Optimized Chiral Separation of 20 Amino Acids Derivatized with 9-Fluorenylmethyl Chloroformate Using Cyclodextrins as Chiral Selectors in Capillary Electrophoresis

H. Wan and **L.G. Blomberg***

Department of Analytical Chemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, S-106 91, Stockholm, Sweden

Abstract

Direct chiral separation of DL-amino acids derivatized with 9-fluorenylmethyl chloroformate (FMOC-amino acids) is accomplished with micellar electrokinetic capillary chromatography. Separation parameters such as sodium dodecyl sulfate (SDS) and cyclodextrin concentrations are optimized by factorial designs. The interaction and effect of these variables on chiral recognition of FMOC-amino acids are presented in optimization charts. The enantioseparations are performed under optimized conditions by using γ-cyclodextrin (γ-CD) or β-cyclodextrin (β-CD) as chiral selectors. By using β-CD as a chiral selector, baseline separation of 20 FMOC-amino acids is achieved under some different conditions; 17 of the amino acids could be separated under the same conditions (50 mM phosphate, 50 mM SDS, 12 mM β-CD, and 15% 2-propanol). Application of γ-CD resulted in chiral separation of 12 FMOC-amino acids. For all FMOC-amino acids tested, the presence of 2-propanol was a prerequisite for a chiral separation when using β-CD as a chiral selector. Similarly, 2-propanol was needed in the majority of cases in which γ-CD was employed. Separation efficiencies are in the range of 10.7–0.9 × 10^5 theoretical plates per meter.

Introduction

Particular attention has been paid to the chiral separation of amino acids because of the different biological activities of their stereoisomers, and there is a growing interest in the application of capillary electrophoresis (CE) for such separations. To enhance detectability, a variety of derivatization reagents for amino acids have been introduced (1). In many cases, such derivatization has led not only to improved detectability, but also improved selectivity. Chiral separation with cyclodextrins as selectors is an example of this. A number of different types of amino acid derivatives have been chirally separated by CE. These include dansyl amino acids (2–6), 2,4-dinitrophenyl (DNP)-amino acids (7), and 1-cyano-2-substituted benz[*L*]isoindole (CBI)-amino acids (8,9).

Various approaches have been taken in the chiral separation of the different amino acid derivatives. Gassmann and coworkers introduced chiral chelating reagents to resolve dansyl DL-amino acids on the basis of a ligand-exchange mechanism in CE (10,11). Using this method, Fanali and coworkers. (12) showed the chiral separation of some amino acids. Snopek and coworkers employed cyclodextrin in micellar electrokinetic capillary chromatography (MEKC) for the separation of optical isomers (13), and similar systems have subsequently been applied to the separation of derivatized amino acids. Guttman and coworkers were able to separate 12 dansyl-amino acids using a gel-filled cyclodextrin column (2). Moreover, some chiral surfactants (14–18) as well as a polysurfactant, poly[sodium(10-undecenoyl)-*L*-valinate] (19) have been employed for chiral separation of phenylhydantoin (PTH)-amino acids and *N*-(3,5)-dinitrobenzoyl-*o*-isopropyl ester derivatized amino acids. The separation of six PTH-amino acids and also *N*-(3,5)-dinitrobenzoyl-*o*-isopropyl ester derivatized amino acids were thus obtained by sodium *N*-dodecanoyl-*L*-valinate (SDVal) and sodium dodecyl sulfate (SDS)(15). In addition, Kuhn and coworkers performed chiral separation of eight underivatized amino acids by using a crown ether as a chiral selector; an ultraviolet (UV) detector was used (20). Some modified cyclodextrins (21,22), dextrin (23), proteins (7), and macrocyclic antibiotics (24) were employed for the separation of dansyl amino acids (7,23) and also for the separation of several structurally related amino acid derivatives (21,24).

