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Electrokinetic Resolution of Amino Acid Enantiomers with Copper(II)–Aspartame Support Electrolyte

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By combination of the separation power of high-voltage capillary zone electrophoresis with the detection sensitivity of laser-induced fluorescence, subfemtomole amounts of racemic mixtures of 14 different dansylated amino acids can be completely resolved in typically less than 12 min. The separation is based on the diastereomeric interaction between DL-amino acids and a chiral Cu(II)–aspartame complex present in the support electrolyte. Effects of electrolyte composition, pH, and temperature are described and discussed as well as linearity and sensitivity of response.

Resolution of racemic mixtures of amino acids (AAs) into their enantiomeric forms continues to be a topic of active research (*1*). Practical uses range from the control of peptide synthesis to geological dating (*2*, *3*). Amino acid racemates are most commonly resolved using high-performance liquid chromatography (HPLC) or gas chromatography (GC). In either case, resolution is made possible by introducing a chiral compound, either in the stationary or in the mobile phase, leading to the formation of diastereomers which are more readily separated. The review of Davankov (*4*) is an excellent guide to this extensive literature. However, such resolution procedures often have drawbacks: the need to use sophisticated chiral stationary phases, large consumption of sometimes expensive and not so readily available chiral mobile phases, applicability of a given technique to a limited number of AA racemates, quantity of racemic mixtures needed, quality of resolution, separation time required, etc.

Recently, Gassmann, Kuo, and Zare (*5*) suggested an alternative procedure based on electrokinetic separations through the combined action of electrophoretic and electroosmotic motions in an open tubular capillary. They applied this technique to the resolution of an AA mixture using diastereomeric interactions with the Cu(II)–L-histidine complex present in the support electrolyte. In the present paper, results using a Cu(II)–aspartame complex, previously introduced by Grushka and co-workers (*6*) for this purpose in HPLC, will be described.

EXPERIMENTAL SECTION

Apparatus and Chemicals. The experimental setup was similar to that used by Gassmann et al. (*5*) and will be reviewed only briefly. A 75-μm-i.d. fused silica capillary has an overall length of 100 cm (75 cm to the detection zone). The injection end was connected to the ground, while the detection end was at high negative voltage, typically −30 kV. Each end of the capillary was immersed together with a Pt electrode into a 4-mL vial containing 2 mL of the electrolyte solution to assure electrical contact. The current was measured through a 1-kΩ resistor in the grounded side of the circuit. UV excitation light at 325 nm (10 mW) from a He–Cd laser (Liconix Model 4050B) was directed perpendicular to the capillary by an 85-μm fused silica optical fiber. The resulting fluorescence was collected at right angles to both the capillary and the excitation direction by a 600-μm glass optical fiber. The fluorescence passed through a 550-nm interference filter (fwhm = 45 nm) (Corion) and a fast (f 3.5) monochromator (ISA Model H10) before striking the face of the photomultiplier tube (PMT) (Centronic Q4249B). The PMT signal was amplified (rise time = 100 ms) and plotted on a chart recorder (Linear Model 585). Electropherograms could also be recorded digitally at 20 Hz by an A/D converter and stored in a computer (IBM PC-XT).

The dansyl-AAs (DNS-AAs) were purchased from Sigma (St. Louis, MO) or prepared by known methods (*7*); the other chemicals were obtained from either Sigma or J. T. Baker (Phillipsburg, NJ, analytical grade reagents) and used without further purification. Water used to prepare the solutions was freshly deionized and distilled with a water purifier (Model LD-2A coupled with a Mega-Pure Automatic Distiller, Corning Glass Works). Stock solutions of 25 mM $CuSO_4 \cdot 5H_2O$, 25 mM aspartame, and 0.1 M NH_4OAc were used for preparing the electrolyte solutions.

Preparation of the Capillary. The formation of a double layer on the capillary inner wall causes an electroosmotic flow to appear when the capillary is subjected to a tangential electric field (*8*). This flow makes it possible to detect all the species, positive, neutral, and negative, at the same end of the capillary. It was found that the double-layer condition was crucial for the AA separation and for run-to-run and day-to-day reproducibility. To prepare the inner surface of the capillary for use, several cleaning procedures were investigated. Our conclusions are the following: very basic solutions (KOH $\geq$ 0.1 M) introduced at room temperature for over 15 min increase the current and the electroosmotic flow, leading to shorter migration times. The resolution is slightly worse but the fluorescence signal and the peak sharpness are both increased to some extent. On the other hand, after treating the capillary with acidic solutions alone (H_3PO_4 or HCl, 0.1 M) without introducing base afterward, no separation is observed. Generally, when a capillary needs to be cleaned or a new one is installed, a solution of 0.1 M H_3PO_4 is first introduced for a few hours in order to remove any metal hydroxide precipitated on the silica walls. Then it is thoroughly rinsed with distilled water, and a solution of 10 mM KOH is introduced for 20 min to reactivate the surface. Finally, the capillary wall is equilibrated with a 10 mM NH_4OAc buffer solution (pH 7.5) for 10 h. After such a treatment, a test sample composed of DNS-L-Arg, DNS-

