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Capillary electrophoretic determination of amino acids Improvement by cyclodextrin additives

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Abstract

Previously, we have developed methods for the capillary electrophoretic determination (without derivatization) of twenty common amino acids with indirect absorbance detection [Y.-H. Lee and T.I. Lin, J. Chromatogr. A, 680 (1994) 287]. Here, we report a further improvement in the resolution, resulting from the use of various cyclodextrin (CD) additives. Capillary electrophoresis (CE) was performed at pH 11.0 with either *p*-aminosalicylic acid (PAS) or 4-(N,N'-dimethylamino)-benzoic acid (DMAB) as the background electrolyte (BGE). The effects on the CE separation brought about by various CD additives, e.g. α- and β-cyclodextrins, methyl-, hydroxypropyl-, 2,6-dimethyl-, and 2,3,6-trimethyl-β-cyclodextrins were investigated. These CD additives form inclusion complexes with the BGE and also with amino acids, altering the migration behavior of the analytes. For some amino acids which have previously proved difficult or impossible to separate, an improvement in the separation selectivity has been attained. Association constants of BGEs for cyclodextrins were determined on the basis of the CE mobility changes caused by the CD additive. The concentration of CD additive needed to achieve a suitable separation was calculated and optimized experimentally. Using 10 m*M* PAS or DMAB, in the presence of 20 m*M* α-cyclodextrin at pH 11.0, eighteen amino acid peaks were baseline-resolved in under 35 min. Leu and Ile could also be separated in the presence of 15–20 m*M* β-CD under similar CE conditions. The performance of CE in separating amino acids in the presence of various CD additives is discussed.

1. Introduction

Amino acids are the building blocks of proteins; determination of the amino acid composition of proteins is often encountered in protein analysis. Amino acids are also present in a wide variety of biological tissues, body fluids, foods, and medicines. Therefore, determination and quantitation of amino acids is of considerable interest. The most efficient and convenient methods for simultaneous determination of amino acids developed in the past have been based on high-performance liquid chromatography (HPLC). Since the majority of amino acids do not absorb light in the UV region, detection is often accomplished by introducing pre- or post-column derivatization of the analytes with fluorescent probes [1–3], the subsequent detection being by fluorescence or laser-induced fluorescence. The fluorescence method has the advantage of high detection sensitivity, reaching an attomolar mass detection limit [3]. However, the derivatization process can be very time-consuming and requires considerable additional work. The process also changes the native electrophoretic mobility of the analytes. Alteration of the electrophoretic mobility of the analyte always occurs because of the modification of physico-

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chemical properties of the analyte by the reactive group of the derivatizing agents.

If capillary electrophoresis (CE) is used, detection of underivatized amino acids can also be accomplished by indirect methods similar to those employed in liquid chromatography. In capillary zone electrophoresis, indirect fluorescence detection has been developed for a variety of analytes, including amino acids [4,5]. To achieve the best detection limits, a laser light source is often required. Another very attractive alternative is the indirect absorbance detection method, since most commercial CE systems are equipped with UV–Vis absorbance detectors and a wide variety of background absorbers are available.

Indirect UV detection in CE has recently become popular, and has been applied to the detection of a wide variety of analytes, e.g. organic acids and bases, inorganic anions and metal cations (for reviews, see Refs. [6,7]). A few studies have been made on the CE of amino acids based on the indirect UV detection scheme [8–10]. In the indirect UV detection method, the choice of background electrolyte (BGE) is of critical importance because it dictates the resolution of the CE separation and the sensitivity. Benzoate, sorbate, salicylate, and quinine sulfate have been used as BGEs for the CE analysis of amino acids and related compounds. Other factors that also affect the CE resolution include pH and additives that modify the electroosmotic flow (EOF). Previously, we have investigated the potential use and suitability of various BGEs for the CE analysis of amino acids with indirect UV detection [11]. Of the nine BGEs studied, we found *p*-aminosalicylic acid (PAS) and 4-(N,N'-dimethylamino)benzoic acid (DMAB) to be most suitable as the carrier electrolytes and background absorbance providers as they have effective mobilities close to the mobilities of most amino acids at alkaline pH. pH also plays a role as it influences the separation behavior of amino acids in CE. Metal cations (e.g. Mg^{2+}) and long-chain cationic surfactants (e.g. cetyltrimethyl ammonium bromide) are effective EOF modifiers (reducing or reversing EOF at high concentration); as buffer additives, they improve the CE resolution [11].

Although 17–19 peaks for twenty common amino acids could be effectively separated in 20–40 min using the method developed previously [11], there is still considerable room for improvement. For example, Phe, Val, and His, Met and Gln, and Ala, Thr, and Asn could not be baseline-resolved under any one single CE condition. An even more serious problem is that Leu and Ile could not be separated at all. Several studies have shown that adding cyclodextrins (CDs) can enhance the selectivity of CE because CDs can form inclusion complexes with a wide variety of guest organic molecules or ions (for review, see Ref. [12]). Selectivity is taken to be a function of whether the guest molecule fits into the CD hydrophobic cavity. Chiral separations of racemic mixtures of D,L-amino acids, peptides, carbohydrates, and various other chiral pharmaceuticals have been aided by the addition of different kinds of CDs and derivatives [13–18]. In the present study, we have investigated the effectiveness of various CDs and derivatives, e.g. α- and β-cyclodextrins, methyl-, hydroxypropyl-, 2,6-dimethyl-, and 2,3,6-trimethyl-β-cyclodextrins, in improving the CE separation of amino acids. Among the various CDs, α-CD gives the best overall performance in terms of CE resolution for all amino acids except for Leu and Ile. On the other hand, complete baseline CE separation for the latter two amino acids could only be achieved by adding β-CD. The performance of CE in separating amino acids in the presence of various CD additives is discussed.

