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Free-solution capillary electrophoretic resolution of chiral amino acids via derivatization with homochiral isothiocyanates. Part I

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Abstract

Within the framework of a more general study aimed at the enantiomeric resolution of non-UV-absorbing chiral amino acids via derivatization with chiral isothiocyanates, we have examined the applicability of two such derivatizing agents, (*S*)-1-(1-naphthyl)ethyl isothiocyanate (SNEIT) and (*S*)-1-phenylethyl isothiocyanate (SAMBI), to the resolution of the enantiomers of alanine, phenylalanine, and valine in free-solution capillary electrophoresis (FSCE). Isothiocyanates have distinct advantages as chiral derivatizing agents in enantiospecific chromatographic analysis, and the two reagents were readily synthesized from commercially available reagents. SNEIT, previously not fully described in the literature, was characterized by rigorous physicochemical and spectroscopic means. The two diastereoisomeric thiourea derivatives of each amino acid were separated by FSCE. Heptakis-2,3,6-tri-O-methyl-β-cyclodextrin was effective in assuring the solubility of the derivatives in the working buffer and was more efficient than β-cyclodextrin in both dissolving the thioureas and improving the electrophoretic resolution. Enantiomeric pairs migrated in the order L before D. Under the conditions used SAMBI-derivatized amino acids had longer elution times than the corresponding SNEIT derivatives. The diastereomeric derivatives of valine and of phenylalanine had larger separation factors α than the corresponding SAMBI derivatives, while the derivatives of alanine had nearly identical α values with the two derivatizing agents. The two reagents may be advantageous in the enantiospecific analysis of amino acids, and it appears that further exploration of these and other similar reagents is warranted.

1. Introduction

Recent studies carried out in our Research Center have been aimed at the enantiomeric separation and identification of chiral amino acids in biological matrices [1] using 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT) as chiral derivatizing agent in conjunction with HPCE. Isothiocyanates react with primary or secondary amines to produce the corresponding thioureas (Fig. 1).

If the isothiocyanate used in the derivatization of enantiomerically related amines is itself chiral and is used in a single-enantiomeric form, the derivative thioureas will be diastereomeric. This is the basis of the so-called "indirect method" in enantiospecific chromatographic analysis [2]. Because diastereomers are characterized by different electrophoretic mobilities, the indirect

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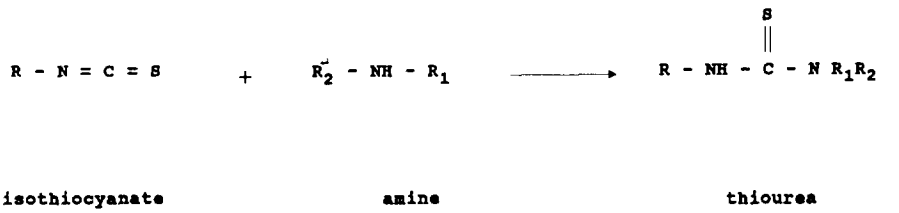

Fig. 1. General reaction scheme for the derivatization of amino groups with isothiocyanates.

method represents an extremely useful tool for the enantiomer separation by HPCE.

A chiral isothiocyanate was chosen for our studies because of the well-documented advantages of this reagent family:

–the reaction with the amino group is rapid and proceeds under mild conditions [2];

–the derivatization reaction can be carried out in aqueous media, a critical advantage in the analysis of amino acids and other polar analytes;

–the derivatization procedure is simpler and more rapid than that with many other chiral derivatizing agents, (see, for instance, Ref. [3] vs. Ref. [4]);

–the diastereomers can often be readily resolved by HPLC [3,5,6] or MEKC [7].

–the thiourea moiety has significant UV absorbing properties, which can enhance detectability;

–chiral isothiocyanates are often less expensive than other commercially available chiral derivatizing agents.

These advantages render chiral isothiocyanates highly useful for enantiospecific analysis and suggested to us that–as an extension and refinement of our above-mentioned work [1] with TAGIT–it would be of interest to investigate the potential applicability of other chiral isothiocyanates to the characterization and chiral separation of amino acids by using HPCE, an analytical technique whose considerable advantages in pharmaceutical/biomedical analysis are very well documented [8].

The state-of-the-art in enantiomeric separations by HPCE has recently been thoroughly discussed by Fanali et al. [9]. From Fanali's review and other technical literature references it emerges that, while several chiral isothiocyanates (in addition to TAGIT) have been used in the HPLC separation of diastereomeric derivatives of enantiomeric compounds [10], these reagents have been nearly completely ignored in HPCE work. The only exception is represented by Nishi et al. [7] who described recently the resolution of the enantiomers of a series of amino acids via derivatization with TAGIT followed by separation using MEKC. In fact, although several papers have dealt with the indirect resolution method in HPCE [11,12], apart from Nishi's work, it appears that no other studies were published on the application of chiral isothiocyanates in HPCE.

