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Control of electroosmotic flow by a cation additive to enhance the separation of amino acids by micellar electrokinetic chromatography

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Abstract

The effect of a divalent cation (Mg^{2+}) and 3 monovalent cations (Na^+, Li^+, and K^+) as buffer additives on the electroosmotic flow (EOF) was investigated in order to improve the separation performance of *p*-acetamidobenzenesulfonyl fluoride (PAABS-F) derivatives of 20 standard amino acids by micellar electrokinetic chromatography (MEKC). The EOF can be decreased with increasing concentration of cations with the order of cations as $Mg^{2+} > K^+ > Na^+ > Li^+$. However, it was found that the resolution cannot be improved easily using a buffer cation which is more capable of decreasing the EOF. Taking the migration time, resolution, and peak shape into account, Na^+ is the best buffer additive, although the EOF decreased most using Mg^{2+}. Using 20 mmol/L borate at pH 9.3 containing 140 mmol/L SDS and 20 mmol/L Na^+ as electrolyte, 20 standard amino acids were successfully separated within 14 min.

Keywords: Micellar electrokinetic chromatography; Amino acids; Electroosmotic flow; Cation additive

1. Introduction

In the past, methods for the determination of amino acids were based mostly on chromatographic techniques, such as gas chromatography (GC) [1,2] and high performance liquid chromatography (HPLC) [3–5]. Recently, capillary electrophoresis (CE) has emerged as one of the most powerful separation techniques due to its fast analysis, small sample consumption, high separation efficiency and applicability to a diversity of analytes [6–10]. However, most amino acids were poorly detected using UV detection because most of them lack of a strong chromophore. Therefore, their precolumn [10–12] or postcolumn [13] chemical derivatization is a common remedy. A lot of derivatization reagents [10,14–23] have been developed to enable the derivatization of amino acids. The most popular derivatization reagents nowadays include *o*-phthalaldehyde (OPA) [14], phenylisothiocyanate (PITC) [15,16], 1-dimethylaminonaphthalene-5-sulphonyl chloride (Dansyl-Cl) [17], 1-fluoro-2, 4-dinitrobenzene (FDNB) [18,19], and some others [10,20–23]. In this article, pre-column *p*-acetamidobenzenesulfonyl fluoride (PAABS-F) labeling was chosen for its benefit in reaction time, cost, and overall utility [10].

Electroosmotic flow (EOF) is a key factor for a successful capillary electrophoresis separation, because it has a strong

Abbreviations: CE, capillary electrophoresis; Dansyl-Cl, 1-dimethylaminonaphthalene-5-sulphonyl chloride; EOF, electroosmotic flow; FDNB, 1-fluoro-2; 4-dinitrobenzene; GC, gas chromatography; HPLC, high performance liquid chromatography; MEKC, micellar electrokinetic chromatography; OPA, *o*-phthalaldehyde; PAABS-F, *p*-acetamidobenzenesulfonyl fluoride; PITC, phenylisothiocyanate

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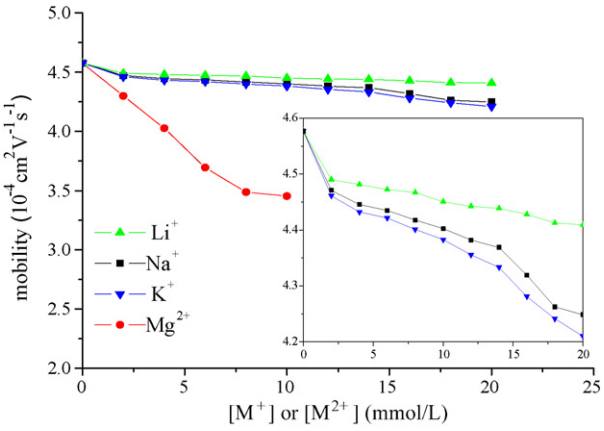

Fig. 1. Effect of monovalent and divalent cations buffer additives concentration on the EOF. Experimental conditions: bare fused silica capillary 60 cm × 50 μm; the effective length of the capillary is 50 cm; 20 mmol/L sodium borate at pH 9.3 containing 140 mmol/L SDS; injection for 5 s at 0.5 psi; running voltage 30 kV; DAD detector at 214 nm. *N*, *N*-dimethylformamide was the EOF marker. Inset: Enlargement of effect of monovalent cations buffer additives concentration on the EOF.