An alternative approach is indirect (diastereomeric) separation of amino acids derivatized with chiral reagents. Some different reagents have been employed for this type of derivatization. These are Marfey's reagent (25), 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) (26), *o*-phthalaldehyde (OPA) with a chiral mercaptan (27), and 1-(9-fluorenyl)-ethyl chloroformate (FLEC) (28). Schützner and coworkers described the diastereomeric separation of tryptophan with the addition of polyvinyl pyrrolidone to the background electrolyte (29). Nishi and coworkers (26) demonstrated the optical resolution of 19 GITC-amino acids; 15 of these were baseline-separated, and 13 GITC-amino acids were simultaneously resolved. Using a chiral FLEC reagent, Wan and coworkers (28) achieved the resolution of 15 FLEC-amino acids and the separation of a

* Author to whom correspondence should be addressed.

mixture of 10 FLEC-amino acids in less than 10 min. Moreover, Bonfichi and coworkers separated 4 amino acids derivatized with (*S*)-(1-naphthyl)ethyl isothiocyanates and (*S*)-phenylethyl isothiocyanates (30). It seems that indirect separation offers advantages such as superior selectivity and the ease of optimization of separation conditions; however, the success of this method relies on the optical purity of the chiral reagents (31).

Although a number of chiral selectors have been successfully applied to many of the above mentioned amino acid derivatives, the chiral separation of all protein amino acids derivatized with a particular reagent has not yet been achieved in CE. Among the different chiral selectors investigated, promising results have been shown with cyclodextrins as chiral selectors. The virtues of cyclodextrins include high selectivity for different enantiomers, simplicity of separation buffers, good stability over a wide range of pH, and no absorption in UV. However, the chiral recognition mechanisms are still not well understood. It may thus be anticipated that the separation of large chiral molecules by means of cyclodextrins would be less successful. Nevertheless, the present authors have demonstrated chiral separation using two types of amino acid derivatives that possess relatively large aromatic moieties. These are 9-fluorenylmethyl chloroformate (FMOC) and 2-(9-anthryl) ethyl chloroformate (AEOC); the separations were achieved with the use of cyclodextrins as chiral selectors in CE (28,32). Under optimized separation conditions, 15 FMOC- and 12 AEOC-amino acids out of 19 tested amino acids could be chirally separated using β-cyclodextrin (β-CD) and a particular organic modifier, 2-propanol. An enhancement of resolution with SDS was observed for the separation of AEOC-amino acids in the presence of 2-propanol (32). Furthermore, the reversal of enantiomeric elution order with organic modifier was found for some AEOC-amino acids when γ-cyclodextrin (γ-CD) was employed for the separation of AEOC-amino acids.

In direct chiral separations, a number of variables have to be considered when optimal conditions are sought. Several of these may be interrelated, and a univariate approach to the optimization, thus trying to optimize one variable at a time, will, in general, not result in optimal conditions. Also, nonlinear models are often required to describe resolution as a function of the separation variables. For these reasons, a multivariate approach must be adopted for the optimization (33).

Table I. Experimental Values for Optimization

Factors	SDS (mM)	γ-CD (mM)
Low	20	6
High	40	14
Exp. 1	20	6
Exp. 2	40	6
Exp. 3	20	14
Exp. 4	40	14
Exp. 5	15.8579	10
Exp. 6	44.1421	10
Exp. 7	30	4.24315
Exp. 8	30	15.6569
Exp. 9	30	10
Exp. 10	30	10
Exp. 11	30	10

The present work is an extension of an earlier investigation of the optimal CE conditions for the separation of FMOC-amino acids with β-CD as a chiral selector (28). A comparison of the application of γ-CD and β-CD as chiral selectors is thus made. The optimization is more comprehensive than before, leading to an improved understanding of the separation system and facilitating the chiral separation of 20 primary protein amino acids derivatized with FMOC.

