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Enhanced capillary zone electrophoretic separation of dinitrophenyl-amino acid derivatives through control of electroosmotic flow by the buffer cation

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Abstract

The effect of Mg^{2+}, Cd^{2+} and Zn^{2+} are evaluated as buffer additives to reduce electroosmotic flow (EOF) to facilitate the capillary zone electrophoretic (CZE) separation of 2,4-dinitrophenyl (DNP) derivatives of amino acids. As the divalent cation concentration increases EOF decreases and reduction of EOF follows the order $Zn^{2+}>Cd^{2+}>Mg^{2+}$. The electrophoretic mobility of the DNP-amino acid derivatives remains constant for a given buffer and pH over a change in divalent cation concentration. The effect of the divalent cation buffer additive is consistent with cation-exchange at the silanol sites on the fused-silica wall. Higher buffer pH favors greater cation-exchange and reduced EOF. Separation of multicomponent mixtures of DNP-L-amino acid derivatives in the presence of Mg^{2+}, Cd^{2+} and Zn^{2+} are compared at pH 7.00. Derivative migration time, resolution and peak shape are enhanced and are best for Mg^{2+} as the buffer additive although Mg^{2+} must be used at a higher concentration than either Cd^{2+} or Zn^{2+}. Raising the buffer pH to 9.25 in the presence of Mg^{2+} increases migration time and resolution but analysis time is also increased. © 1997 Elsevier Science B.V.

Keywords: Electroosmotic flow; Buffer composition; Amino acids

1. Introduction

Amino acids are structural units in peptides and proteins, precursors in many metabolic pathways, indicators of physiological disorders, and important components in foods and food products. Therefore, the analysis of amino acids is a major analytical problem that is encountered across most scientific, medicinal and industrial areas. Modern quantitative methodology for the determination of amino acids is based largely on chromatographic techniques, namely, high-performance liquid chromatography (HPLC) [1–4]. While free amino acid mixtures can be separated, usually on ion-exchange columns, and subsequently detected and determined [1,2], conversion of amino acids into a derivative [2–5] is often preferred. The derivative strongly influences the separation because of a greater compatibility with reversed-phase columns and because a highly chromophoric or fluorophoric group is introduced, which improves detection limit. Typical amino acid derivatives in which the derivatization chemistry is well understood include the formation of *o*-phthaladehyde (OPA), 5-dimethylamino-1-naphthalenesulfonic (dansyl), 2,4-dinitrophenyl (DNP), (9-fluorenylmethoxy)carbonyl (Fmoc), phenyl iso-

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thiocyanate- (PITC) and phenylthio-hydatoin (PTH) amino acid derivatives as well as others [2–5].

Capillary zone electrophoresis (CZE) is a viable separation strategy for free amino acids [6–21] but is applied most often to the separation of amino acid derivatives [22–24] because of enhanced resolution and sensitivity in detection. Procedures for CZE and micellar electrokinetic capillary chromatographic (MEKC) separations involving dansyl [13,22,25–37], 4-dimethylamino-azobenzene-4′-sulfonyl (dabsyl) [38], PTH [26,39–43], fluorescein isothiocyanate (FITC) [10,14,44–50], OPA [7,14,38,51], naphthalene-2,3-dicarboxaldehyde (NDA) [10,52,53], Fmoc [14], dimethylaminoazobenzene isothiocyanate [48], fluorescamine [14,22,44], N-3,5-dinitrobenzoylated-amino acid-O-isopropylester [54], 3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde [55], and O-acetyl-β-D-glucopyranosyl isothiocyanate [56] amino acid derivatives have been reported. Detection at an extraordinarily low detection limit is also possible since the many derivatives are highly fluorescent and can be detected by laser detection strategies. For example, detection limits for FITC [45] and dabsyl [40] amino acid derivatives were reported to be at the sub-amol level. For FITC-Phe, detection limit was 0.4 amol and is more sensitive than for NDA- or OPA-Phe [10]. Albin et al. [14] compared CZE fluorescence detection limits for FITC, FMOC, OPA and fluorescamine amino acid derivatives and suggested that FMOC in pre-capillary and OPA in post-column derivatization were the most sensitive using Gly as the test analyte. Amino acid post-column derivatization has also been successful in CZE separations [6,7,9,10,14].

CZE and MEKC can be used to resolve racemic mixtures of amino acids. While several free D,L-amino acids were resolved by CZE using the crown ether, 18-crown-6-tetracarboxylic acid [57], most amino acid-enantiomeric separation procedures rely on derivative formation. For example, dansyl-D,L-amino acid derivatives were separated into their enantiomeric forms using a chiral selector, such as Cu^{2+}-L-histidine [58], Cu^{2+}-aspartame [28], a bile acid [31,59], or a cyclodextrin [13,60,61] in the buffer.

A successful CZE separation requires control of electroosmotic flow (EOF) because of its influence on migration time, resolution and efficiency [62]. Derivatizing the fused-silica capillary wall, adding surfactants, an organic modifier, or altering buffer pH, components, or concentration are key factors that will modify and even reverse EOF. The buffer cation and its concentration will affect EOF [20,63–68]. Recently, an EOF model was reported for singly charged electrolytes that yielded a linear correlation between capillary wall zeta potential and log cation activity which suggests that the fused-silica surface acts like an ion-selective electrode towards the monovalent cations [68]. Divalent cations [67] have a greater affect than monovalent cations [64,67] and increasing the concentration of either decreases EOF. When different cations were compared the effect followed cation-exchange selectivity between the mono- and divalent inorganic cations and the silanol sites [67]. Optimization of the cation and its concentration enhances resolution in the CZE separation of anionic surfactants [67], free amino acids [20] and olignucleotides [63].