effect on migration time, resolution and efficiency [24]. Some methods such as the addition of surfactants [25], organic modifiers [26], changing buffer pH or concentration [27] and some times derivatizing the capillary wall can modify or even reverse the EOF [28,29]. Some articles reported that a buffer cation and its concentration can affect the EOF [30–33] and consequently improve the resolution of free amino acids by means of CE [33]. Pietrzyk et al. [33] have researched the effect of adding divalent cations (Mg^{2+}, Cd^{2+}, and Zn^{2+}) to the electrolyte on the CE separation of amino acids, and they found that the best cation additive was Mg^{2+}. However, up till now no research work has been carried out on the effect of monovalent cations on the separation of amino acids by means of micellar electrokinetic chromatography (MEKC). In this work, we compared one divalent cation (Mg^{2+}) with three other monovalent cations (Na^+, Li^+, and K^+) to demonstrate which buffer cation is the best to improve the performance of the MEKC separation of 20 standard PAABS-F labeled amino acids.

2. Experimental

2.1. Apparatus

A Beckman MDQ P/ACE system (Beckman Fullerton, CA, USA) with an on-column DAD detector was used for all experiments. The Beckman 32 Karat software Version 4.01 (1999–2000 Beckman-Coulter) allowed instrument control and data analysis. Bare fused silica capillaries (50 μm i.d., 365 μm o.d.) were obtained from Yongnian Optic Fiber (Hebei, China). The total length of the capillary was 60 cm, with an effective length of 50 cm. The temperature of the capillary was

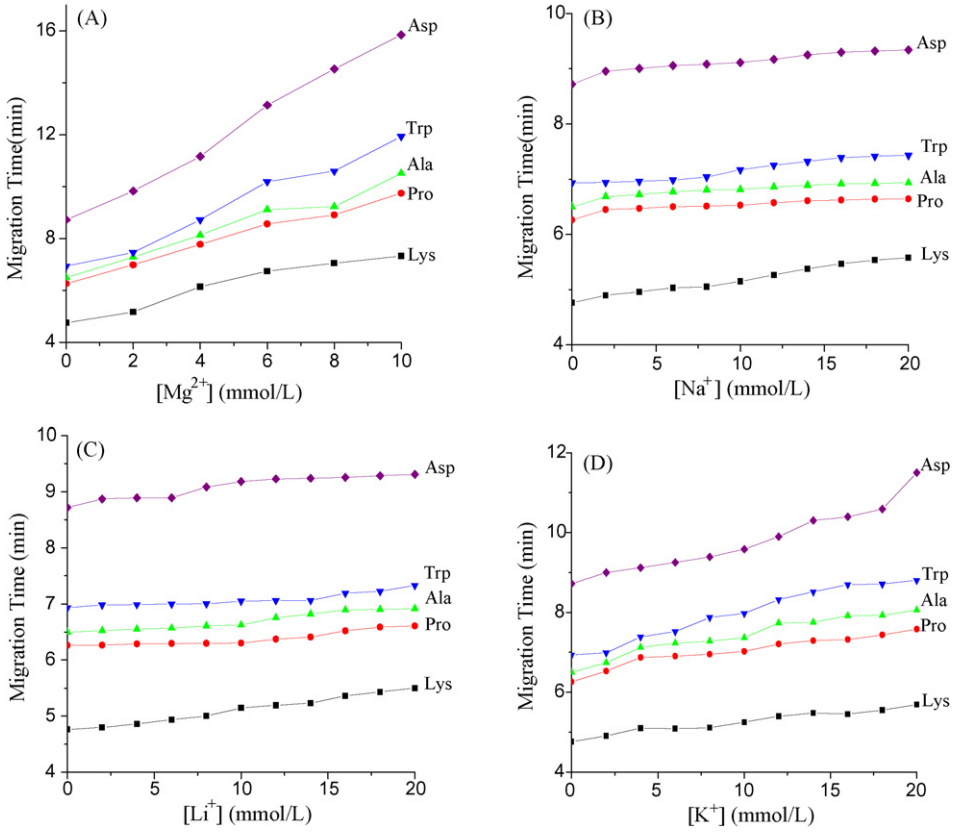

Fig. 2. Effect of monovalent and divalent cations concentration on the migration time for 5 PAABS- amino acid derivatives. Detection at 254 nm. (A) Mg^{2+} concentration is varied in 20 mmol/L borate at pH 9.3 containing 140 mmol/L SDS buffer; in (B), (C), and (D) the same buffer was used with varying Na^+, Li^+, and K^+ concentrations, respectively. All other conditions were the same as in Fig. 1.