Experimental

Apparatus

All CE separations were carried out on a Prince (Lauerlabs, Emmen, The Netherlands) CE instrument equipped with a UV detector (256 nm), CV[4] (ISCO, Lincoln, NE) and a high voltage supply (0–30 kV). An untreated fused-silica capillary column (67 cm × 25-µm i.d., 45 cm to detector window) obtained from Polymicro Technologies (Phoenix, AZ) was employed as a separation column. After each run, the column was cleaned for 5 min with 0.2M NaOH to which 10% (v/v) methanol had been added, followed by cleaning with water for 5 min. Prior to sample introduction, the column was equilibrated with running

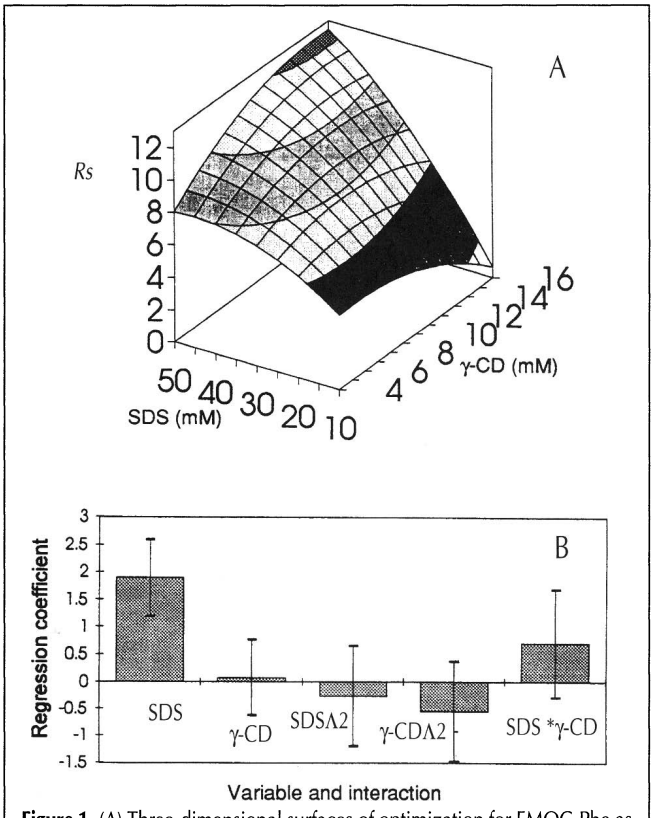

Figure 1. (A) Three-dimensional surfaces of optimization for FMOC-Phe as a function of SDS and γ-CD concentrations. (B) Chart showing the interaction of variables in the optimization of separation of FMOC-Phe. Conditions: buffer, 50 mM phosphate (pH=7.5), 15% 2-propanol (v/v); column, 67 cm × 25-µm i.d. (45 cm to detector). 25 kV; temperature, 25°C; UV detection at 256 nm.

buffer for 8 min. The temperature of the column was controlled at 25°C. All buffer concentrations, SDS concentrations, and β-CD or γ-CD concentrations are given as they were before the addition of organic modifiers. In all cases, 50 mM phosphate was used as a background electrolyte. The data were collected by using an ELDS 900 laboratory data system (Chromatography Data Systems, Kungshög, Sweden).

Reagents

A set of 20 DL-amino acids (see Table II), β-CD, and γ-CD was from Sigma (St. Louis, MO). SDS and the derivatization reagent FMOC were obtained from Fluka (Buchs, Switzerland). Other chemicals used in this work were of analytical grade.

Derivatization

The procedure for derivatization of amino acids with FMOC was as described earlier (28). Derivatization with the FMOC reagent was rapid and simple. Good stability of the derivatives was observed; almost identical electropherograms were achieved before and after storage of the derivatives for more than six months in a buffer solution (pH = 8–9) at 4°C. The concentration of injected FMOC-amino acids was approximately 100 µM.

Table II. Separation Data of 20 DL-FMOC-Amino Acids with γ-CD as a Chiral Selector