For the present study we chose (*S*)-1-(1-naphthyl)ethyl isothiocyanate (SNEIT, Fig. 2) and (*S*)-α-methylbenzyl isothiocyanate (SAMBI, Fig. 2), primarily because of the presence in these molecules of highly lipophilic organic moieties and UV-absorbing chromophores. Further-

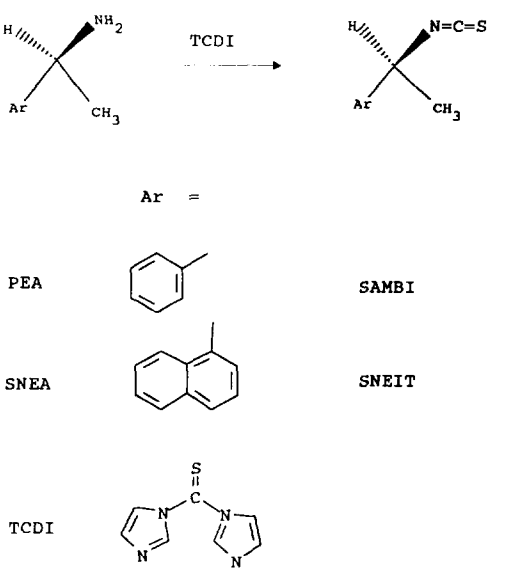

Fig. 2. Reaction scheme for the synthesis of the chiral isothiocyanates SAMBI and SNEIT obtained from the corresponding precursor chiral primary amines.

more, both SNEIT and SAMBI could be readily synthesized in one step from the corresponding precursor primary amines which are commercially available reagents.

In this report we describe our findings on the applicability of the two chiral isothiocyanate reagents to the enantiomeric resolution of selected amino acids by free-solution capillary electrophoresis (FSCE).

2. Experimental

2.1. Instrumentation

All separations were carried out on a SpectraPhoresis 1000 (Thermo Separation Products, CA, USA) instrument equipped with a programmable, high-speed scanning, multiple-wavelength UV–Vis detector. Data acquisition, handling and reporting were supported by the multi-tasking SpectraPhoresis CE 1000 version 1.05c and by the PC1000 version 2.0 software packages running on a Spectra 386E/25MHz personal computer equipped with a Hewlett-Packard (Avondale, CA, USA) laser jet Model IIp printer.

Elemental analysis was performed on an EA 1106 EAGER 200 instrument (Carlo Erba, Milan, Italy); differential scanning calorimetry was carried out using a DSC 910 TA2000 instrument (DuPont, Wilmington, DE, USA); ^{1}H NMR spectra were recorded in $CDCl_3$ on an AM 500 NMR spectrometer (Bruker, Karlsruhe, Germany); mass spectra were obtained using an EI-TSQ 700 instrument (Finnigan Mat, San Jose, CA, USA), with a source temperature of 150°C, electron energy: 70 eV; filament current: 200 mA. IR spectra were recorded in nujol using an IFS 48 FT-IR spectrometer (Bruker, Karlsruhe, Germany); optical rotations were obtained using a Model 241 polarimeter (Perkin-Elmer, Palo Alto, CA, USA).

2.2. Reagents and materials

(*S*)-(−)-1-(1-Naphthyl)ethylamine, (*S*)-(−)-α-methylbenzylamine, and N,N′-thiocarbonyldiimidazole were obtained from Aldrich (Milwaukee, WI, USA); all the solvents used were of HPLC grade (Carlo Erba, Milan, Italy); the salts were reagents of analytical grade (Carlo Erba). Water was obtained from a Milli-Q Plus purification system (Millipore, Bedford, MA, USA). Silica gel HPTLC plates, 60 F_{254}, 5 × 10 cm, layer thickness 0.25 mm, were from Merck (Darmstadt, Germany).

2.3. Synthesis and characterization of (S)-1-(1-naphthyl)ethyl isothiocyanate (SNEIT)

To a stirred solution of N,N′-thiocarbonyldiimidazole (TCDI, 500 mg, 2.8 mmol) in dichloromethane (20 ml) in a 100-ml round-bottomed flask was added dropwise over 3.5 h a solution of (*S*)-1-(1-naphthyl)ethyl amine (SNEA) (411 mg, 2.4 mmol) in dichloromethane (30 ml) in an argon atmosphere at room temperature [13]. The reaction was monitored by TLC with an eluent of *n*-hexane–methanol (99:1, v/v); SNEIT $r_F = 0.68$. After completion of the addition vigorous stirring was continued for an additional hour to assure completion of the reaction. The reaction mixture was then transferred into a separatory funnel and washed with a 5% aqueous solution of sodium bicarbonate (3 × 30 ml), followed by water (3 × 30 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent evaporated in vacuo on a rotary evaporator. The yellow solid obtained was purified by recrystallization from absolute ethanol to obtain the expected product in 85% yield. Found: C: 72.9%; H: 5.2%; N: 6.5%; S: 14.2%; calculated for; $C_{13}H_{11}SN$: C: 73.2%; H: 5.2%; N: 6.6%; S 15.0%.