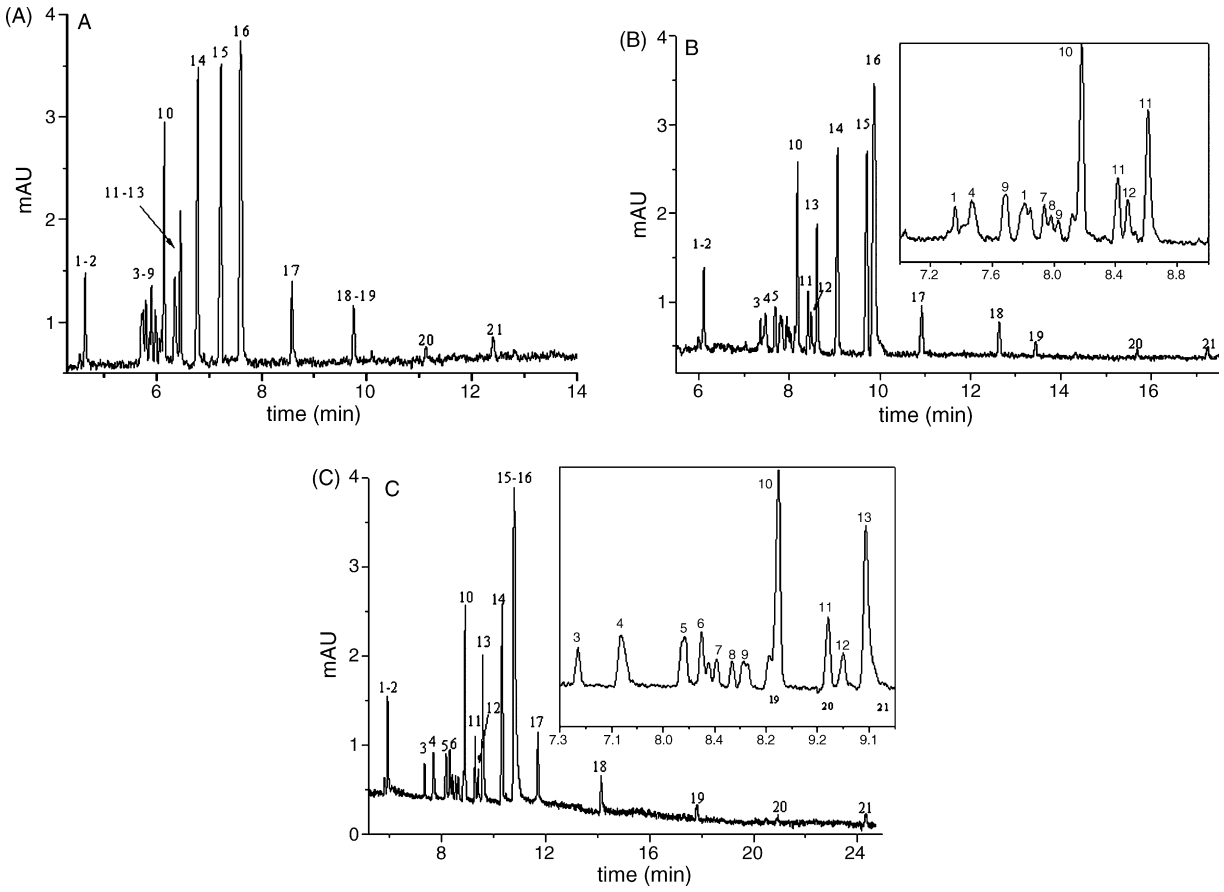

Fig. 3. Effect of Mg^{2+} on the resolution of a mixture of PAABS- amino acid and glycine derivatives. The electrolyte is 20 mmol/L borate at pH 9.3 containing 140 mmol/L SDS. (A) In the absence of Mg^{2+}, (B) with 5 mmol/L Mg^{2+}, and (C) with 10 mmol/L Mg^{2+}. Analyte peak numbering: 1, Lys; 2, Gln; 3, Thr; 4, Asn; 5, Ile; 6, Leu; 7, Val; 8, Ser; 9, Met; 10, Pro; 11, Ala; 12, Gly; 13, Phe; 14, Trp; 15, Cys; 16, PAABS-OH; 17, Asp; 18, Arg; 19, Glu; 20, His; and 21, Tyr. All other conditions were the same as in Fig. 1.