In this paper, we report our studies on the CZE separation of 2,4-dinitrophenyl (DNP) derivatives of amino acids using an inorganic cation as a buffer additive to enhance migration time and improve resolution. The LC separation of DNP-amino acid mixtures has been reported [69,70], but, this derivative has not been widely used in CZE and MEKC [71,72] primarily because other amino acid derivatives possess a fluorophore which provides very low detection limits. However, CZE separation of DNP-amino acid derivatives can be useful in many applications when the most sensitive detection is not required because the derivatives are easily detected by absorption at favorable detection limits due to the 2,4-dinitrophenyl chromophore, are readily available as standards, are stable and the chemistry for derivative formation is well understood.

2. Experimental

2.1. Chemicals

DNP-Amino acid derivatives were obtained from Sigma. Mesityl oxide was purchased from Aldrich while all inorganic salts, acids, and bases were obtained from Fisher Scientific, Aldrich and Mallinckrodt. Water used for sample and buffer solutions

was freshly prepared by passing in-laboratory distilled water through a Milli-Q-Plus water treatment unit followed by a 0.2 μm filter.

2.2. Instrumentation

All measurements were made with a 48 cm fused-silica capillary from Polymicro Technologies that was 50 μm I.D.×375 μm O.D. The optical window was prepared at 8 cm from the capillary outlet end to provide a 40 cm effective capillary length. A Waters Quanta 4000 CE instrument equipped with a filter fixed-wavelength UV detector, an automatic injection unit, an air cooled system, a d.c. maximum 30 kV power supply and an automatic operation program was used. Separation data were collected and manipulated with a Spectra Physics M-4270 integrator controlled by Spectra Physics Autolab software and spread sheet software.

2.3. Procedures

The fused-silica capillary was pre-conditioned by pulling an alkaline solution through the capillary by a vacuum pump for about 20 min followed by a 5 min wash with water. The background buffer of interest was drawn into the capillary and the capillary was stored overnight prior to its first use. Thereafter, the capillary was stored containing the buffer of interest when not in use. Prior to making measurements a new buffer solution of interest with or without the cation additive was introduced into capillary, and the voltage, typically +25 kV except where noted, was applied until a constant EOF as measured with mesityl oxide was obtained. During the measurements the buffer was replaced in the reservoirs after 3–4 runs. In studies with divalent cation additives the capillary was treated with a 0.5 *M* $(NH_4)_2$citrate, pH 6.0 buffer to ensure cation removal prior to changing to a new buffer condition. Capillary performance was checked during the measurements with a mesityl oxide analyte in a CH_3CN–water (1:4) solution (EOF marker) using a pH 7.00, 20 m*M* NH_4OAc buffer solution. When broadening of the EOF marker peak or an appreciable change in its migration time occurred the capillary was discarded for a new capillary. All other buffer and analyte solutions used in the studies were aqueous solutions.

Stock solutions of individual DNP-L-amino acids of about $5 \cdot 10^{-5}$ *M* or their mixtures were prepared in a pH 7.00, 10 m*M* NH_4OAc buffer solution. Samples were introduced into the CE system by the hydrostatic method for 5 to 45 s and the volume injected varied between 3 to 20 nl depending on the study. At 25 kV the current was typically about 22 μA for buffer only and 25 to 26 μA when the buffer contained 1.0 m*M* Mg^{2+}. All measurements were made at ambient temperature, 25°C, and represent averages of usually more than three measurements for a given condition. All data were also verified by additional runs with new capillaries, buffer and standard solutions. Identity of individual peaks in the mixtures was verified by spiking the mixture repeatedly with known, standard DNP-L-amino acid derivatives.

3. Results and discussion

3.1. CZE conditions

Optimum buffer, buffer concentration, voltage and fused-silica capillary dimensions were established in preliminary experiments. A 20 m*M* NH_4OAc buffer at pH 7.00 provided the best conditions. This pH ensures appreciable dissociation of the terminal carboxyl group of the DNP-L-amino acid derivatives while the 20 m*M* NH_4OAc concentration provides suitable buffer capacity, permits appropriate sample loading and produces sharp and symmetrical peaks in the electropherogram without excessive current being passed. The NH_4OAc does not interfere in the detection of the DNP-L-amino acid derivatives nor does it cause precipitation at the concentrations of Mg^{2+}, Zn^{2+} and Cd^{2+} examined as buffer additives.

As expected, as applied voltage was increased current increased in the absence and presence of Mg^{2+} in the buffer. For example, current for a 20 m*M* NH_4OAc, pH 7.00 buffer increased but even at 30 kV current was only 27 μA. For 4.0 m*M* Mg^{2+} the current increased to 43 to 46 μA at 30 kV. Migration times for DNP-L-amino acid derivatives decreased as voltage increased with the rate of decrease being small above 20 kV. However, the best

peak shape in terms of efficiency (about $3.1 \cdot 10^5$ plates/column) and symmetry was obtained from 25 to 30 kV. To avoid excessive heat while still maintaining the best peak shape 25 kV was used for all the studies. All experiments were carried out using a 50 μm I.D. fused-silica capillary and resolution and analysis time increased as effective capillary length (capillary inlet end to detection region) was increased. To avoid a long analysis time a 40 cm effective capillary length was used.