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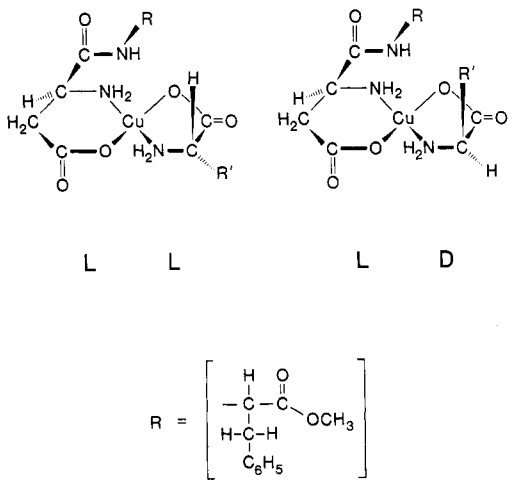

Figure 1. Configuration of the Cu(II) ternary complex formed with the aspartame and an AA. Note that specific hydrophobic interactions between the aspartame phenylalanine residue (R) and the AA side chain (R′) can occur only in the L–D configuration.

DL-Val, and DNS-DL-Asp is run in order to check the capillary conditions.

Injection. The capillary is first filled with the electrolyte (typically a solution of 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc adjusted to pH 7.5 with 0.1 M NH_4OH) using a 0.5-mL disposable syringe. When the electrolyte composition is changed, the new solution is left in the tube for at least $^1/_2$ h to equilibrate with the capillary walls. The sample is then injected using the electroosmotic flow to transfer a small volume of the DNS-AA mixture diluted in the electrolyte. Typical injection conditions are 6 s at 10 kV; the estimated volume injected is approximately 20 nL. After each experiment, the capillary is washed with the electrolyte. At the end of each day or when the capillary is not used for an extended period of time, a buffer solution (10 mM NH_4OAc, pH 7.5) is introduced.

RESULTS AND DISCUSSION

Resolution Characteristics. The AA resolution is based on the formation of a ternary complex of Cu(II) in the electrolyte solution. In the presence of the dipeptide L-aspartyl-L-phenylalanine methyl ester (aspartame), a commercial sweetener, chelation of the metal ions most likely occurs through the formation of a six-membered ring formed by the Cu(II), the α-amino, and the β-carboxy groups of the aspartyl residue of the aspartame molecule (see Figure 1), as suggested by Touche and Williams (*9*). Such a six-membered ring is less stable than the five-membered ring involving the α-amino and α-carboxy groups of an AA (*10*). Thus, when an AA is added to the electrolyte solution containing the $Cu^{II}(aspartame)_2$ complex, it can replace one aspartame ligand, leading to the formation of a ternary complex.

This formation is essential not only for the separation of the different amino acids but also for the resolution of the enantiomeric forms. Indeed, as shown in Figure 1, specific hydrophobic interactions between the aspartame phenylalanine residue and the AA side chain can take place, leading to slightly different stability constants for each enantiomeric form of the AA. Typically, such stabilizing hydrophobic interactions occur preferentially with the D enantiomer, since the interacting groups are closer in this complex than in that formed with the L enantiomer. It must be noted that the dansyl group of the AA derivative can also take part to an unknown extent in these interactions.

Under neutral pHs, the Cu(II) ternary complex is positively charged and will move faster than the neutral free AAs due to the electrophoretic action superimposed on the electroosmotic motion. Thus, the Cu(II) ternary complex stability will determine the electrokinetic migration times of the two enantiomers and hence the selectivity of the enantiomeric resolution. Moreover, in the case of the positively charged AAs (i.e., those with a basic side chain) or the negatively charged AAs (i.e., those with an acidic side chain), an additional separation factor occurs because of different ionic mobilities in an electric field.

