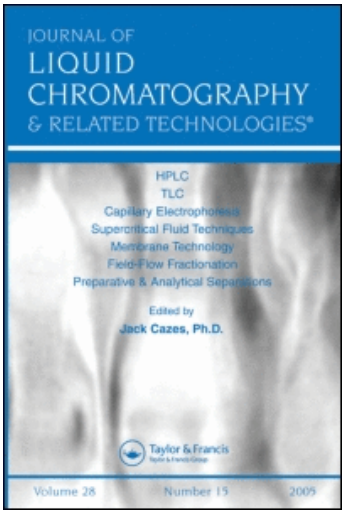

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DETERMINATION OF AMINO ACIDS IN URINE BY CAPILLARY ELECTROPHORESIS WITH INDIRECT UV DETECTION

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ABSTRACT

This study has described a relatively simple method for determination of 14-18 amino acids (AA's) by CE with indirect UV detection using both myristyltrimethyl-ammonium bromide (MTAB)-coated and uncoated capillaries. The method used either salicylate or benzoate as UV-absorbing additive in carbonate buffer and the non-absorbing AA's were detected indirectly at 230 nm. The optimization of the assay conditions was demonstrated with an MTAB-coated capillary using salicylate-carbonate as separation buffer. Factors that affect indirect signal response and migration behavior of AA's such as buffer concentration, pH, and the relative amount of UV-absorbing additive in buffer have been studied. Comparison of MTAB-coated and uncoatd capillaries on the analysis of AA's

has been made. It has been found that the limits of detection of 18 AA's by the method were in the order of 53.6 to 785 pg injected. The method developed was used in the determination of AA's in urine sample and preliminary results were provided.

INTRODUCTION

Amino acids (AA's) are important biological molecules. Determination of AA's in proteins, peptides, and biological fluids may help us, not only in mapping the structures of these macromolecules, but in understanding their metabolic pathways as well in diagnosing diseases. There are more than 50 hereditary diseases of AA metabolism that have been identified today.[1] Diseases like aminoacidurias (e.g., phenylketonuria, branched-chain ketoaciduria also known as maple syrup urine disease, histidinemia and so forth), marked by high levels of urinary AA's can be diagnosed through screening of AA's in urine specimens.[2]

Determination of AA's have been accomplished by liquid chromatography (LC) and capillary electrophoresis (CE).[3-10] Because most AA's, except the aromatic ones, lack absorptivity of UV and visible radiation that can be used for their sensitive detection, pre- or post-column derivatization reaction of AA's with a fluorophore or a chromophore is often employed. However, the derivatization of AA's in LC and CE methods is limited by many factors, such as the availability of derivatizing agent, the reaction rate, the stability, and the solubility of product, the ability of derivatizing agent to react with both primary and secondary AA's, and the absorption and emission wavelengths of derivatized product in regard to the outputs of the instrument.

CE is a micro-analytical technique with unrivaled separation efficiency that is well suited for the determination of AA's in biological fluids. Detection of AA's in CE can also be done by indirect approaches, similar to those used in LC. For example, indirect fluorescence detection of AA's has been reported.[11,12] To achieve low limits of detection, a laser light that ensures a tightly focused beam is essential for efficient fluorescence detection. Alternatively, indirect UV detection is more practical if the limits of detection is not a major concern, since most commercial CE instruments are equipped with UV detectors.

Indirect UV detection in CE may be characterized by monitoring of the UV absorbance of the separation buffer, where background UV-absorbing additive is displaced by zones of non-UV-absorbing solute ions and resulted in

negative peaks. A few studies on indirect UV detection of AA's by CE have been reported, including the detection of 7 AA's with salicylate,[13] 3 AA's with quinine sulfate,[14] and 18 AA's with either *p*-aminosalicylic acid or 4-(N,N'-dimethylamino)benzoic acid in the presence of α-cyclodextrin.[15]

In this study we have developed a simple CE method for the analysis of 14-18 AA's, using either salicylate or benzoate as UV-absorbing additives in carbonate buffer by MTAB-coated and uncoated capillaries. Factors that affect indirect signal response and migration behavior of AA's, such as buffer concentration, pH, and the relative amount of UV-absorbing additive in buffer have been studied. The potential use of the method in the analysis of urinary AA's has been investigated and preliminary results are presented.

EXPERIMENTAL

Chemicals and Solutions

All AA's used in this study were purchased from Sigma (St. Louis, MO). Myristyltrimethylammonium bromide (MTAB) was obtained from Aldrich (Milwaukee, WI). Sodium salicylate, sodium benzoate, sodium carbonate (anhydrous), sodium hydroxide, and hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA).

Aqueous solutions were prepared with deionized water (Barnstead/Thermolyne NANOpure system, Dubuque, IA). Stock standard solutions of AA's were prepared in deionized water at concentrations of 1.0 mg/mL and 0.1 M. Prior to CE analysis, fresh working solutions of various AA's were prepared by serial dilutions from the stock solutions with deionized water. 0.1 M sodium salicylate and sodium benzoate were prepared in deionized water and used as stock solutions of UV-absorbing additives. 10 mM MTAB was prepared in deionized water and used as stock coating solution. The separation buffers were prepared by dissolving the appropriate amount of sodium carbonate and 0.1 M UV-absorbing additive stock solutions, as well as 10 mM MTAB stock solution if the dynamic coating was being used, in deionized water.

