# Enantioseparation on HPLC Chiral Stationary Phases

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*Received March 12, 1992*

The chromatographic separation of the stereoisomers of the N-(3,5-dinitrobenzoyl) derivatives of fifteen dipeptide methyl esters and nine dipeptide alkyl esters was investigated on three different chiral stationary phases derived from N-acylated α-arylalkylamines. Two of these CSPs contain second stereogenic centers. These secondary stereogenic centers of CSPs were proposed to provide secondary effects in terms of chiral recognition. From the elution orders of the four dipeptide stereoisomers and the separation factors of the enantiomeric pairs of the N-(3,5-dinitrobenzoyl) derivatives of the dipeptide alkyl esters having different alkoxy substituents, it was proposed that the intercalation of the alkoxy substituents of dipeptide derivatives between the connecting arm of CSPs may control the magnitude of chiral separations of dipeptide derivatives.

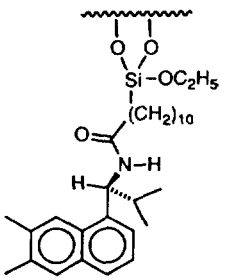
## Introduction

Because of the significance of optically active small peptides as therapeutic and sweetening agents,[1,2] techniques for the easy determination of enantiomeric and diastereomeric purity of small peptides have been required in the field of peptide and related chemistry. Previously, several studies concerning the resolution of enantiomeric dipeptides using GC and HPLC have been reported.[3] However, such separations are not yet routine. In this area, we also reported the separation of enantiomeric dipeptide methyl ester derivatives on CSP **1**[4,5] and we proposed the operation of the two competing "opposite-sense" chiral recognition processes to explain the elution orders of four stereoisomers on CSP **1**.[5]

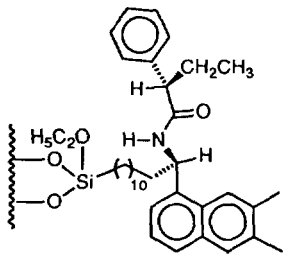
Recently, we prepared CSPs **2** and **3** which have a second stereogenic center.[6] The direction of connecting arm of these CSPs is different from that of CSP **1**. Conceivably the chromatographic behavior of the four stereoisomers of N-(3,5-dinitrobenzoyl)dipeptide alkyl esters on CSPs **2** and **3** might be different from that on CSP **1** based on the proposed two competing chiral recognition processes.[5] CSPs **2** and **3** might also show different degree of chiral recognition for the four stereoisomers of N-(3,5-dinitrobenzoyl)dipeptide alkyl esters since the added stereogenic unit would have a differential suppressing effect on the two competing chiral recognition processes proposed.[5] This paper presents such a study and provides additional evidence for the operation of the two competing chiral recognition processes during the resolution of enantiomeric dipeptide derivatives on CSPs derived from α-arylalkylamines.

## Experimental

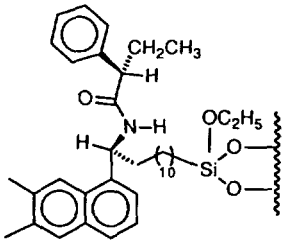
All chromatograms were obtained with a Waters Model 510 pump, Waters Model U6k Universal Liquid Chromatograph Injector, Waters Model 441 Absorbance Detector with 254 nm UV filter and Waters Model 740 Data Module Recorder. The mobile phase used in this study was 2-propanol-hexane mixed solvent (10 : 90) and the flow rate was 2 ml/min.



(S)-CSP 1



(R,S)-CSP 2



(S,S)-CSP 3

Melting point determination was performed by using Rigaku Thermal Analyzer TAS 100. $^1$H-NMR spectra were obtained on a Jeol JNN-FAX 100 spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Mattson Polaris FT-IR spectrometer.

N-(3,5-Dinitrobenzoyl) derivatives of dipeptide methyl esters were available from the previous study.[5] As an example for the preparation of N-(3,5-dinitrobenzoyl) derivatives of dipeptide alkyl esters, the synthesis of N-(3,5-dinitrobenzoyl)-(DL)-valinyl-(L)-valine *n*-butyl ester is described in the following. The other N-(3,5-dinitrobenzoyl) derivatives of dipeptide alkyl esters were prepared by the same method described in the following. All samples thus prepared were found