2. Experimental

2.1. Chemicals

BGEs, 20 common amino acids, DM-β-CD [heptakis(2,6-di-O-methyl-β-CD)] and TM-β-CD [heptakis(2,3,6-tri-O-methyl-β-CD)] were from Sigma. HP-α-CD (hydroxypropyl-α-CD, average molar substitution (MS) = 0.6), HP-β-CD (hydroxypropyl-β-CD, MS = 0.6), and methyl-β-CD (average degree of substitution = 1.8) were from Aldrich (Milwaukee, WI, USA). α-CD and β-CD were obtained from two manufacturers: Sigma (St. Louis, MO, USA) and

Janssen (Geel, Belgium); each brand gave very different CE results (see Results and Discussion). These chemicals were used as received without further purification. All other chemicals used were of analytical or reagent grade from several manufacturers. Doubly deionized water prepared from a Milli-Q system (Millipore, Bedford, MA, USA) or doubly deionized distilled water was used exclusively for all solutions. Infusion fluids containing amino acids (Aminol Infusion and Aminol-12X Injection) were supplied by Sintong Chem. (Tauyen, Taiwan).

Samples, buffers and pH adjustment

BGE solutions containing 10 m*M* of *p*-aminosalicylic (PAS) or 4-(N,N′-dimethylamino)benzoic (DMAB) acids, the standard solution of 20 amino acids and their mixture were prepared according to the procedure published previously [11]. The pH of the running BGE solution was adjusted by adding aliquots of 1 or 0.1 *M* NaOH to the desired pH, depending on the experiments, from 10 to 11.2 as specified in the figures. As noted previously, the solution had a rather high pH and if exposed to air, the pH could be lower by CO_2 dissolution. Thus, the vial must be capped tightly immediately after use. The original pH could be maintained for 3–4 days. The pH of the solution was checked periodically and readjusted if necessary. For the CD experiments, 20–30 m*M* solutions were made containing 10 m*M* BGE and then diluted to the desired concentration.

2.2. Apparatus

CE experiments were carried out in a fully automated Spectra Phoresis Model 1000 instrument (Thermo Separation Products, Fremont, CA, USA) as described previously [11]. The detector wavelength was fixed at 266 nm for PAS and at 288 nm for DMAB. In indirect detection, peaks in the electropherogram appeared originally as negative peaks but were inverted to positive peaks by using the vendor's software. The capillaries (bare fused-silica) from Polymicro Technologies (Phoenix, AZ, USA) were 75 μm I.D. (365 μm O.D.) × 70 cm (63 cm to the detector) for the determination of the mobilities of BGEs and 75 μm I.D. (365 μm O.D.) × 90 cm (83 cm to the detector) for the separation of mixtures of amino acids. For the determination of the molar absorptivities for the various BGEs, the UV–Vis absorption spectra of BGEs (10^{-4} *M* in 10 m*M* phosphate buffer, pH 11.0) were measured, using 0.5-cm quartz cuvettes (190–400 nm), by means of a double-beam scanning spectrophotometer (Hitachi U-2000, Tokyo, Japan). Electrospray ionization (ESI) mass spectrometric analysis of CD was performed in a Fisons Plateform quadrupole mass spectrometer (Manchester, UK) and it was detected as $[M + H]^+$. Experimental procedures for ESI-MS were similar to those previously published [19].

2.3. Electrophoretic procedures

Pretreatment of new capillaries and wash protocols for subsequent runs were carried out according to the established procedure [11]. The standard solution containing a mixture of 20 amino acids, each with a final concentration of $5 \cdot 10^{-4}$ *M*, was prepared from stock solutions of amino acids (10 m*M*, stored at 4°C) in deionized water. Sample injection was effected in the hydrodynamic (HD) mode for 1 s. The separation run was carried out at +20 kV constant voltage, at 25°C constant temperature, and with a current of ca. 10 μA. Other CE procedures for the determinations of amino acids were the same as described previously [11]. Peak identification for each analyte was carried out by spiking with the known standards and the peaks with increased height were identified. For the analysis of the infusion fluids containing amino acids, the real samples were suitably diluted, filtered, and injected direct into the capillary.

2.4. Electrophoretic mobility determination

Benzyl alcohol or dimethyl sulfoxide (DMSO) was added to samples as a neutral marker for the electrophoretic mobility determination. The mobilities of various BGEs under the specified CE conditions were determined in the buffer containing 20 m*M* sodium phosphate at pH 11.0. A mixture of all BGEs, 0.1 m*M* each in deionized

water at pH 11.0, was injected in HD mode for 1 s. The CE voltage applied was +15 kV. Detection was made by a rapid scanning of absorbance from 200 to 350 nm, which allowed a positive identification of the background provider. The electroosmotic mobility, μ_{eo}, and the electrophoretic mobility of the BGE, μ_e, were calculated as described previously [11].

2.5. Determination of the binding constant of CD with BGE

The binding constants for the various BGEs with CDs were determined by the CE method. The CE method is based on the changes in BGE electrophoretic mobility caused by the addition of CD, which formed a complex with the BGE. In the CE experiments, the analytes were prepared in 0.1 m*M* containing 0.05% DMSO as the EOF (neutral) marker. Various amounts of CD (0–20 m*M*) were added to the analyte solutions and the capillary electrophoreses were carried out in 20 m*M* phosphate buffer, pH 11.0, at 25°C. Samples were injected via the HD mode; the separation voltage was +20 kV. The BGE peak was detected by a rapid absorption spectral scan from 200–350 nm. The mobility was determined as described in Section 2.4.