The pH values of the buffers were adjusted to the desired values with 0.1 M HCl solution. In this work, all buffers were filtered through 0.45-μm cellulose-acetate-membrane syringe filters (Alltech, Deerfield, IL) before use.

CE Instrument

A Beckman (Fullerton, CA) P/ACE 2050 CE instrument and an IBM PC with System Gold software were used for this study. On-column UV absorption was detected at 230 nm and the capillary temperature was maintained at 25°C. Sample injection was done by pressure at 0.5 psi for 5 s. If an uncoated capillary was used, the cathode was placed in the outlet of the capillary and the anode was placed in the inlet. If MTAB had been added in separation buffer, the polarity of the electrodes had to be reversed because the surfactant dynamically coated the inner surface of the capillary through the formation of a bilayer (with cationic heads facing outwards into the aqueous solution), and reversed the direction of electroosmotic flow. The details of experimental conditions can be found in the figure legends.

Capillary Columns

Open tubular fused-silica capillaries (360 μm O.D., 100 μm I.D.), with polyimide coating on the outer surface, were purchased from Polymicro Technologies (Phoenix, AZ). A new capillary was conditioned by rinsing sequentially with 0.1 M NaOH, deionized water and separation buffer, and the capillary was used as uncoated capillary. An MTAB-coated capillary was prepared by flushing the uncoated capillary with salicylate-carbonate (or benzoate-carbonate) buffer containing 0.15 mM MTAB at 0.5 psi for 3 min. Between runs, the capillary was rinsed for 90 s using the separation buffer.

Urine Sample Pretreatment

Urine samples used in this study were collected from a healthy volunteer. All samples were first filtered through 0.45-μm cellulose-acetate-membrane syringe filters (Alltech), and then diluted four times by deionized water (1 portion of urine to 4 portions of water) prior to the CE analyses.

RESULTS AND DISCUSSION

The signal response of indirect UV detection is affected by many factors, including the relative concentration of UV-absorbing additive in the separation buffer, the charge ratio of UV-absorbing additive to analyte ions, and the pH and concentration of the separation buffer. In this study, we have investigated

Table 1

Properties of the Non-UV Absorbing AA's and UV Additives*

Amino Acids	M.W.	pKa_1(COOH)	$pKa_2(NH_3^+)$	pKa_3(R)	pI
Aspartic acid (Asp)	133.10	1.88	9.60	3.65 (COOH)	2.77
Glutamic acid (Glu)	147.13	2.19	9.67	4.25 (COOH)	3.22
Cysteine (Cys)	121.16	1.96	10.28	8.18 (SH)	5.07
Glycine (Gly)	75.07	2.34	9.60		5.97
Alanine (Ala)	89.09	2.34	9.69		6.00
Valine (Val)	117.15	2.32	9.62		5.96
Leucine (Leu)	131.17	2.36	9.60		5.98
Isoleucine (Ile)	131.17	2.36	9.60		6.02
Serine (Ser)	105.09	2.21	9.15		5.68
Threonine (Thr)	119.12	2.09	9.10		5.60
Asparagine (Asn)	132.12	2.02	8.80		5.41
Glutamine (Gln)	146.15	2.17	9.13		5.65
Proline (Pro)	115.13	1.99	10.60		6.30
Hydroxyproline (OH-Pro)	131.13	1.92	9.73		5.83
Citrullline (Cit)	175.19	NA	NA		NA
Ornithine (Orn)	132.16	NA	NA		NA
Methionine (Met)	149.21	2.28	9.21		5.74
Lysine (Lys)	146.19	2.18	8.95	10.53 (NH_3^+)	9.74
Arginine (Arg)	174.20	2.17	9.04	12.48 (NH_3^+)	10.76
Histidine (His)	155.16	1.82	9.17	6.00 (NH_3^+)	7.59
Uric acid (UA)	168.11			5.40 (NH)	
Salicylic acid (SA)	138.12	2.97		13.40 (OH)	
Benzoic acid (BE)	122.12	4.19			

* See reference 22.

pKa_1, the dissociation constant for the α-COOH group.

pKa_2, the dissociation constant for the α-NH_3^+ group.

pKa_3, the dissociation constant for the R group present in the molecule.

pI, isoelectric point.

NA, not available.