FMOC-amino acids	0% IPA			15% IPA		
	t (min)*	*Rs*	$N \times 10^5$/m	*t* (min)†	*Rs*	$N \times 10^5$/m
Ala	10.58	0	2.28	15.60	0.89	8.00
Arg	20.85	0	6.22	28.27	1.08	8.97
Asn	11.15	0	4.36	18.82	0	3.16
Asp	10.38	0	4.71	17.48	0	3.24
Gln	10.82	0	5.73	18.33	0	3.24
Glu	10.07	0	3.91	17.13	0	2.16
His	22.42	0	4.51	17.04	3.11	10.0
Ile	14.78	2.14	6.93	18.41	1.93	9.73
Leu	17.18	0	2.38	19.85	1.46	10.7
Lys	28.27	0	6.98	71.59	4.17	5.13
Met	14.39	0.97	5.96	16.78	1.97	9.07
Nleu	17.43	0.82	5.93	21.15	2.58	8.42
Nval	13.59	0	5.20	18.16	< 0.5	2.13
Phe	18.31	0	5.47	21.27	10.35	10.3
Pro	12.65	0	0.31	17.20	0	0.64
Ser	10.24	0	7.07	15.22	0	7.78
Thr	10.30	0	8.58	14.58	2.09	9.33
Trp	22.68	< 0.5	1.51	29.54	0.58	4.00
Tyr	13.24	2.14	8.53	15.88	7.34	9.73
Val	11.66	< 0.5	1.78	16.08	2.94	8.64

* Conditions: buffer, 50 mM phosphate, pH = 7.5, 50 mM SDS, 12 mM γ-CD; 25 kV; current, 18 µA; capillary temperature, 25°C; detection at 256 nm.
† 15% 2-propanol (IPA) (v/v); 11 µA; otherwise same as (*).

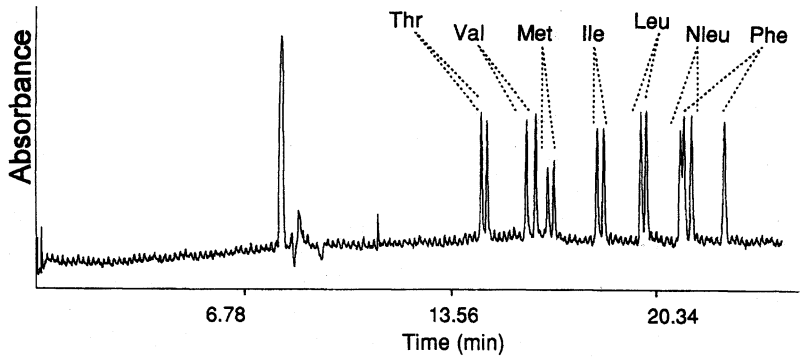

Figure 2. Micellar electrokinetic chromatogram showing D/L separation of 7 FMOC-amino acids. Conditions: buffer, 50 mM phosphate (pH=7.5), 50 mM SDS, 12 mM γ-CD, 15% (v/v) 2-propanol; current, 11 µA; other conditions as in Figure 1.

Calculation of parameters

Resolution (*Rs*) was calculated by conventional methods. Statistical experimental design for the optimization experiments was done in Codex (Sum IT System AB, Sollentuna, Sweden). For simplicity, separation factor α was not calculated. To facilitate comparison with previous work, separation efficiency is expressed as the number of theoretical plates per meter.

Results and Discussion

Optimization of separation with γ-CD

It has been demonstrated that optimization of separation variables by means of statistical techniques is an efficient method for enhancing separation (28). In general, prior to the optimization of separation conditions, a group of scouting experiments must be performed to find conditions that provide selectivity. Moreover, the scouting experiments indicate which parameters primarily influence the chiral separation. In fact, without scouting, the optimization efforts could be directed toward less important variables, and optimal conditions may not be found. Optimization of key parameters can undoubtedly lead to increased resolution. In addition, an improved understanding of the interactions between these parameters can be achieved from the optimization.