Mathematical models describing the pH and concentration dependence of the electrophoretic mobilities during CE have been developed [20]. It has been demonstrated that K_2 can be determined from the following equations [21]:

$$\mu_{eff} = \frac{\mu_B + \mu_{BCD} K_2[CD]_0}{1 + K_2[CD]_0 + ([H_3O^+]/K_a)(1 + K_1[CD]_0)} \quad (1)$$

where $[CD]_0$ is the initial CD concentration added to the BGE solution, μ_{eff}, μ_B, and μ_{BCD} are the effective mobility of all BGE species, the mobilities of BGE in the absence of CD, and of the BGE–CD complex, respectively. K_1 and K_2 are the binding constants of CD with the unionized and ionized form of BGE, and K_a is the acid dissociation constant of BGE.

In the absence of CD, Eq. 1 is reduced to

$$\mu_{eff} = \frac{\mu_B}{1 + [H_3O^+]/K_a} \quad (2)$$

When $pH >> pK_a$, Eq. 1 can be approximated by

$$\mu_{eff} = \frac{\mu_B + \mu_{BCD} K_2[CD]_0}{1 + K_2[CD]_0} \quad (3)$$

The binding constant K_2 can be determined from Eq. 3 by the nonlinear least-squares curve fit technique. The equation was solved by adjusting the values of μ_B, μ_{BCD}, and K_2 (three-parameter curve fit) until the best fit of the calculated curve with the experimental data was obtained, using the Sigma Plot for Windows version 1.01 software (Jandel Scientific, Corte Madera, USA) on a 486 personal computer. The mobility μ_B can also be determined experimentally, by using Eq. 2, from the mobility measurement of BGE in the absence of CD. We found that μ_B determined by the curve fit technique was in good agreement with the value obtained experimentally.

3. Results and discussion

3.1. CE determination of the binding constant of CD with BGE

As described above, addition of CD to the BGE solution affects the mobility of BGE when a complex is formed by CD and the BGE. Figs. 1a and b show the effects of various CDs on the electrophoretic mobilities of PAS and DMAB, respectively. The mobility is expressed in negative values because of the negative charges carried by the BGE. Since CE was performed with positive voltage (at the injection side), the anions migrated opposite to the EOF; as the BGE formed a complex with CD, its mobility decreased (smaller negative value). Therefore, the complexed form of BGE actually migrated faster toward the cathode (the detector side) than the uncomplexed form. As the CD concentration is increased, the observed mobility of the BGE decreases as more BGE anions form

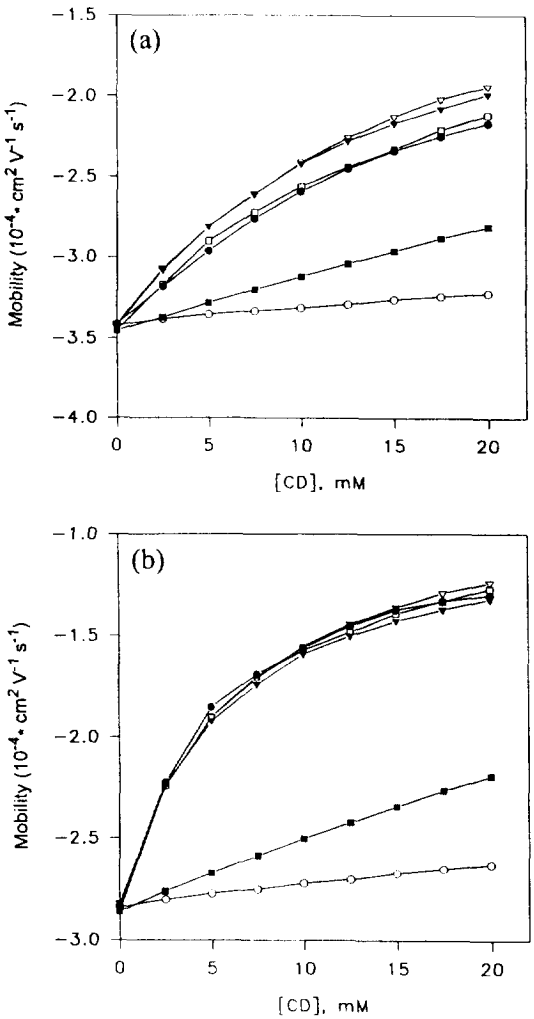

Fig. 1. Effects of concentration of various CDs on the electrophoretic mobilities of selected BGEs in (a) PAS and (b) DMAB. ○ = α-CD; ● = β-CD; ▽ = HP-β-CD; ▼ = methyl-β-CD; □ = DM-β-CD; ■ = TM-β-CD. Buffer: 20 m*M* phosphate at pH 11.0. Other conditions as in Experimental.

complexes with CD. The binding constants determined by the CE method for the various CDs with PAS and DMAB are listed in Table 1.