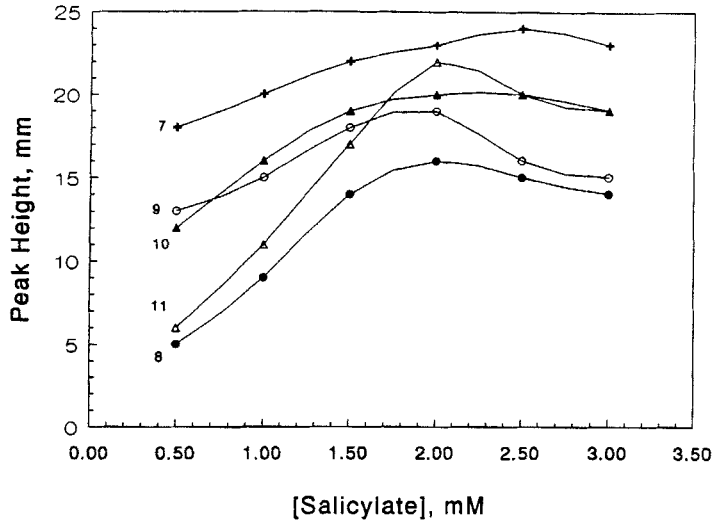

Figure 1. The influence of salicylate concentrations on the indirect UV responses. Experimental conditions: uncoated capillary, 77 cm x 100 μm (i.d.); temperature, 25°C; UV filter, 230 nm; separation potential, 15 kV; separation buffers, 5.0 mM carbonate/0.5-3.0 mM salicylate/0.15 mM MTAB at pH 10.7; injection pressure/time, 0.5 psi/5 s, AA's, 10 μg/mL. The identities of AAs are given in Table 2.

the factors that affect the sensitivity of indirect UV detection and the migration characteristics of selected AA's using an MTAB-coated capillary. The analytical performances of MTAB-coated and uncoated capillaries on the separation of AA's were compared. The method developed was applied to the determination of AA's in urine samples.

Optimization of the Separation Conditions and the Sensitivity of Indirect UV Detection

UV-absorbing additives

The selection of UV-absorbing additive is of the utmost importance in indirect UV detection. According to the previous studies,[16-18] an effective UV-absorbing additive ought to provide a high UV absorbance at a wide range of wavelengths, and a close match of ionic mobility with those of analyte ions. Unmatched ionic mobilities between UV-absorbing additive and analyte ions may result in low detection sensitivity (due to the low displacement ratio of UV additive to analytes), poor peak symmetry, and reduced separation efficiency.

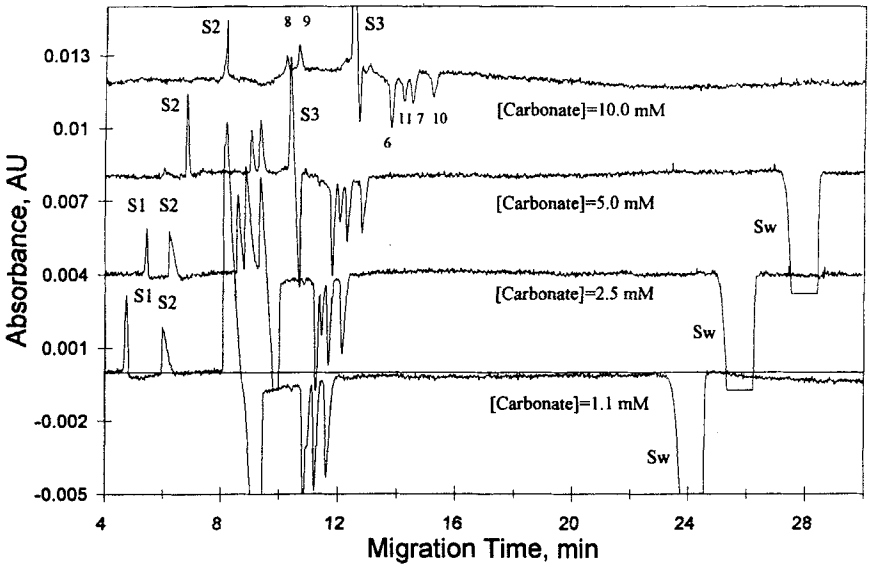

Figure 2. The influence of carbonate concentrations on the indirect UV responses and the migration times of AA's. Separation buffers: 1.1-10 mM carbonate/2.0 mM salicylate/0.15 mM MTAB at pH 10.7. The other experimental conditions were the same as those in Figure 1. Peak identities: S_1, system peak 1 (chloride), S_2, system peak 2 (carbonate/bicarbonate), S_3, system peak 3 (salicylate), S_w, system peak (water), the other peak identities: see Table 2.

In this work, salicylate was used as a UV-absorbing additive in the studies of signal response, separation efficiency, and analytical performances of the MTAB-coated and uncoated capillaries because it has a dissociation constant and a molecular mass compatible to those of AA's being studied (Table 1). Benzoate was also used as UV-absorbing additive for the reason of having compatible mobility and molecular mass to those of AA's. The detection wavelength of both salicylate and benzoate was chosen at 230 nm rather than 200 nm, as interference experienced from urine samples was much less at this wavelength.