kept constant at 25 °C. The capillaries were conditioned by washing consecutively with 0.1 mol/L NaOH (5 min, 20 psi), doubly distilled water (2 min, 20 psi) and the separation buffer (2 min, 20 psi). Each separation was initiated with a rinse with a 0.1 mol/L NaOH (3 min, 20 psi), followed by a rinse with doubly distilled water (2 min, 20 psi) and separation buffer (2 min, 20 psi). *N*,*N*-dimethylformamide (detection at 214 nm) was chosen as the EOF marker.

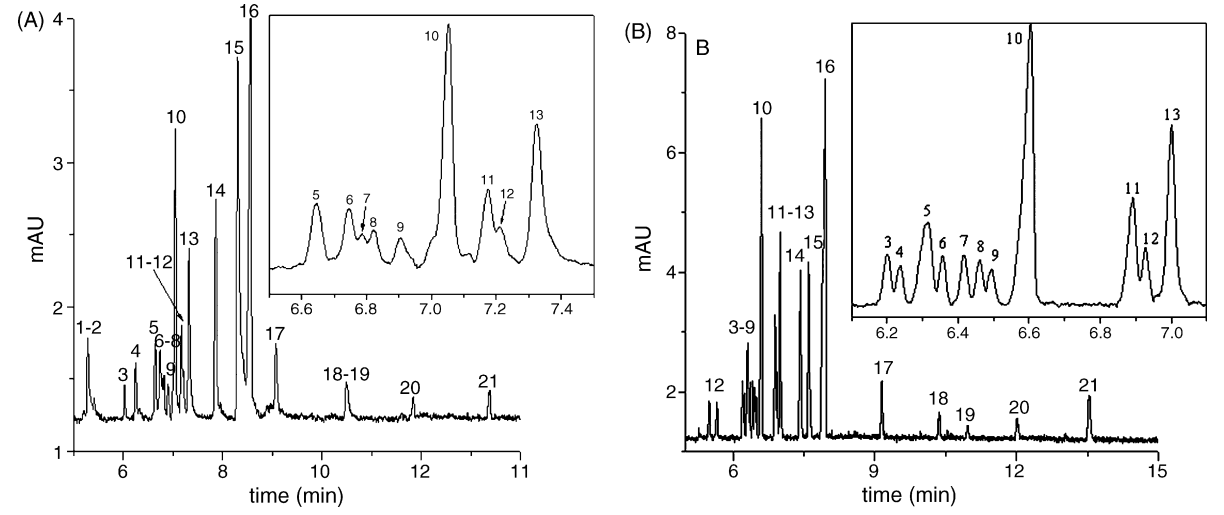

Fig. 4. Effect of Na^{+} concentration on the resolution of a mixture of PAABS-amino acid derivatives. The buffer was 20 mmol/L borate at pH 9.3 with 140 mmol/L SDS buffer (A) with 10 mmol/L Na^{+} and (B) with 20 mmol/L Na^{+}. All other conditions were the same as in Fig. 1.