From the table and the two titration curves in Figs. 1a and b, it is obvious that α-CD has the lowest affinity for both BGEs. Similarly, the affinity of TM-β-CD is not as strong for either BGE as that of the other β-CDs. The affinities

Table 1
Determination of binding constants of PAS and DMAB with various CDs

Compound	Binding constant, K_2 (M^{-1})	
	PAS	DMAB
α-CD	na[a]	na
β-CD	42 ± 4	195 ± 10
DM-β-CD	52 ± 2	178 ± 5
TM-β-CD	na	na
Methyl-β-CD	61 ± 3	174 ± 8
HP-β-CD	53 ± 2	159 ± 8

[a] na: not applicable.

of DMAB for the other four types of β-CD are almost identical and are also the strongest. DMAB has a higher affinity than PAS, presumably because it possesses a hydrophobic dimethylamino group (whereas PAS has *p*-amino); therefore it is easier for DMAB to enter the hydrophobic cavity of the CD. The binding constant of TM-β-CD is smaller, probably because of the steric hindrance caused by its bulky trimethyl group. Both BGEs have the same poor affinity for α-CD because of the negative charge carried by the carboxyl group. In general, α-CD forms a complex with a neutral molecule better than with a negatively charged molecule. The binding constants of α-CD for the neutral and negatively charged molecules can sometimes differ by an order of magnitude or more [22].

3.2. Selection of the BGE and CE of the twenty common amino acids in the presence of α-CD

There are some drawbacks when using a CD that forms a strong complex with the BGE. Firstly, complex formation between the BGE and the CD could decrease the mobility of the BGE, reducing the EOF and thus increasing the CE run time. Secondly, when the BGE forms a strong complex with a CD, there are less CD molecules available for the analytes, lessening the role that CD plays in enhancing the selectivity of CE separation. Comparing the two BGEs,

PAS seems to work better with the CD additives. Our previous study showed that DMAB had a lower mobility than PAS [11], and that the EOF was smaller and the CE analysis time longer when DMAB was used as the BGE. Moreover, the binding constants of DMAB with most β-CDs are about three times as high as their PAS counterparts. The EOF in the PAS–CD solution is moderately reduced and the analytes have sufficient time to interact with CD. For these reasons, we chose PAS as the preferred BGE for the present method (see also Ref. [11] for other advantages). Fig. 2 compares the electropherograms of 20 common amino acids in 10 m*M* PAS (a) and 10 m*M* DMAB (b) at pH 11.0 in the presence of 20 m*M* α-CD (Sigma).

Nineteen amino acid peaks are identified. The Arg peak merged with the system peak (the leading peak), thus is not shown. The order of amino acid migration is as follows: system peak (Arg), (1) K (Lys), (2) P (Pro), (3) L (Leu), I (Ile) (not resolved), (4) W (Trp), (5) F (Phe), (6) M (Met), (7) H (His), (8) V (Val), (9) Q (Gln), (10) T (Thr), (11) N (Asn), (12) A (Ala), (13) S (Ser), (14) G (Gly), (15) Y (Tyr), (16) C (Cys), (17) E (Glu), (18) D (Asp). Note that the order in which some amino acids migrate is different in the absence of CD (cf. Ref. [11]). Trp migrates faster than Leu and Ile, the order for Met and His is reversed, and Val migrates faster than Met. The changes in migration order suggest that α-CD interacts more favorably with Leu, Ile, and Met, decreasing their mobilities.

Using PAS as the BGE, 18 amino acid peaks could be resolved in less than 35 min. Using DMAB, it would take about 50 min to resolve these peaks; however, the resolution would be better. Using PAS, only 16 peaks could be baseline-resolved. The Phe and Met peaks, and the Asn and Ala peaks could not be completely resolved. Using DMAB, on the other hand, the Asn and Ala peaks can be baseline-resolved. A drawback associated with using DMAB is that the last three tailing peaks are broader (peak width at half height is in the range of 0.23–0.76 min in PAS but 0.34–1.38 min in DMAB) and have poorer reproducibility.

3.3. Effect of impurities in the CD source

The impurities in the commercial sources of CD vary considerably. For example, mass spectrometric analysis (data not given) shows that a batch of α-CD from Sigma (purity 99%) contains only a small amount of m/z 506 impurity, whereas in a batch of α-CD from Janssen (labeled purity $>98\%$) several times as much of the same impurity is found. The relative intensity ratios of the m/z 506 impurity peak to α-CD (m/z 974) peak were 0.30 and 1.58 for the Sigma and Janssen brands, respectively. The electropherogram of the twenty amino acids obtained with the α-CD from Janssen (Fig. 3) looks very different from that of the Sigma α-CD (Fig. 2a) under identical CE conditions (except for the concentration of CD). When CE was performed using the Janssen batch, which contains a higher impurity level, the EOF was lower and the analysis time longer. Note that for Fig. 3, a much lower α-CD concentration (2 m*M*) was used; even so, it extended the analysis time to almost 55 min. The result suggests that the reduction in EOF and greater CE run time are due to the impurities in the Janssen brand altering the surface properties of the capillary. Note also that the migration order changed; most noticeably, for Trp, Met, and Thr. This is due in part to the difference in the CD concentration used in the two experiments. The impurity may be cyclic or acyclic dextrin which adsorbed to the capillary surface, thus reducing the EOF. It has been reported that several kinds of dextrin and dextran [23,24] could be used to enhance the CE separation of chiral compounds. The effect of impurities on the CE observed here seems to be similar to that caused by dextrin or dextran.