Indirect signal response

In indirect UV detection, the concentration ratio of UV additive to co-ions in the separation buffer, determines the sensitivity of the signal response. As shown in Figures 1 and 2, the indirect responses of five AA's increased either with an increase in salicylate concentration (Figure 1), or with a decrease in

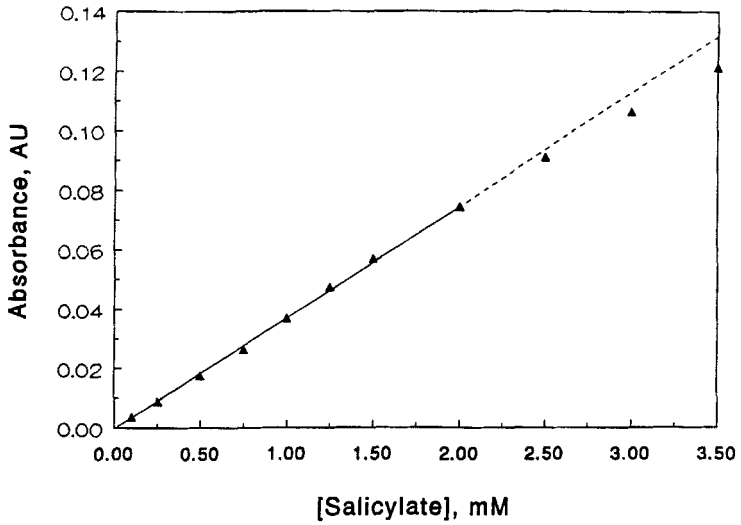

Figure 3. The UV absorbance of salicylate at different concentrations. Experimental conditions: uncoated capillary, 77 cm x 100 μm (i.d.); temperature, 25°C; UV filter, 230 nm; separation buffers, 5 mM carbonate/0.1 mM-3.5 mM salicylate at pH 10.7. The experiment was done by sequentially pushing eleven buffers containing various amounts of salicylate through the separation capillary.

carbonate concentration in separation buffer (Figure 2). These results reveal that the response in indirect UV detection is related to the relative displacement of UV-absorbing additive to co-ions by the analyte ions. Furthermore, if the concentration of the UV-absorbing additive (salicylate) exceeded its upper linear dynamic range of detection (2.0 mM) in CE (Figure 3), the responses of the analyte ions (AA's) either decreased or reached plateaus (Figure 1), because the displacement of the UV-absorbing additives with the analyte ions no longer follows the same relationship.[19] Accordingly, 2.0 mM salicylate was chosen as the concentration of UV-absorbing additive for later work in order to retain a linear detection response with maximum sensitivity of detection.

The pH influences the degrees of dissociation of buffer ions and UV additive, as well as of AA's; therefore, it affects the apparent mobilities of ionic species in CE and the signal responses of indirect detection. As shown in Figure 4, when the pH of buffer solution was equal to or less than 10.3, it not only caused the low-signal responses of AA's but also worsened the separation efficiencies due to the diminishing distinction in apparent mobilities among the AA's. However, at pH values equal to or greater than 10.7, the signal

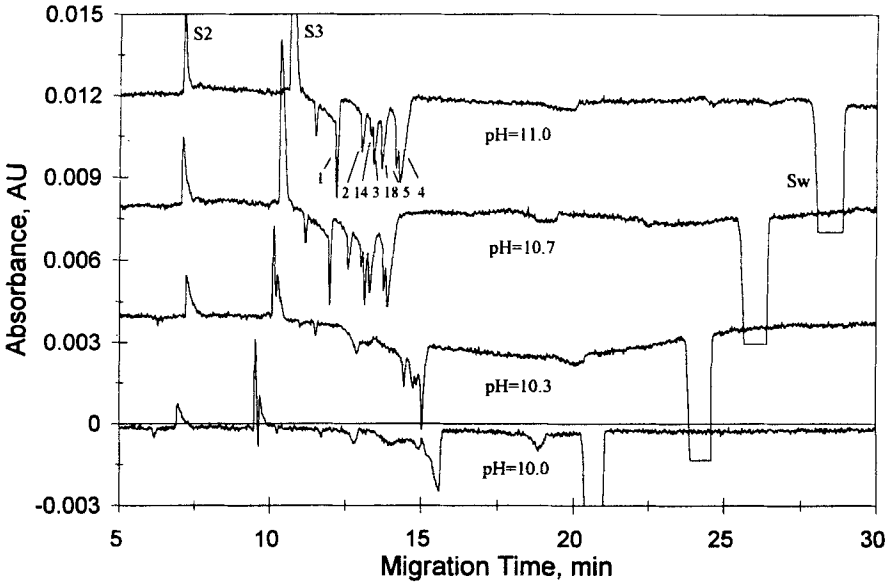

Figure 4. The influence of the buffer pH on the indirect UV responses and the migration times of AA's. Separation buffers: 5.0 mM carbonate/2.0 mM salicylate/0.15 mM MTAB at pHs 10.0-11.0. The other experimental conditions and peak identities were the same as those in Figures 1 and 2.

responses and the separation efficiencies of the AA's had both increased and improved because the zwitterionic AA's were converted to anions at the higher pHs and these UV-transparent anions displaced more UV-absorbing additive anions with more distinctive mobilities.