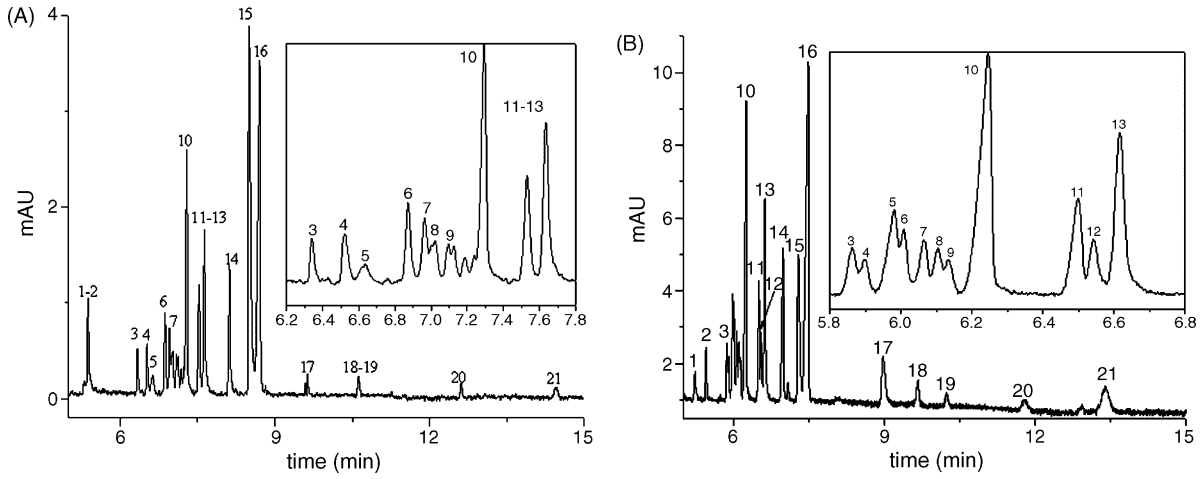

Fig. 5. Effect of K^+ and Li^+ on the resolution of a mixture of PAABS-amino acid derivatives. (A) With 10.0 mmol/L K^+ and (B) with 20.0 mmol/L Li^+. All other conditions were the same as in Fig. 1.

2.2. Reagents

PAABS-F was synthesized in our laboratory [10]. Sodium dodecyl sulfate (SDS, 99%) was purchased from Alfa Aesar (St. Louis, MO, USA). Twenty biochemical grade amino acids (Lys, Gln, Thr, Asn, Ile, Leu, Val, Ser, Met, Pro, Ala, Phe, Trp, Cys, Asp, Arg, Glu, His, Tyr, and Gly), analytical grade hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), potassium chloride (KCl), lithium chloride (LiCl), magnesium chloride ($MgCl_2$), boric acid, *N*, *N*-dimethylformamide and methanol were purchased from Chemical Reagent Co. (Shanghai, China).

The amino acid standard stock solutions (10 mmol/L of each component) were prepared by accurately weighing and were dissolved in sodium borate buffer (10 mmol/L, pH 9.3).

Sodium borate buffer was prepared by dissolving boric acid in water and adjusting the pH value to 9.3 by NaOH (0.1 mol/L). SDS and salts were dissolved in the buffer solution to obtain a final electrolyte solution containing 140 mmol/L SDS and salts varying between 2 and 20 mmol/L.

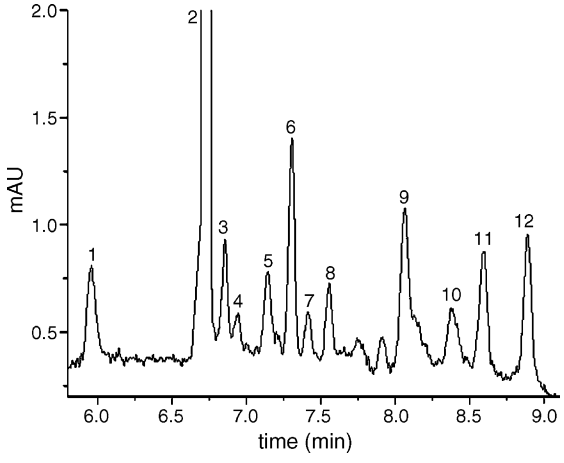

Fig. 6. Electropherogram obtained from a beer sample of Heineken. The buffer was 20 mmol/L borate at pH 9.3 with 126 mmol/L SDS and 20 mmol/L Na^+. Peak numbering: 1, Lys; 2, Thr; 3, Ile; 4, Leu; 5, Val; 6, Ser; 7, Met; 8, Pro; 9, Ala; 10, Gly; 11, Phe; and 12, Trp. All other conditions were the same as in Fig. 1.