3.2. Effect of buffer cation

Analyte anion migration time increases as monovalent inorganic or organic cation concentration in the buffer increases and follows the order $K^+ > NH_4^+ > Na^+ > Li^+$ [64,67] which also correlates to the cation-exchange selectivity exhibited by chromatographic silica [67,68,73]. That is, the larger the cation-exchange selectivity is, the greater the increase in the migration time for the analyte. A divalent cation, for example Mg^{2+}, has a greater effect on migration time and a lower divalent cation buffer concentration can be used to obtain the migration time change exhibited by the monovalent cation [67]. For this reason only data for the effect of inorganic divalent cation buffer additives on DNP-L-amino acid derivative migration times are reported here.

EOF was determined for an aqueous 20 m*M* NH_4OAc, pH 7.00 buffer as a function of Mg^{2+}, Cd^{2+} and Zn^{2+} concentration as chloride salts using mesityl oxide (detection at 214 nm) as the analyte marker. Precipitation was not observed under these conditions. The voltage was 25 kV and the current for Mg^{2+}, Cd^{2+} and Zn^{2+} ranged from 22 to 35 μA. When the cation buffer additive concentration increases, see Fig. 1, EOF decreases with the major

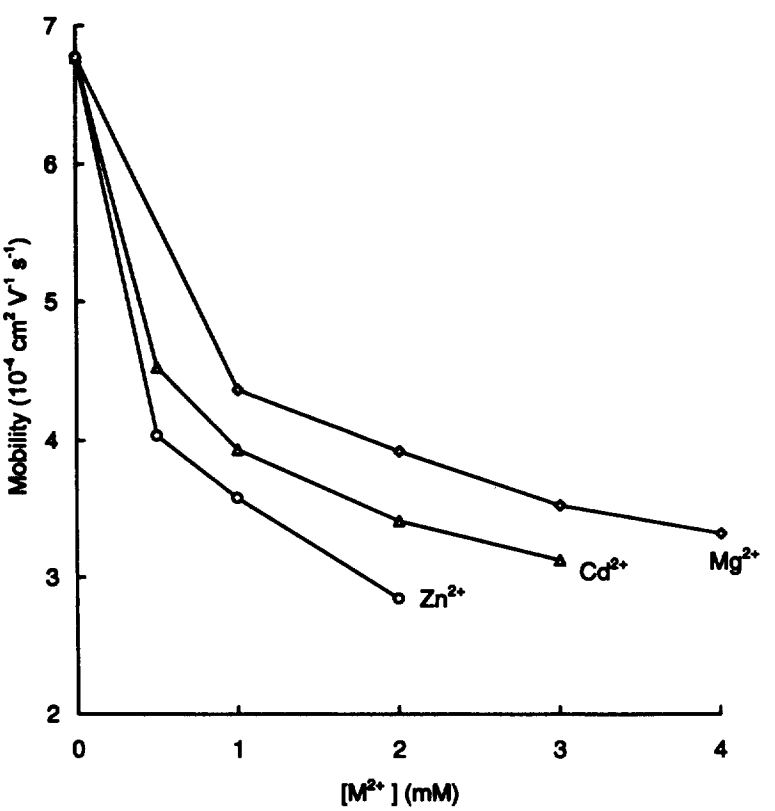

Fig. 1. Effect of divalent cation buffer additive concentration on electroosmotic flow. Mesityl oxide was the analyte and the buffer was 20 m*M* NH_4OAc, pH 7.00.

change occurring up to 1 to 1.5 m*M* M^{2+} and the reduction in the EOF follows the order $Zn^{2+}>Cd^{2+}>Mg^{2+}$. Although not shown in Fig. 1, monovalent cations also have a pronounced effect on reducing the EOF. A Mg^{2+} concentration of about four times the Zn^{2+} concentration is required to produce an EOF decrease that is equivalent to that obtained with Zn^{2+}. When a divalent cation, for example Mg^{2+}, is compared to a monovalent cation the latter must be over ten times the Mg^{2+} concentration in order to produce about the same decrease in EOF [67].

3.3. 2,4-Dinitrobenzene derivatives of amino acids

Amino acids undergo a reaction with 2,4-dinitrofluorobenzene at the α-amino group to form the DNP derivative and the net charge of the DNP-L-amino acid derivative is determined by dissociation of the terminal carboxyl group of the amino acid unless the side chain also contains an ionizable group. DNP-L-Arg, DNP-L-Trp, DNP-L-Thr, DNP-L-Asn and DNP-L-Ala were selected as test analytes to evaluate the effect of divalent cations on derivative migration time.

Migration time was determined for the five DNP-L-amino acids in a pH 7.00, 20 m*M* NH_4OAc buffer at 25 kV as a function of Mg^{2+}, Cd^{2+} and Zn^{2+} concentration. In the absence of the cation migration times for the DNP-L-amino acids are within 1 min of each other. When the divalent cation is present migration time sharply increases and continues to increase as the cation concentration increases. The migration time change for the five DNP-amino acid derivatives is shown in Fig. 2A and B for Mg^{2+} and Zn^{2+}, respectively, as a function of their concentration. For Cd^{+2} migration time is intermediate to Mg^{2+} and Zn^{2+} and thus the increase follows the order $Zn^{2+}>Cd^{2+}>Mg^{2+}$ at comparable cation concentration. The order for the migration time shift for the DNP-amino acids is the same for each of the three cations and follows the order DNP-L-Ala> DNP-L-Asn > DNP-L-Thr>DNP-L-Trp>DNP-L-Arg.