Migration and separation of AA's

Once a surfactant known as myristyltrimethylammonium bromide (MTAB) was added to the salicylate-carbonate buffer and filled in an uncoated capillary, the positively charged heads of the surfactant molecules dynamically formed ion-pairs with the negatively charged silanol ($Si\text{-}O^-$) groups on the inside wall of the fused silica capillary at pH > 2.[20] If the amount of surfactant exceeded a monolayer coverage of the capillary surface, the hydrophobic chain of the surfactant in buffer would be pushed to the hydrophobic chain on the surface by the polar medium, resulting in a self-assembled molecular bilayer of the surfactant, which has the positive heads facing outwards into the aqueous

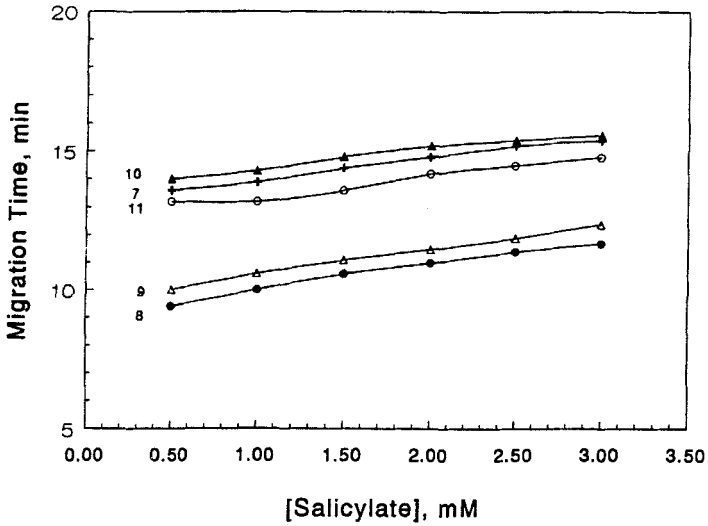

Figure 5. The influence of salicylate concentrations on the migration times of AA's. Experimental conditions were the same as those in Figure 1.

buffer. Because of the dynamic coating of MTAB on the inside surface of the capillary, the surface charge of the uncoated capillary had changed from negative to positive, and the electroosmosis adopts the same flow direction as the electrophoresis of anionic AA's.

Migration and separation of AA's are influenced by the buffer concentration. Experimental results show that the migration time of AA's decreased with decreasing carbonate concentration (Figure 2), and salicylate concentration (Figure 5).

Although higher signal responses were obtained when lower carbonate concentrations and a fixed salicylate concentration were used (Figure 2), the best separation efficiency was achieved when carbonate concentrations were between 2.5 and 5.0 mM and salicylate concentrations were between 1.5 and 2.0 mM.

Based on the studies shown in Figures 1 to 5, the optimum conditions for separation and indirect detection of AA's in MTAB-coated capillary were those in which the salicylate concentration was at 1.5 mM to 2.0 mM; the carbonate concentration, 2.5 mM to 5.0 mM; and the buffer pH, 10.7 to 11.0.

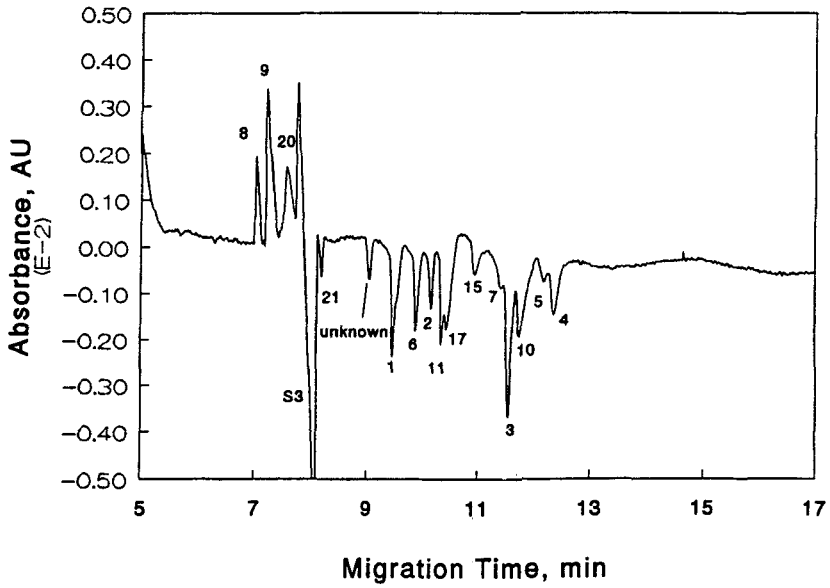

Figure 6. The electropherogram of AA's using an MTAB-coated capillary capillary with salicylate as a UV absorbing additive. Separation buffer: 5.0 mM carbonate/2.0 mM salicylate/0.15 mM MTAB at pH 10.7. The other experimental conditions and the peak identities were the same as those in Figures 1 and 2.

Analytical Performance

Under the optimum conditions, separations of a mixture of 14-18 AA's were accomplished in MTAB-coated capillaries using either salicylate (Figure 6) or benzoate (Figure 7) as UV-absorbing additives. The results were compared with those obtained from an uncoated capillary (Figure 8). As slab gel electrophoresis, CE exploits differences in the acid-base behavior of the different AA's, i.e., differences in the sign and magnitude of their net electric charges at a given pH, which are predictable from their pK_a values (Table 1). In other words, the migration orders of AA's in Figures 6 to 8 were governed by the apparent mobilities of AA's in the different systems, which were the vector sums of electrophorectic and electroosmotic mobilities of the individual AA's. In this work, the peak identities in Figures 6 to 8 were assigned by comparisons of electropherograms of the mixture to those of the individual AA's and by stepwise addition of known AA's to the mixture.