3.4. Effects of concentration of the various CDs on CE

The effects of concentration of various CDs on the electrophoretic mobilities of selected amino acids are shown in Figs. 4–6. HP-α-CD has a more pronounced effect on the mobilities of Met, aliphatic (Leu, Ile) and aromatic (Trp, Phe,

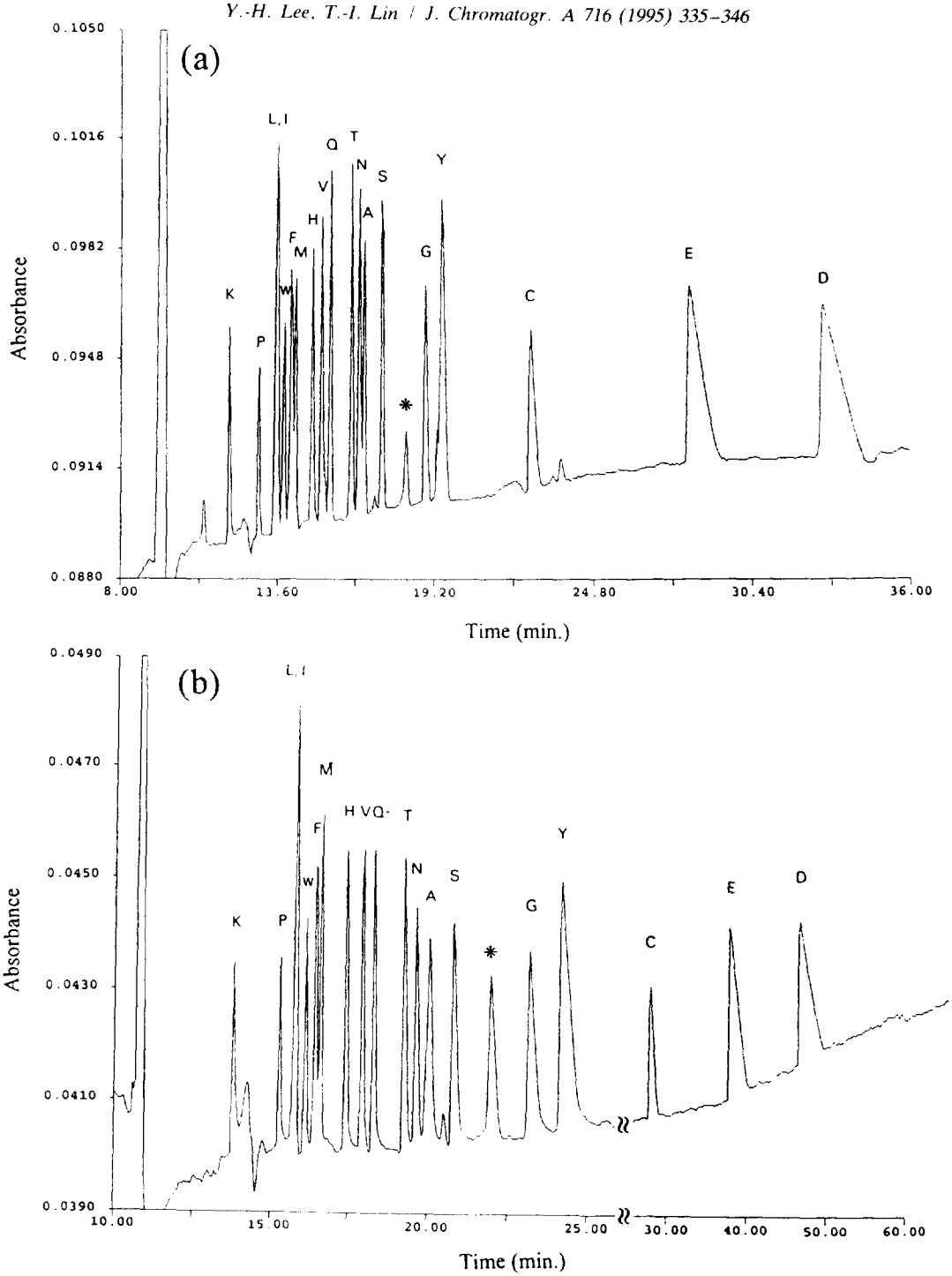

Fig. 2. Electropherograms of 20 common amino acids in 10 m*M* (a) PAS and (b) DMAB in the presence of 20 m*M* α-CD (Sigma) at pH 11.0. Concentration of amino acids, 0.5 m*M* each. Peak identification: K = Lys, P = Pro, L = Leu, I = Ile (not resolved), W = Trp, F = Phe, M = Met, H = His, V = Val, Q = Gln, T = Thr, N = Asn, A = Ala, S = Ser, G = Gly, Y = Tyr, C = Cys, E = Glu, D = Asp. Arg is merged with the system peak (the first peak), * is an unknown peak.