When γ-CD was utilized as a chiral selector to examine the separation of FMOC-amino acids, a number of scouting experiments were performed in which four parameters were considered: pH, SDS, γ-CD, and organic modifier (2-propanol). Two sets of scouting experiments were performed: one was performed at a pH of 6.0 in a capillary zone electrophoresis (CZE) system in the presence and absence of 15% 2-propanol; the other set was performed at a pH of 7.5 in MEKC in the absence and presence of 15% 2-propanol. Two FMOC-

amino acids with different hydrophobicity, Ala and Phe, were used as test samples. The results in both CZE and MEKC modes showed that for Ala, selectivity was observed only in the presence of 2-propanol. Methanol, acetonitrile, and tetrahydrofuran (THF) were also tested, but no chiral recognition was achieved with them. Furthermore, the presence of SDS and 2-propanol in MEKC resulted in improved resolution for both Ala and Phe. These results were in accordance with those obtained from the separation of AEOC-amino acids in which β-CD and γ-CD were used as chiral selectors (32). In our experience, an organic modifier concentration of 15% (v/v) usually results in the best separation for the types of systems examined here, provided that its presence is necessary for chiral recognition. Therefore, an optimization involving SDS and γ-CD, including a total of 11 experiments, was performed at pH 7.5 in the presence of 15% (v/v) 2-propanol. Discrete concentration data are given in Table I. The response surface for the resolution of FMOC-Phe is shown in Figure 1A, from which it is apparent that the resolution of FMOC-Phe was enhanced with increasing SDS concentrations and that the γ-CD concentration did not affect the resolution to a large degree. It should be noted that an increase in γ-CD concentration resulted in decreased migration times. Furthermore, separation efficiency increased with γ-CD concentration.

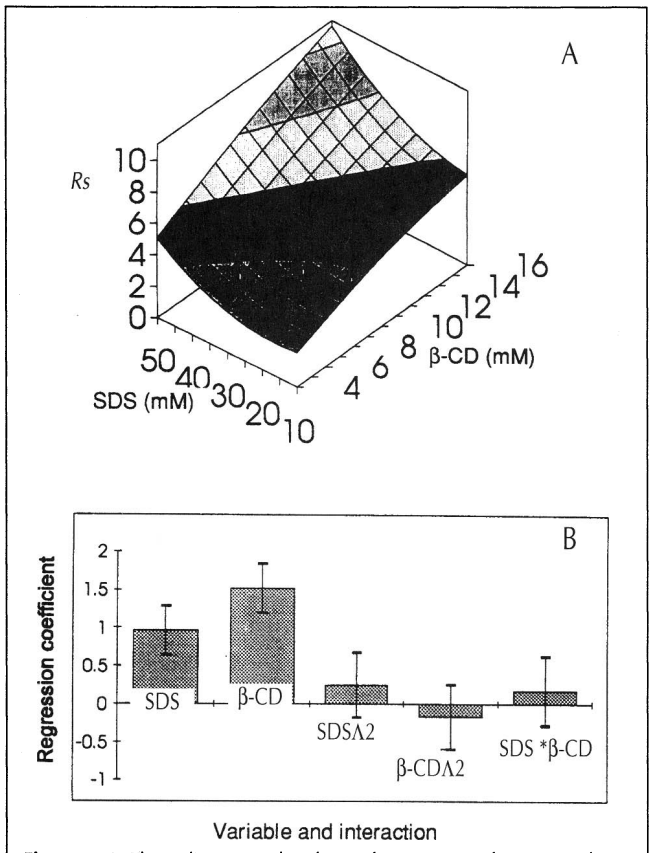

Figure 3. (A) Three-dimensional surfaces of optimization for FMOC-Phe as a function of SDS and β-CD concentrations. (B) Chart showing the interaction of variables in the optimization of separation of FMOC-Phe. Conditions: buffer, 50 mM phosphate (pH=7.5), 15% 2-propanol (v/v); other conditions as in Figure 1.

The results of the optimization indicated that SDS concentration was a key variable, but γ-CD concentration was not (see Figure 1B). Also, there was no mutual interaction between SDS and γ-CD concentrations. A 50-mM phosphate buffer containing 50 mM SDS, 12 mM γ-CD, and 15% 2-propanol was considered optimal to attempt separation of all FMOC-amino acids. Moreover, the same buffer in the absence of organic modifier was used to examine the selectivity for 20 FMOC-amino acids. The results shown in Table II indicate that only two FMOC-amino acids, Ile and Tyr, were well-separated, and five other FMOC-amino acids, Ala, Met, Nleu, Trp, and Val, were partially or near-baseline separated in the absence of 2-propanol; the D-form eluted first in all cases. On the other hand, the addition of 15% 2-propanol resulted in a considerable improvement of the chiral resolutions of the major part of the FMOC-amino acids. As a result, a baseline separation of 10 FMOC-amino acids could be achieved (see Table II). The D-form eluted first in all cases, which indicated that the stability of the complex D-isomer–CD was stronger than that of L-isomer–CD. The chiral separation of a mixture of 7 FMOC-amino acids is shown in Figure 2.

