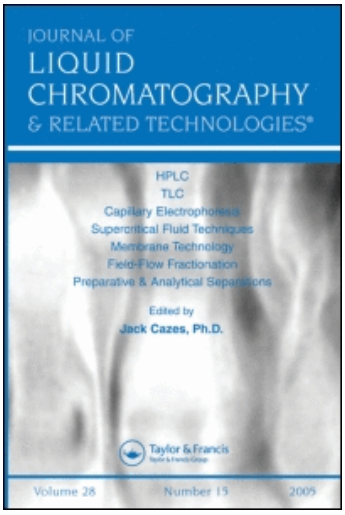

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B. Soontornniyomkij[a]; K. Scandrett[b]; D. J. Pietrzyk[b]
[a] Department of Chemistry Warinchamrap, Ubon Ratchathani University, Ubon Ratchathani, Thailand
[b] Department of Chemistry, University of Iowa, Iowa City, IA

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CAPILLARY ZONE ELECTROPHORESIS SEPARATION OF AMINO ACID ENANTIOMERS AS DANSYLATED DERIVATIVES THROUGH CONTROL OF ELECTROOSMOTIC FLOW

B. Soontornniyomkij[1,†], K. Scandrett,[2] D. J. Pietrzyk[2,*]

[1] Ubon Ratchathani University
Department of Chemistry
Warinchamrap
Ubon Ratchathani, 34190 Thailand

[2] University of Iowa
Department of Chemistry
Iowa City, IA 52242

ABSTRACT

D,L-Amino acid enantiomers are separated by capillary zone electrophoresis (CZE) as 5-dimethylamino- 1-naphthalene sulfonyl (dansyl) derivatives using a Cu^{2+}-L-aspartyl-L-phenylalanine methyl ester (aspartame) complex as a chiral selector in the buffer. Only partial enantiomeric resolution is obtained for several dansyl-D,L-amino acids and for the most favorable cases resolution approaches about 1.4. Increasing Mg^{2+}, Cd^{2+}, or Zn^{2+} concentration in a 10 mM NH_4OAc, 2.5 mM Cu^{2+}, 5.0 mM aspartame, pH = 7.40 buffer increases dansyl-D,L-amino acid migration time, increases migration time difference between the D-enantiomer, which appears first in

the electropherogram, and the L-enantiomer, and resolves the enantiomers of most dansyl-D,L-amino acids with resolution values in the range of 1.4 to >5.0 depending on the derivative and buffer conditions. An increase in the buffer divalent cation concentration reduces the electroosmotic flow (EOF) while the electrophoretic mobilities of the dansyl-D,L-amino acid enantiomers do not undergo significant change as the divalent cation concentration increases. The improved resolution between the dansyl-D,L-amino acid enantiomers is due to the reduced EOF and small differences in the ternary complex formed between the dansyl-D,L-amino acid enantiomers and the Cu^{2+}-aspartame complex.

INTRODUCTION

Successful enantiomeric separations in capillary zone electrophoresis (CZE) are often determined by the appropriate selection of a chiral selector to include in the buffer. No one selector is universal for all enantiomeric separations and several different classes of selectors have been employed in CZE enantiomeric separations. The major ones include chiral metal complexes, cyclodextrins, chiral crown ethers, chiral micelles, and certain proteins and glycoproteins capable of chiral recognition.

Resolution of amino acids into their enantiomeric forms continues to be a significant problem faced within the biological sciences and related areas. Often the amino acids are first converted into derivatives and these chiral derivatives are subsequently separated. This approach has been successful in the separation of amino acid enantiomers by high performance liquid chromatography (HPLC) and is now being applied to CZE and related strategies.

Many derivatizing reagents have been used in the CZE separation of amino acids[1] but not all are useful in the separation of chiral amino acid derivatives. Racemic mixtures of amino acids as 5-dimethyl-amino-1-naphthalene sulfonyl (dansyl) derivatives were resolved by CZE using the chiral selector Cu^{2+}-L-histidine complex,[2] Cu^{2+}-aspartame (L-aspartyl-L-phenylalanine methyl ester) complex,[3] a bile acid,[4,5] a cyclodextrin,[6-8] or antibiotics such as vancomycin,[9] teicoplanin,[10] or ristocetin[11] in the buffer. Phenyl isothiocyante- (PTH) amino acid derivatives were separated into enantiomers by micellar electrokinetic capillary chromatography (MEKC) with sodium dodecyl sulfate (SDS)[12] or by SDS/sodium-N-dodecanyl-L-serine[13] as

the buffer additive. SDS/sodium-N-dodecanyl-L-valine facilitated the enantiomeric resolution of N-3,5-dinitrobenzoylated-D,L-amino acid-O-isopropyl ester derivatives.[14] A SDS buffered system aided the MEKC resolution of D,L-amino acid-O-acetyl -β-D-glucopyransoyl isothiocyanate derivatives[15] while naphathalene-2,3-dicarboxaldehyde-(NDA)-D,L-amino acid derivatives[16] were resolved by MEKC with cyclodextrin as the buffer additive.

Derivatization is not always required and CZE resolution of racemic mixtures of free amino acids is possible by including 18-crown-6-tetracarboxylic acid[17,18] as a chiral selector in the buffer.

In addition to the chiral selector, its concentration, and the derivatizing reagent, other factors such as the buffer pH, ionic strength, micelle reagents, electrolytes, or nonelectrolytes and their concentration must be optimized to obtain good resolution in the enantiomeric CZE separation of D,L-amino acids.