Of the derivatives in the test group only DNP-L-Arg has a reduced net charge due to a basic side chain group and migrates with the EOF. The other

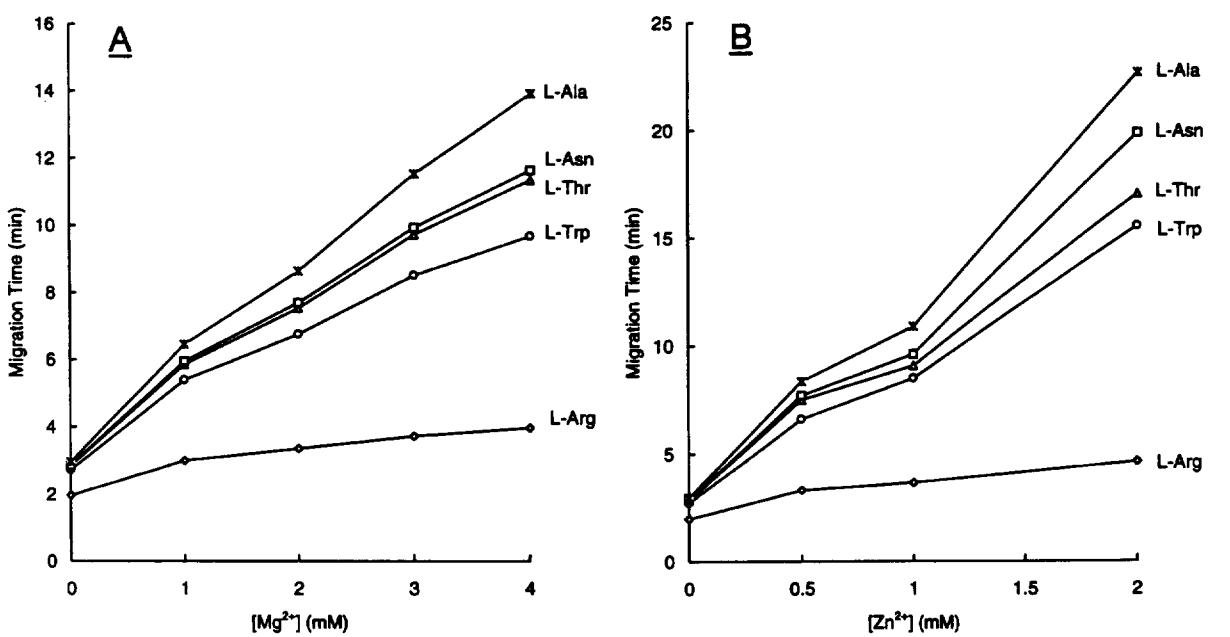

Fig. 2. Effect of divalent cation buffer additive concentration on migration time for several DNP-L-amino acid derivatives. (A) Mg^{2+} concentration is varied in a 20 m*M* NH_4OAc, pH 7.00 buffer and (B) the same buffer with varied Zn^{2+} concentration at 25 kV.

four derivatives have neutral side chains, are therefore anionic, and have an electrophoretic mobility towards the anode. The migration order of the four derivatives follows the side chain size with DNP-L-Trp with the largest side chain group having the least negative electrophoretic mobility and hence the lowest migration time of the four derivatives. However, the rate of increase in migration time, see Fig. 2, is greatest for the derivative with the smallest neutral side chain group as the M^{2+} concentration increases.

3.4. Electrophoretic mobility of DNP-L-amino acid derivatives

The migration velocity of an analyte depends on the analyte electrophoretic mobility, μ_{ep}, and the electroosmotic mobility, μ_{eo}. The electrophoretic mobility was determined for several DNP-amino acid derivatives as a function of divalent cation concentration in the 20 m*M* NH_4OAc, pH 7.00 buffer. Table 1 lists the μ_{ep} values determined for DNP-L-Trp, DNP-L-Thr and DNP-L-Ala and the μ_{eo} values as a function of Mg^{2+} concentration. While μ_{eo} decreases significantly as Mg^{2+} concentration increases, the μ_{ep} value remains constant at all Mg^{2+} concentrations studied.

A similar result was obtained when Cd^{2+} or Zn^{2+} concentration was varied in the buffer. This is shown for Zn^{2+} in Fig. 3 where μ_{ep} values for the three DNP-L-amino acids are plotted in the lower half and the μ_{eo} values are plotted in the upper half as a function of Zn^{2+} concentration. Although not shown, a constant μ_{ep} and a decreasing μ_{eo} was also determined for DNP-Gly and DNP-L-Val as a function of Mg^{2+} concentration in a 20 m*M* borate, pH 9.25 buffer.

It can be concluded that the decrease in net velocity of the DNP-amino acids and their enhanced migration time in the presence of the divalent cation is due to the change in the surface charge at the fused-silica wall because of cation-exchange at the silanol sites which causes the sharp drop in EOF. Furthermore, since μ_{ep} values are constant for all three cations, association between the DNP-amino acid derivatives and Mg^{2+}, Cd^{2+} or Zn^{2+} does not take place or is present as a very weak association which has little effect on the μ_{ep} of the M^{2+}-DNP-amino acid complex.