2.3. Derivatization procedure

The derivatization procedure is performed according to our previous work [10] and described as follows: solid PAABS-F was dissolved in ethanol to prepare a 0.05 mol/L solution. Reactions were performed in a 2 mL vial. To a vial containing the appropriate volume of amino acid standard solution, 240 μL of 0.05 mol/L PAABS-F and 700 μL borate buffer (20 mmol/L, pH 9.3) were added, followed by the addition of 1 μL methanol to the solution. Derivatization was carried out at 35 °C during 30 min. Before the mixture was injected into the analytical system, it was filtered through a 0.22 μm membrane filter.

Heineken^{TM} beer was purchased from a food store. Before analysis, the beer was filtered through a 0.22 μm membrane filter and titrated to NaOH (0.1 mol/L) with pH 9.3. To a 2 mL vial containing 800 μL sample and 200 μL of 0.05 moll/L PAABS-F, 1 μL methanol was added. The reaction was carried out at 35 °C during 30 min.

3. Results and discussion

3.1. MEKC conditions

Borate was chosen as the running buffer because it gives a reproducible EOF and provides high speed and good reproducibility for the separation of amino acids [7]. SDS is an anionic surfactant that has been widely used in MEKC for amino acid and peptide analysis [34]. A 20 mmol/L borate buffer at pH 9.3 with 140 mmol/L SDS was proved to be the best condition because a relative small current was generated and high separation efficiency can be gained at this optimum condition.

In MEKC, the migration times are reduced with increasing applied voltage while current increases at the same time, both in the absence and presence of cations in the buffer. The noise of the baseline also increases with increasing concentration of cations.

Table 1
Resolution (R) of PAABS-F labeled amino acids using optimal concentrations of various salts as buffer additive

	Asn-Thr	Val-Asn	Ile-Val	Met-Ile	Ser-Met	Leu-Ser	Pro-Leu
20 mmol/L Na^+	0.80	1.2	1.9	1.6	6.8	1.0	2.2
10 mmol/L K^+	0.98	0.91	1.0	0.54	1.2	0.95	1.1
20 mmol/L Li^+	0.63	1.2	0.64	1.1	0.76	0.60	1.7
5 mmol/L mg^{2+}	2.3	5.8	1.6	0.8	0.6	0.5	1.9

The currents produced at 30 kV were greater than 100 μA when the salts solution (concentrations of more than 20 mmol/L) were added into the buffer. Heat generation at such high current is significant and thus decreases the resolution. As a result, high concentration of cations cannot be used as buffer additive, even if they are more capable to decrease the EOF.

3.2. Effect of buffer cation

Pietrzyk et al. [33] reported that the addition of some inorganic divalent cations (Mg^{2+}, Cd^{2+}, and Zn^{2+}) to the buffer could improve the resolution and peak shape in the separation of amino acids and found that the best cation was Mg^{2+} [33]. In this work, we choose Mg^{2+} and three monovalent cations, Na^+, Li^+, and K^+, and determined which is the best cation additive for the separation of amino acids.

The EOF was determined with an electrolyte containing 20 mmol/L borate buffer at pH 9.3, 140 mmol/L SDS and different concentrations of Mg^{2+} (ranging from 2 to 10 mmol/L. When the Mg^{2+} concentration was higher than 10 mmol/L, the resolution decreased greatly because of high separation current generation. Considering the same reason, the Na^+, Li^+ or K^+ concentration ranged from 2 to 20 mmol/L were used in the followed experiments). Under these conditions, no precipitation occurred. In Fig. 1, it can be seen that the EOF decreased with increasing concentration of cations and the reduction of the EOF followed the order $Mg^{2+} > K^+ > Na^+ > Li^+$. The order of $K^+ > Na^+ > Li^+$ correlated to the cation-exchange selectivity exhibited by chromatographic silica [32,35]. From Fig. 1, it could also be observed that the reduction of the EOF by adding Mg^{2+} is significantly higher than any other monovalent cation such as K^+, Na^+, or Li^+. However, the reduction of the EOF with the addition of monovalent cations was only slightly decreased with increasing concentrations of them. This indicated that the resolution improvement when adding K^+, Na^+, and Li^+ might be lower than that when adding Mg^{2+}. The analysis time, however, when adding Mg^{2+} might be longer than that when adding K^+, Na^+, and Li^+. Therefore, if the overall separation performance (resolution, analysis time, and efficiency) is considered, attention should be paid to select the types and concentrations of cations in order to gain highest separation performance.

