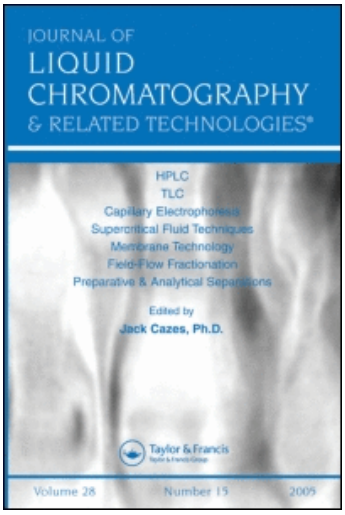


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Enantiomeric Separation of Synthetic Amino Acids Using Capillary Zone Electrophoresis

C. Jiang[a]; Daniel W. Armstrong[a]; A. W. Lantz[b]; A. Péter[c]; G. Tóth[d]

[a] Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, Texas [b] Department of Chemistry, Iowa State University, Ames, Iowa [c] Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungry [d] Institute of Biochemistry, Biological Research Center, Szeged, Hungry

To cite this Article Jiang, C. , Armstrong, Daniel W. , Lantz, A. W. , Péter, A. and Tóth, G.(2007) 'Enantiomeric Separation of Synthetic Amino Acids Using Capillary Zone Electrophoresis', Journal of Liquid Chromatography & Related Technologies, 30: 9, 1421 — 1436

To link to this Article: DOI: 10.1080/10826070701277018

URL: http://dx.doi.org/10.1080/10826070701277018

Journal of Liquid Chromatography & Related Technologies®, 30: 1421–1436, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701277018

Enantiomeric Separation of Synthetic Amino Acids Using Capillary Zone Electrophoresis

C. Jiang and D. W. Armstrong
Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, Texas

A. W. Lantz
Department of Chemistry, Iowa State University, Ames, Iowa

A. Péter
Department of Inorganic and Analytical Chemistry, University of Szeged, Szeged, Hungry

G. Tóth
Institute of Biochemistry, Biological Research Center, Szeged, Hungry

Abstract: Three chiral selectors, sulfated α-cyclodextrin (SAC), sulfated β-cyclodextrin (SBC), and carboxymethyl β-cyclodextrin (CMBC) were examined as run buffer additives for the separation of sixteen racemic synthetic amino acids and three prepared mixtures of chiral synthetic amino acids, using capillary zone electrophoresis. Seventeen of the nineteen synthetic amino acids were enantiomerically separated and fourteen of them were optimized to baseline using one or more chiral running buffer additives. SAC, with eleven baseline and three partial separations, and SBC, with ten baseline and four partial separations, were found to be more broadly useful than CMBC. Increasing the chiral selector concentration improved the enantioresolution, but also produced longer analyses times. Addition of organic modifier (ethanol) increased migration times and decreased enantiomeric resolution. Increasing the pH

Address correspondence to Dr. Daniel W. Armstrong, Department of Chemistry and Biochemistry, University of Texas at Arlington, Arlington, TX 76019-0065. E-mail: sec4dwa@uta.edu

of the run buffer decreased analyses time as well as resolution. Decreasing the applied voltage generally improved resolution.

Keywords: Capillary electrophoresis (CE), Capillary zone electrophoresis, Amino acid, Enantiomeric separation, Chiral separation, Sulfated cyclodextrins, Carboxymethyl cyclodextrin

INTRODUCTION

Enantioseparation of chiral compounds has attracted considerable interest during the past two decades due to the different biological and pharmaceutical properties of enantiomers.[1] As building blocks of peptides, proteins, and other important biological molecules, amino acids are very important compounds.[2] Amino acids also play an important role in the design of new pharmacons.[3,4] What's more, unnatural amino acids and their derivatives are commonly used as building blocks in the synthesis of stereochemically pure compounds in pharmaceutical discovery programs.[5]

Enantiomeric separation techniques, such as high performance liquid chromatography (HPLC),[6–12] gas chromatography (GC),[13] and supercritical fluid chromatography (SFC)[14] have been reported for the separation of amino acids enantiomers and their derivatives. Capillary electrophoresis (CE) also is a powerful analytical separation technique due to its high efficiency, low consumption of analytes, and buffers.[2,5,15,16] Enantiomeric separations of amino acids by CE using different chiral selectors have reported, including a metal chelate,[17–22] cyclodextrins, [23–29] and crown ethers.[30–32] Among them, cyclodextrins (CDs) and their derivatives are the most prevalent and broadly useful class of chiral selectors in CE.[5,33–37] A review on enantioselective separations by CE using CDs has been published.[35]

In this work, sixteen racemic synthetic α-amino acids and three prepared mixtures of chiral synthetic α-amino acids were examined via CZE with three anionic chiral selectors: sulfated α-cyclodextrin (SAC), sulfated β-cyclodextrin (SBC), and carboxymethyl β-cyclodextrin (CMBC). Phenylalanine was also examined as a reference. The elution orders of two of the prepared mixtures were determined by spiking with enantiomers of known absolute configuration into the mixture. The amino acids studied in this paper were also evaluated previously by HPLC using macrocyclic glycopeptide chiral stationary phase (CSP).[3,38]