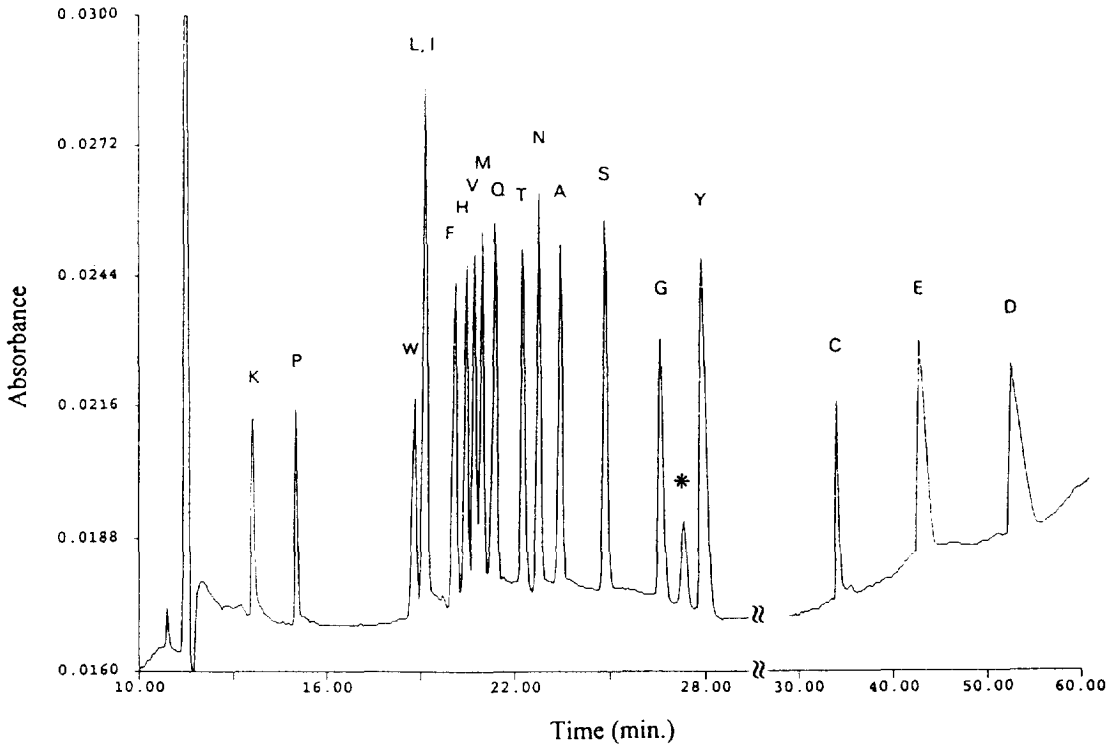

Fig. 3. Effects of impurities in 2 m*M* α-CD (Janssen) on the electropherogram of 20 common amino acids. For other CE conditions see Fig. 2a.

Tyr) amino acids (Fig. 4b). The effect of α-CD is similar (Fig. 4a) except for Trp. Since Trp has a larger indole ring, it may be unable to enter the small α-CD cavity. The effects on Met and Leu/Ile are the most dramatic, changing their migration order. This is presumably because the long aliphatic chain enables these residues to exert a stronger interaction with α-CD. However, Leu and Ile still could not be separated, presumably because the interactions of CD with these two amino acids are similar. β-CD and HP-β-CD affect Tyr and Phe most significantly, changing their migration orders (Fig. 5). It is also noteworthy that with high β-CD concentrations, Leu and Ile could be separated. Comparing the effects of α-CD and HP-α-CD on Tyr, the hydroxyl groups on either HP-α-CD or Tyr seem to be responsible for the stronger interaction. This suggestion is consistent with the similar result found for β-CD and HP-β-CD. The effects of DM-β-CD and methyl-β-CD (Fig. 6) are most noticeable on the aromatic amino acids but are less pronounced than those exerted by β-CD and HP-β-CD. In particular, the interaction of DM-β-CD with Tyr is less strong (Fig. 6). Presumably, the extra methyl and dimethyl groups cause some steric hindrance to the aromatic amino acids entering the DM-β-CD cavity.

3.5. CE separation of Leu and Ile in the presence of various CDs

Separation of Leu and Ile was not successful using the method we developed previously [11]. Because of the structural similarity of Leu and Ile, their separation by CE is a most challenging problem. Among the various CDs that have been investigated, we found that while α-CD was ineffective, several kinds of β-CD, including β-CD, HP-β-CD, and DM-β-CD, were promising. Fig. 7 depicts the effects of various CDs on the CE resolution (R_S) between the Leu and Ile

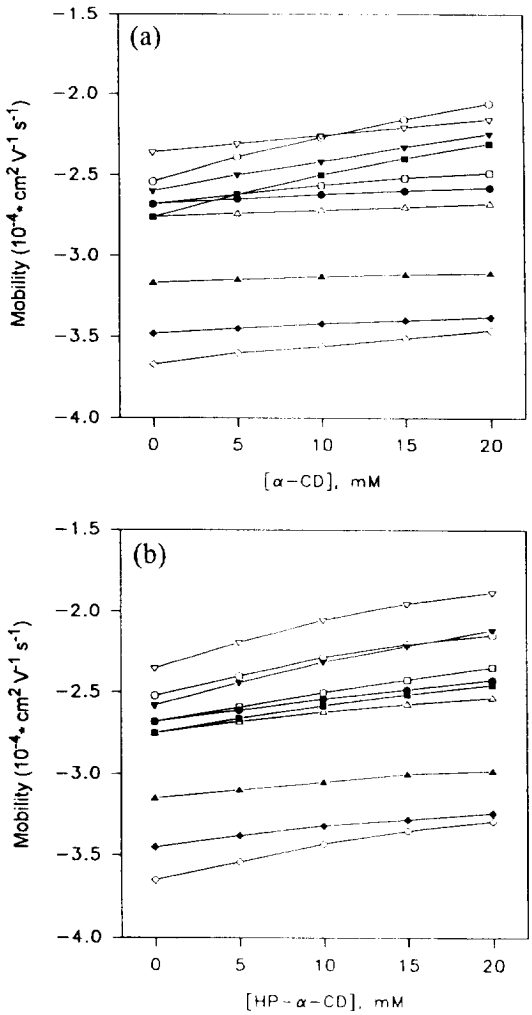

Fig. 4. Effects of concentration of (a) α-CD and (b) HP-α-CD on the electrophoretic mobilities of selected amino acids. ○ = Leu/Ile; ● = Val; ▽ = Trp; ▼ = Phe; □ = His; ■ = Met; △ = Gln; ▲ = Ser; ◇ = Tyr; ◆ = Gly. For other CE conditions see Fig. 2.