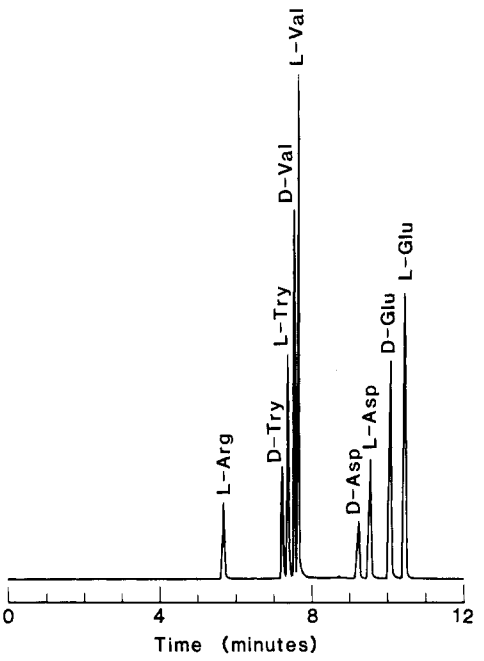

Figure 2. Electropherogram of a mixture of four DNS-DL-AAs. DNS-L-Arg is used as an internal standard. Electrolyte composition is as follows: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc, pH 7.4; capillary, 75 μm i.d., 100 cm (75 cm to the detection zone); applied voltage, −30 kV; current, ≈33 μA.

The resolution of each AA is characterized by the entity R_s defined by

$$R_s = 2(t_L - t_D)/(w_L + w_D) \quad (1)$$

and where t_L and t_D are the migration times and w_L and w_D are the peak bandwidths of the L and D enantiomers, respectively. Base-line resolution is generally observed when R_s exceeds unity.

Figure 2 shows a typical electropherogram obtained with an electrolyte solution of 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc at pH 7.4; the concentration of each pair of DNS-DL-AAs in the sample is 2.5×10^{-4} M. It can be seen that the peak sharpness and the resolution are clearly improved, even with shorter migration times, in comparison with the HPLC results of Grushka and co-workers (*6*). In addition, the laser-induced fluorescence detection considerably reduces the background signal, resulting in a nearly flat base line in Figure 2.

Table I presents the resolution of 18 racemic DNS-DL-AAs. For each AA, the migration times of the D and L enantiomeric forms and the resolution R_s, calculated from eq 1, are reported together with the value of $\Delta t = 2(t_L - t_D)/(t_L + t_D)$ previously introduced by Gassmann et al. (*5*). The electrolyte solution was 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc at pH 7.5. Usually, DNS-L-Arg is used as an internal standard since it migrates faster than the other AAs. In every case, the L form has been identified by adding the corresponding DNS-L-AA to the racemic mixture. The migration order is the same for all of the AAs studied: the D isomer appears first. This observation suggests that the DNS-D-AA is more strongly bound to the Cu(II)(aspartame) than the corresponding DNS-L-AA. This is supported by a more effective quenching of the fluorescence in this diastereomeric Cu(II) complex, leading to a slightly weaker signal for the DNS-D-AA than for the corresponding DNS-L-AA.

Table I. Migration Times, t_D and t_L, Resolution, R_s, and Δt Values of 18 DNS-DL-AAs[a]

DNS-DL-AA	t_D	t_L	R_s	$100\Delta t$
Arg	5.55	5.55	0	0
Try	7.06	7.22	1.56	2.32
Met	7.25	7.37	0.91	1.70
Phe	7.25	7.44	1.56	2.51
Ser	7.28	7.46	1.70	2.43
Thr	7.28	7.45	1.67	2.34
Tyr	7.36	7.46	1.12	1.40
Norval	7.38	7.47	0.83	1.14
Norleu	7.40	7.40	0	0
Leu	7.45	7.45	0	0
α-ab[b]	7.47	7.60	1.38	1.78
Ala	7.61	7.72	0.97	1.45
Val	7.65	7.81	1.47	2.07
Pro	7.80	7.80	0	0
$(Cys)_2$	8.17	8.36	1.34	2.22
Asp	9.43	9.75	1.65	3.34
Cys ac[c]	10.02	11.10	5.57	10.2
Glu	10.40	10.89	2.61	4.60

[a] Each pair of DNS-DL-AA enantiomers (5×10^{-4} M) is run separately. We used a 75-μm-i.d. untreated fused silica capillary column, 100 cm long (75 cm to the detection zone); applied voltage, −30 kV. [b] α-ab = α-aminobutyric acid. [c] Cys ac = cysteic acid.

We note that the $|\Delta t|$ values obtained here are larger by a factor of at least 50% than those obtained previously with a Cu(II)–L-histidine complex (*5*). DNS-DL-Ser, for example, could not be resolved with the Cu(II)–L-histidine complex.

From Table I, it can be seen that the main disadvantage of the method is that all the neutral AAs, i.e., those having an aliphatic or an aromatic side chain, have almost the same migration times. This means that, although they could be resolved when run separately, they are not readily separated in a mixture.