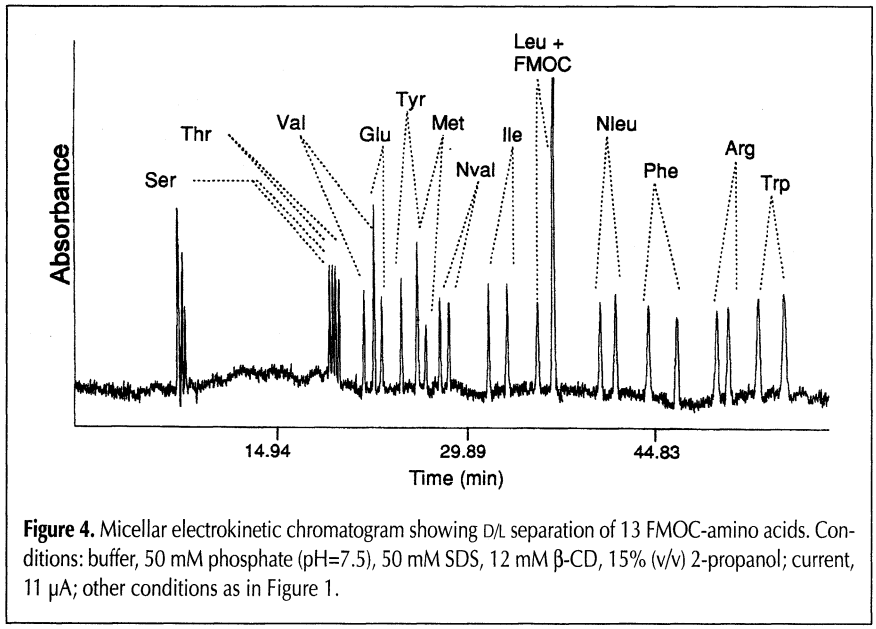

Figure 4. Micellar electrokinetic chromatogram showing D/L separation of 13 FMOC-amino acids. Conditions: buffer, 50 mM phosphate (pH=7.5), 50 mM SDS, 12 mM β-CD, 15% (v/v) 2-propanol; current, 11 μA; other conditions as in Figure 1.

Enantioselectivity of β-CD toward FMOC-amino acids

For the separation of FMOC-amino acids with β-CD as chiral selector, β-CD concentration and the presence of an organic modifier, 2-propanol, were found to be the main factors influencing chiral recognition (28). In the present work, we wanted to compare the results that were obtained with β-CD and γ-CD. Thus, an optimization was performed concerning the same variables as in the optimization for γ-CD as a selector. The variables were SDS and β-CD concentration, and the 2-propanol concentration was maintained at 15%. The optimization of two FMOC-amino acids, Ala and Phe, gave similar results. A response surface of the optimiza-

tion of FMOC-Phe separation is shown in Figure 3A. It is clear from the optimization that higher SDS and β-CD concentrations result in a higher resolution of FMOC-Phe. It should be noted that β-CD has a relatively limited water solubility of approximately 16 mM (34). On the other hand, solubility was improved in the presence of SDS. Thus, the β-CD was fully soluble under the applied conditions.

Figure 3B shows that SDS and β-CD concentrations are key variables. This is in contradiction to earlier work in which SDS concentration was found to be of less importance (28). The explanation for this difference is that the range of SDS concentrations that were examined was too narrow (5–18 mM)(28). In the present work, the range was 10–50 mM SDS, which gave a more accurate picture of the importance of the variable. This illustrates an important aspect of the optimization.