In general these factors influence electroosmotic flow (EOF) and since the mobility difference between D- and L-amino acid enantiomers is small, resolution is often enhanced by manipulation of EOF. Coating or chemical derivatization of the silica capillary wall can also produce a change in EOF.[19,20]

In this paper, we report our studies on using inorganic cations as buffer additives to reduce EOF and subsequently to enhance the resolution of dansyl-D,L-amino acid derivatives using a Cu^{2+}-aspartame complex as the chiral selector in the buffer.

Previous studies have shown that migration times and subsequently resolution for the CZE separation of anionic surfactants,[21] organic acids and small chain peptides,[22] and for 2,4-dinitrophenyl-amino acid derivatives[1,23] increase as buffer cation concentration increases.

These studies[1,21-23] also demonstrated that increasing the buffer cation concentration decreases EOF due to cation exchange at the fused silica silanol sites which alters the surface zeta potential. Since analyte electrophoretic mobilities are unaffected, the improved CZE separation is due to the EOF change. Furthermore, ionic strength plays only a minor role and the major influence on migration time and resolution is cation dependent with divalent cations being more effective than monovalent cation.

Optimization of the buffer cation and its concentration significantly enhances migration time and resolution in the CZE separation of these analytes and, therefore, has a significant impact on analysis time.

EXPERIMENTAL

Chemicals

Free D- and L-amino acids, 5-dimethylamino-1-naphthalene sulfonyl chloride, 5-dimethylamino-5-naphthalene sulfonyl-D,L- and L-amino acid (dansyl-D,L-amino acids) derivatives, and L-aspartyl-L-phenylalanine methyl ester (aspartame) were obtained from Sigma Chemical Co. Mesityl oxide, used as a neutral marker for the determination of EOF, was obtained from Aldrich Chemical Co. All inorganic salts, acids, and bases were purchased from Mallinckrodt Chemical Works, Aldrich Chemical Co., and Fisher Scientific as analytical reagent grade and used as received. Freshly purified water, obtained by passing in house distilled water through a Milli-Q-Plus water treatment unit with 2μm filtration, was employed for the preparation of all sample and buffer solutions.

Instrumentation

A 60.2 cm, 50 μm i.d., and 375 μm o.d. fused silica capillary from Polymicro Technologies was used for all measurements. An optical window was prepared at 20 cm from the capillary outlet end to yield a 40.2 cm effective capillary length. A lab-assembled CE instrument composed of a Spellman High Voltage DC Power Supply Model UHR (0 to 30 kV), a Spectra Physics Spectra Focus 100 variable wavelength detector equipped with an on-column capillary accessory Model 9550-01555 to accommodate the capillary, platinum electrodes to connect the power supply with the buffer reservoirs, and a multimeter to monitor current flow was used for all measurements. Both electrode reservoirs were enclosed in a Plexiglas safety box with an interlock system. Separation data were collected with a Spectra Physics M-4270 integrator controlled by Spectra Physics Autolab and were processed by Microsoft Excel software.

Procedures

The fused silica capillary was preconditioned for 20 min by pulling a NaOH solution through the capillary by vacuum followed by a 5 min wash with water. The buffer was drawn into the capillary and the capillary containing the buffer was stored overnight prior to its first use. Capillary storage thereafter was in the presence of the buffer. Measurements were made after the capillary

delivered a constant, reproducible EOF, determined with mesityl oxide as the marker and +25 kV applied to the capillary, and at 25°C. During the studies the buffer in the reservoirs was replaced with new buffer after 3 to 4 runs. When switching between divalent cations as the buffer additive the capillary was treated with 0.5 M $(NH_4)_2$citrate, pH = 6.0 buffer prior to using a new buffer condition. Capillary performance was monitored during the study with a mesityl oxide analyte in a 1:4 CH_3CN:H_2O solution and a 20 mM NH_4OAc, pH = 7.0 buffer. When analyte peak broadening or an appreciable change in migration time (EOF) occurred the capillary was reconditioned or discarded for a new capillary of the same dimensions.

The chiral buffer solution was prepared by combining aliquots of aqueous 0.5 M NH_4OAc and 0.2 M $CuSO_4$ stock solutions and a known weight of aspartame to yield a final solution that is 10 mM NH_4OAc, 2.5 mM $CuSO_4$, and 5 mM aspartame with the pH adjusted to pH = 7.40 with dilute ammonia prior to dilution to the final volume. Standard solutions of 0.1 to 1.0 mM of each dansyl-D,L,-amino acid and dansyl-L-amino acid were prepared by dissolving the derivative in a 2.5 mM $CuSO_4$, 10 mM NH_4OAc, pH = 7.40 buffer solution. For mixtures of dansyl amino acids, known quantities of the more concentrated dansyl amino acid standard solutions were combined to give a final concentration of 0.1 mM in each dansyl amino acid. Dansyl amino acid derivatives were prepared according to the procedure described by Tapuhi, et al.[24] The room temperature reaction mixture containing a known weight of amino acid and derivatizing reagents was terminated after one hr with 100 μL of a 2.0 % ethylamine solution and diluted to a known volume with a 2.5 mM $CuSO_4$, 10 mM NH_4OAc, pH = 7.40 buffer to yield a solution that is 0.1 mM of the dansyl amino acid derivative. A blank solution containing all the reagents except the amino acid was prepared in the same way to identify extraneous peaks.

Samples were introduced into the CE instrument by a hydrostatic method for 5 to 45 sec depending on the sample with the volume injected being 3 to 15 μL. Applied voltage was typically + 30 kV and the measured current typically varied between 16 to 30 μA depending on the buffer cation concentration. Detection of the dansyl derivatives was at 325 nm. All measurements were made at ambient temperature, 22.5°C. The data reported here represent averages of usually more than three measurements and repetitions of each study where new capillaries, dansyl amino acid standard solutions, and buffer solutions were employed. Identity of individual dansyl amino acid enantiomers was verified by comparison of migration times and spiking the mixture repeatedly with known, standard dansyl-D- (not all D-derivatives were available) and L-amino acid derivative samples.