In order to increase the differentiation of these neutral AAs, we have employed a micellar electrolyte solution. The use of micelles in capillary zone electrophoresis has already been reported by Terabe et al. (*11*); micelles add another dimension to the separation, in that each AA will partition itself to a different extent between the aqueous and the micellar phases (*12*). Since the micelle is negatively charged at the pH conditions of the experiment, the separation time scale of the neutral AAs will broaden. For this purpose, we added 20 mM sodium tetradecyl sulfate (STS) as a surfactant to the conventional Cu(II) electrolyte solution. Figure 3 shows the advantages of this micellar solution, since the three DNS-DL-AAs tested could not be completely resolved with the previous nonmicellar electrolyte. It can also be seen from Figure 3 that DNS-L-Arg now has a longer migration time than the neutral DNS-DL-AAs, corresponding to a preferential partitioning in the micellar phase. Moreover, the fluorescence signal of the first AA isomer is, in certain cases, no longer the weaker one, probably due to a more complicated quenching phenomenon involving the intimate presence of the micelle.

The linearity and the sensitivity of the method were investigated by using three DNS-L-AAs with a laser power of 16 mW. Similarly to the analysis Scott did for the approximately linear detectors in liquid chromatography (*13*), we can use the function

$$y = C[\mathrm{AA}]^n \qquad (2)$$

where y is the PMT output signal, [AA] is the DNS-L-AA concentration, C is a constant, and n is the response index to describe the response of our present detection system. Thus, from the logarithmic form of eq 2, which is linear with a slope equal to n, we can deduce the actual response index for each compound. Note that the value $n = 1$ corresponds to a perfectly linear detector. The results of the linear least-squares fit for the three DNS-DL-AAs tested are given in Table II. Although n deviates slightly from unity, we believe the detection system is linear because the coefficient of correlation is very close to unity. Furthermore, it should be noted that the linearity of the graphs covers 4 orders of magnitude of concentration of the DNS-L-AAs. The 10^{-3} M upper limit is due to solubility restrictions of the dansyl-AAs in the electrolyte solution, while the lower limit of 10^{-7} M corresponds to a $S/N \simeq 3$. With an estimated detection volume of about 0.5 nL, based on the geometrical configuration of the capillary and the excitation optical fiber, about 50 amol can be detected with a satisfactory S/N ratio.

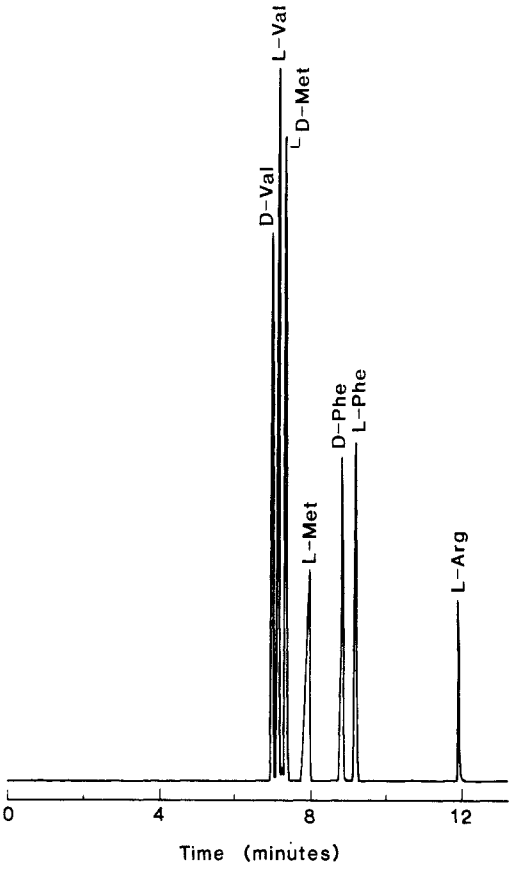

Figure 3. Electropherogram of a mixture of three DNS-DL-AAs. DNS-L-Arg is used as an internal standard. Electrolyte composition is as follows: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, 10 mM NH_4OAc, and 20 mM STS, pH 7.81. Same capillary as in Figure 2: applied voltage, −30 kV; current, ≈38 μA.

Table II. Linearity Test: Response Index, n, and Correlation Coefficient, r, for Three DNS-L-AAs[a]

DNS-L-AA	n	r
Arg	0.94	0.999
Glu	0.85	0.998
Val	0.95	0.998

[a] Electrolyte composition: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc, pH 7.4; laser power, 16 mW; same capillary as in Figure 2.