Although SNEIT has been previously used in the HPLC resolution of enantiomeric amines [14,15], a search of the literature produced no published data on its rigorous identification and physicochemical characterization. In order to fully characterize the reagent, therefore, we obtained, in addition to its elemental analysis (see under Experimental), its NMR, IR, and mass spectra, and examined its DSC. The NMR spectrum [δ, ppm; $\delta_{TMS} = 0$, 7.95–7.84 (2 H, m), 7.849 (1 H, d), 7.645 (1 H, d), 7.585–7.268 (3

H, m), 5.723 (1 H, q), 1.862 (3 H, d)], the IR spectrum [main absorption bands: 3051 (ν arom C–H); 2127 (ν S=C=N); 1597, 1512 (ν C=C); 805, 798 (γ arom C–H) cm^{-1}], and the mass spectrum [m/z 213 ($M^{+\cdot}$); m/z 155 ($M^{+\cdot}$ – SCN)] were in agreement with the structure of SNEIT. DSC analysis confirmed that the sample obtained was highly crystalline and showed a melting peak (T_{onset} 45.36°C, T_{final} 46.54°C; $\Delta H_f = 82.61$ J/g) typical of a compound of high purity. Decomposition occurred above 200°C.

The specific rotation of SNEIT was measured: $[\alpha]_D^{20} = +114.2$ ($CHCl_3$; concentration 0.5% w/v). Thus, the starting levorotatory amine of *S* configuration yields the dextrorotatory isothiocyanate of *S* configuration, i.e. the direction of rotation is reversed between starting material and product; the reaction does not affect the actual configuration of the stereogenic center, and there is no change in the Cahn–Ingold–Prelog priority order of substituents at the stereogenic center.

2.4. Synthesis and characterization of (S)-(+)-α-methylbenzyl isothiocyanate (SAMBI)

The reaction was carried out and worked up as described above for SNEIT and using the same molar ratios, with (*S*)-(−)-α-phenylethyl amine (SPEA) instead of SNEA. The reaction was monitored using the TLC system used in the synthesis of SNEIT; SAMBI $R_F = 0.74$. The yellow oil obtained in the reaction was purified by distillation, bp 114–116°C, yielding the title compound as a colourless oil in 51% yield.

SAMBI [(*S*)-(+)-1-phenylethyl isothiocyanate] has been previously characterized [16]. The NMR and IR spectra (data not shown) of our sample of SAMBI were in agreement with the expected structure and with the literature [17,18], and its mass-spectrum [m/z 163 ($M^{+\cdot}$); m/z 104 ($M^{+\cdot}$ – SCN)] and elemental analysis was also in agreement with the expected structure. Our specific rotation for SNEIT in chloroform ($[\alpha]_D^{20} = +14.6$ ($CHCl_3$; 0.5% w/v)), was different from the specific rotation obtained in toluene ($[\alpha]_D^{20} = +53.1$) [18]. For SAMBI also, the direction of optical rotation is reversed between the precursor (*S*)-(−)-1-phenylethylamine and the isothiocyanate.

2.5. Background electrolyte (BGE) and working buffer (WB) preparation

The BGE used during this study consisted of a 50 m*M* $Na_2B_4O_7$ solution at pH 10.

Within the context of this study, if not otherwise stated, "working buffer" (or WB) is intended to indicate a 20 m*M* solution of heptakis-2,3,6-tri-O-methyl-β-cyclodextrin in the BGE.

2.6. Derivatizing solutions

SNEIT or SAMBI (20 mg) was diluted to 1 ml with acetonitrile. The solution was swirl-mixed until complete dissolution and then directly used. Care was taken to protect the vial from light by covering the container with aluminium foil. The reagents in the derivatizing solutions were stable for several weeks provided the vial was tightly closed and protected from light.

2.7. Amino acid reference solutions

For each of the amino acids L-valine, D,L-valine; L-alanine, D,L-alanine; L-phenylalanine, D,L-phenylalanine, a reference solution was prepared by diluting ca. 5 mg of the amino acid to 1 ml with H_2O–CH_3CN (50:50, v/v) containing 0.4% (w/v) of triethylamine (TEA).

2.8. Derivatizations with the chiral isothiocyanates

SAMBI: Into a 300-μl glass vial were placed, in order, 50 μl of amino acid standard solution (equivalent to 2.1 μmole of amino acid), and 20 μl of SAMBI solution in CH_3CN (equivalent to 2.4 μmole of SAMBI). After swirl-mixing, the vial was transferred into a Thermoline dry bath (Thermolyne Corp., Dubuque, IA, USA) thermostatted at 60°C and left to stand for 30 min. A 25-μl aliquot of working buffer was then added and the mixture was swirl-mixed for a few seconds. The vial was then transferred into the satellite carousel of the autosampler and the

sample injected according to the method specifications given below.

SNEIT: The procedure described above for SAMBI was also used for SNEIT, with two exceptions: (a) the aliquot of derivatizing agent was 25 μl of SNEIT solution (equivalent to ca. 2.3 μmole of SNEIT), and (b) in a few cases it was necessary to dilute the final contents of the vial 1:1 with MeOH to dissolve the resulting thioureas.

2.9. Operation conditions

Unless otherwise stated, the electropherograms reported in this work were obtained using the following conditions:

–Capillary: l = 440 mm (370 mm from injector to detector), I.D. 50 μm

–Applied voltage: 10 kV for 20 min.

–Prefill of the capillary: 1 min with the working buffer.

–Injection: It was performed hydrodynamically for 1 s. By using the Poiseuille equation [19], it can be estimated that, in the assumption of $\eta = 1$ cP, the amount of sample directly injected into the capillary was ca. 1.8 nl, the total capillary volume being $\simeq 864$ nl.