In the presence of 15% 2-propanol, baseline separation of 17 FMOC-amino acids was achieved by using the optimized conditions (see Table III); the D-form eluted first in all cases. Optimal SDS concentration was different for different amino acids. Thus, early-eluting amino acids were separated only in buffers that contained relatively high SDS concentrations, and low SDS concentration was needed for the late-eluting amino acids. This is illustrated in Figure 4, which shows the separation of a mixture of relatively hydrophilic amino acids. To separate the first peaks, Ser and Thr, a relatively high SDS concentration of 50 mM, was applied. As a consequence, the migration time of the last peak, Trp, was relatively long, (approximately 55 min). In an earlier paper (28), we were aiming at the separation of a mixture containing amino acids that were more hydrophobic (e.g., Glu and Asp). Therefore, only 15 mM SDS was employed in that case, and the elution time of Trp was then much shorter (25 min).

It can be seen from Figure 4 that the L-FMOC-Leu coeluted with the residues of the derivatization reagent FMOC, but this peak could be separated from the FMOC reagent peak by means of a minor alteration of either the SDS or β-CD concentrations. Moreover, Ala was near-baseline separated, and Pro was partially resolved. However, no peak was observed for Lys, even after 80 min. It can be postulated that stronger interaction could occur between FMOC-Lys and SDS due to the fact that Lys possesses two primary amino groups and, consequently, could form a doubly labeled FMOC-derivative (35). In addition, it can been seen from Figure 3B that β-CD affected the chiral separation more than SDS, but that these two factors did not interact with each other. Thus, it could be expected that further optimization of SDS or β-CD concentrations might result in improved chiral separation of Ala, Pro, and Lys. By following this approach when decreasing the SDS concentration to 25 mM, the baseline separation of Lys was achieved (see Table III). Alternatively, when the β-CD concentration was increased to 20 mM, Ala and Pro were chirally baseline separated.

Table III. Separation Data of 20 DL-FMOC-Amino Acids with β-CD as a Chiral Selector

FMOC-amino acids	0% IPA			15% IPA		
	t (min)*	*Rs*	$N \times 10^5$/m	*t* (min)†	*Rs*	$N \times 10^5$/m
Ala	15.96	0	4.80	21.72	0.90	2.51
Ala‡	–	–	–	28.43‡	1.85	2.58
Arg	23.06	0	2.33	50.89	2.38	2.87
Asn	13.24	0	3.24	24.88	2.17	5.58
Asp	22.82	0	4.37	24.18	1.57	2.52
Gln	13.22	0	3.33	24.15	2.43	6.33
Glu	22.85	0	4.23	23.55	3.32	2.88
His	20.57	0	4.23	25.50	4.24	2.40
Ile	18.72	1.62	4.00	32.61	6.52	3.14
Leu	20.88	0	3.20	36.60	3.85	2.10
Lys§	26.63	0	4.91	60.45	1.51	1.28
Met	17.40	0	3.27	26.94	3.02	2.58
Nleu	20.93	0	3.49	41.62	3.72	2.88
Nval	16.51	0	2.92	28.72	2.84	2.34
Phe	22.87	0	3.21	45.43	5.60	2.20
Pro	15.24	0	0.81	23.98	< 0.5	0.56
Pro‡	–	–	–	28.74‡	1.49	0.40
Ser	12.78	0	4.62	20.03	1.80	3.21
Thr	12.34	0	2.05	20.50	2.14	3.56
Trp	24.26	0	4.06	54.25	4.62	2.49
Tyr	14.64	0	1.29	25.67	5.82	2.52
Val	15.20	0	2.53	22.75	4.53	2.75

* Conditions: buffer, 50 mM phosphate, pH = 7.5, 50 mM SDS, 12 mM β-CD; 25 kV; current, 18 μA; capillary temperature, 25°C; detection at 256 nm.
† 15% 2-propanol (IPA) (v/v); 11 μA; otherwise same as (*).
‡ 15% 2-propanol (IPA) (v/v); 20 mM β-CD; otherwise same as (*).
§ 15% 2-propanol (IPA) (v/v) and 20 mM SDS; current, 8.8 μA; otherwise same as (*).