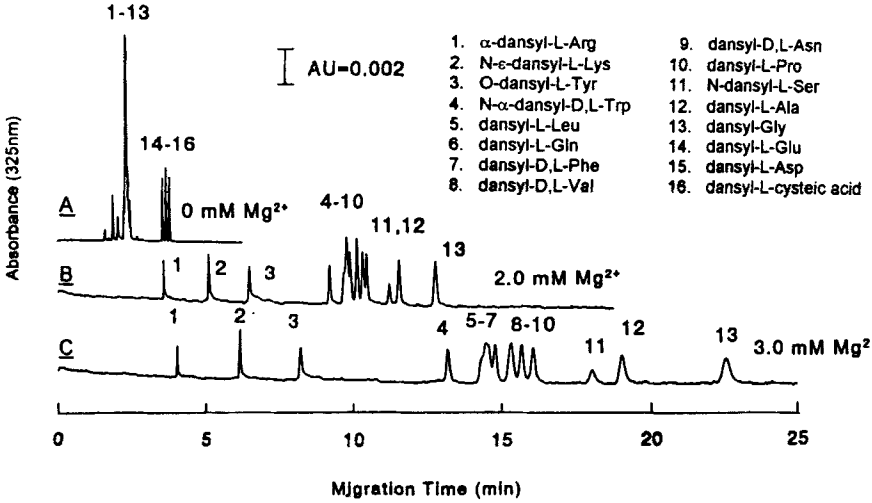

Figure 1. The separation of dansyl-amino acid derivatives in the presence of Mg^{2+} and the absence of a chiral selector in the buffer. The buffer is 20 mM borate, pH = 9.25 (A) in the absence of Mg^{2+}, (B) with 2.0 mM Mg^{2+}, and (C) with 3.0 mM Mg^{2+}

RESULTS AND DISCUSSION

Dansyl-D,L-Amino Acids

In a basic, aqueous buffer most dansyl-D,L-amino acids, which are predominately anions, except those that have a basic side chain such as dansyl-D,L-Arg and dansyl-D,L-Lys, can be separated by CZE. The derivatives have different migration times because of electrophoretic mobility differences due to dissimilarity in the amino acid side chain and to EOF. This is illustrated in Figure 1 where 16 different dansyl amino acid derivatives are separated in a 20 mM borate, pH = 9.25 buffer with Mg^{2+} as a buffer EOF modifier (36,37). As Mg^{2+} concentration increases, EOF decreases, migration time increases, and the electrophoretic mobility for each derivative remains nearly constant. Thus, resolution is improved because of the decrease in the EOF. The decreased EOF is due to cation exchange between the Mg^{2+} and the fused silica free silanol sites which changes the silica wall surface charge.[1,21-23] Even though CZE provides high efficiency, dansyl-D,L-amino acid enantiomeric separation is not observed and the dansyl-D,L-amino acid derivative enantiomers coelute as shown in Figure 1.

If a chiral selector is included in the buffer, separation of the enantiomers is possible. Both Cu^{2+}-L-His[2] and Cu^{2+}-aspartame[3] complexes, the latter being more effective, have been employed as chiral buffer additives to achieve enantiomeric resolution in the CZE separation of dansyl-D,L-amino acids.

The 1:2 Cu^{2+}:aspartame complex is reported to form a six membered ring between Cu^{2+} and the α-amino group and β-carboxyl group of the aspartyl residue and when the dansyl-D,L-amino acid enantiomers are introduced into the buffer containing the chiral selector, each enantiomer replaces one of the aspartame molecules forming a more stable five membered ring.[3] This results in a diastereomeric ternary complex that forms dynamically in the CZE buffer system.

It is also suggested[3] that the ternary complex stability constant is influenced by additional hydrophobic interactions between the dansyl amino acid side chain and the aspartame L-Phe residue. The dansyl group may also be involved in the interactions. From models the dansyl-D-amino acid enantiomer appears to have slightly more favorable interactions and should, therefore, have a slightly higher complex stability constant. Thus, its net velocity in a CZE separation should be greater than the ternary complex of the corresponding L-enantiomer and, therefore, the latter should have a longer migration time resulting in resolution of the two enantiomers.

Enantiomer migration order depends on both the chiral selector as well as the chiral selection mechanism. For example, when Cu^{2+}-D-His in a 1:2 ratio is used the D-enantiomers of the dansyl-D,L-amino acid derivatives have the longer migration time while if the Cu^{2+}-L-His is used the reverse order is obtained.[2] In this study only the L-aspartyl-L-phenylalanine methyl ester (aspartame) was used and no attempt was made to use the D-,D-, the D-,L-, or the L-,L-aspartame diastereomer as the chiral selector.

CZE Conditions

The buffer, its concentration and pH, voltage, and fused silica capillary dimensions were optimized by preliminary experiments and from previously reported results.[1,21-23] The 20 mM borate, pH = 9.25 buffer used for the CZE separation of the dansyl-D,L-amino acid derivatives in Figure 1 is not compatible with the Cu^{2+}-aspartame and the divalent cation additives that were used in this study. Thus, it was subsequently shown that a more favorable buffer was a 10 mM NH_4OAc, 2.5 mM Cu^{2+}-aspartame, pH = 7.40 buffer solution this was used throughout the study.