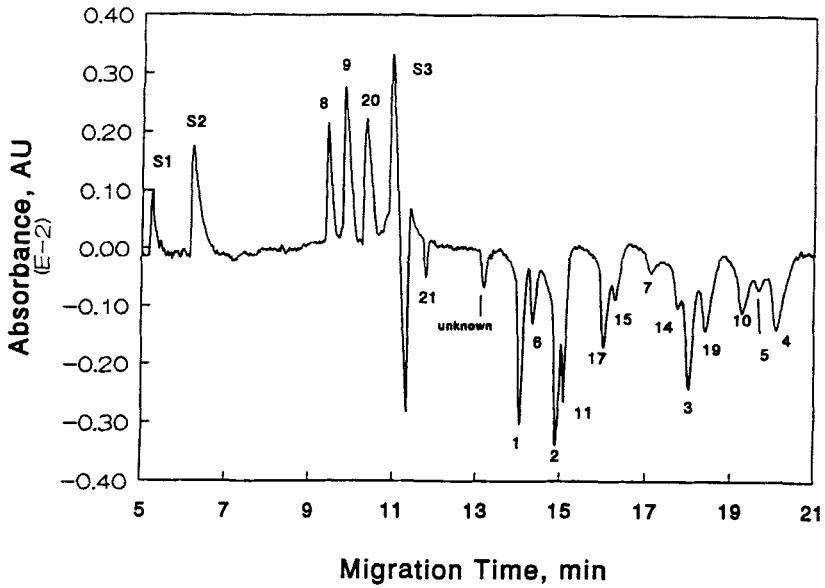

Figure 7. The electropherogram of AA's using an MTAB-coated capillary with benzoate as a UV absorbing additive. Separation buffer: 5.0 mM carbonate/2.0 mM benzoate/0.15 mM MTAB at pH 10.7. The other experimental conditions and the peak identities were the same as those in Figures 1 and 2 except the S_3 peak is the peak of benzoate.

In an MTAB-coated capillary, the electroosmotic flow of the buffer solution had the same direction as the electrophoretic flow of the AA's under the separation conditions employed and moved toward the anode at the capillary outlet. The migration order of AA's depended solely on the electrophoretic mobility of AA's because the contribution of electroosmotic mobility to the apparent mobility of AA's remained constant.

According to the equation:[20] $\mu_{ep} = \frac{Q}{6\pi\eta r}$ electrophoretic mobility (μ_{ep}) is proportional to the charge of the molecule (Q), and inversely proportional to the viscosity of the separation media (η) and the hydrodynamic radius (r) of the molecule. Because the hydrodynamic radius of an AA is related not only to the mass and the shape of the molecule but also to the degree of hydration of the molecule,[21] it is rather difficult to estimate its relative magnitude by comparing its mass with those of AA's in the mixture. However, it is relatively easy to

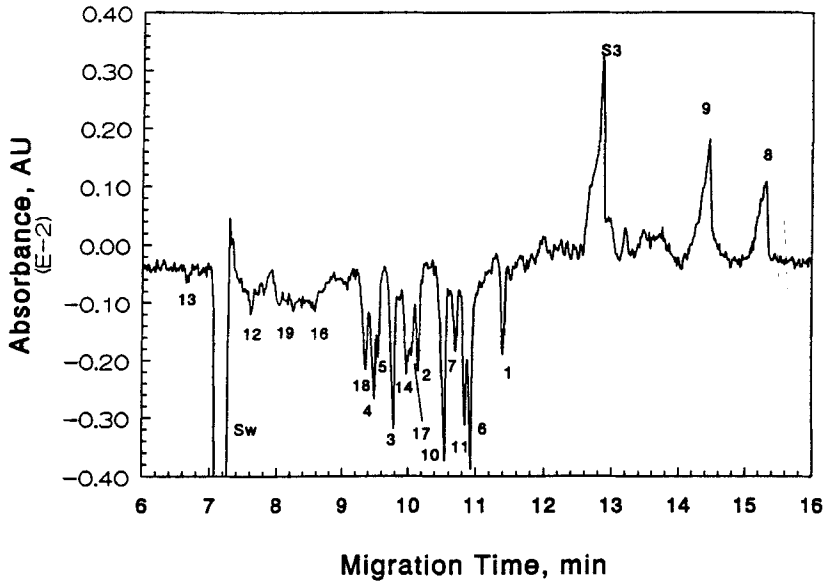

Figure 8. The electropherogram of AA's using an uncoated capillary. Separation buffer: 5.0 mM carbonate/2.0 mM salicylate at pH 10.7. The other experimental conditions and the peak identities were the same as those in Figures 1 and 2.