3.5. Effect of pH in the presence of the cation buffer additive

Because the silanol sites are weakly acidic with a pK_a of about 5.9 [68], depending on the buffer [74] and cation additives undergo cation-exchange at this site, the magnitude of the EOF should be sensitive to both concentration of H^+ and the cation additive. While most of our studies were at a buffer pH 7.00, the effect of pH was also studied. Other workers [22,74] have shown that the μ_{eo} value for fused-silica increases rapidly in the pH 4 to 6 region where the silanol sites begin to dissociate and levels off at pH>8 where silanols are more fully dissociated. The pH region of greatest change depends on whether the buffer pH in the capillary is raised or lowered [74]

Table 1
Effect of Mg^{2+} concentration on electrophoretic mobility of DNP-L-amino acid derivatives

Mg^{2+} (m*M*)	Mobility ($\cdot 10^{-4}$ cm^2 V^{-1} s^{-1})[a]			
	μ_{eo}[b]	μ_{ep}[c]		
		DNP-L-Trp	DNP-L-Thr	DNP-L-Ala
0	6.77±0.02	−1.88±0.02	−2.09±0.03	−2.28±0.02
1.0	4.36±0.02	−1.93±0.02	−2.12±0.02	−2.33±0.02
2.0	3.91±0.02	−1.97±0.01	−2.17±0.01	−2.39±0.01
3.0	3.52±0.02	−1.98±0.01	−2.17±0.02	−2.39±0.02
4.0	3.32±0.02	−1.95±0.02	−2.15±0.02	−2.37±0.02

[a] A 20 m*M* NH_4OAc, pH 7.00 buffer with and without Mg^{2+} at 25 kV.
[b] Average of 6 measurements.
[c] Average of 3 measurements.

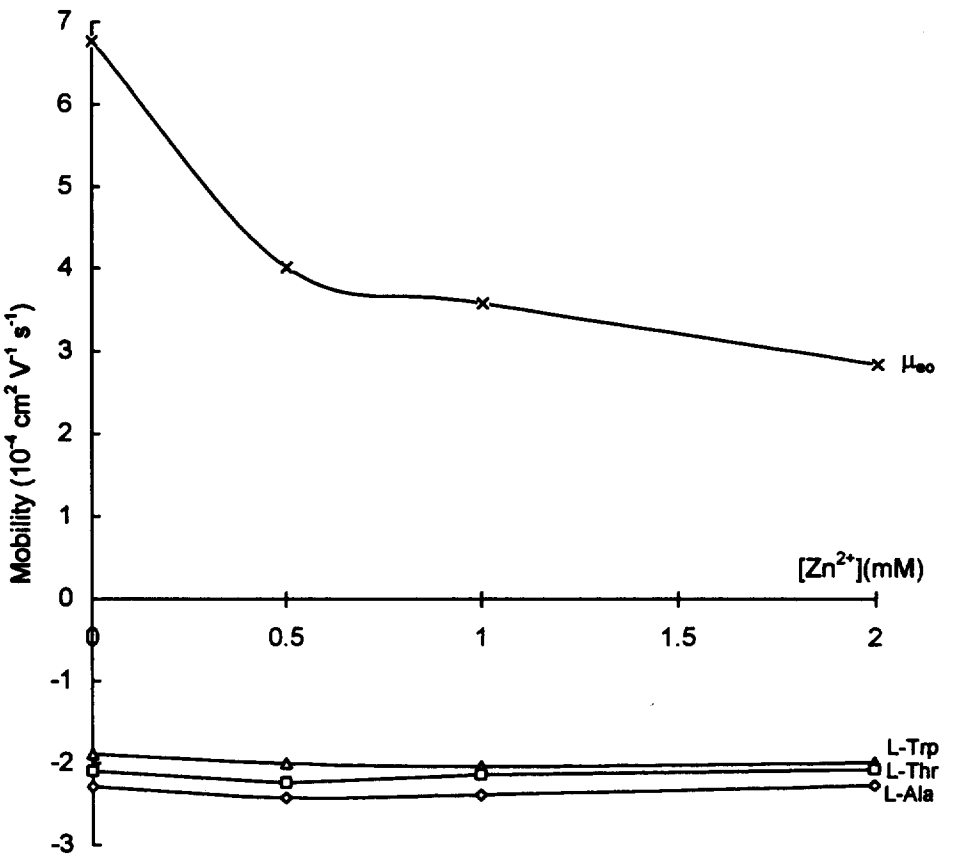

Fig. 3. Effect of Zn^{2+} concentration as a buffer additive on the electrophoretic mobility of several DNP-L-amino acid derivatives. The μ_{ep} values for the three derivatives are in the lower graph for the 20 m*M* NH_4OAc, pH 7.00 buffer containing Zn^{2+} while the μ_{eo} values for the same conditions are listed in the upper half of the graph.

and how the capillary is conditioned [68] prior to the μ_{eo} measurement. The role of Mg^{2+} at 2.0 m*M* in a buffer which also contained 15 m*M* NaCl and approximately 20 m*M* NH_4OAc where the NH_4^+-to-OAc^- ratio was changed to provide a pH over the range of pH 4.85 to 8.50, was determined. As shown in Fig. 4, μ_{eo} is nearly constant over the pH range of 4.5 to 7.5 and sharply decreases as pH is increased. This μ_{eo} decrease is in contrast to the increase in μ_{eo} observed for a fused-silica capillary when only a monovalent cation salt such as NaCl or KCl is in the buffer for ionic strength control. Fig. 4 also shows how μ_{eo} changes at lower ionic strength for a fused-silica capillary that is exposed to an increase in buffer pH [74].