While the solution is chromophoric due to the Cu^{2+}-aspartame complex the dansyl-D,L-amino acid derivatives are still detected with good sensitivity at 325 nm where the derivatives absorb; this wavelength was used for all the studies reported here.

A 40.2 cm effective fused silica capillary length with a 50 μm i.d. was used in all the studies. Increasing the length increases resolution but also increases analysis time. The applied voltage was + 30 kV and the current never exceeded 30μA even at high concentrations of divalent cation as the buffer additive and was often less depending on the conditions. Peak shapes were well-defined at this voltage and efficiency was typically about 3 X 10^5 plates per column.

Effect of Buffer Cation

When mono- and divalent cation concentration is increased in the buffer, migration time and resolution in the CZE separation of dansyl-D,L-amino acids increases. This is illustrated in Figure 1 where Mg^{2+} buffer concentration is increased up to 3.0 mM. When different types of cation additives are compared at equivalent ionic strengths the effect on migration time and resolution is cation dependent with divalent cations having a greater affect than monovalent cations (the co-anion has little effect).[1,21-23]

When cation concentration is increased in the presence of the chiral selector, Cu^{2+}-aspartame, migration time of the dansyl-D,L-amino acids also increases but the increase differs for the D and L enantiomers. This results in a significant increase in resolution of the enantiomers compared to the resolution obtained[2,3] in the absence of Mg^{2+} (see also Figure 1A). Figure 2 shows the electropherograms for the enantiomeric separation of dansyl-D,L-Phe in the absence of Mg^{2+} and as Mg^{2+} buffer concentration is increased. In all cases buffer and chiral selector concentration are constant and the only variable is the $MgCl_2$ concentration. As shown in Figure 2 the D-enantiomer has the smaller migration time as Mg^{2+} concentration increases but its rate of change is less than observed for the L-enantiomer. Resolution increases sharply and almost doubles as Mg^{2+} is increased from 0 to 1.0 mM in the buffer and reaches a value of 3.45 at 5.0 mM Mg^{2+} even though peaks are broader at the higher migration times.

The migration velocity of the dansyl-D- and -L-Phe enantiomers is dependent on their electrophoretic mobility, μ_{ep}, and the electroosmotic mobility, μ_{eo}, for the buffer system. Table I lists μ_{ep} values determined for the

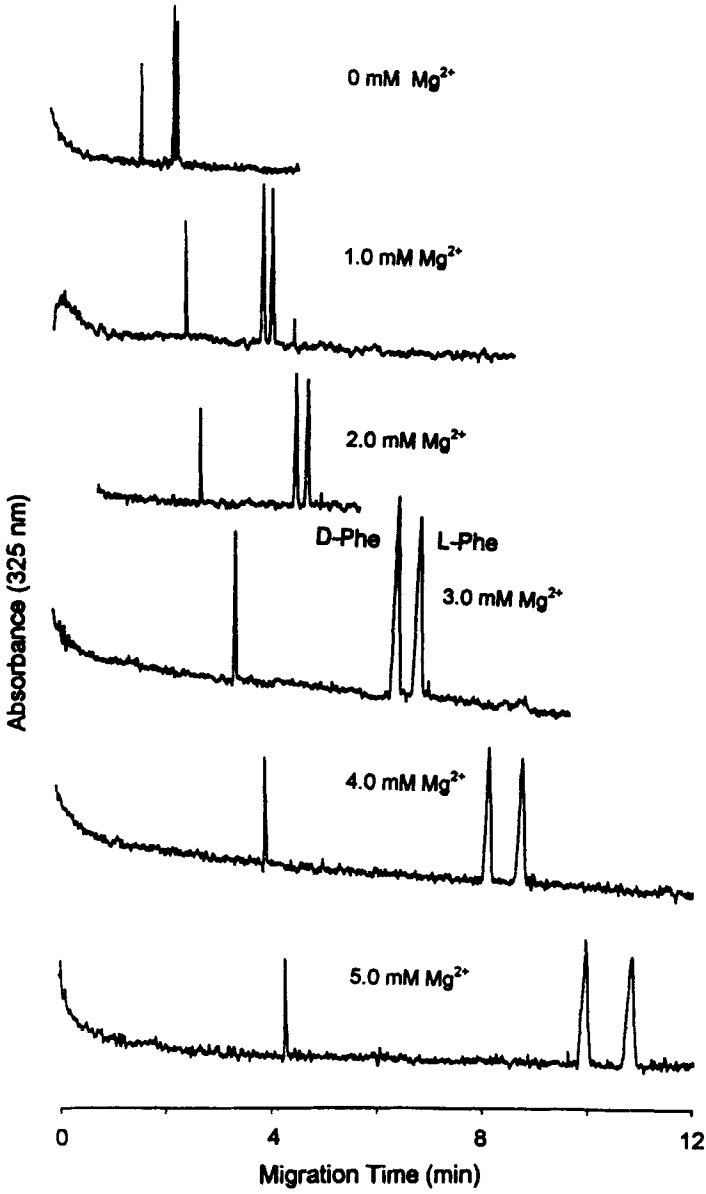

Figure 2. Effect of buffer Mg^{2+} concentration on the migration time and resolution of dansyl-D- and -L-Phe. The buffer is 10 mM NH_4OAc, 2.5 mM $CuSO_4$, 5.0 mM aspartame, pH = 7.40 with and without Mg^{2+}.