–Detection: UV at 210 and 254 nm. While both wavelengths were recorded, only 210 nm is plotted in the present report.

3. Results and discussion

At the outset of our studies we focused on SNEIT in view of its previously demonstrated utility in chiral chromatographic separations and of its properties [14,15] and its other advantages (see Introduction). Once this choice was made, we also selected SAMBI, a closely related reagent that appeared convenient. It was important to retain the same relative configuration (i.e. *S*) between the two reagents, in light of the additional chiral component in our system which could be a complicating factor (see below). Both SAMBI and its *R*-enantiomer are commercially available (Trans World Chemicals, Rockville, MD, USA), but the quoted purity is only 98–99%, and we therefore decided to synthesize SAMBI in our laboratory. The *R*-enantiomer of SAMBI has previously been used in the HPLC analysis of the enantiomeric composition of amino compounds [17,18,20,21].

In the preliminary experiments during the development of the analytical method, a poor solubility was observed for some thioureas, formed after derivatization, in the BGE. Among the several additives added to the BGE in order to overcome this problem, β-cyclodextrin seemed, at the first sight, to be the most effective and, therefore, the WB containing β-cyclodextrin was also used to dilute the thioureas obtained from derivatization. However, in spite of the presence of β-cyclodextrin in the WB, the clear derivatization medium still became turbid in 5–10 min. Fig. 3 shows the electropherogram of the SNEIT-derivative of D,L-threonine separated by using a 20 m*M* solution of β-cyclodextrin in the BGE as working buffer and by applying 20 kV for 10 min, the rest of the operative conditions being unchanged. Under these conditions $\Delta t_R = 0.05$ min and the resolution, R_s, was 0.9 as calculated by:

$$R_s = (t_M^2 - t_M^1)/\tfrac{1}{2}(w_b^2 + w_b^1) \quad (1)$$

where t_M^2 and t_M^1 are the migration times of components 2 and 1, and w_b^2 and w_b^1 are the mean peak base widths relative to components 2 and 1

To improve the solubility of the derivatization mixture in the working buffer and the quality of the separations, experiments were performed with varying concentrations of β-cyclodextrin in the working buffer or by diluting the final derivatization mixture 1:1 with methanol, but none of these attempts lead to any significant change in the separation although the solubility was improved. However, by replacing β-cyclodextrin with heptakis-2,3,6-tri-O-methyl-β-cyclodextrin in the working buffer and keeping the concentration unchanged (i.e., 20 m*M*) the resolution, R_s, of the enantiomeric pair was improved by a factor >2: $R_s = 2$ (Fig. 4) vs. $R_s = 0.9$ (Fig. 3). Furthermore, a definite improvement in the solubilization of the reaction product thioureas

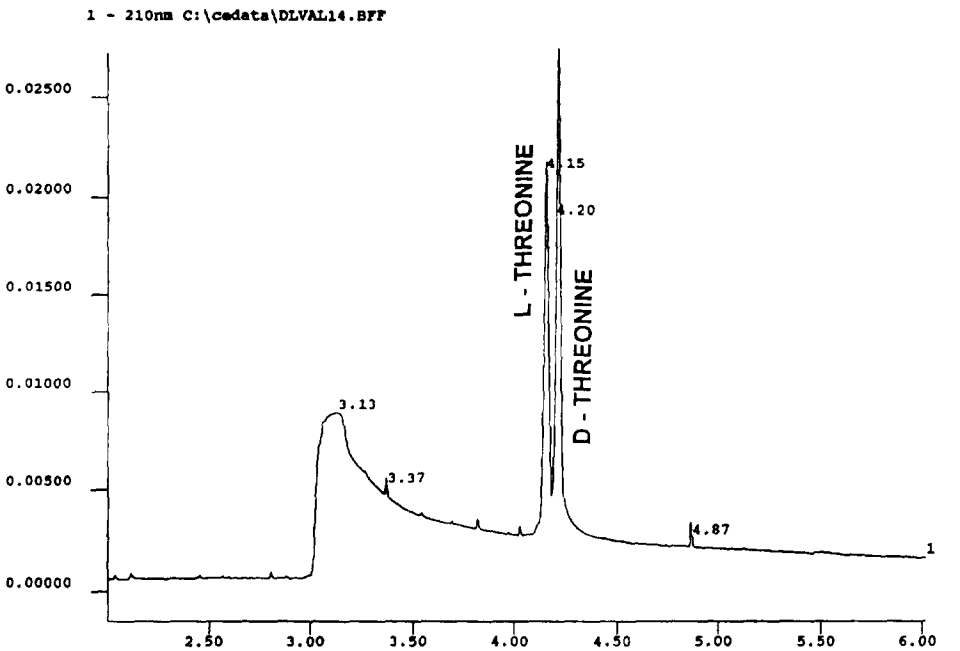

Fig. 3. Electropherogram of the SNEIT-derivatives of D,L-threonine. Sample, 50 μl D,L-Thr solution + 25 μl SNEIT solution; derivatization, 60°C for 30 min; + 25 μl WB; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* β-CD solution in BGE; voltage, 20 kV for 10 min.

in the working buffer was also achieved in this manner. Indeed, the addition of heptakis-2,3,6-tri-O-methyl-β-cyclodextrin instead of β-cyclodextrin, to the derivatization mixture led to clear solutions. These findings prompted us to use in all further experiments heptakis-2,3,6-tri-*O*-methyl-β-cyclodextrin instead of β-cyclodextrin in the working buffer.