To determine the optimal conditions for the resolution, we studied the influence of three variables (pH, [Cu(II)] to [aspartame] ratio, and temperature) on the resolution of some selected DNS-DL-AAs.

pH Effects. Initially, we tried to use a nonbuffered electrolyte solution containing only the $Cu^{II}(aspartame)_2$ complex adjusted at various pHs between 3.5 and 8.0, but this led to very poor separation. Since the presence of a buffered solution seems to be critical for the separation process and/or the double-layer formation, we chose the ammonium acetate

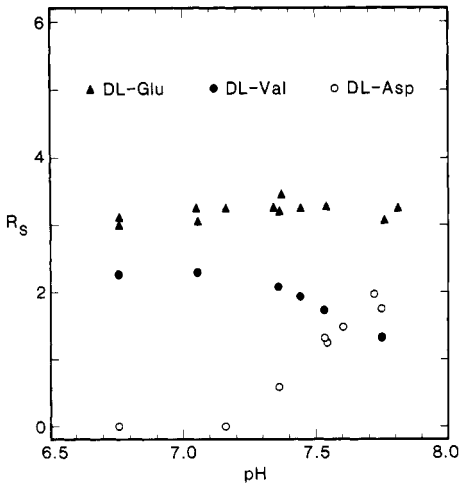

Figure 4. Resolution (R_s) of three DNS-DL-AAs vs. the pH of the electrolyte solution (2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc). Same capillary as in Figure 2.

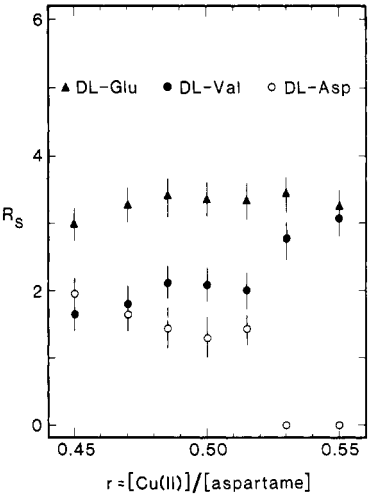

Figure 5. Resolution (R_s) of three DNS-DL-AAs vs. the [Cu(II)] to [aspartame] ratio, r. Electrolyte composition is as follows: ≈2.5 mM $CuSO_4 \cdot 5H_2O$, ≈5.0 mM aspartame, and 10 mM NH_4OAc, pH 7.5; same capillary as in Figure 2.

buffer since it works satisfactorily at neutral pH and does not precipitate the Cu(II).

Figure 4 shows the resolution, R_s, as a function of the pH obtained in the resolution of DNS-DL-Val, DNS-DL-Asp, and DNS-DL-Glu using the same electrolyte composition adjusted to different pH values in the range $6.8 < pH < 7.8$ by adding 0.1 N NH_4OH. The pH effects were not studied over a larger pH range since the maximum buffer capacity of the NH_4OAc lies around pH 7.0. It can be seen that the pH has practically no effect on the resolution of DL-glutamic acid; in contrast, the resolution of DL-aspartic acid is sensitive to the pH since it is unresolved at a pH lower than 7.3, but above this value, the higher the pH, the better the resolution. We cannot explain this difference of behavior between these two amino acids both having acidic side chains, although the formation of a six-membered ring in the complexation of the aspartic acid by Cu(II) is a possibility to keep in mind. DNS-DL-Val, however, is somewhat more poorly resolved when the pH is increased above 7.3, which may be the result of a simultaneous augmentation of the ternary complex stability at higher pH.

Effect of the [Cu(II)] to [Aspartame] Ratio. In an approach similar to the one used by Grushka (*14*) in reversed-phase HPLC, the effect of the [Cu(II)] to [aspartame] ratio was studied. Perturbations were made to the stoichiometric ratio of one Cu(II) ion for each two aspartame ligands. Preliminary results have indeed shown that the AA resolution is sensitive to this ratio, necessitating meticulous preparation of the electrolyte from the $CuSO_4 \cdot 5H_2O$ and aspartame stock solutions. Figure 5 shows the R_s values of three different DNS-DL-AAs as a function of r, where r is the [Cu(II)] to [aspartame] ratio, in the range $0.45 < r < 0.55$. The ammonium acetate concentration is maintained constant at 10 mM and the pH at 7.5. Contrary to what Gilon, Leshem, and Grushka (*14*) found, we do not observe a linear dependence of the separation as a function of r for $r < 0.5$. Here again, this ratio is crucial for the resolution of DL-aspartic acid, since a large excess of Cu(II) dramatically reduces its resolution; however, an excess of aspartame increases its R_s value. The two other AAs studied are almost insensitive to r, although a slight decrease of the resolution can be observed with an increase of aspartame concentration.

The pH and [Cu(II)] to [aspartame] ratio effects on resolution can in fact be related through the solubility product of the Cu(II) hydroxide in water ($K_{sp} = 2.2 \times 10^{-20}$) (*15*); an increase in the pH is possibly followed by some precipitation of $Cu(OH)_2$, causing the [Cu(II)] to [aspartame] ratio to be lowered. This may explain the observation that the electrolyte solutions, when stored for several days at room temperature, produce a better resolution of DL-aspartic acid, possibly due to a slow precipitation of copper hydroxide. A dilution factor of 10 in the concentrations of the Cu(II) and aspartame results in worse separations of the three DNS-DL-AAs tested. This may be explained by a competitive binding of hydroxide to the Cu(II).