EXPERIMENTAL

Materials

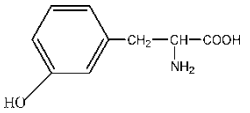
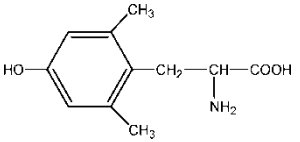
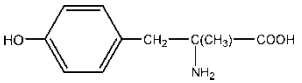
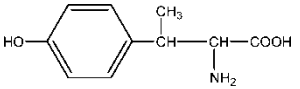
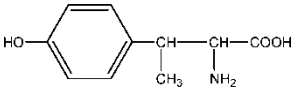
Most of the amino acids were synthesized in our laboratory in Szeged, except for compounds **1**, **3**, and **13**, which were purchased from Aldrich (Steinheim,

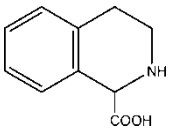
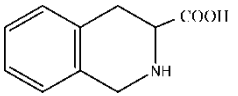
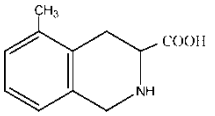
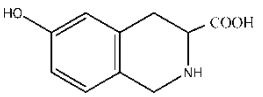
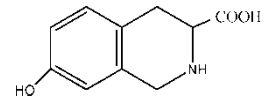
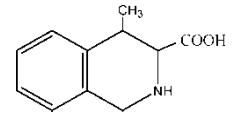
Germany). The other seventeen amino acids were either produced as racemates or enantiomerically enriched via asymmetric synthesis.[3,38] These unusual amino acids are listed as follows (See Table 1 and Table 2 for structures): **1**. *m*-tyrosine (*m*-Tyr), **2**. 2′,6′-dimethyltyrosine (2′,6′-diMeTyr), **3**. α-methyltyrosine (α-MeTyr), **4**. *erythro*-(2*S*,3*S* and 2*R*,3*R*)-β-methyltyrosine (*erythro*-β-MeTyr), **5**. *threo*-(2*S*,3*R* and 2*R*,3*S*)-β-methyltyrosine (*threo*-β-MeTyr), **6**. 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (Tic1), **7**. 1,2,3,4-tetrahydroisoquino-line-3-carboxylic acid (Tic3), **8**. 5′-methyl-1,2,3,4-tetrahydro- isoquinoline-3-carboxylic acid (5′-MeTic3), **9**. 6′-hydroxy-1,2,3,4-tetrahydroisoquino- line-3-carboxylic acid (6′-HO-Tic3), **10**. 7′-hydroxy -1,2,3,4-tetrahydroisoquinoline- 3-carboxylic acid (7′-HO-Tic3), **11**. *erythro*-(2*S*,3*S* and 2*R*,3*R*)-4-methyl-1,2,3,4-tetra hydroisoquinoline-3-carboxylic acid (*erythro*-β-MeTic3), **12**. *threo*-(2S,3S and 2*R*,3*R*)-4-methyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (*threo*-β-MeTic3), **13**. Phenylalanine (Phe) **14**. 2′-methylphenylanaline (2′-MePhe), **15**. 4′-methylphenylanaline (4′-MePhe), **16**. *o*-methyltyrosine **17**. 2′,6′-dimethylphenyl- alanine (2′,6′-diMePhe), **18**. α-methyl- phenylalanine (α-MePhe) **19**. *erythro*-(2*S*,3*S* and 2*R*,3*R*)-β-methylphenylalanine (*erythro*-β-MePhe) **20**. *threo*-(2*S*,3*S* and 2*R*,3*R*)-β-methylphenylalanine (*threo*-β-MePhe). Among them, **7**, **10**, **17** are artificial mixtures of the two enantiomers while the rest of them are obtained as racemates. α-Cyclodextrin, hydrate, sulfated, sodium salt (SAC) and β-cyclodextrin, hydrate, sulfated, sodium salt (SBC) with a degree of substitution of 7–11 moles/mole β-cyclodextrin were obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Carboxymethyl β-cyclodextrin (CMBC) was obtained from American Maize Products (Hammond, IN, USA). Sodium phosphate, monobasic, sodium phosphate, dibasic, HPLC grade ethanol, phosphoric acid, and sodium hydroxide were all purchased from Fisher Scientific (St. Louis, MO, USA). The fused silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

Method

A Beckman P/ACE 2050 CE instrument (Fullerton, CA, USA) was used for all CE separations with on-column UV detection. The capillary used for all separations had a length of 37 cm (30 cm from inlet to detection window) and a 50 μm inner diameter. The detection window was created by burning the polyamide coating of the desired length from the capillary. Before the first use, the capillary was rinsed with deionized water for 5 minutes and then 1 M sodium hydroxide for 5 min. Before each experiment, the capillary was rinsed with 1 M sodium hydroxide for 30 seconds, deionized water for 1 minute, then running buffer for 3 minutes to ensure reproducible EOFs.[37] All the samples were prepared by dissolving 2 mg/mL analytes in