calculate the net charges of AA's by considering AA's that have a single α-amino group, a single carboxyl group, and an R group that does not ionize as diprotic acids (H_2L^+), and AA's with an ionizable R group as triprotic acids (e.g., H_3L^+ for AA's with negatively charged R groups, and H_3L^{2+} for AA's with positively charged R groups). Quantitatively, the net charges of AA's ($Q_{H_2L^+}$) be calculated either as diprotic AA's:

$$Q_{H_2L^+} = \alpha_{H_2L^+} Z_{H_2L^+} + \alpha_{HL} Z_{HL} + \alpha_{L^-} Z_{L^-} \qquad (1)$$

(where $\alpha_{H_2L^+}$, α_{HL} and α_{L^-} are the fractions in forms of H_2L^+, HL and L^-, and $Z_{H_2L^+}$, Z_{HL} and Z_{L^-} are the charges on these species) or as triprotic AA's with a negatively charged R group:

$$Q_{H_3L^+} = \alpha_{H_3L^+} Z_{H_3L^+} + \alpha_{H_2L} Z_{H_2L} + \alpha_{HL^-} Z_{HL^-} + \alpha_{L^{2-}} Z_{L^{2-}} \qquad (2)$$

(where $\alpha_{H_3L^+}$, α_{H_2L}, α_{HL^-} and $\alpha_{L^{2-}}$ are the fractions in forms of H_3L^+, H_2L, HL^- and L^{2-}, and $Z_{H_3L^+}$, Z_{H_2L}, Z_{HL^-} and $Z_{L^{2-}}$ are the charges on these species) or as triprotic AA's with a positively charged R group:

$$Q_{H_3L^{2+}} = \alpha_{H_3L^{2+}} Z_{H_3L^{2+}} + \alpha_{H_2L^+} Z_{H_2L^+} + \alpha_{HL} Z_{HL} + \alpha_{L^-} Z_{L^-} \qquad (3)$$

(where $\alpha_{H_3L^{2+}}$, $\alpha_{H_2L^+}$, α_{HL} and α_{L^-} are the fractions in forms of H_3L^{2+}, H_2L^+, HL and L^-, and $Z_{H_3L^{2+}}$, $Z_{H_2L^+}$, Z_{HL} and Z_{L^-} are the charges on these species). Since the pI values of AA's were below the pH (10.70) of the separation buffer; therefore, all AA's in Figure 6 carried net negative charges. The order of migration is Asp (the charge-to-mass ratio: -1.447×10^{-2}), Glu* (-1.301×10^{-2}), Cys (-1.422×10^{-2}), salicylate* (-7.256×10^{-3}), urate* (-5.949×10^{-3}), unknown peak, Gly (-1.234×10^{-2}), Ser* (-9.255×10^{-3}), Ala (-1.023×10^{-2}), Gln* (-6.663×10^{-3}), OH-Pro* (-6.889×10^{-3}), His* (-6.261×10^{-3}), Thr (-8.189×10^{-3}), Val (-7.877×10^{-3}), Asn (-7.475×10^{-3}), Ile (-7.063×10^{-3}), Leu (-7.063×10^{-3}). It is noted that the species with an asterisk (*) did not follow the sequence of their charge-to-mass ratios due to the differences in the shape and the degree of hydration. Using an MTAB-coated capillary with salicylate as the UV absorbing additive, 14 AA's could be separated within 13 min. In another experiment benzoate was used to substitute salicylate, 16 AA's could be resolved in less than 21 min with an order of migration (Figure 7) similar to that of Figure 6.

In an uncoated capillary, the electroosmotic flow of buffer solution moving toward the cathode was greater than the electrophoretic flow of the negatively charged AA's moving toward the anode.[20] The migration order of the AA's in an uncoated capillary (Figure 8) was basically a reversal of those in the MTAB-coated capillaries (Figures 6 and 7) because of the combined effect of the two opposite flows. With salicylate as the UV absorbing additive, 18 AA's could be separated in less than 16 min in an uncoated capillary.

It is worth noting that the AA's with pIs close to the buffer pH showed low responses in the indirect detection signal because these AA's carried less negative charges and accordingly they displaced less UV absorbing additives. Table 2 shows the detection limits (defined as signal/noise = 3) of the AA's being studied by both MTAB-coated and uncoated capillaries, and they ranged from 53.6 to 785 pg. For the AA's that have large differences between pIs and buffer pH (e.g., Glu, Asp, etc.), the detection limits were lower than those AA's that have small differences (e.g., Lys, Arg, etc.), due to the greater charge densities on the former ions.

Table 2

The Detection Limits of AA's in Different Separation Conditions[a]

Peak No.[b]	Amino Acid	Uncoated Capillary $N^c = 1.73\times10^5$ Salicyate	MTAB-Coated Capillary $N = 1.67\times10^5$ Salicyate	MTAB-Coated Capillary $N = 1.67\times10^5$ Benzoate
		Limit of Detection[d], pg		
1	Gly	119	73.2	65.4
2	Ala	125	146	68.7
3	Val	74.2	62.8	106
4	Leu	137	169	172
5	Ile	687	549	687
6	Ser	61.0	110	196
7	Thr	162	628	458
8	Asp	131	91.6	85.8
9	Glu	94.7	53.6	65.4
10	Asn	58.4	146	275
11	Gln	78.5	137	110
12	Lys	391	439	NA
13	Arg	687	517	NA
14	Met	196	NA	687
15	His	NA	220	275
16	Pro	687	NA	NA
17	OH-Pro	275	110	137
18	Cit	131	NA	162
19	Orn	785	439	NA
20	Cys	NA	157	85.8
21	UA	NA	200	275

[a] The separation conditions were the same with Figures 6-8.