D,L-Alanine, D,L-valine and D,L-phenylalanine were chosen as test compounds, primarily because they are primary protein α-amino acids and are characterized by a progressively increasing degree of lipophilicity. Furthermore, alanine and valine are non-UV-absorbing amino acids. All the three enantiomeric pairs were tested with both SNEIT and SAMBI. The separations of the diastereoisomeric SAMBI derivatives of alanine and those of phenylalanine are compared in Fig. 5, and the corresponding SNEIT-based separations are shown in Fig. 6. In Table 1 are reported the capacity ratios, k', and the separation factors, α, of the SNEIT- and SAMBI-derivatives of the amino acids studied. Fig. 7 shows the electropherograms that demonstrate the lack of a significant effect of diluting the derivatization mixture of L-alanine and SNEIT 1:1 with methanol. Such dilution was necessary in some cases to assure complete solubility of the derivatives; as seen in the example in Fig. 7, the dilution did not affect significantly the migration times.

The stereoisomeric elution order was assessed by comparing the electropherograms of the isothiocyanate-derivatized D,L-amino acid with that obtained for the corresponding isothiocyanate-derivatized L-amino acids, as reported for L-alanine in Fig. 8. It was thus found that for all the enantiomeric pairs studied and for both chiral derivatizing agents used, the L-enantiomer always migrates first. Interestingly, this stereoisomeric migration order was also found for TAGIT-derivatized amino acids when analyzed by reversed-phase HPLC [6] or by MEKC [7]. In the indirect method of separating enantiomeric analytes the stereoisomeric order of elution can be reversed if the chiral derivatizing agent is

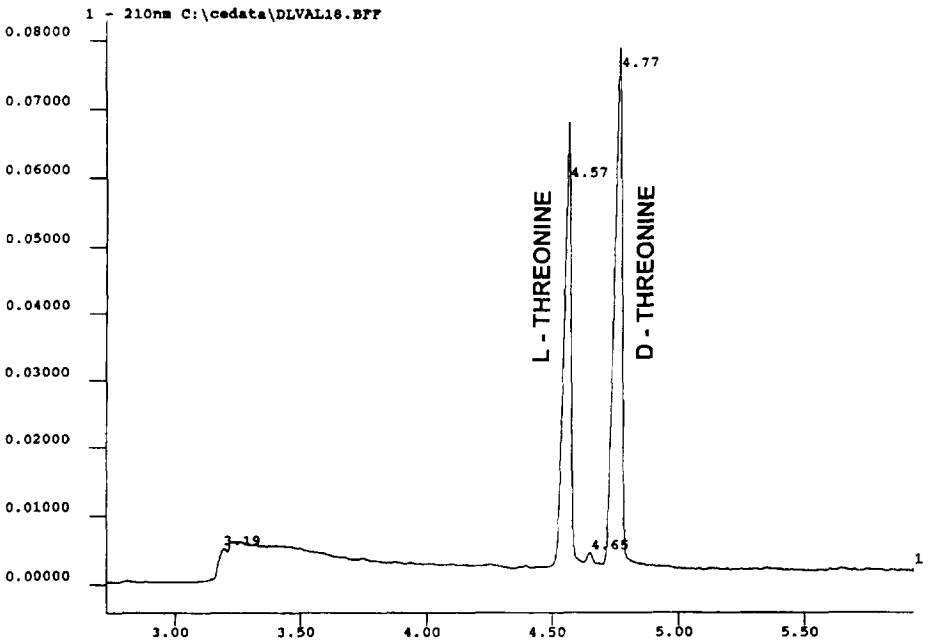

Fig. 4. Electropherogram of the SNEIT-derivatives of D,L-threonine. Sample, 50 μl D,L-Thr solution + 25 μl SNEIT solution; derivatization, 60°C for 30 min; + 25 μl WB; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* heptakis-2,3,6-tri-O-methyl-β-CD solution in BGE; voltage, 20 kV for 10 min.

available in both enantiomeric forms, and such choice in the elution order is a significant advantage if one of the enantiomers in the mixture is present in much higher concentration than the antipode, i.e. it is an advantage to have the lesser component elute before the predominant enantiomer. In this context it is important to note that for both SAMBI and SNEIT the precursor amines are available in both enantiomeric forms and that for SAMBI the *R*-enantiomer itself is also available commercially. It must be pointed out however, that the presence in our system of an additional chiral element, i.e. the cyclodextrin, suggests that without experimental determination of the stereoisomeric elution order as a function of the configuration of the derivatizing agent, caution must be exercised in its prediction. Such predictions may, however, be made with greater confidence if the mechanism of diastereoselectivity in the separation is known in detail.

Fig. 8 also illustrates two other observations: (a) during the derivatization no racemization of the amino acids or epimerization of the derivative occurs; (b) the enantiomeric purity of the chiral reagents was found to be >99%, inasmuch as a 1% enantiomeric contamination would have been detected under our conditions.