Table 1

Electrophoretic Mobility for Dansyl-D, L-Phe and Electroosmotic Flow as a Function of Mg^{2+} Buffer Concentration[a]

	μ_{eo} X 10^4 $cm^2s^{-1}V^{-1}$	μ_{ep} X 10^4 $cm^2s^{-1}V^{-1}$	
Mg^{2+} (mM)		D-Phe	L-Phe
0	7.62	-1.99	-2.15
1.0	5.25	-1.91	-2.04
2.0	4.74	-1.85	-1.98
3.0	3.84	-1.82	-1.94
4.0	3.35	-1.73	-1.85
5.0	3.07	-1.75	1.85

[a] A 10 mM NH_4OAc, 2.5 mM $CuSO_4$, 5.0 mM aspartame, pH = 7.40 buffer with Mg^{2+} as a buffer additive.

dansyl-D- and -L-Phe enantiomers and the μ_{eo} values found as the buffer Mg^{2+} concentration increases. While migration times for the two enantiomers increases as Mg^{2+} concentration increases and EOF decreases, the difference in migration time and mobility between the dansyl-D-Phe enantiomer, which has the higher velocity or lower migration time, and the dansyl-L-Phe enantiomer increases. For example, in Table 1, in the absence of Mg^{2+} in the buffer, the time difference is 4.2 sec, the mobility difference is 0.16 X 10^{-4} $cm^2s^{-1}V^{-1}$, and the enantiomers are barely resolved. At 5.0 mM Mg^{2+} the time difference is 52.2 sec, the mobility difference is 0.10 X 10^{-4} $cm^2s^{-1}V^{-1}$, and the enantiomers are easily resolved because of the significant change in the EOF.

Including Mg^{2+} in the Cu^{2+}-aspartame buffer increases migration time, migration difference, and resolution of other dansyl-D,L-amino acids. This is shown in Figure 3 where migration times for three other dansyl-D,L-amino acids are compared to dansyl-D,L-Phe. In all cases the D-enantiomer migrates faster than the L-enantiomer and has the smaller migration time; migration time for both enantiomers increases with Mg^{2+} concentration, and migration time for a basic side chain amino acid (dansyl-L-Arg in Figure 3) derivative is the least affected because of dissociation at the side chain. Dansyl-L-Arg has a near zero net charge and moves with the EOF as do the other basic side chain dansyl D,L-amino acids.

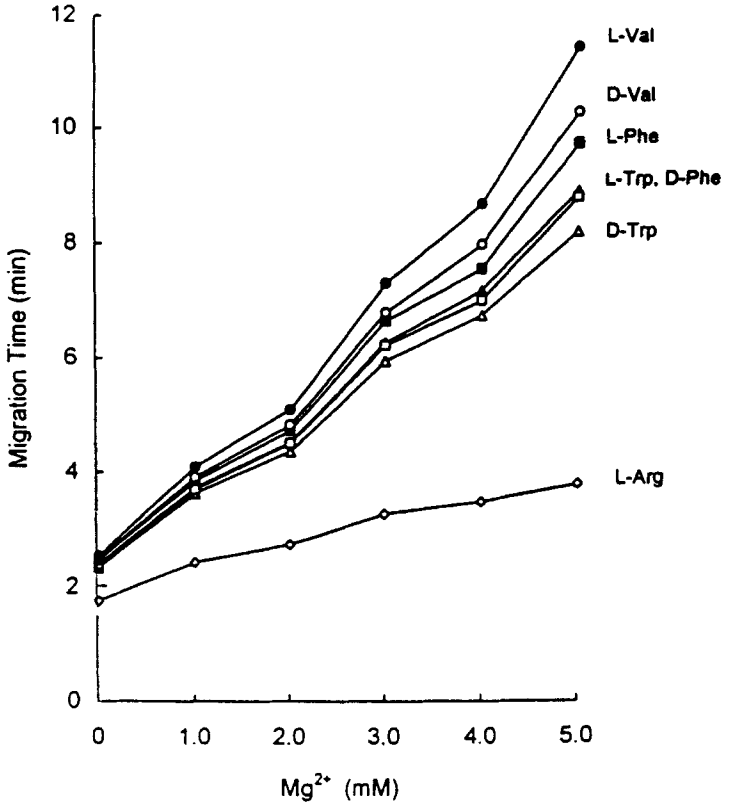

Figure 3. Effect of buffer Mg^{2+} concentration on migration time for several dansyl-D,L-amino acid derivatives. The buffer is the same as in Fig. 2 with increasing Mg^{2+} concentration.

Other cation additives affect dansyl-D,L-amino acid migration time and resolution. Divalent cations have a greater effect than monovalent cations and to achieve the change illustrated in Figures 1 to 3 and Table 1 monovalent inorganic cation as a buffer additive would have to be well over ten times the Mg^{2+} concentration.

The effects of Cd^{2+} and Zn^{2+} concentration on dansyl-D,L-amino acid migration time are summarized in Figure 4. As with Mg^{2+} as the buffer additive, migration time is increased, the D-enantiomer has a lower time,