[b] The peak numbers were the same as those used for peak identities in all figures.

[c] The theoretical plate numbers (N, plates/m) of the capillary columns were calculated using valine peaks in Figures 6 and 8.

[d] Limit of detection was defined as the injection volume (57 nL) times the concentration of AA at S/N = 3.

NA, not available.

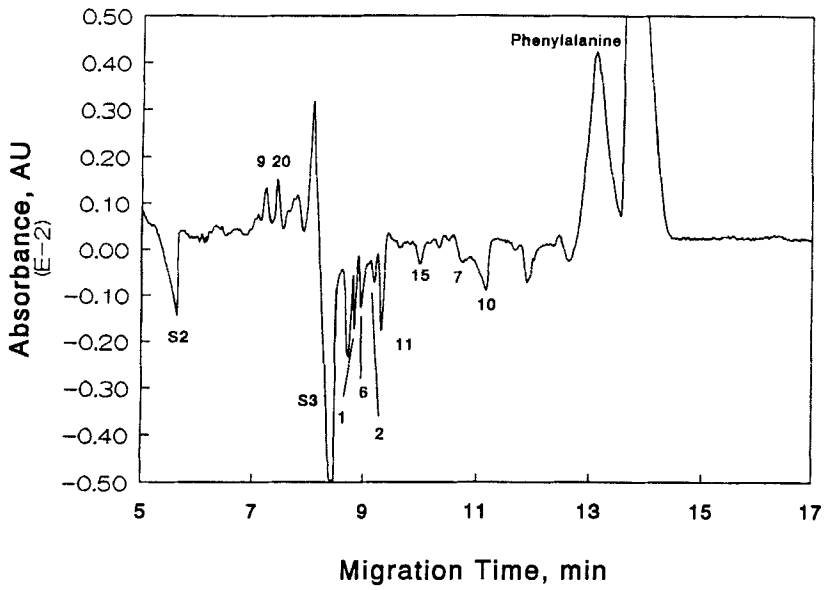

Figure 9. The electropherogram of human urine sample. Experimental conditions were the same as those in Figure 6.

Determination of AA's in Urine Sample

The developed method using MTAB-coated capillary was applied to the analysis of AA's in human urine sample. The sample pretreatment procedure consisted of a filtration step with a 0.45-μm cellulose-acetate-membrane syringe filter and a fourfold dilution with deionized water (1 portion of urine to 4 portions of water). As shown in Figure 9, eight AA's, i.e., Glu, Cys, Gly, Ser, Ala, Gln, Thr, and Asn were detected under the chosen conditions. The peak identities of AA's in the urine sample were verified by sequential addition of known AA's to the test sample. The concentrations of AA's in urine could be found from the linear calibration curves of the AA's ($r \geq 0.996$), which were constructed using various concentrations of the AA's (from 25 to 100 μM), versus the peak heights of the AA's (the calibration curves are not shown). The results are summarized in Table 3, together with the measured values and the normal values cited from the literature. All the values of AA's reported from our preliminary analysis are reconciled with the normal range reported, with the exceptions of cysteine and asparagine in urine.

Table 3

The Levels of Eight Amino Acids in Urine Detected by CE

Amino Acids (Calibration Equation[b] and Regression Coefficient)	Urinary AA (μM) CE Method	Normal Range[a]*
Glu (y = 5.0 + 0.280x, r = 1.0000)	71.4	37-180
Cys (y = 0.5 + 0.172x, r = 0.9984)	319.8	13 - 87
Gly (y = -2.5 + 0.412x, r = 0.9960)	248.8	108 - 2800
Ser (y = -1.0 + 0.336x, r = 0.9983)	193.5	108 - 467
Ala (y = 1.0 + 0.292x, r = 0.9994)	119.9	40 - 533
Gln (y = 1.0 + 0.496x, r = 0.9992)	241.9	93 - 573
Thr (y - 0.228x, r = 0.9991)	98.7	57 - 293
Asn (y = 2.5 + 0.344x, r = 0.9997)	152.6	180 - 467

* See reference 23.

[a] Assuming the average volume of urine for a 70 kg adult is 1500 mL/24 hrs.

[b] In the equations, y is the peak height (mm) and x is the concentration of AA in μM.

CONCLUSIONS

This study has shown a relatively simple method for determination of 14-18 AA's by CE with MTAB-coated and uncoated capillaries. The optimization of the analysis conditions was demonstrated with an MTAB-coated capillary using salicylate as the UV absorbing additive in carbonate buffer. Factors that affect indirect signal response and migration behavior of AA's, such as buffer concentration, pH, and UV absorbing additive were studied. The separations of AA's in both MTAB-coated and uncoated capillaries were compared. The detection limits of 18 AA's found in this method were in the order of 53.6 to 785 pg injected. The method developed has been applied to the determination of AA's in urine sample and preliminary results has been presented.

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