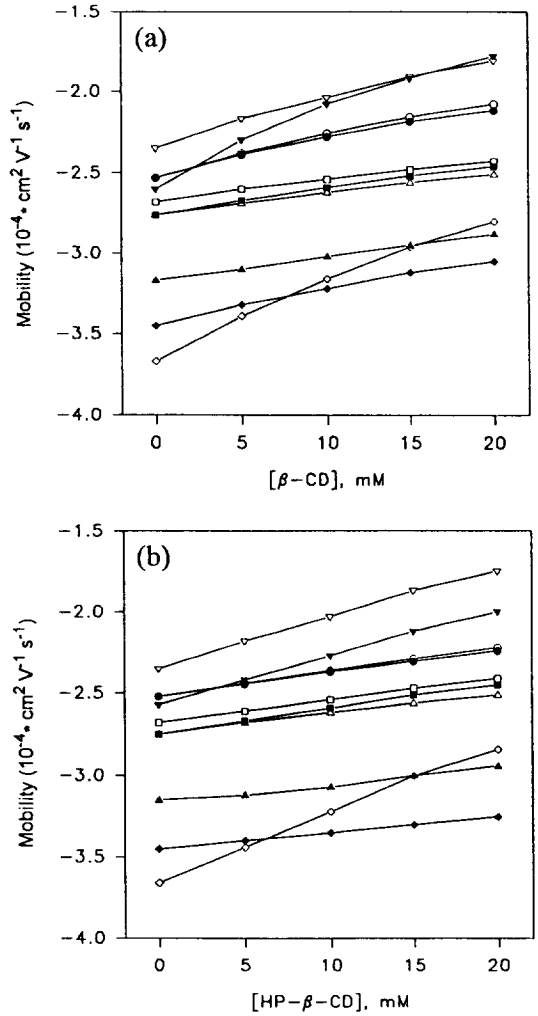

Fig. 5. Effects of concentration of (a) β-CD and (b) HP-β-CD on the electrophoretic mobilities of selected amino acids. ○ = Leu; ● = Ile; ▽ = Trp; ▼ = Phe; □ = His; ■ = Met; △ = Gln; ▲ = Ser; ◇ = Tyr; ◆ = Gly. For other CE conditions see Fig. 2.

peaks. Although both HP-β-CD and DM-β-CD do improve the separation of the two analytes to a certain extent, the improvement is less than ideal, even at the high concentration of 20 m*M*. On the other hand, β-CD could give a resolution better than one, even at a lower concentration of 15 m*M*. One note of caution, however, is that under the conditions where the best resolution of Leu and Ile could be obtained, the separation for several other amino acids became rather poor (data not shown). Thus, so far there is still no method available that can separate all twenty amino acids in one single CE run. Further studies are needed in order to achieve this goal.

3.6. Analyses of real samples

To demonstrate the applicability of the present method to real samples, two infusion fluids containing several amino acids from a local

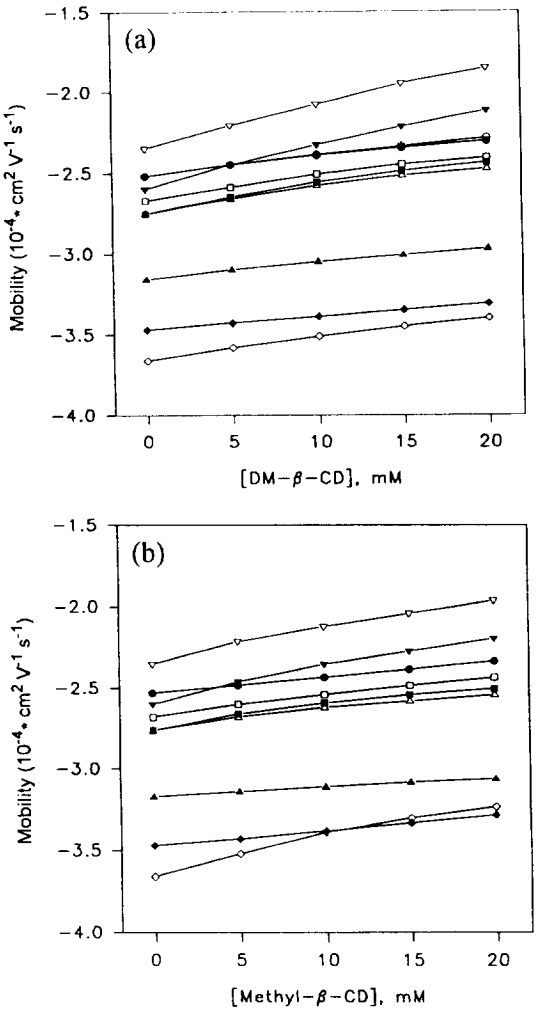

Fig. 6. Effects of concentration of (a) DM-β-CD and (b) methyl-β-CD on the electrophoretic mobilities of selected amino acids. ○ = Leu; ● = Ile; ▽ = Trp; ▼ = Phe; □ = His; ■ = Met; △ = Gln; ▲ = Ser; ◇ = Tyr; ◆ = Gly. For other CE conditions see Fig. 2.

pharmaceutical company have been analyzed. Fig. 8 displays the electropherograms of the two infusion fluids. Thirteen and sixteen components were identified in the two samples, respectively, and the results agreed (qualitatively) with the ingredient labels. As noted previously, under the conditions best suited to the CE separation of most amino acids, Leu and Ile could not be separated. Thus, a separate analysis would be

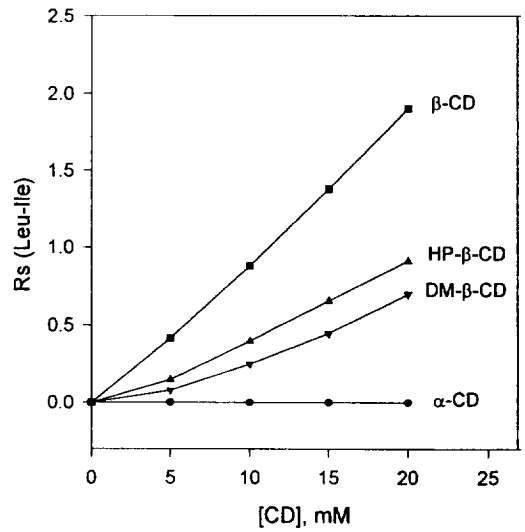

Fig. 7. Effects of concentration of various CDs on the resolution of Leu and Ile.

required for a complete determination of all twenty amino acids.