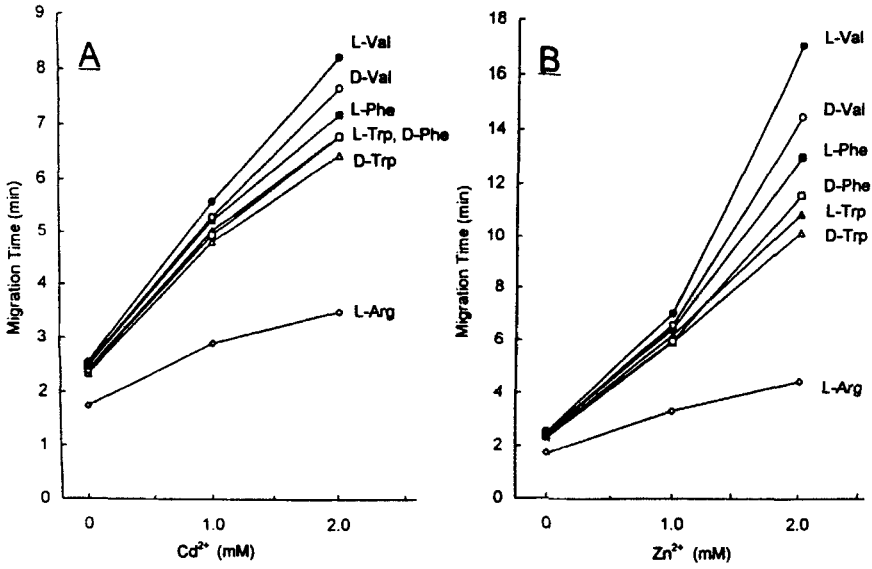

Figure 4. Effect of buffer (A) Cd^{2+} and (B) Zn^{2+} concentration on migration time for several dansyl-D,L-amino acid derivatives. The buffer is the same as in Fig. 2 with increasing Cd^{2+} or Zn^{2+} concentration.

migration time difference between the D- and L-enantiomers increases, and resolution increases as the Cd^{2+} and Zn^{2+} concentration is increased. When the three divalent cations are compared at equivalent concentrations (and ionic strength) migration time is highest for Mg^{2+} and lowest for Zn^{2+}.

The Zn^{2+}-aspartame complex has been reported to be a chiral selector like the Cu^{2+}-aspartame complex.[25] In our studies described here, when the buffer contained both Cu^{2+} and Zn^{2+} and a controlled amount of aspartame, multiple complex formation equilibria are possible.

If Cu^{2+} is replaced by Zn^{2+} in the complex, the EOF should decrease, and perhaps even reverse, as Cu^{2+} would undergo cation exchange at the silanol sites.[1] The magnitude and the change of the observed EOF and the migration time and order for the dansyl-D,L-amino acid derivatives suggests that the Cu^{2+} complex is the primary chiral selector and the Zn^{2+} serves only as the cation additive.

Table 2

Effect of Mg^{2+} and Zn^{2+} Concentration on Electrophoretic Mobility of Dansyl Amino Acid Derivatives[a]

	Mobility (X 10^4 $cm^2s^{-1}V^{-1}$)			
	μ_{eo}	μ_{ep}		
Mg^{2+} (mM)		D-Trp	D-Ser	D-Met
0	7.63 ± 0.02	-1.89 ± 0.03	-2.06 ± 0.04	-2.12 ± 0.02
1.0	5.47 ± 0.02	-1.81 ± 0.02	-1.91 ± 0.02	-1.95 ± 0.02
2.0	4.87 ± 0.02	1.80 ± 0.04	-1.89 ± 0.04	-1.99 ± 0.07
3.0	4.06 ± 0.02	-1.85 ± 0.04	-1.94 ± 0.04	-2.00 ± 0.02
4.0	3.82 ± 0.03	-1.80 ± 0.04	-1.94 ± 0.03	-1.9 ± 0.03
5.0	3.51 ± 0.04	-1.90 ± 0.04	-2.0 ± 0.05	-2.08 ± 0.04
Zn^{2+} (mM)				
0	7.63 ± 0.02	-1.89 ± 0.03	-2.06 ± 0.04	-2.12 ± 0.02
1.0	3.99 ± 0.04	-1.72 ± 0.09	-1.78 ± 0.05	-1.79 ± 0.08
2.0	3.03 ± 0.08	-1.70 ± 0.02	-1.70 ± 0.08	-1.85 ± 0.09

[a] A 10 mM NH_4OAc, 2.5 mM $CuSO_4$, 5.0 mM aspartame, pH = 7.40 buffer with Mg_{2+} or Zn^{2+} as a buffer additive.

Electrophoretic Mobility of Dansyl-D,L-Amino Acid Derivatives

Electrophoretic mobilities were determined for dansyl-D-Trp, -D-Ser, and -D-Met, as a function as a function of Mg^{2+}, Cd^{2+}, and Zn^{2+} concentration and Table 2 lists the values determined for Mg^{2+} and Zn^{2+} as the additive. When Mg^{2+} and Zn^{2+} concentration are increased μ_{eo} decreases sharply and is more than halved by a change from 0 to 5.0 mM Mg^{2+} or 0 to 2.0 mM Zn^{2+}.

In contrast μ_{ep} for the dansyl amino acid derivatives remains constant when the Mg^{2+} concentration is increased from 0 to 5.0 mM. For Zn^{2+} a small decrease in μ_{ep} was found over the 0 to 2.0 mM change in Zn^{2+} concentration. Although not shown, a trend similar to Zn^{2+} was found when Cd^{2+} was used as the buffer cation additive.[1,23]

Table 3

Migration Time and Resolution for Dansyl-D- and L-Amino Acid Derivatives in the Presence of a Mg^2, Cd^{2+}, or Zn^{2+} Buffer Additive