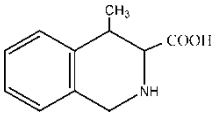
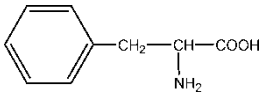
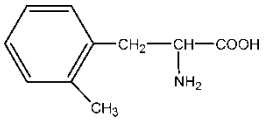
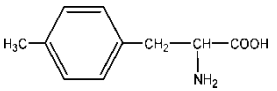
Table 1. Structure and separations of synthetic amino acids using SAC, SBC, and CMBC. Separation conditions: SAC: 90 mg/mL SAC in 5 mM sodium phosphate buffer, pH 8.0; +8 kV except for 15 and 19 at +6 kV with different batch of SAC. SBC: 120 mM SBC in 5 mM sodium phosphate buffer, pH 8.0; +4 kV. CMBC: 65 mM CMBC in 5 mM sodium phosphate buffer, pH 8.0; +7 kV. Other details refer to experimental part

		90 mg/mL SAC(a)			120 mM SBC(b)			65 mM CMBC		
	Structure	t_{m1} (min)	t_{m2} (min)	Rs	t_{m1} (min)	t_{m2} (min)	Rs	t_{m1} (min)	t_{m2} (min)	Rs
1.		Not separated			Not separated			Not separated		
2.		11.59	11.73	0.4	31.99	38.56	5.9	Not separated		
3.		Not separated			Not separated			Not separated		
4.		Not separated			25.87	26.63	1.3	Not separated		
5.		14.95	16.56	4.7	26.00	27.63	2.8	Not separated		

6.		13.02	13.75	3.0	Not separated			24.44	25.12	1.0
7.		19.45	20.95	2.8	30.00	31.51	2.1	Not separated		
8.		16.46	16.83	1.0	32.02	36.21	5.2	Not separated		
9.		8.70	8.89	0.9	37.79	38.79	1.1	Not separated		
10.		16.15	17.20	2.1	37.96	48.68	11	Not separated		
11.		16.92	17.44	1.5	25.09	27.80	4.7	34.7	35.8	1.4

(*continued*)

Table 1. Continued

Structure	90 mg/mL SAC(a)			120 mM SBC(b)			65 mM CMBC		
	t_{m1} (min)	t_{m2} (min)	Rs	t_{m1} (min)	t_{m2} (min)	Rs	t_{m1} (min)	t_{m2} (min)	Rs
12. 	15.33	19.24	7.5	26.75	36.32	13	40.74	48.01	3.3
13. 	Not separated			Not separated			Not separated		
14. 	17.77	19.02	2.9	32.01	34.74	3.7	27.26	27.83	0.4
15. 	13.44	14.01	2.2	28.48	29.12	1.0	36.8	37.6	0.6

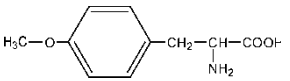
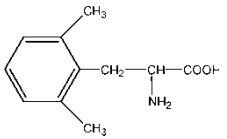
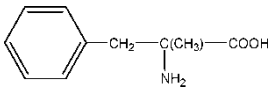
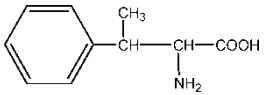
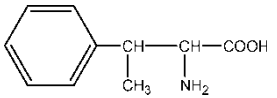
16.		18.84	20.01	2.9	27.29	27.68	0.6	30.84	31.60	0.5
17.		Not separated			29.20	31.68	3.6	Not separated		
18.		14.89	16.27	1.6	Not separated			Not separated		
19.		Not separated			Not separated			35.40	36.30	0.6
20.		16.92	17.44	3.3	25.25	26.09	1.5	37.27	38.35	0.6

Table 2. Effect of addition of organic modifier ethanol on the separation of amino acid 12. Separation conditions: 60 mM SBC in 5 mM sodium phosphate buffer, pH 7.2; +5 kV

EtOH V/V	t_{m1} (min)	t_{m2} (min)	t_{eo} (min)	Rs	N	t_{m2}/t_{m1}
0%	11.97	13.51	10.45	6.6	47000	1.13
5%	14.71	16.35	12.90	5.7	47000	1.11
10%	17.56	19.22	15.50	5.0	47000	1.09

deionized water. Samples were injected hydrodynamically. All the analytes were detected by UV absorbance at 214 nm. Sodium phosphate (monobasic) and sodium phosphate (dibasic) were dissolved in deionized water to make 5 mM solutions and then mixed in a 1:1 ratio. This solution was then adjusted to desired pH using 5 mM phosphoric acid or 1 M sodium hydroxide to desired pH. The chiral selectors were added into this mixture and used as running buffer. When utilized, the organic modifier was added on a volume based ratio prior to the addition of chiral selectors.