Temperature Effects. The passage of a current through the capillary causes a temperature rise from joule heating. Effective cooling must occur in order to prevent severe band broadening by convective mixing. Most often, cooling depends on heat transfer between the outside capillary walls and the surrounding air layer. The efficiency of this type of heat exchange is undoubtedly improved by using smaller capillary diameters. In addition, the radial temperature gradient inside the capillary (*16*, *17*) is also reduced by the analyte motion back and forth across the capillary inner diameter. Several attempts were made to measure the temperature of the electrolyte solution inside the capillary by means of a tiny thermocouple introduced at the grounded capillary end. All of the trials failed, mainly because of the liberation of gas bubbles on the thermocouple surface. It was possible, however, to measure the temperature increase on the capillary outer surface as a function of the power dissipated per unit length. In this case, a type-E thermocouple (80 μm in diameter) was attached to the capillary surface with a thermoconductive paste. It was found that the temperature rise (ΔT, in °C) vs. the power dissipated per unit length (P_1 in mW/cm) obeyed the equation $\Delta T = 2.13 P_1^{0.88}$ in the range $0 < \Delta T < 70$ °C. Thus, under the conditions of a typical electrophoresis experiment (30 kV and 33 μA), a temperature rise of about 16 °C was observed on the capillary outer walls. This corresponds to a higher temperature for the electrolyte solution inside the capillary because of the presence of a temperature gradient across the fused silica walls, which are good insulators.

Since most of the experimental parameters (such as the ionic mobility, the electrolyte viscosity, and the ternary complex stability) are temperature dependent, we attempted to control the temperature during some of our experiments by using a water-cooled capillary column. Sixty centimeters of the capillary length preceding the detection zone is introduced into a 4-mm-i.d. Teflon tube through which a water/2-propanol mixture is being pumped by a refrigerated bath circulator. To our knowledge, this is the first time that results have been published using a capillary with the temperature controlled by contact with a bath.

The plot in Figure 6 shows how the resolution, R_s, of four DNS-DL-AAs varies with temperature. Although the resolu-

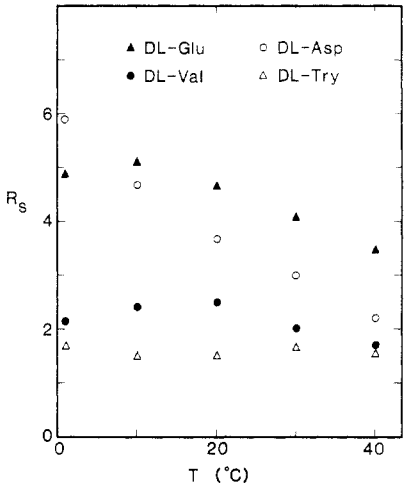

Figure 6. Resolution (R_s) of four DNS-DL-AAs vs. the water cooling temperature. Electrolyte composition is as follows: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc, pH 7.5 (measured at 22 °C); capillary, 75 μm i.d., 100 cm (75 cm to the detection zone); 60 cm of the capillary before the detection zone is water-cooled; applied voltage, −30 kV.

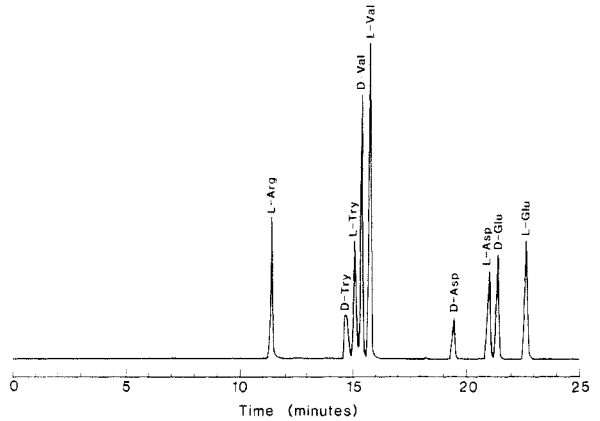

Figure 7. Electropherogram of a mixture of four DNS-DL-AAs. DNS-L-Arg is used as an internal standard. Electrolyte composition is as follows: 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc, pH 7.5 (measured at 22 °C). Same capillary as in Figure 6 with the water cooling at 1 °C; applied voltage, −30 kV.

tion of DNS-DL-Try and DNS-DL-Val is almost insensitive to the temperature, this is not the case for DNS-DL-Asp, which is better resolved at lower temperatures. This last observation is consistent with the results presented in Figures 4 and 5. The [Cu(II)] to [aspartame] ratio can be affected by the temperature dependence of the $Cu(OH)_2$ solubility. The resolution of DNS-DL-Glu shows the same behavior to a lesser extent.