	Migration Time (Min) and Resolution											
	No Cation Additive			2.0 mM Mg^{2+}			0.5 mM Cd^{2+}			0.5mM Zn2+		
Amino Acid	D	L	R_s	D	L	R_s	D	L	R_s	D	L	R_s
Ala	3.02	3.03	0.11	8.38	8.61	0.84	6.12	6.26	0.70	5.01	5.08	0.39
Val	2.74	2.79	0.83	6.72	7.01	1.61	5.81	6.03	1.63	4.79	4.90	0.92
Leu	2.75	2.75	0	6.75	6.80	0.33	5.96	5.96	0	4.82	4.82	0
Ile	2.78	2.83	0.91	6.34	6.56	1.47	5.47	5.90	3.58	4.58	4.69	1.10
Phe	2.60	2.68	1.14	6.60	6.45	2.52	5.58	5.86	2.00	4.43	4.63	1.82
Trp	2.54	2.61	1.40	5.78	6.10	1.94	5.28	5.56	2.07	4.30	4.49	1.58
Tyr	2.69	2.76	1.17	5.89	6.15	2.08	5.46	5.68	2.00	4.44	4.60	1.45
Ser	2.68	2.73	0.56	5.89	6.17	1.33	5.45	5.69	1.10	4.40	4.54	1.27
Thr	2.71	2.77	0.75	5.99	6.26	1.35	5.55	5.79	1.37	4.61	4.77	1.39
Met	2.74	2.78	0.57	6.02	6.21	1.00	5.60	5.75	1.00	4.54	4.60	0.48
Asp	3.38	3.45	1.00	11.0	11.7	5.07	7.57	8.29	4.80	6.78	6.89	1.11
Glu	3.78	3.96	1.80	14.8	17.2	5.18	10.7	12.2	5.76	8.96	9.78	3.81
Asn	2.94	3.02	1.14	6.74	7.11	2.11	5.40	5.71	1.44	4.34	4.50	0.80
Gln	3.02	3.02		7.25	7.42		5.61	5.72		4.82	4.89	
Lys	2.05	2.12	0.93	3.61	3.67	1.20	3.20	3.20	0	2.80	2.81	0.20
L-Arg[b]		1.90			3.23			3.17			2.73	

[a] A 1 mM NH_4OAc, 2.5 mM $CuSO_4$, 5.0 mM aspartame, ph = 7.40 buffer with or without divalent cation buffer additive.
[b] Used as the internal standard.

CZE Separation of Dansyl-D,L-Amino Acid Derivatives

Adding a divalent cation to the buffer increases the migration time, migration difference, and the resolution for the CZE enantiomeric separation of all dansyl-D,L-amino acid derivatives. This is illustrated in Table 3 where the migration time data for 16 dansyl-D- and -L-amino acid derivatives are listed for buffers containing 2.0 mM Mg^{2+}, 0.5 mM Cd^{2+}, or 0.5 mM Zn^{2+}. The divalent cation concentrations in Table 3 represent optimum migration time, resolution, peak shape, and analysis time.

In all cases the D-enantiomer appears before the L-enantiomer derivative, the migration time increases in the presence of the divalent cation, and the migration time difference between the D- and L-enantiomer dansyl derivatives is buffer cation and cation concentration dependent.

Resolution of the D- and L-enantiomeric dansyl derivatives is also significantly improved when the divalent cation is in the buffer even though peaks, while still well-defined, are broader compared to the absence of the cation. Resolution data for each of the D- and L-enantiomeric dansyl derivatives are listed in Table 3 in the absence and presence of the divalent cation. Without the cation additive baseline resolution is obtained for only a few cases and in only a third of the cases does resolution exceed 1.0. In the presence of each of the three cation buffer additives baseline resolution is obtained in most cases and often reaches a resolution value of 5.0 for the conditions used in Table 3.

While the amino acid side chain in the derivative plays a major role in the selectivity and the resolution of the D,L-enantiomeric dansyl derivatives, the trends do not appear to be completely systematic. For example, for hydrophobic side chain amino acid derivatives migration time decreases with increase in side chain hydrophobicity but resolution of the D- and L-enantiomers varies. In Table 3 dansyl-D,L-Val is enantiomerically resolved while dansyl-D,L-Ile is partially resolved , and dansyl-D,L-Leu is not resolved. For polar amino acid side chain dansyl-D,L-amino acids resolution, whether in the absence or presence of the divalent cation buffer additive where it is sharply increased, does not appear to follow a structural trend. Apparently, subtle differences in hydrophobic/hydrophilic interactions between the amino acid side chain and the Cu^{2+}-aspartame ternary complex account for the differences.

Figure 5 shows the electropherograms for the CZE separation of a chiral mixture containing eight enantiomeric pairs of dansyl-D,L-amino acid derivatives (about 0.36 pmol in each derivative) in the absence and presence of 3.0 mM Mg^{2+} in the buffer. In the absence of Mg^{2+} only dansyl-D,L-Asp and dansyl-D,L-Glu are enantiomerically resolved because of the acidic amino acid side chain, which at the buffer pH used, these analytes exist significantly as divalent anions and have low migration velocities. The remaining dansyl-D,L-amino acid derivatives in Figure 5A are monovalent anions, have greater migration velocities, and are poorly enantiomerically resolved in the absence of the Mg^{2+}. When the buffer contains 3.0 mM Mg^{2+}, see Figure 5B, enantiomeric resolution of each chiral dansyl-D,L-amino acid derivative is accomplished but the overall separation is affected by overlap of individual dansyl-D- and -L-amino acid enantiomers. For example, dansyl-D,L-Trp or dansyl-D,L-Ser derivatives are enantiomerically resolved , however, when they are combined in the sample dansyl-L-Trp and dansyl-D-Ser overlap. In Figure 5B peaks for dansyl-D- and -L-Glu, which are easily resolved, are not shown because of their large migration times and all basic side chain amino acid dansyl derivatives travel with the EOF (only data for dansyl-L-Arg is shown in Figure 5B or