All the data were analyzed with Beckman System Gold Software. The resolution was calculated as $Rs = 2(t_{m2} - t_{m1})/(w_1 + w_2)$, the selectivity α was calculated as $\alpha = t_{m2}/t_{m1.}$ The apparent mobility, the efficiency N was calculated as $N = 16 \cdot (t_{m1}/w_1)^2$, where t_{m1} and t_{m2} are the migration time of the first and second observed peak, while w_1 and w_2 are the extrapolated peak width at baseline.

RESULTS AND DISCUSSION

Enantiomeric Separation of Synthetic Amino Acids using CZE

Anionic cyclodextrin derivatives have been one of the most broadly useful types of chiral selectors for CE.[33–40] In the CE normal polarity mode, the bulk solution moves toward the cathode due to electroosmotic flow (EOF) while the anionic cyclodextrin chiral selectors move toward the anode due to electrophoretic movement.[37,39,40] Neutral enantiomers (with no electrophoretic mobility themselves) have different distributions between these two countercurrent moving phases, leading to different mobilities and possible enantiomeric separations.[33–37,39,40] Synthetic amino acids, which have two ionizable groups with pKas of around 3 and 8, will exist mainly as zwitterions between the two pKas. Therefore, experimental conditions analogous to those used for nonionizable compounds can be used for enantiomeric separation in the appropriate pH range.[39]

The structures of all compounds used in this study, as well as their migration times and resolutions under optimized conditions, are given

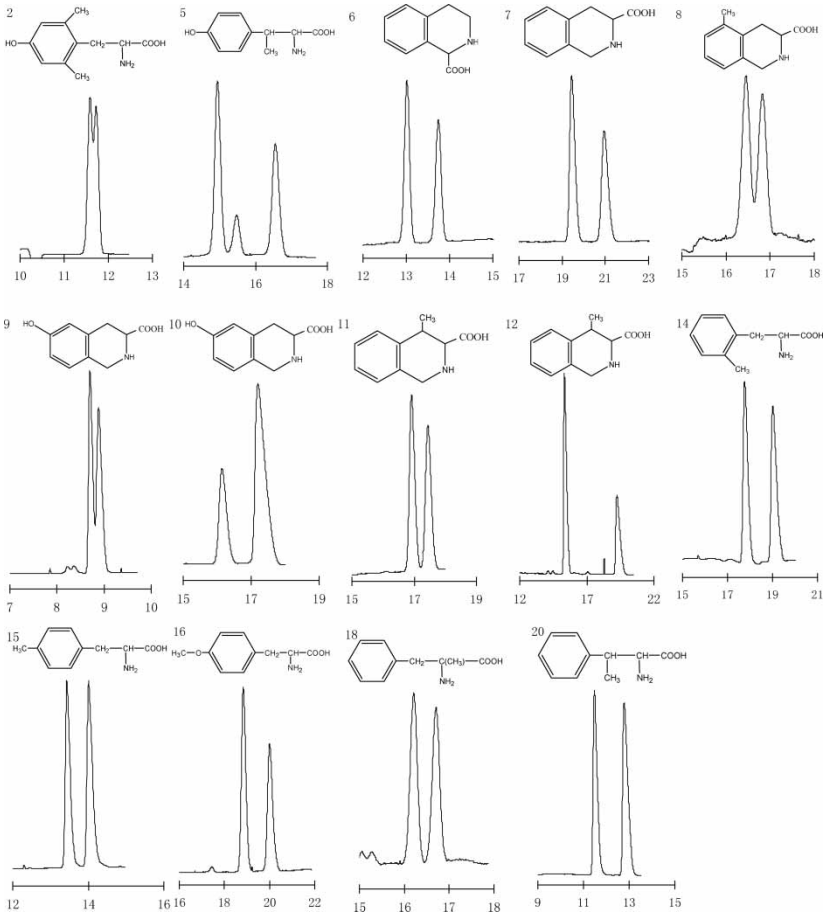


Figure 1. Electropherograms of synthetic amino acids that are separated by SAC under optimized conditions. Experimental conditions: 90 mg/mL SAC in 5 mM phosphate buffer, pH 7.2; +8 kV except 15 and 19, +6 kV. Details refer to experimental part.

in Table 1. All of the corresponding electropherograms are given in Figures 1–3. Among these analytes, compounds **4** and **5**, **11** and **12**, **19** and **20** have two stereogenic centers, and therefore, they can exist as two pairs of enantiomers. However, the samples in this study consist of a single pair of enantiomers (see Experimental). Seventeen of the nineteen synthetic amino acids were enantiomerically separated. Among them, fourteen were optimized to baseline using one or more chiral running buffer additives. Eleven baseline and three partial separations were obtained using SAC (Table 1). Ten baseline and four partial separations were obtained with SBC (Table 1). Many fewer compounds were separated by CMBC, and of these compounds all except compound **19** could be separated by SAC and/or