Typical electropherograms of four DNS-DL-AAs taken at 1 and 40 °C are shown in Figures 7 and 8, respectively; in both cases, the electrolyte composition and the pH are the same. Except for DNS-DL-Asp (whose temperature behavior has already been discussed), it can be seen that a low temperature does not increase the AA resolution, since the electropherogram recorded at 40 °C seems to be nothing more than a "time-compressed" copy of the one taken at 1 °C.

Our impressions are confirmed by the observation of a constant theoretical plate number, N = 130 000, for DNS-DL-Val as a function of the temperature. The value of N is found either by a statistical moments calculation (*18*) or from the establishment of breakthrough curves (*18*). Both methods give the same results. When only molecular diffusion contributes to band broadening, Jorgenson (*19*) has shown that the theoretical plate number, N, is given by

$$N = \mu V/2D \qquad (3)$$

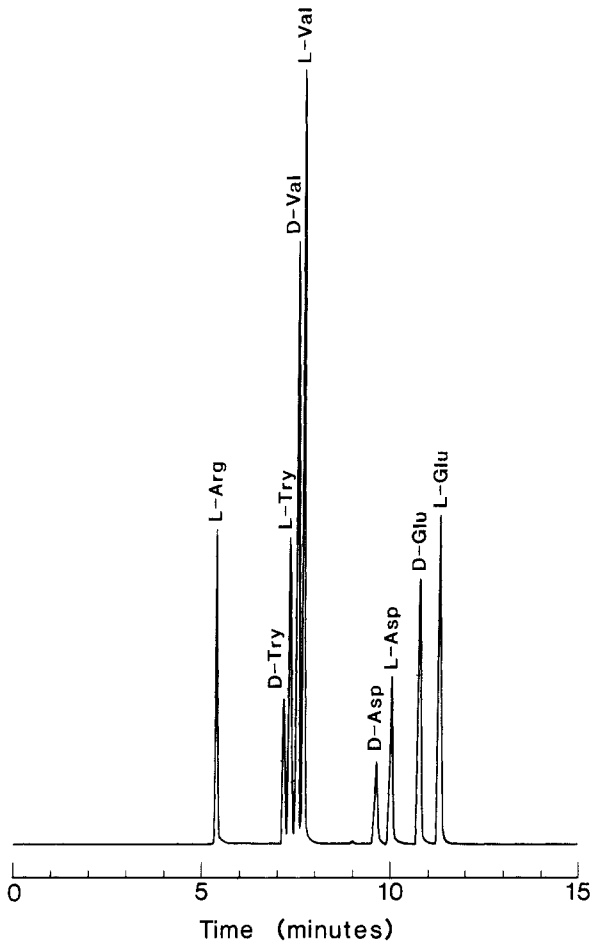

Figure 8. Electropherogram of a mixture of four DNS-DL-AAs. The conditions are the same as in Figure 7 except that the water cooling temperature is at 40 °C.

where μ is the sum of the electrophoretic and electroosmotic mobilities, V is the applied voltage, and D is the diffusion coefficient. From eq 3, some predictions can be formulated: (1) the higher the voltage, the better the resolution; (2) separation is independent of the column length; and (3) because N is proportional to the μ/D ratio, the temperature will not significantly affect the separation when μ and D have approximately the same temperature dependence. The theoretical plate number and particularly the resolution are, in fact, affected much more by the intrinsic values of D and, chiefly, μ than by temperature.

CONCLUSION

We have shown that the resolution of racemic mixtures of dansylated AAs is possible using readily available chemicals and relatively simple instrumentation. Fourteen out of a list of 18 DNS-DL-AAs were completely resolved with migration times shorter than 12 min. Moreover, it has been shown that the Cu(II)–aspartame complex significantly improves the electrokinetic resolution in comparison with the Cu(II)–L-histidine complex. Generally, the variables such as the pH, the [Cu(II)] to [aspartame] ratio, and the temperature do not markedly influence the DNS-DL-AA resolution. Indeed, the use of a 2.5 mM $CuSO_4 \cdot 5H_2O$, 5.0 mM aspartame, and 10 mM NH_4OAc electrolyte solution adjusted to pH 7.2 ± 0.2, and with a [Cu(II)] to [aspartame] ratio fixed at 0.5 ± 6%, gives excellent results in an air-cooled capillary. The sensitivity of laser-induced fluorescence detection combined with the small amount of sample needed in capillary zone electrophoresis should allow the use of this method in analyzing minute samples such as those encountered in many biological assays.