Based on the α-values given in Table 1 it can be seen that SNEIT gives a better separation of the diastereomeric derivatives, i.e. greater diastereoselectivity, for phenylalanine and valine than does SAMBI. In the case of alanine, however, the two chiral reagents produce nearly identical α values (Table 1). It is well-known that cyclodextrins can form inclusion complexes with suitable molecules and that such inclusion complexation may play a fundamental role in chromatographic separations mediated by cyclodextrins [9]. The structural features of β-cyclodextrin are such that when inclusion complexations do occur, the naphthalene ring is often well accommodated within the cavity of the cyclodextrin and its presence in the analyte may be crucial for chromatographic separations to occur; it might be attractive to conjecture, there-

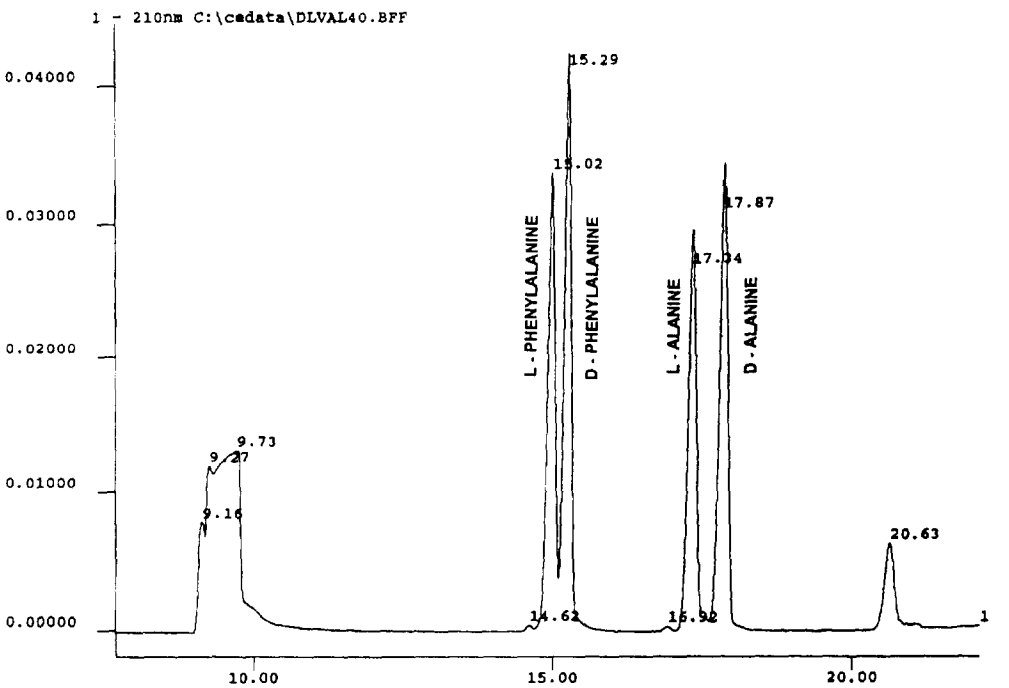

Fig. 5. Electropherogram of the SAMBI-derivatives of D,L-phenylalanine and D,L-alanine. Sample, 50 μl D,L-PhAla solution + 50 μl D,L-Ala solution + 50 μl SAMBI solution; derivatization, 60°C for 30 min; + 50 μl WB; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* heptakis-2,3,6-tri-O-methyl-β-CD solution in the BGE; voltage, 10 kV for 20 min; resolution: R_s (D-PhAla, L-PhAla) = 0.9; R_s (D-Ala, L-Ala) = 1.74; R_s (L-Ala, L-PhAla) = 7.6; R_s (D-Ala, D-PhAla) = 8.5.

fore, that the better diastereoselectivity provided by SNEIT, a naphthalene derivative, in two of the three cases could indicate that inclusion complexation is an important contributor to the overall diastereoselectivity. However, the small number of amino acids studied and the failure of the alanine derivatives to follow the pattern suggest that it is premature to draw such conclusions on the role of the cyclodextrin in the separations of the diastereomers obtained in our studies. In this regard it is important to recall that stereoisomer separations by cyclodextrins may also occur by mechanisms other than inclusion complexation [9].

From a practical viewpoint it is of interest to examine the resolution factor R_s for the separation of the diastereomeric amino acid derivatives. This parameter provides an indication of the extent of overlap of two peaks, and a value of 1.5 corresponds to essentially baseline separation of two equal-sized peaks [22]. For the separations shown in Fig. 5 (i.e. SAMBI derivatives) the R_s values for the diastereomeric pairs are as follows (calculated according to Eq. 1): D-PhAla, L-PhAla = 0.9; D-Ala, L-Ala = 1.74; for the separations shown in Fig. 6 (i.e. SNEIT derivatives) the R_s values are: D-PhAla, L-PhAla = 3.1; D-Ala, L-Ala = 1.72. Thus, under the conditions used the separation of the diastereomeric derivatives of phenylalanine is considerably greater with SNEIT than with SAMBI; since the migration times are not too dissimilar between Figs. 5 and 6 for the phenylalanine derivatives it can be readily concluded that the better separation seen for the SNEIT derivatives is a reflection of the differences between the α-values (Table 1) for SNEIT vs. SAMBI. Similarly, when alanine is considered, the nearly identical separation factors α for SAMBI vs. SNEIT (Table 1) coupled with the rather similar