This difference can be explained by the fact that Mg^{2+} has a much higher cation-exchange selectivity at the silanol sites compared to Na^+ or K^+. In the presence of only Na^+ or K^+ at a constant concentration, even though the H^+ concentration is decreased, the cation-exchange equilibrium does not favor Na^+ or K^+ unless the concentration of Na^+ or K^+ is very high. Therefore, at such a high pH of 10 to 12 the number of SiO^- sites remains at a maximum value which is indicated by the increase in the μ_{eo}. On the other hand, in the presence of Mg^{2+}, as the concentration of H^+ in the buffer is decreased from 10^{-4} to 10^{-6} *M*, the number of silanols dissociating is increased, but H^+ is readily replaced by Mg^{2+} because of its high cation-exchange selectivity. Therefore, μ_{eo} hardly changes over the pH range of 4.5 to 7.5. But, as H^+ is decreased to 10^{-10}

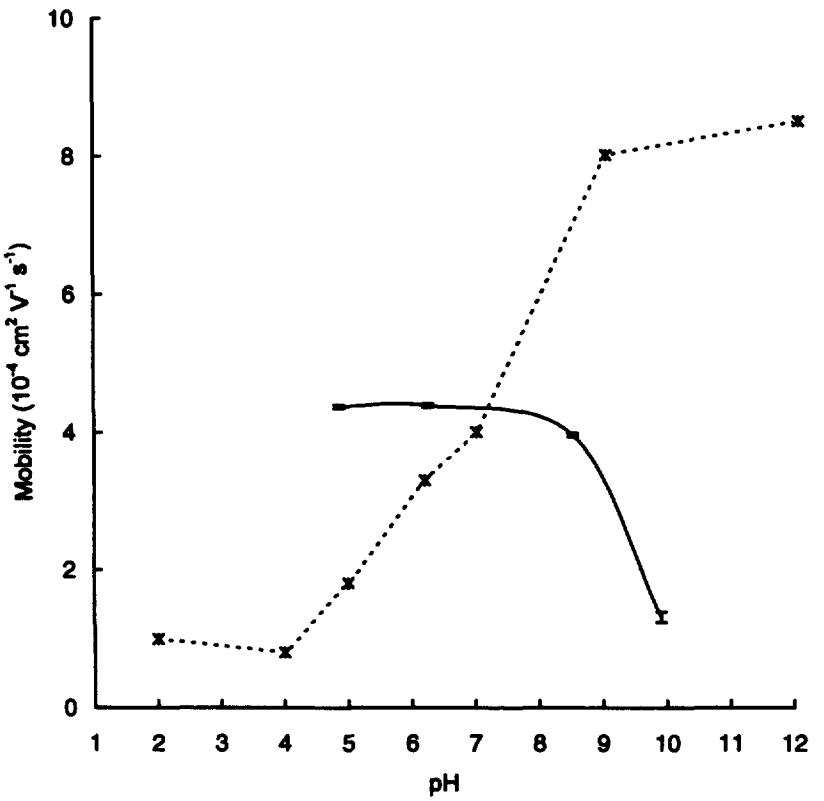

Fig. 4. Effect of buffer pH on electroosmotic flow. Mesityl oxide was the analyte and the buffer contained different ratios of NH_4^+ to OAc^- at constant 15 m*M* NaCl and 2.0 m*M* $MgCl_2$. The change in electroosmotic flow as a function of pH in the absence of Mg^{2+} is shown as the dotted line and is taken from Ref. [74].

M, more SiO^- sites become available for Mg^{2+} exchange. As the Mg^{2+} balances the larger number of the negative charges, the capillary wall zeta potential decreases, and therefore the μ_{eo} decreases as shown in Fig. 4.

For the pH range used in Fig. 4 all DNP-L-amino acid derivatives studied were fully dissociated. When μ_{ep} values were determined for these derivatives over this pH range, μ_{ep} for each derivative was constant over the entire pH range. This is consistent with the major effect being cation-exchange at the silanol sites. Evidence of precipitation prevented studies of Cd^{2+} and Zn^{2+} at the higher buffer pH values.

Since μ_{ep} values for the DNP-L-amino acids are constant but the μ_{eo} decreases at high pH in the presence of Mg^{2+}, migration time for the DNP derivatives will increase as pH increases in the basic side. This was demonstrated by determining migration time for the five DNP-L-amino acid derivatives in a 2.0 m*M* Mg^{2+}, 20 m*M* NH_4OAc, 15 m*M* NaCl buffer as the buffer pH was increased. The rate of change increased the greatest for the slower moving derivatives as buffer pH was increased. Above pH 9.0 the increase is very rapid and migration times become excessive particularly for the more slowly moving DNP-L-amino acid derivatives.

DNP-L-Arg with a basic side chain migrates with the EOF even at high pH values. For DNP-L-Asp and DNP-L-Glu, both of which contain an acidic side chain and provide an additional anionic site at high pH, their migration at the high pH is towards the anode and are therefore not detected under the conditions used. This migration was confirmed by switching the power supply polarity so that the two derivatives would pass through the detection zone.

3.6. CZE separation of DNP-L-amino acid derivatives

Fig. 5 illustrates the CZE separation of nineteen DNP-L-amino acid derivatives in a 20 m*M* NH_4OAc, pH 7.00 buffer as a function of Mg^{2+} concentration. In Fig. 5A EOF is high, or about $7 \cdot 10^{-4}$ cm^2 V^{-1} s^{-1} in the absence of Mg^{2+} and migration times for all derivatives are short. Even acidic side chain derivatives, DNP-L-Asp and DNP-L-Glu, which are appreciably in a divalent anion form, appear in less than 10 min. DNP-L-Arg and DNP-L-Lys with their basic side chain and near zero charge migrate with the EOF. When Mg^{2+} is increased (Fig. 5B,C), migration time increases and resolution is significantly improved. The basic side chain DNP-L-amino acids move with the EOF even at 4.0 m*M* Mg^{2+} while the acidic side chain derivatives migrate toward the anode and are not detected in Fig. 5C because EOF is so low. Also, in Fig. 5 L-Try, L-His and L-Cys are derivatized at two positions and this also influences migration time.