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Registry No. DL-Arg, 7200-25-1; D-Arg, 157-06-2; L-Arg, 74-79-3; DL-Try, 54-12-6; D-Try, 153-94-6; L-Try, 73-22-3; DL-Met, 59-51-8; D-Met, 348-67-4; L-Met, 63-68-3; DL-Phe, 150-30-1; D-Phe, 673-06-3; L-Phe, 63-91-2; DL-Ser, 302-84-1; D-Ser, 312-84-5; L-Ser, 56-45-1; DL-Thr, 80-68-2; D-Thr, 632-20-2; L-Thr, 72-19-5; DL-Tyr, 556-03-6; D-Tyr, 556-02-5; L-Tyr, 60-18-4; DL-Norval, 760-78-1; D-Norval, 2013-12-9; L-Norval, 6600-40-4; DL-Norleu, 616-06-8; D-Norleu, 327-56-0; L-Norleu, 327-57-1; DL-Leu, 328-39-2; D-Leu, 328-38-1; L-Leu, 61-90-5; DL-α-ab, 2835-81-6; D-α-ab, 2623-91-8; L-α-ab, 1492-24-6; DL-Ala, 302-72-7; D-Ala, 338-69-2; L-Ala, 56-41-7; DL-Val, 516-06-3; D-Val, 640-68-6; L-Val, 72-18-4; DL-Pro, 609-36-9; D-Pro, 344-25-2; L-Pro, 147-85-3; DL-$(Cys)_2$, 923-32-0; D-$(Cys)_2$, 349-46-2; L-$(Cys)_2$, 56-89-3; DL-Asp, 617-45-8; D-Asp, 1783-96-6; L-Asp, 56-84-8; DL-Cys ac, 3024-83-7; D-Cys ac, 35554-98-4; L-Cys ac, 498-40-8; DL-Glu, 617-65-2; D-Glu, 6893-26-1; L-Glu, 56-86-0; Cu(II), 7440-50-8; aspartame, 22839-47-0.

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Photoelectrochemical Detection of Benzaldehyde in Foodstuffs

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Photoelectrochemical detection (PED) coupled with high-performance liquid chromatography was used to quantitatively determine benzaldehyde in extracts, beverages, and foodstuffs. Photoelectrochemical detection is responsive to alkyl and aryl ketones and aldehydes and offers the advantages of 2-3 orders of magnitude linearity, 5-1-ng limits of detection, and a high degree of selectivity without chemical derivatization. This is the first application of the PED to sample analysis.

High-performance liquid chromatography (HPLC) with electrochemical detection (EC) has gained widespread analytical use over the past decade (*1*). This has been due, in part, to its high sensitivity and selectivity for specific functional groups. The photoelectrochemical detector (PED), recently introduced by LaCourse and Krull, expands the normal range of electrophores to include alkyl and aryl ketones and aldehydes (*2*, *3*).

Photoelectrochemical detection takes advantage of the fact that optical energy is converted to electrochemical energy via electronic promotion. The excited state may be a better oxidizing agent (an electron may fill the lower energy, empty orbital) or a better reducing agent (the electron is more easily removed from its higher energy orbital) depending on the molecule. Thus, the electrochemical properties of the excited state should be different from the ground state. The PED uses a conventional thin-layer amperometric detection system, which has been modified such that the working electrode can be continuously irradiated with high-intensity ultraviolet light. As the analyte passes over the working electrode surface, it is optically transformed to the excited state. The PED is designed to detect molecules or species derived from photogenerated excited states (singlets and/or triplets), intermediates, and/or products. A detailed discussion of the mechanism of detection will be covered in a forthcoming publication (*4*).

HPLC-PED combines the selectivity of a commercial chromatographic system with the high sensitivity and additional selectivity of electrochemical detection. Under optimized conditions of flow rate, gasket thickness, solvent composition (organic component and electrolyte), light intensity, and applied potential, the responses of over 50 compounds have been characterized. The responsive compounds were all carbonyl compounds or carbonyl derivatives. It is the goal of this paper to demonstrate the analytical utility of the PED by applying it to "real-world" applications, namely, the analysis of benzaldehyde in a variety of sample matrices.

Production of benzaldehyde, or oil of bitter almonds, is in the millions of pounds per year (*5*). About 50% of the produced benzaldehyde has been used in the manufacture of odorants and flavoring agents. These compounds are used by the soap, perfume, and food industries. Thirty percent is used in the synthesis of various dyes. The remainder is accounted for as an intermediate in the manufacture of pharmaceuticals (analgesics, antipyretics, and antispasmodics) and fine organics. It may also be employed as an ingredient in compounding and dispensing, as well as a flavoring agent in pharmaceuticals. Benzaldehyde is a skin irritant and, in high enough quantities, a central nervous system (CNS) depressant (*6*).

Since benzaldehyde is found in so many products, accurate and sensitive monitoring procedures are needed. In spite of

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