3.3. Separation of PAABS-F labeled amino acids

In order to evaluate the effect of monovalent and divalent cations on the migration time of the derivatives, PAABS-Lys, PAABS-Pro, PAABS-Ala, PAABS-Trp, and PAABS-Asp were selected as the representative test analytes. Migration times were determined at 30 kV in 20 mmol/L borate buffer pH 9.3 containing 140 mmol/L SDS as a function of the Mg^{2+}, K^+, Na^+, and Li^+ concentration. Fig. 2 shows the effect of different concentrations of monovalent and divalent cations on the migration time of five PAABS-amino acid derivatives. It can be found from Fig. 2 that the migration time of these five PAABS-amino acid derivatives increased with increasing concentration of buffer cations. The migration time increased sharply with increasing concentration of Mg^{2+} (Fig. 2A), whereas only a small increase was seen with the monovalent cations such as Na^+ (Fig. 2B), K^+ (Fig. 2C), and Li^+ (Fig. 2D). The elution order for the PAABS-amino acids was the same for

Table 2
Quantitative data and for amino acid derivatives

Amino acid	Correlation coefficient	Linear range (μmol/L)	CDL[a] (μmol/L)	%RSD[b] (time)	%RSD[b] (area)	Average theoretical plate height[b] (plates/m)
Lys	0.991	200–3000	155	0.52	1.55	103,200
Thr	0.986	40–2000	37.9	0.49	1.38	199,600
Ile	0.999	50–2000	44.2	0.64	2.56	375,400
Leu	0.999	55–2000	52.7	0.82	3.01	215,600
Val	0.999	30–2000	26.2	0.76	2.12	229,700
Ser	0.998	40–2000	37.7	0.53	1.97	239,800
Met	0.992	65–2000	61.1	0.50	2.89	246,600
Pro	0.996	25–2000	21.1	0.68	1.45	335,400
Ala	0.992	70–2000	68.6	0.44	2.06	155,700
Gly	0.999	25–2000	21.9	0.58	2.75	102,500
Phe	0.999	25–2000	21.8	0.69	2.34	211,700
Trp	0.999	15–2000	10.8	0.72	1.76	226,800

[a] CDL, concentration detection limit.
[b] Calculated for six consecutive runs at 1 mmol/L of each amino acid derivative.

each of the four cations, PAABS-Asp > PAABS-Trp > PAABS-Ala > PAABS-Pro > PAABS-Lys. However, it must be noted that although the migration time of all the derivatives increased with increasing concentrations of cations, the interval between any two derivatives did not increase at the same time. This meant that the resolution could not be improved easily by only using a buffer cation which can decrease the EOF.

3.4. MEKC separation of PAABS-amino acid derivatives

In order to demonstrate which cations were the best buffer cations to improve the resolution, we tested the four cations with varying concentrations. Fig. 3 demonstrates the MEKC separation of 20 amino acid derivatives as a function of the Mg^{2+} concentration. Fig. 3A shows the electropherogram of 20 standard amino acid derivatives in absence of Mg^{2+}. The migration times for all derivatives were short because no reduction of the EOF occurred. When the Mg^{2+} concentration increased (Fig. 3B), the migration times of all the derivatives increased and the resolution improved. However, when a high Mg^{2+} concentration (10 mmol/L) was used, the resolution did not improve anymore, it reduced slightly while the migration time was sharply increased to 25 min. The baseline noise also increased clearly (Fig. 3C). This indicated that the joule heat generated during the separation process was high, and reduced the resulting resolution. That meant that the resolution can only be improved limitedly by adding a suitable concentration of Mg^{2+}.