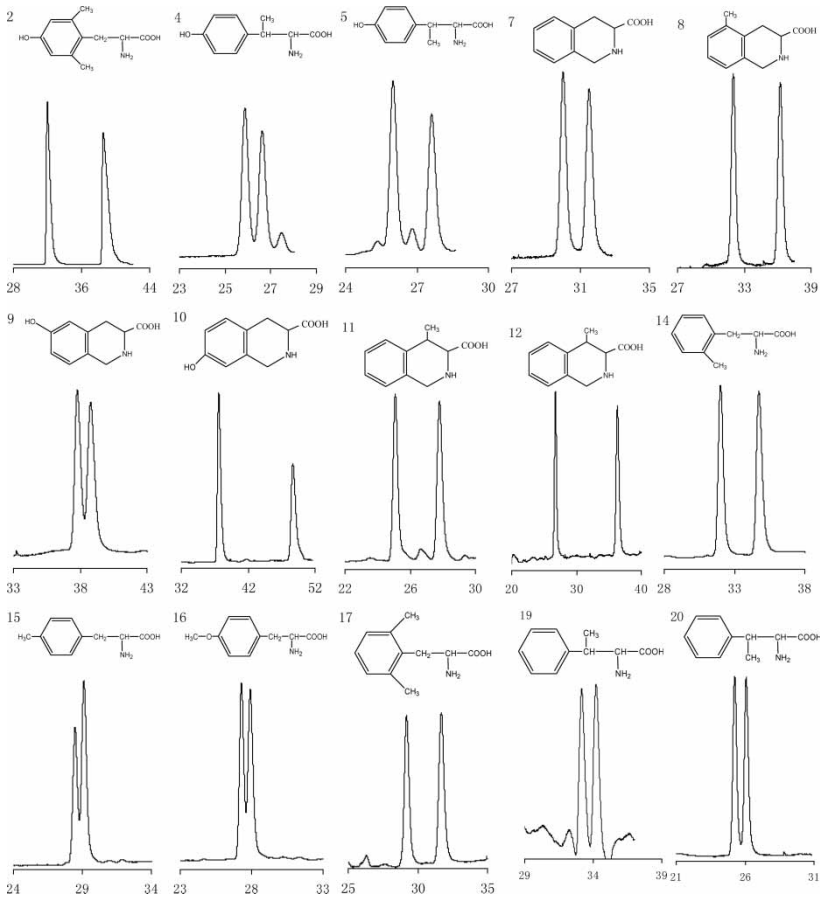


Figure 2. Electropherograms of synthetic amino acids that are separated by SBC under optimized conditions. Experimental conditions: 120 mM SBC in 5 mM phosphate buffer, pH 7.2; +4 kV except compound 19, +5 kV. Details refer to experimental part.

SBC with much higher resolutions. Also, it was found that, the electromigration order of both compounds **7** and **10** were reversed when using SAC and SBC (see electropherograms of **7** and **10** using SAC and SBC in Figures 1 and 2). Reversal of the migration orders can be important in the determination of enantiomeric impurities.[41,42]

Effect of Chiral Selector Concentration

As reported in many previous studies, the concentration of a chiral selector can have a significant effect on enantiomeric separations.[33,37,39,40,43,44] The

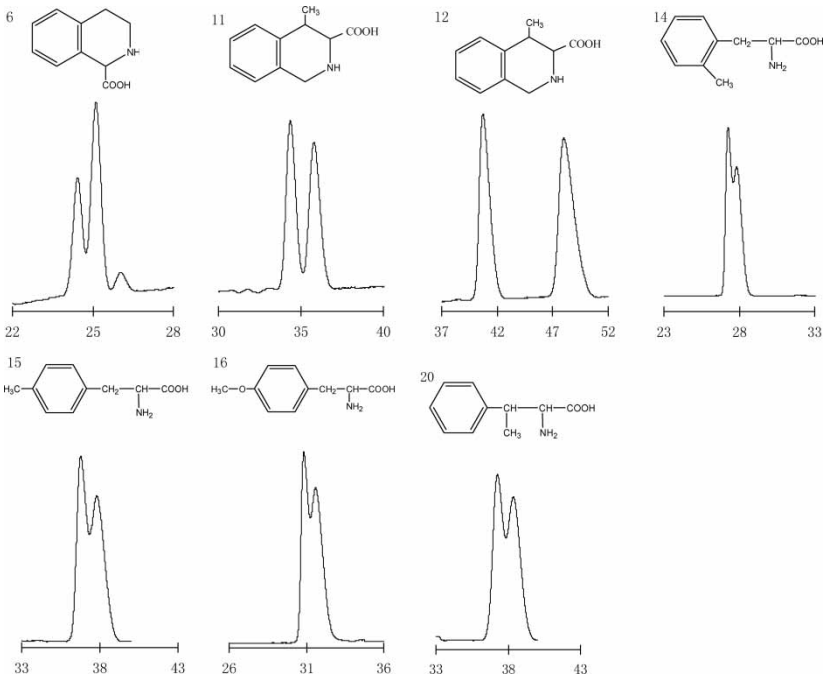


Figure 3. Electropherograms of synthetic amino acids that are separated by CMBC under optimized conditions. Experimental conditions: 65 mM CMBC in 5 mM phosphate buffer, pH 7.2; +7 kV. Details refer to experimental part.