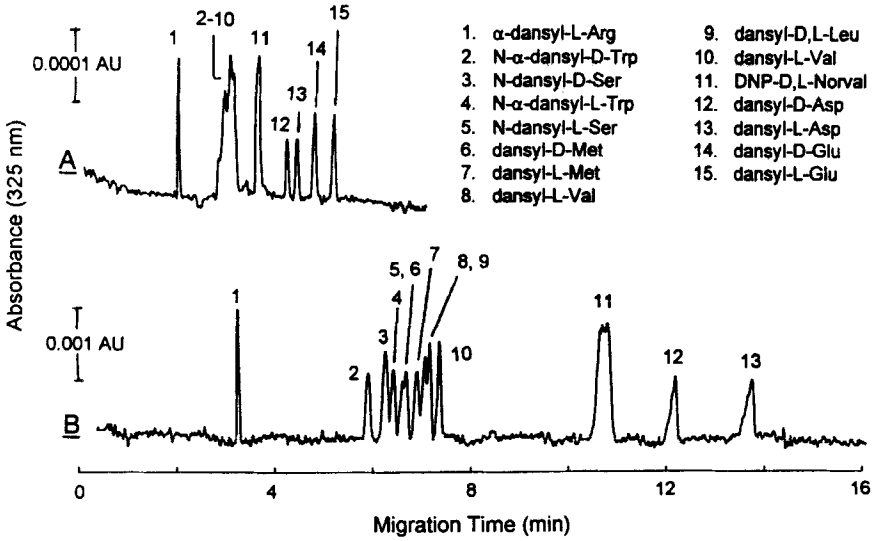

Figure 5. Comparison of the resolution for a mixture of dansyl-D,L-amino acid derivatives in the (A) absence and (B) presence of 3.0 mM Mg^{2+} as the buffer additive. The buffer is the same as in Fig. 2.

Table 3). DNP-D,L-norvaline was included in the mixture to demonstrate that the DNP-D,L-amino acid derivatives are not resolved by the Cu^{2+}-aspartame complex even in the presence of the Mg^{2+} (or other divalent cation) as a buffer additive.

Figure 6 compares resolution obtained in the absence and presence of 0.5 mM Cd^{2+} or Zn^{2+} as a buffer additive. The trends, in general, are similar to using Mg^{2+} (Figure 5B) with the major difference being that less Cd^{2+} or Zn^{2+} is required to bring about the same level of resolution. Regardless of the divalent cation employed migration order remains the same.

Our studies indicate that the enhanced migration times, resolution, peak shapes, and analysis time are best overall when using Mg^{2+} as the buffer additive even though a larger concentration of Mg^{2+} is required. Furthermore, the solubility range for the Mg^{2+} buffer is much greater.

However, for the enantiomeric separation of specific dansyl-D,L-amino acids better resolution may be obtained with either Cd^{2+} or Zn^{2+} as the additive (see Table 3).

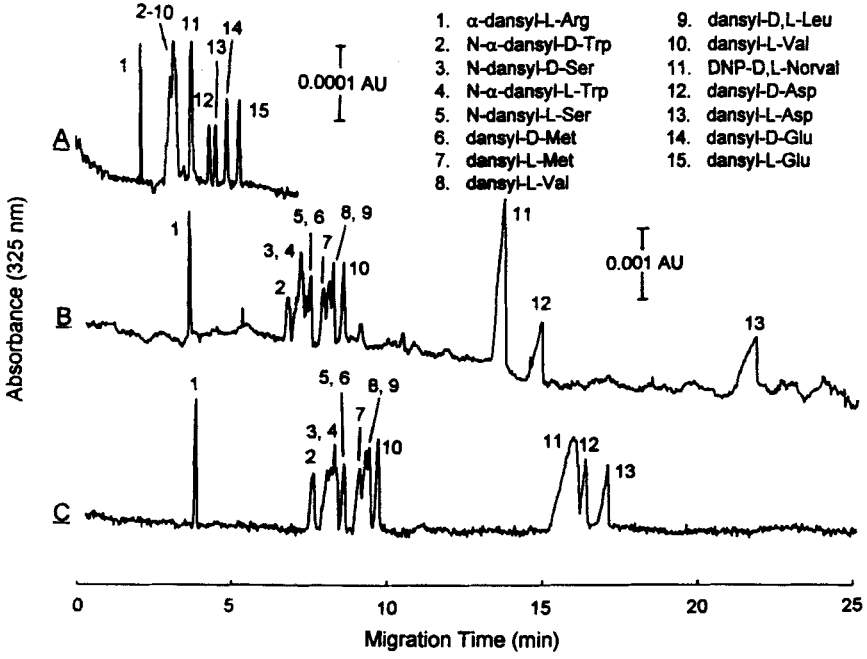

Figure 6. Comparison of the resolution for a mixture of dansyl-D,L-amino acid derivatives in the (A) absence and presence of (B) 0.50 mM Cd^{2+} or (C) 0.50 mM Zn^{2+} as the buffer additive. The buffer is the same as in Fig. 2.

CONCLUSION

Resolution of dansyl-D,L-amino acids enantiomers is significantly improved by adding Mg^{2+}, Cd^{2+}, or Zn^{2+} to a buffer containing Cu^{2+}(aspartame)$_2$ as the chiral selector. Improved resolution is cation and cation concentration dependent. Increasing the divalent cation concentration decreases EOF while electrophoretic mobility of the dansyl-D,L-amino acids remains constant. Migration time and migration difference between the dansyl-D- and -L-amino acid enantiomers increases as the divalent cation concentration increases causing a marked improvement in resolution. Although Mg^{2+} must be employed at a higher concentration than Cd^{2+} and Zn^{2+}, Mg^{2+} is preferred as the EOF modifier because of better solubility, good peak shape, and favorable analysis time. Adding a divalent cation to the buffer to reduce EOF is a strategy that can be used to improve resolution in many other enantiomeric CZE separations.

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