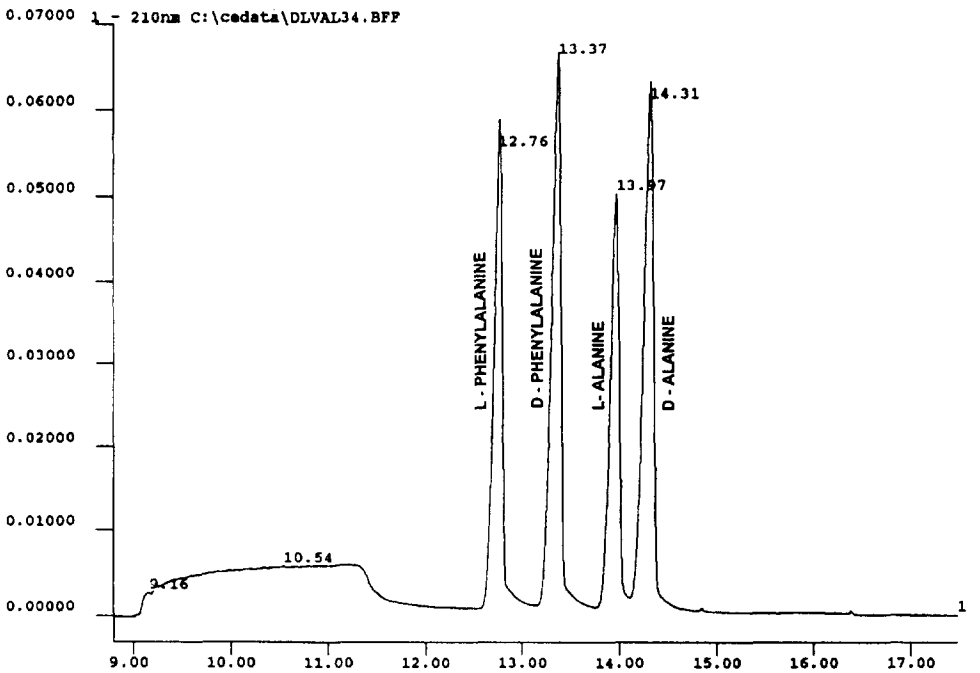

Fig. 6. Electropherogram of the SNEIT-derivatives of D,L-phenylalanine and D,L-alanine. Sample, 50 μl D,L-PhAla solution + 50 μl D,L-Ala solution + 50 μl SNEIT solution; derivatization, 60°C for 30 min; + 50 μl WB; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* heptakis-2,3,6-tri-O-methyl-β-CD solution in the BGE; voltage, 10 kV for 20 min; resolution: R_s (D-PhAla, L-PhAla) ≈ 3.1; R_s (D-Ala, L-Ala) ≈ 1.72; R_s (L-Ala, L-PhAla) ≈ 6.5; R_s (D-Ala, D-PhAla) ≈ 4.5.

Table 1
Capacity ratios, k', and separation factors, α, of the SNEIT- and SAMBI-derivatives of D-, L-phenylalanine; D-, L-valine and D-, L-alanine

Derivatizing agent	SNEIT		SAMBI	
	k'[a]	α[b]	k'	α
L-Phenylalanine	0.37	1.17	0.62	1.03
D-Phenylalanine	0.44	1.01	0.64	1.13
L-Valine	0.46	1.22	0.72	1.07
D-Valine	0.56	0.95	0.77	1.12
L-Alanine	0.50	1.06	0.86	1.07
D-Alanine	0.54		0.92	

[a] k' = capacity ratio = $(t_M - t_0)/t_0$. Acetone was used as neutral marker for t_0 determination (t_0 = 9.30 min).
[b] α = separation factor = k'_2/k'_1.