separation of amino acid **12** using SBC was used as an example to study this effect (see electropherograms and other separation parameters in Figure 4). Experimental factors, such as buffer concentration, pH, and applied voltage were kept the same except for SBC concentration. As shown in Figure 4, when the chiral selector (SBC) concentration was increased from 5 mM to 40 mM, a pronounced improvement in the enantiomeric separation was observed, from a shoulder (at 5 mM SBC) to a greater than baseline separation with a resolution of 6.1 (at 40 mM SBC). However, the analysis time also was significantly increased from 8 minutes to 20 minutes. This is due to the fact that, as more SBC was present, a higher percentage of analyte was associated with the cyclodextrin pseudophase. This not only gave the analyte an increased electrophoretic mobility toward the anode (which increased analysis time), but also accentuated the mobility difference between the two enantiomers. Also, since the ionic strength was higher when more SBC was present, the EOF magnitude was decreased.[45] This also contributed to longer analysis times.

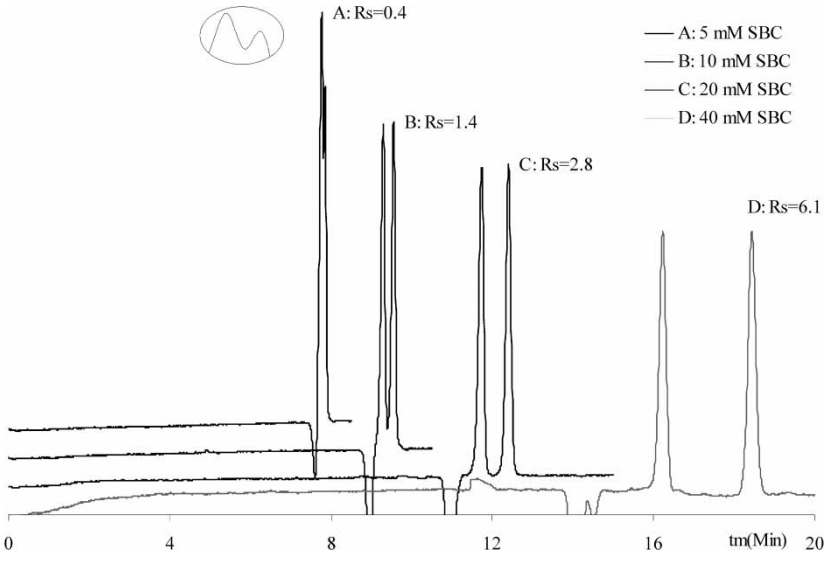


Figure 4. Electropherograms of amino acid **12** at different SBC concentrations. Separation conditions: SBC were dissolved in 5 mM phosphate, pH = 7.2, +5 kV. Other details refer to experimental part.

Effect of Organic Modifier

The effect of added organic modifiers on the enantioseparations using dissolved cyclodextrin chiral selectors has been reported to be very complicated, as an organic modifier can alter several parameters. These include the stability constants for the inclusion complexes, the EOF, the conductivity of the BGE, etc.[2,35,44] In some cases, added organic modifiers can improve enantiomeric resolution.[46,47] However, in most cases when using CDs as chiral selectors, the addition of organic modifier to the running buffer decreases the enantioselectivity.[33,37,44] Organic modifier is known to compete with the analyte for binding with the CD cavity. Eventually it decreases the binding constants between the analyte and CD.[33,35,37] Table 2 shows the experimental data when using 60 mM SBC to separate amino acid **12** at different volume percentages of ethanol. Upon the addition of ethanol, the EOF was slowed and the analysis time was increased. At a higher percentage of ethanol, both the selectivity (α) and the resolution (Rs) were decreased.

Effect of Running Buffer pH

Varying the pH of running buffer is known to be an effective way to control the magnitude of the EOF.[45] In the normal polarity mode, higher pHs

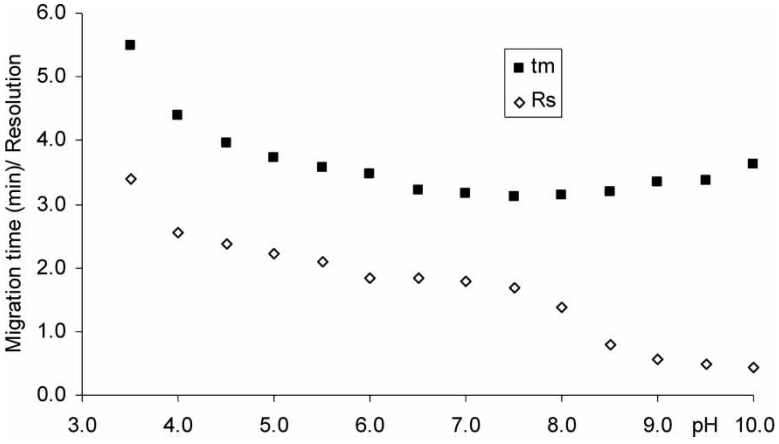


Figure 5. Effect of buffer pH when separating amino acid **12** using SBC. Conditions: 20 mM SBC in 5 mM phosphate buffer, +10 kV. Details refer to experimental part.