4. Conclusion

CE methods for effective separation and detection of all twenty common amino acids with indirect UV absorbance detection have been developed using commercial CE instruments. By using 10 m*M* PAS (as the BGE), 20 m*M* α-CD (to reduce EOF and enhance selectivity) at pH 11.0, all twenty amino acids except Leu and Ile were separated in less than 35 min. As an alternative, an even better resolution was obtained by replacing PAS with the same concentration of DMAB; however, a longer analysis time (55 min) was then required. Complete CE separation of Leu and Ile could only be achieved by replacing α-CD with 15 or 20 m*M* β-CD, which gave inferior results for several other separations. Thus, at present, using the indirect absorbance detection method, two separate runs are still required for complete CE determination of all twenty amino acids.

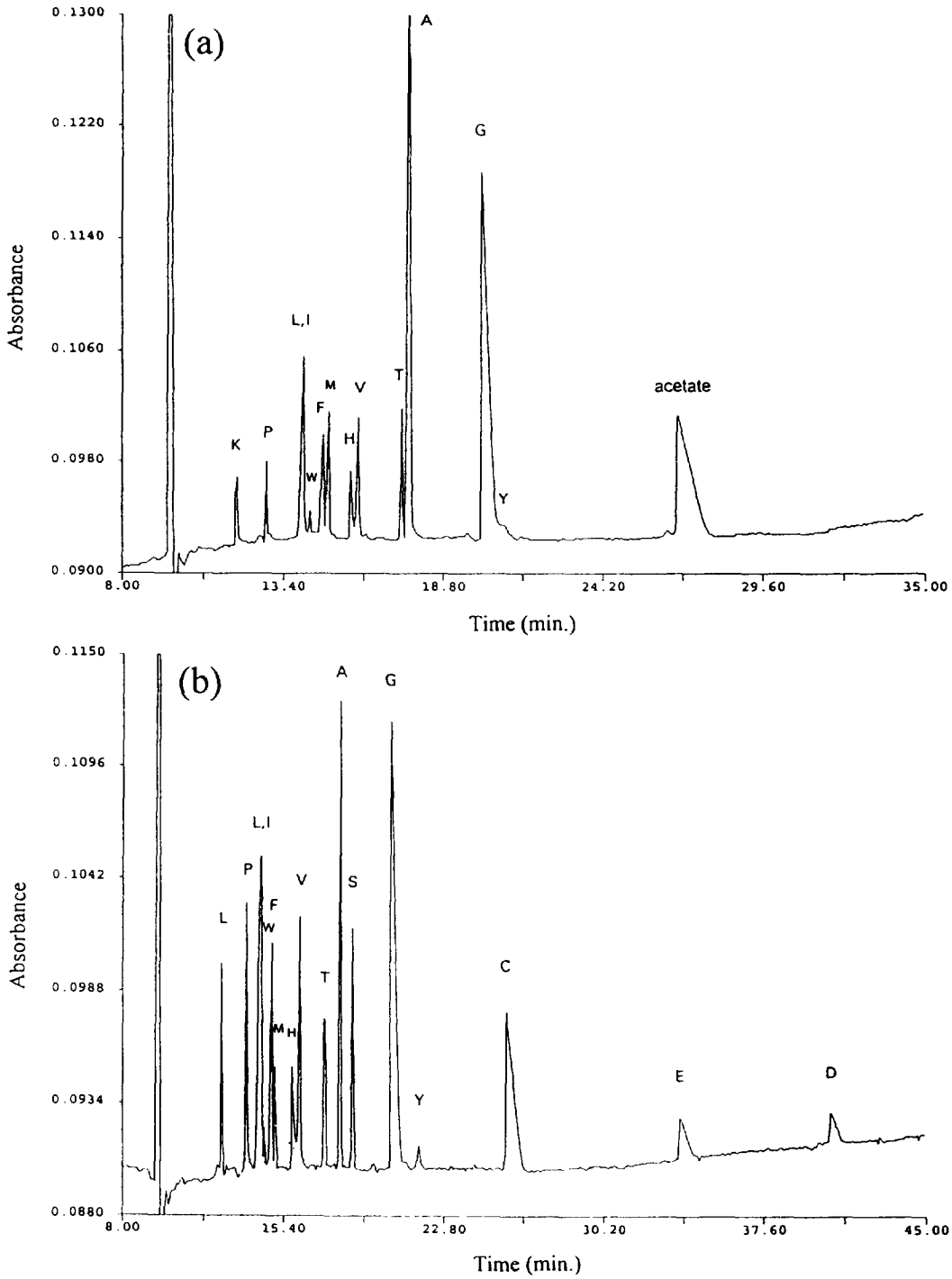

Fig. 8. Electropherograms of two infusion fluids containing amino acids. (a) Aminol Infusion and (b) Aminol-12X injection. (Both samples from Sintong Chem., Taiwan.) For CE conditions see Fig. 2.

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