Fig. 6 compares Cd^{2+} at 1.0 m*M* (Fig. 6B) and Zn^{2+} at 1.0 m*M* (Fig. 6C) as a buffer additive, which are optimum concentrations when taking into account resolution, peak shape, analysis time and solubility, to the absence of the cation (Fig. 6A) for the CZE separation of the DNP-amino acid derivatives. In contrast, as shown in Fig. 5C, Mg^{2+} at 4.0 m*M* is required to achieve a similar resolution. Since the EOF decrease follows the order $Zn^{2+} > Cd^{2+} > Mg^{2+}$, Zn^{2+} can be used at the lowest concentration to produce a low EOF and a large increase in migration time. The best peak efficiency is with

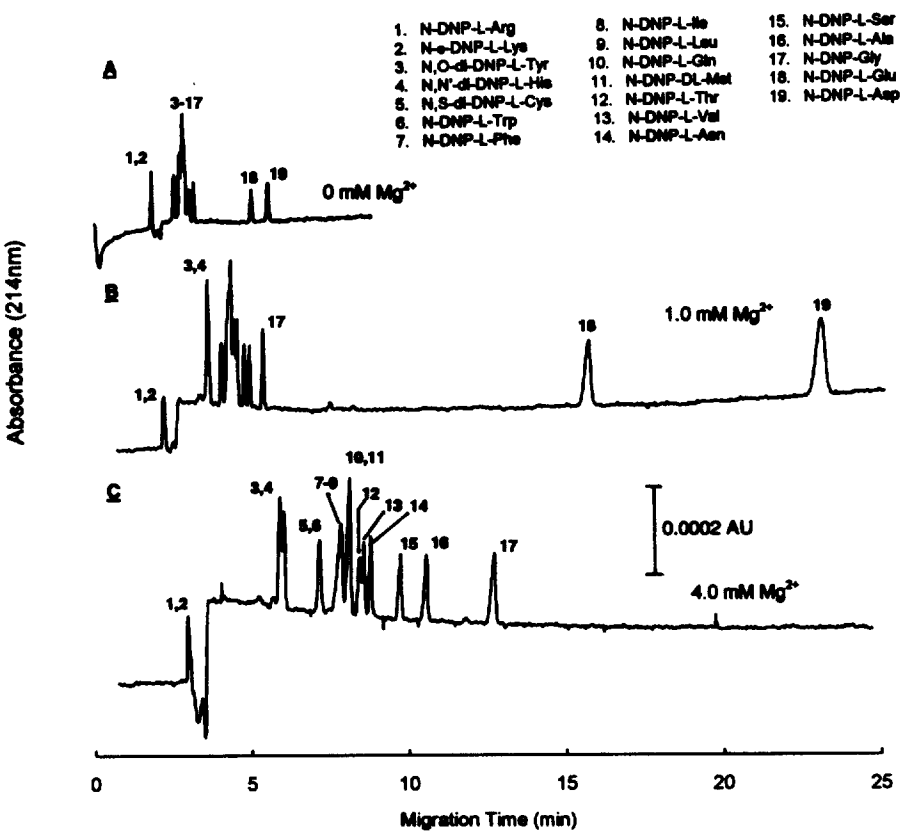

Fig. 5. Effect of Mg^{2+} on the resolution of a mixture of DNP-amino acid derivatives. The buffer is 20 m*M* NH_4OAc, pH 7.00 (A) in the absence of Mg^{2+}, (B) with 1.0 m*M* Mg^{2+} and (C) with 4.0 m*M* Mg^{2+} where the votage is 30 kV and the current varies from 27 to 46 μA.

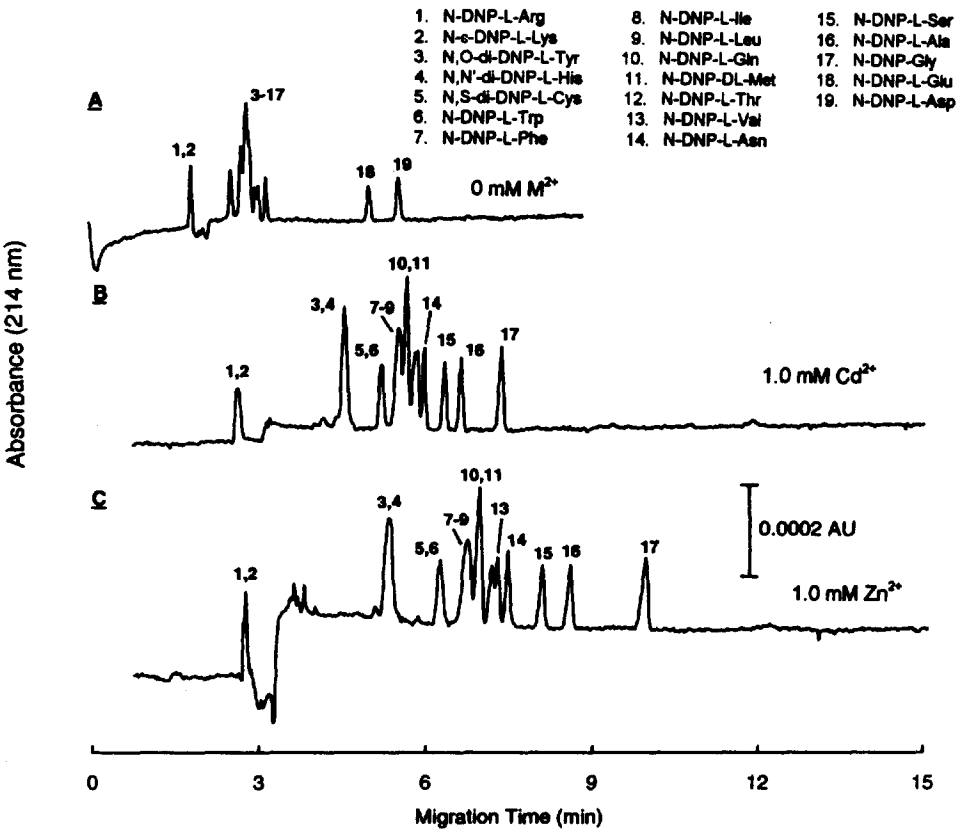

Fig. 6. Effect of Cd^{2+} and Zn^{2+} on the resolution of a mixture of DNP-amino acid derivatives. The buffer is 20 m*M* NH_4OAc, pH 7.00 (A) in the absence of the cation additive, (B) with 1.0 m*M* Cd^{2+} and (C) with 1.0 m*M* Zn^{2+} where the voltage is 30 kV and the current varies from 27 to 35 μA.