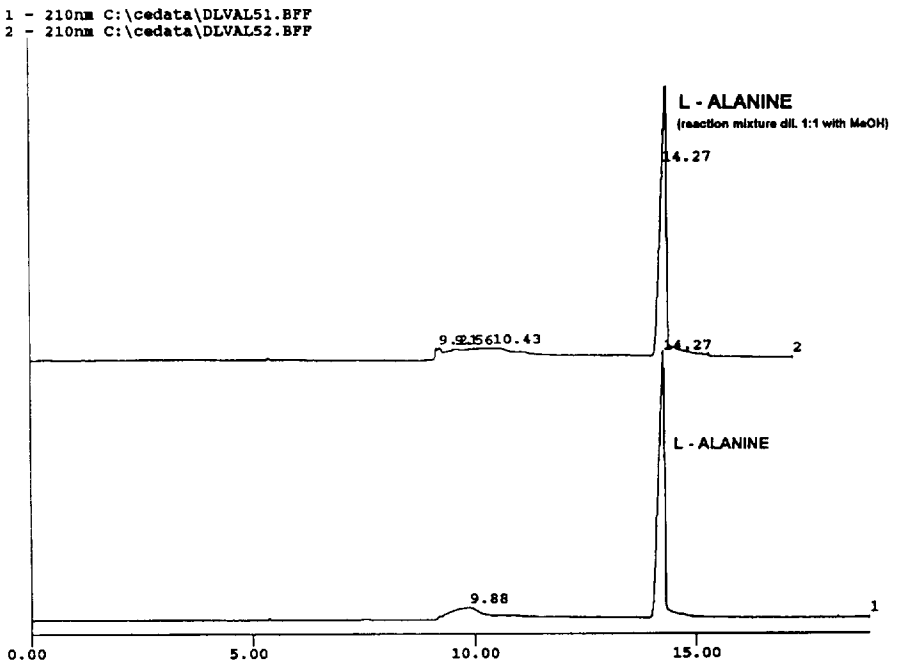

Fig. 7. Comparison between the electropherograms of the SNEIT-derivative of L-alanine when the derivatization mixture (50 μl L-Ala solution + 25 μl SNEIT solution; derivatization, 60°C for 30 min; + 25 μl WB = derivatization mixture) was directly injected or further diluted 1:1 with MeOH; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* heptakis-2,3,6-tri-O-methyl-β-CD solution in the BGE; voltage, 10 kV for 20 min.

migration times under the two sets of conditions (i.e. Figs. 5 and 6) translate into nearly identical R_s values (1.72 vs. 1.74).

The order of elution of the individual amino acids was, for both the SNEIT and SAMBI derivatives: phenylalanine, valine, alanine (Table 1). Interestingly, this order is the opposite of that of the corresponding TAGIT derivatives in both HPLC [6] and MEKC [7]. It appears then, that under the conditions used, the migration order of the amino acids is the inverse of their order of lipophilicity.

Enantiospecific analysis of amino acids often involves the simultaneous presence of several amino acids as analytes in a mixture. In such situations there is a requirement not only for the separation of the enantiomers of each amino acid, but also for the separation of each amino acid from all of the others present in the mixture. The limited number of amino acids studied does not permit us to draw extensive conclusions in this regard, but it is noteworthy that the three amino acids were, in general, better separated via derivatization with SAMBI. It is interesting to note in this context that in a previous study on the separation of amino acid enantiomers as TAGIT derivatives with MEKC the derivatives of D-alanine and L-valine were not separable [7].

In conclusion, it is clear that the chiral isothiocyanates SNEIT and SAMBI may be useful in the enantiospecific analysis of amino acids via FSCE. These reagents are easily prepared (the *R*-form of SAMBI is commercially available) and have distinct advantages. Additional studies to delineate the scope and mechanisms of such analyses are progress in our laboratory.

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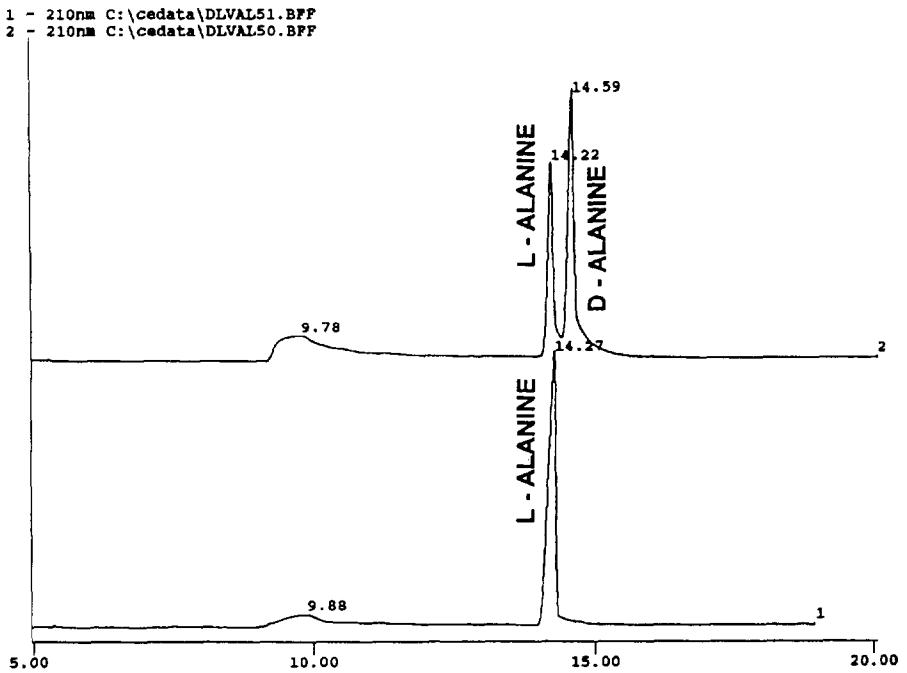

Fig. 8. Comparison between the electropherograms of the SNEIT-derivatives of D,L-alanine (curve 2) and L-alanine (curve 1). Sample, 50 μl D,L-Ala (or L-Ala) solution + 25 μl SNEIT solution; derivatization, 60°C for 30 min; + 25 μl WB; BGE, 50 m*M* $Na_2B_4O_7$ pH 10; WB, 20 m*M* heptakis-2,3,6-tri-O-methyl-β-CD solution in the BGE; voltage, 10 kV for 20 min.

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