produce faster EOFs, resulting in shorter analysis time. The running buffer pH also controls the charge state of ionizable analytes and chiral selectors. According to the results shown in Figure 5, between the pHs of 3.5 and 7.5, higher pHs accelerated analysis at the price of a slight decrease in resolution. As the pH was increased further, the migration times of the two peaks increased while the resolution dropped abruptly. This can be explained by the following facts. The amine group of the analyte starts to be deprotonated at pH 7.5, which gives the analyte a negative charge, and in turn a slower velocity toward the detection window (longer migration time). Also the binding of the anionic amino acid can differ from that of its zwitterionic form.

Effect of Applied Voltage

The effect of applied voltage also was studied using the separation of amino acid **12** as an example. The experimental data is summarized in Table 3. When using 20 mM SBC to separate amino acid **12**, as the voltage decreased, the analysis time and migration time of EOF marker increased significantly. The separation also improved from a partial separation with a resolution of 0.7 to a baseline separation with a resolution of 2.1. The efficiency reached an optimum at a voltage of 10 kV, while selectivity increased as voltage was decreased in the voltage range studied. These results are consistent with the result we obtained for β-lactams in a previous study.[39]

Table 3. Effect of the applied voltage on the separation of amino acid 12. Separation conditions: 20 mM SBC in 5 mM sodium phosphate buffer, pH 7.2

Voltage (kV)	t_{m1} (min)	t_{m2} (min)	t_{eo} (min)	Rs	N	t_{m2}/t_{m1}
16	1.56	1.59	1.49	0.7	16000	1.02
15	1.62	1.66	1.55	0.7	17000	1.02
14	1.93	1.98	1.84	1.1	28000	1.03
13	2.21	2.27	2.10	1.2	33000	1.03
12	2.56	2.64	2.43	1.4	35000	1.03
11	2.96	3.06	2.82	1.6	36000	1.03
10	3.40	3.53	3.24	1.7	38000	1.04
9	3.94	4.10	3.75	1.8	35000	1.04
8	4.59	4.77	4.36	1.9	36000	1.04
7	5.44	5.67	5.17	1.9	35000	1.04
6	6.45	6.73	6.13	1.9	33000	1.04
5	7.52	7.84	7.14	1.9	32000	1.04
4	10.37	10.85	9.83	2.1	34000	1.05

CONCLUSION

Nineteen synthetic chiral amino acids were analyzed using CZE and three different anionic cyclodextrins as chiral selectors. SAC and SBC were found to be effective chiral selectors for this series of compounds, each separating about 80% of the analytes with 70% showing a baseline separation. Increasing the chiral selector concentration was found to be the most effective way to improve enantioresolution. Raising pH generally increases analysis time at the expense of a slight loss of resolution, when the charge state of both analyte and chiral selector remain the same. Addition of organic modifier usually hurts the resolution and increases the analysis time. Higher resolutions are generally obtained at lower voltages, albeit at the expense of longer analysis times.

ACKNOWLEDGMENTS

We gratefully acknowledge the support of National Institute of Health (NIH 5 ROICM053825–11) and Hungarian National Science Foundation Grant OTKAT 042451.

REFERENCES

1. Amini, A. Electrophoresis **2001**, *22*, 3107.
2. Wan, H.; Blomberg, L.G. J. Chromatogr. A **2000**, *875*, 43.
3. Péter, A.; Török, G.; Armstrong, D.W. J. Chromatogr. A **1998**, *793*, 283.