As shown in Tables II and III, chiral recognition was not obtained for any of the 20 examined FMOC-amino acids in the absence of 2-propanol, except for Ile, when β-CD was used as a chiral selector. Ile is an exception because it occurs as diastereomers. Evidently, the organic modifier, 2-propanol, played an important role in the chiral separation of these FMOC-amino acids. The influence of organic modifiers on chiral separation in CE has been demonstrated in the literature (36–38). In the present case, 2-propanol probably modified the cavity of CDs (39) or degraded the interaction between CDs and the fluorene moiety, the hydrophobic part of FMOC-amino acids; this could have been achieved by loosening the hydrogen bonding between the hydroxyls of CDs and the amide bonds of FMOC-amino acids. As a result, a better fit or improved steric interaction could have occurred between analytes and CDs. This can be supported by recent computational studies on the dynamic properties of CDs. It was proposed that CDs should not be considered to occur as rigid cones but rather as flexible, twisting baskets (40). It has been suggested that the hydrophobic chain of SDS could enter the cavity of CDs (41) and modify the cavity of cyclodextrin. However, in the present systems, no mutual interactions between SDS and β- and γ-CD that affect resolution occurred (Figures 1B and 3B). The optimization showed that the SDS

concentration had a positive effect on the increase of selectivity for FMOC-amino acids. The observed selectivity enhancement with increasing SDS concentrations could be attributed to the decreased interaction between SDS monomers and CDs as a consequence of the decreased polarity of the buffer system, which resulted in improved interaction between CDs and FMOC-amino acids. In addition, it is evident from Tables I and II that the presence of 2-propanol in general led to increased separation efficiency for FMOC-amino acids when γ-CD was applied as a chiral selector, but that the efficiency was decreased in buffers that contained β-CD. This suggests that the speed of dynamic exchange between FMOC-amino acids and CDs differed as a consequence of the differences in CD cavity sizes. It may be speculated that there is a difference in 2-propanol–CD interaction for the two types of cyclodextrins. Furthermore, it should be noted that the presence of 2-propanol did not lead to the reversal of enantiomeric elution order for any of the FMOC-amino acids examined, as was the case in the separation of AEOC-amino acids in MEKC with γ-CD as a chiral selector (32). In their studies of the interaction mechanisms of alanine β-naphthylamide and different types of CD, Tanaka and coworkers demonstrated that the active mechanisms of interaction were highly dependent on the type and substitution of the CD (42). Similarly, the effects of 2-propanol on the chiral recognition of FMOC-amino acids and AEOC-amino acids could be quite different, although these two types of derivatives have similar structures and dimensions.

Conclusion

By means of optimization, it has been demonstrated that the selectivity for FMOC-amino acids could be enhanced with increasing SDS concentrations, using either β- or γ-CD as chiral selectors. Furthermore, the β-CD concentration was found to be the dominating factor influencing the chiral separation of FMOC-amino acids. In contrast, γ-CD concentration had an insignificant effect on chiral recognition of FMOC-amino acids. Simultaneous optimization of multiple separation variables provided an improved understanding of the interaction between the different variables. Therefore, the separation systems could be efficiently controlled by varying key separation factors to attain the desired separations for different FMOC-amino acids according to the optimization results. However, it should be noted that the selection of the experimental domain is of crucial importance; application of a domain that is too small can lead to misleading results.

Chiral baseline separations of all 20 FMOC-amino acids examined were successfully achieved using β-CD as a chiral selector. Evidently, β-CD offered better selectivity than γ-CD for chiral resolution of all FMOC-amino acids. However, somewhat lower efficiency was obtained with β-CD than with γ-CD; this is probably due to the relatively slow kinetics of the interaction between FMOC-amino acids and β-CD. An organic modifier, 2-propanol, was found to be indispensable for the chiral recognition of all 20 FMOC-amino acids when β-CD was employed as a chiral selector. Similarly, when γ-CD was used as a chiral selector, the application of 2-propanol resulted in a substantial improvement in the resolution of most FMOC-amino acids.

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