It was reported that the addition of Na^+ could change the size and shape of micelles [36]. Considering that the resolution can also be changed by adding Na^+, it was tested for improving the separation performance of amino acids. Fig. 4 shows the MEKC separation of 20 standard amino acid derivatives as a function of the Na^+ concentration varied by adding different amount NaCl into buffer solution. It is true that the Na^+ concentration also can be increased by adding more SDS or Sodium borate into the buffer since Na^+ is the component of them. However, the experimental results showed that the resolution cannot be improved by adding more SDS or sodium borate into the buffer when optimal concentration of them were added into the buffer (data not shown). It is probably due to the anions contained in these two substances that made some negative contribution to the separation. When comparing Fig. 3A with Fig. 4A, it can be seen that the resolution was improved and that 11 amino acids were successfully separated when 10 mmol/L Na^+ was added to the buffer. In contrast with the effect of high Mg^{2+} concentrations on the resolution, the resolution was improved significantly when the concentration of Na^+ was further increased to 20 mmol/L (Fig. 4B). Twenty standard amino acid derivatives were all successfully separated at these conditions. It can also be seen from Fig. 4B that using a Na^+ concentration up to 20 mmol/L did not have a negative impact on the baseline. Two reasons might contribute to achieve the good resolution obtained with high Na^+ concentration: (1) the separation current when Na^+ was used as buffer cation was lower than that using Mg^{2+}; (2) monovalent cations such as Na^+ have a stronger ability to change the size and shape of micelles and increase the hydrophobicity of surfactant micelles more than divalent cations such as Mg^{2+} [37,38].

Table 3
Mean recoveries ($n = 6$) of each amino acids added at different concentrations[a] to beer samples

Amino acid	Recovery (%)	RSD%
Lys	95	2.7
Thr	97	2.1
Ile	103	3.3
Leu	86	3.8
Val	106	4.2
Ser	101	2.6
Met	92	2.2
Pro	93	2.5
Ala	104	4.4
Gly	108	4.6
Phe	95	2.0
Trp	92	2.3

[a] Amino acids were spiked at 0. 5, 1.0, and 1.5 mmol/L.

K^+ and Li^+ were also tested for their effect on the performance of MEKC separation. Fig. 5 compares K^+ at 10 mmol/L (Fig. 5A) and Li^+ at 20 mmol/L (Fig. 5B) as a buffer additive, which were optimum concentrations when taking into account the resolution, peak shape and analysis time of the 20 amino acid derivatives. Table 1 shows the resolution of PAABS-F labeled amino acids using optimal concentrations of various salts as buffer additive. As shown in Fig. 5 and Table 1, K^+ required 10 mmol/L while Li^+ requires 20 mmol/L to gain a better resolution, because the EOF decrease follows the order $K^+ > Li^+$. Thus, K^+ can be used at lower concentration to produce a slower EOF. However, the resolution gained using these two cations is less than that using Na^+, as can be seen from the comparison of Fig. 4B with Fig. 5A and B. This indicated that Na^+ had the strongest ability to improve the resolution not only through decrease of the EOF but also via strong interaction with the micelles. When 20 mmol/L Na^+ was used as buffer cation, 20 standard amino acids were well separated within 14 min. This method was also applied to separate the amino acids in beer and 12 amino acids were found (Fig. 6). Quantification of amino acids in beer was based on the calibration curves of peak areas against analytes concentration. The limit of detection (LOD) was determined as three times the signal-to-noise ratio. The precision of the method was evaluated by injecting standard amino acid consecutively six times. The RSDs of the areas are between 1.38 and 3.01, and the RSDs of the migration time are between 0.44 and 0.82. These results are shown in Table 2. It also can be found from Table 2 that the theoretical plate heights are between 102,500 and 375,400 plates/m. It indicates that the joule heating generated during the separation dose not make some negative effect on separation performance. To determine the accuracy, each amino acid was added to the bear sample. The average recovery for the impurities ranged from 86 to 108% (Table 3). Therefore, this method can be potentially be used to determine amino acids in clinical practice and scientific research.

4. Conclusion

This work studied carefully the effect of monovalent and divalent cations as buffer additives on the MEKC separation of 20

standard amino acid derivatives. It was found that although the electroosmotic flow could be decreased by adding Na^+, Li^+, K^+, or Mg^{2+} into the buffer, the resolution could only be improved limited by increasing the concentration of the buffer cations. To gain a high separation performance, the effect of selected buffer cations on the migration time, resolution, and peak shape (efficiency) of the amino acids derivatives should all be considered carefully. From the comparison of a divalent cation with three monovalent cations, 20 mmol/L Na^+ was found as the best buffer cation for the separation of amino acids. Using proposed method, 20 standard amino acids were successfully separated by capillary electrophoresis within 14 min.

Acknowledgements

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