4. Hruby, V.J.; Al-Obeidi, F.; Kazmierski, W. Biochem. J. **1990**, *268*, 249.
5. Perrin, C.; Vargas, M.G. Vander Heyden, Y.; Maftouh, M.; Massart, D.L. J. Chromatogr. **2000**, *883*, 249.
6. Lingenfelter, D.; Helgeson, R.; Cram, D. J. Org. Chem. **1981**, *46*, 393.
7. Armstrong, D.W.; Yang, X.; Han, S.M.; Menges, R.A. Anal. Chem. **1987**, *59*, 2594.
8. Armstrong, D.W.; Yiu, Y.; Ekborg-Ott, K.H. **1995**, *7*, 474.
9. Petterson, C. J. Chromatogr. **1984**, *316*, 553.
10. Chang, S.C.; Wang, L.R.; Armstrong, D.W. J. Liq. Chromatogr. & Rel. Technol. **1992**, *15*, 1411.
11. Esquivel, J.B.; Sanchez, C.; Fazio, M.J. J. Liq. Chromatogr. & Rel. Technol. **1998**, *21*, 777.
12. Chen, C.C.; Lin, C.E. J. Chromatogr. Sci. **1995**, *33*, 229.
13. Yasuhara, F.; Tekada, M.; Ochiai, Y. Chem. Lett. **1992**, *2*, 251.
14. Hara, S.; Dobashi, A.; Kinoshita, K.; Hondo, T.; Saito, M.; Senda, M. J. High Resolut. Chromatogra. A. **1997**, *792*, 269.
15. Rizzi, A. Electrophoresis **2001**, *22*, 3097.
16. Yoshinaga, M.; Tanaka, M. J. Chromatogr. A. **1994**, *679*, 359.
17. Gassmann, E.; Kou, J.E.; Zara, R.N. Science **1985**, *230*, 813.
18. Gozel, P.; Gassmann, E.; Michelsen, H.; Zara, R.N. Anal. Chem. **1987**, *59*, 44.
19. Wu, B.; Wang, Q.; Liu, Q.; Xie, J.; Yun, L. Electrophoresis **2005**, *26*, 1013.
20. Hizuru, N.; Katasuhiko, K.; Shen, H.; Nakagama, T.; Katsumi, U. Anal. Sci. **2005**, *21*, 67.
21. Zhao, S.; Liu, Y.M. Anal. Chim. Acta **2001**, *426*, 65.
22. Akos, V.; Schmid, M.G.; Kilar, F.; Guebitz, G. Electrophoresis **1998**, *19*, 2109.
23. Terabe, S.; Miyashita, Y.; Ishihara, Y.; Shibata, O. J. Chromatogr. **1993**, *636*, 47.
24. Ueda, T.; Kitamura, F.; Mitchell, R.; Metcalf, T.; Kuwana, T.; Nakamoto, A. Anal. Chem. **1991**, *636*, 47.
25. Jin, L.J.; Rodriguez, I.; Li, S.F.Y. Electrophoresis **1999**, *20*, 1538.
26. Miura, M.; Kawamoto, K.; Funazo, K.; Tanaka, M. Anal. Chim. Acta **1998**, *373*, 47.
27. Branch, S.K.; Holzgrabe, U.; Jefferies, T.M.; Mallwitz, H.; Oxley, F.J.R. J. Chromatogr. **1997**, *758*, 277.
28. Wang, F.; Khaledi, M.G. J. Chromatogr. A. **1998**, *817*, 121.
29. Francotte, E.; Brandel, L.; Jung, M. J. Chromatogr. A. **1997**, *792*, 379.
30. Kuhn, R.; Erni, F.; Bereuter, T.; Hausler, J. Anal. Chem. **1992**, *64*, 2815.
31. Walbroehl, Y.; Wagner, J. J. Chromatogr. A. **1994**, *685*, 321.
32. Yoshihiro, M.; Kyoji, U.; Tsuneji, U. J. Chromatogr. A. **1997**, *757*, 328.
33. Egger, M.D.; Liu, Y.; Sevčík, J.; Tesařová, E.; Rozhkov, R.; Larock, R.C.; Armstrong, D.W. Electrophoresis **2003**, *24*, 2650.
34. Lantz, A.; Rozhkov, R.V.; Larock, R.C.; Armstrong, D.W. Electrophoresis **2004**, *25*, 2727.
35. Fanali, S. J. Chromatogr. A. **2000**, *875*, 89.
36. Fanali, S. J. Chromatogr. **1989**, *474*, 441.
37. Rodriguez, M.A.; Liu, Y.; McCulla, R.; Jenks, W.S.; Armstrong, D.W. Electrophoresis **2002**, *23*, 1561.
38. Péter, A.; Török, G.; Armstrong, D.W.; Tóth, G.; Tourwé, D. J. Chromatogr. A. **2000**, *904*, 1.
39. Jiang, C.; Armstrong, D.W.; Péter, A.; Fülöp, F. Submitted to J. Liq. Chromatogr. Rel. Technol.
40. Gratz, S.; Stalcup, A.M. Anal. Chem. **1998**, *70*, 5166.

41. Armstrong, D.W.; Lee, J.T.; Chang, L.W. Tetrahedron: Asymm. **1998**, *9*, 2034.
42. Armstrong, D.W.; He, L.; Yu, T.; Lee, J.T.; Liu, Y. Tetrahedron: Asymm. **1999**, *10*, 37.
43. Wren, S.; Rowe, R. J. Chromatogr. A **1992**, *603*, 235.
44. Wren, S.; Rowe, R. J. Chromatogr. A **1992**, *609*, 363.
45. Grossman, P.D.; Colburn, J.C. *Capillary Electrophoresis, Theory and Practice*; Academic Press, 1992.
46. Vako, I.E.; Billiet, H.A.H.; Frank, J.; Luyben, K.C.A.M. Chromatographia **1994**, *38*, 730.
47. Gareil, P.; Gramond, J.P.; Guyon, F. J. Chromatogr. **1993**, *615*, 317.

Received January 10, 2007
Accepted January 31, 2007
Manuscript 6026