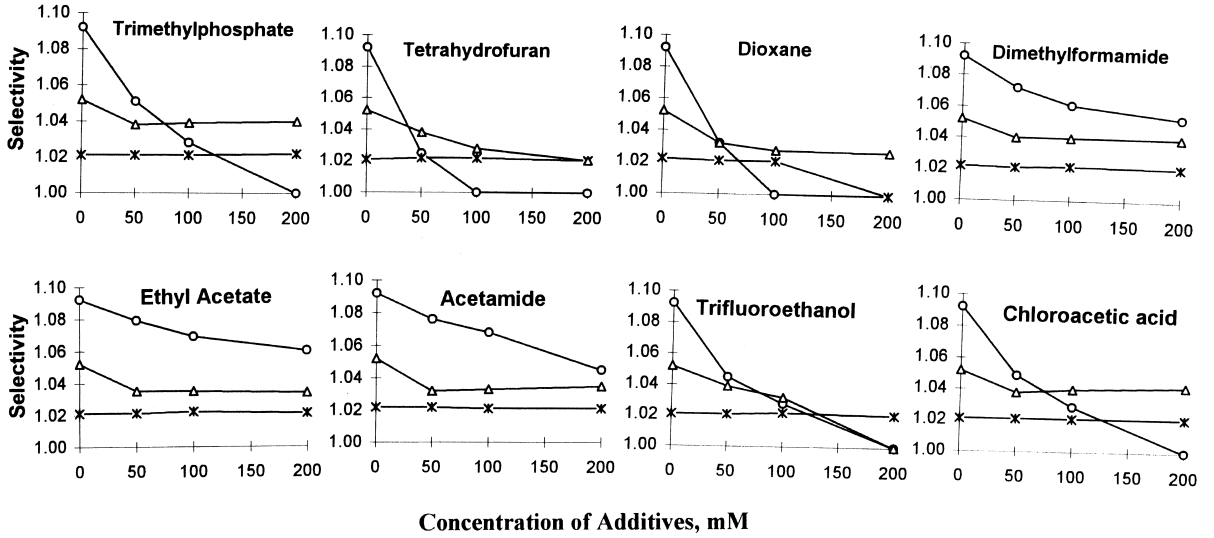

Fig. 4. Effect of additives on chiral selectivities. Buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate, pH 2.5. O = Dansyl-phenylalanine; Δ = dansyl-leucine; * = propranolol. Capillary: 72 cm × 50 μm, voltage: 25 kV, temperature: 25°C.

additives in buffer. It clearly shows that chiral selectivities of dansyl-phenylalanine decreased significantly by adding these additives. The selectivities decreased as the additive concentration increased. For example the selectivity of dansyl-phenylanine in the buffer without any additives is 1.09. It decreased to 1.00 in buffers with 200 m*M* additives. The effect of these additives on chiral selectivities of dansyl-leucine are much weaker. In fact, there are no differences in selectivities of dansyl-leucine in buffers with 50, 100, and 200 m*M* additives. Selectivities of propranolol remained unchanged upon adding all these additives.

Fig. 5 shows the selectivities of dansyl-phenylalanine vs. concentrations of different additives with different β values and zero in α values. It shows that hydrogen bond basicity of the additive is also a significant factor in chiral separation by capillary electrophoresis. Trimethylphosphate which has the highest β value had the most significant effect, while ethylacetate which has the lowest β value had the least effect on selectivities with the exception of tetrahydrofuran and dioxane. The electropherograms in Fig. 6 show the decreases of chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol in buffers with 50, 100, and 200 m*M* of trimethylphosphate comparing to the buffer with no additives.