Mg^{2+} and why peak broadening is observed with Cd^{2+} and Zn^{2+} is not clear. It may be that very weak association between Cd^{2+} or Zn^{2+} and the DNP-amino acid derivative is a factor even though μ_{ep} values for the derivatives in the presence of the two cations are constant when cation concentration is varied. Also, since the conditions used are close to a condition where precipitation will occur, low level hydrolysis at the capillary wall surface may be present.

Raising the buffer pH in the presence of Mg^{2+} increases migration time and also improves resolution since the rate of change increases for the DNP-L-amino acid derivatives with the longer migration time. Fig. 7 illustrates the CZE separation of an 18 component DNP-L-amino acid derivative mixture in a 20 m*M* borate, pH 9.25 buffer as a function of Mg^{2+} concentration. Resolution is improved even at a lower Mg^{2+} concentration, (compare Fig. 7C for 2.0 m*M* Mg^{2+} at pH 9.25 to Fig. 5C for 4.0 m*M* Mg^{2+} at pH 7.00) but analysis time is also sharply increased. No attempt was made to use Cd^{2+} or Zn^{2+} at the higher pH because of precipitation.

4. Conclusions

Electroosmotic flow is reduced when Mg^{2+}, Cd^{2+} or Zn^{2+} is added to the CZE buffer. As the cation concentration is increased in a 20 m*M* NH_4OAc, pH 7.00 buffer, EOF decreases and follows the order $Zn^{2+} > Cd^{2+} > Mg^{2+} \gg M^{+}$. When DNP-L-amino

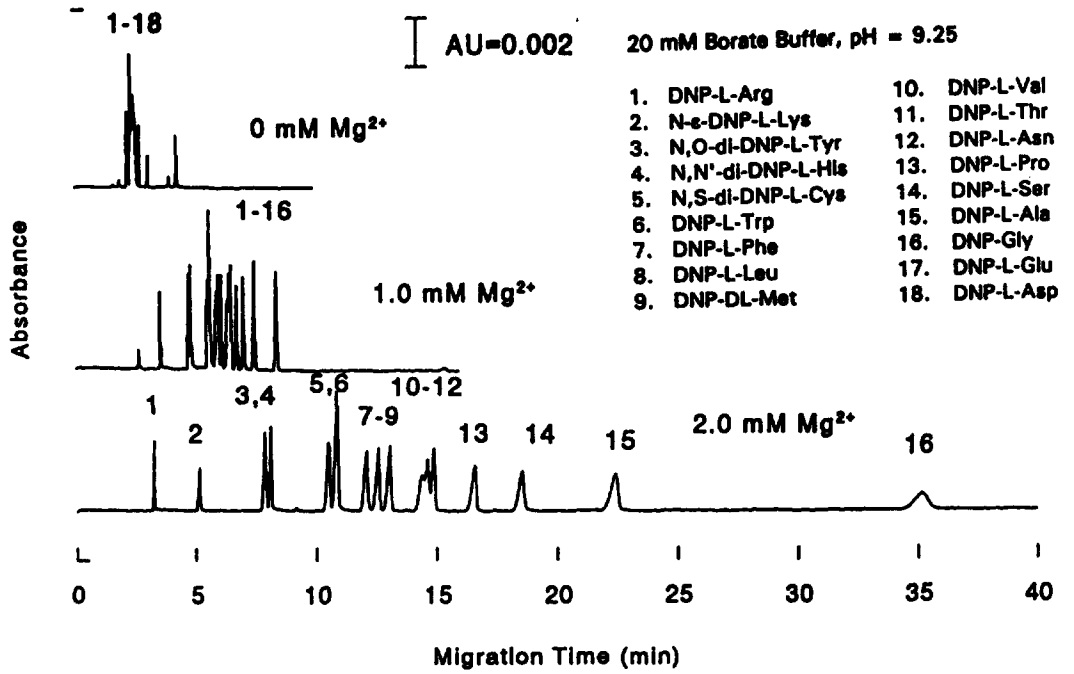

Fig. 7. Separation of DNP-amino acid derivatives at higher pH in the presence of Mg^{2+}. The buffer is 20 m*M* borate, pH 9.25 (A) in the absence of Mg^{2+}, (B) with 1.0 m*M* Mg^{2+} and (C) with 2.0 m*M* Mg^{2+}.

acid derivatives are the analytes, their μ_{ep} values remain constant for a given buffer and pH as the cation concentration is increased in the buffer. The increased migration time for the DNP-L-amino acid derivatives is due to a reduction in EOF rather than to an interaction between the derivative and the buffer cation additive. In addition, the cation buffer additive causes a significant improvement in resolution in the CZE separation of complex mixtures of DNP-L-amino acid derivatives. Peak shape is best for Mg^{2+} as the additive and resolution can also be improved by raising the buffer pH to above 9.

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