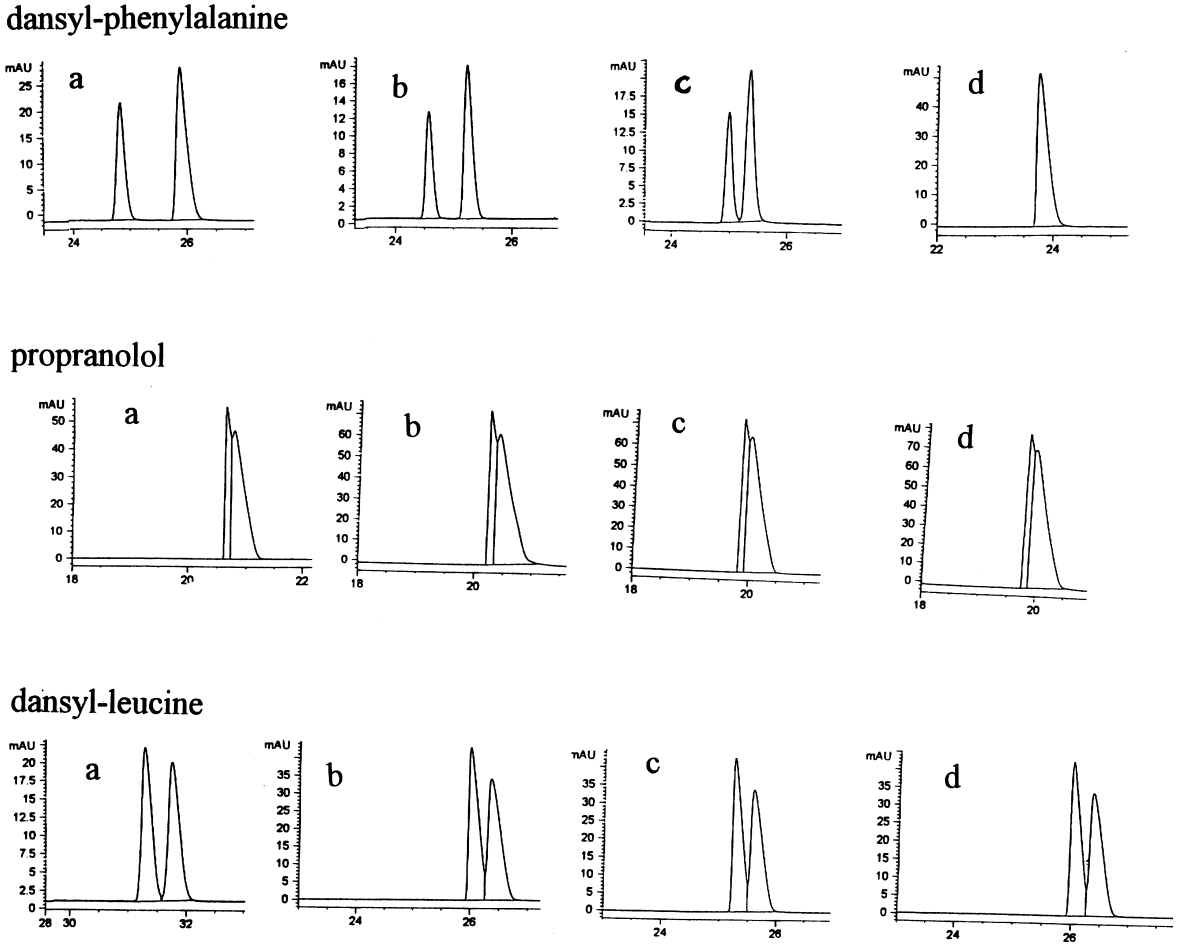

Fig. 6. Effect of 50, 100, and 200 m*M* trimethylphosphate on chiral separation of dansyl-phenylalanine, dansyl-leucine, and propranolol. (a) No trimethylphophate added, (b) 50 m*M* trimethylphosphate, (c) 100 m*M* trimethylphosphate, (d) 200 m*M* trimethylphophate. Buffer: 10 m*M* β-cyclodextrin in 50 m*M* sodium phosphate at pH 2.5. Capillary: 72 cm×50 μm, voltage: 25 kV, temperature: 25°C.

The decreases in chiral selectivities with the additives listed in Table 2 are probably due to the decreases in hydrogen bond strengths between β-cyclodextrin and analytes, since higher β values means stronger hydrogen bond-accepting abilities. The distinct effect in chiral selectivities by tetrahydrofuran and dioxane is probably due to the ring structures of these two additives. They may also be included in the hydrophobic cavity of β-cyclodextrin and hence likely acted as competitive complexing solutes with β-cyclodextrin.

It is difficult to address the effect of hydrogen bond acidity on chiral selectivity since there are not many solvents which have the same β or $\log L^{16}$ but different α values. Most importantly water has a very high hydrogen bond acidity and a rather weak hydrogen bond basicity. Consequently, when there is a great deal of water present, it is expected that additives with lower hydrogen bond acidities should not increase the hydrogen bonding between cyclodextrin and analytes and consequently chiral selectivity. This speculation was proven in reversed-phased liquid chromatography where there is a lack of dependence of the solute's retention on its α value [10].

4. Conclusions

Chiral selectivities of dansyl-phenylalanine, dansyl-leucine, and propranolol by β-cyclodextrin in capillary electrophoresis were measured to relate to two solvatochromic parameters, buffer additive's $\log L^{16}$ and their hydrogen bond basicity β values. For the systems added C_1–C_6 alcohols, C_1–C_5 amines, and C_1–C_5 acids, chiral selectivities decreased with the increased additive's $\log L^{16}$. The chiral selectivities also decreased significantly with additive's increasing hydrogen bond basicity. The decreased selectivities of additives on dansyl-phenylalanine is much greater than on dansyl-leucine. The chiral selectivities of propranolol remained unchanged with all the additives in the buffer.

References

[1] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 603 (1992) 235.
[2] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 609 (1992) 363.
[3] I.E. Valko, H. Siren, M.L. Riekkola, J. Chromatogr. A 737 (1996) 263.
[4] F. Wang, M.G. Khaledi, Anal. Chem. 68 (1996) 3460.
[5] S. Fanali, J. Chromatogr. A 735 (1996) 77.
[6] T.J. Ward, Anal. Chem. 66 (1994) 633.
[7] M. Novotny, H. Soini, M. Stefansson, Anal. Chem. 66 (1994) 646.
[8] C.E. Dalgliesh, J. Chem. Soc. 132 (1952) 3940.
[9] D.W. Armstrong, T.J. Ward, R.D. Armstrong, T.E. Beesley, Science 232 (1986) 1132.
[10] P.W. Carr, Microchem. J. 48 (1993) 4.
[11] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, M.H. Abraham, Anal. Chem. 57 (1985) 2971.
[12] M.H. Abraham, P.L. Grellier, R.A. McGill, J. Chem. Soc. Perkin Trans. II (1987) 797.
[13] J.H. Park, P.W. Carr, J. Chromatogr. 465 (1989) 123.
[14] S.G. Penn, G. Liu, E.T. Bergstrom, D.M. Doodall, J.S. Loran, J. Chromatogr. A 680 (1994) 147.
[15] M.W. Matchett, S.K. Branch, T.M. Jefferies, J. Chromatogr. A 705 (1995) 351.
[16] A. Guttman, N. Cooke, J. Chromatogr. A 680 (1994) 157.