**Table 1.** The Resolution of N-(3,5-Dinitrobenzoyl) Derivatives of Enantiomeric Dipeptide Methyl Esters on CSP 2

| Entry | N-DNB-a-b-$OCH_3$ a-b | Elution order | (SS)/(RR) Pair | | (SR)/(RS) Pair | |
|---|---|---|---|---|---|---|
| | | | $\alpha^a$ | $k_1{}^b$ | $\alpha^a$ | $k_1{}^c$ |
| 1 | Ala-Val | (SS)(SR)(RR)(RS) | 2.11 | 4.51 | 2.08 | 6.90 |
| 2 | Ala-Leu | (SS)(SR)(RR)(RS) | 2.34 | 3.20 | 2.14 | 5.08 |
| 3 | Ala-Pheala | (SS)(SR)(RR)(RS) | 1.78 | 6.79 | 1.77 | 10.06 |
| 4 | Val-Val | (SS)(SR)(RR)(RS) | 2.93 | 1.88 | 2.66 | 2.99 |
| 5 | Val-Leu | (SS)(SR)(RR)(RS) | 2.97 | 1.48 | 2.42 | 2.25 |
| 6 | Val-Pheala | (SS)(SR)(RR)(RS) | 2.23 | 2.19 | 1.93 | 4.38 |
| 7 | Leu-Ala | (SS)(SR)(RR)(RS) | 2.53 | 3.60 | 1.78 | 5.13 |
| 8 | Leu-Val | (SS)(SR)(RR)(RS) | 2.10 | 2.50 | 1.70 | 4.11 |
| 9 | Leu-Leu | (SS)(SR)(RR)(RS) | 2.30 | 1.90 | 1.66 | 3.10 |
| 10 | Leu-Pheala | (SS)(RR)(SR)(RS) | 1.64 | 3.25 | 1.48 | 6.53 |
| 11 | Phegly-Ala | (SS)(RR)(SR)(RS) | 1.38 | 8.01 | 1.00 | 14.26 |
| 12 | Phegly-Val | (SS)(RR)(RS)(SR) | 1.21 | 6.61 | −1.07[d] | 12.74 |
| 13 | Phegly-Leu | (SS)(RR)(RS)(SR) | 1.26 | 5.38 | −1.18 | 9.00 |
| 14 | Phegly-Pheala | (SS)(RS)(SR)(RR) | 1.00 | 9.40 | −1.17 | 13.75 |
| 15 | Phegly-Phegly | (SS)(RS)(RR)(SR) | 1.13 | 11.50 | −1.14 | 12.45 |

[a] Separation factor. [b] Capacity factor for the first eluted enantiomer of the (SS)/(RR) pair. [c] Capacity factor for the first eluted enantimer of the (SR)/(RS) pair. [d] Negative value indicates that the elution order departs from the usual elution order.

to be pure enough by thin layer chromatography and used for the HPLC analysis without further purification or characterization.

**(L)-Valine *n*-Butyl Esters.** To a 50 m*l* round-bottom flask equipped with an anhydrous calcium chloride tube was added 10 m*l* of *n*-butyl alcohol. After cooling this flask to −10℃ in an ice-salt bath, 1 m*l* of thionyl chloride was added slowly and the mixture was stirred. After 20 min, 0.4 g of (L)-valine was added and the ice bath was removed. Then, the mixture was heated at 60-70℃ for 24 hours. The completion of reaction was checked by silica gel thin layer chromatography (ninhydrin, mobile phase = methanol/chloroform : 1/5). After checking the completion of the reaction, *n*-butyl alcohol was removed under reduced pressure. After purification by flash chromatography (silica gel, ethyl acetate/hexane), white crystalline (L)-valine *n*-butyl ester hydrochloride was obtained. Yield: 98%, mp.: 63.5-66.5℃, $^1$H-NMR ($CDCl_3$) δ 0.97 (t, 3H), 1.12 (d, 3H), 1.16 (d, 3H), 1.25-1.80 (m, 4H), 2.30-2.62 (m, 1H), 3.80-4.02 (m, 1H), 4.21 (t, 2H), 8.86 (broad s, 3H), IR (KBr) $cm^{-1}$ 3450, 2980, 1750.

**N-(3,5-Dinitrobenzoyl)-(DL)-Valinyl-(L)-Valine *n*-Butyl Ester.** This dipeptide derivative was obtained by coupling N-(3,5-dinitrobenzoyl)-(DL)-valine and *n*-butyl ester of (L)-valine in the presence of the coupling agent, 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) via the method described previously.[5] The spectroscopic and physical data are presented in following. mp.: 149.5-153.5℃, $^1$H NMR ($CDCl_3$) δ 0.80-1.10 (m, 15H), 1.20-1.80 (m, 4H), 2.00-2.40 (m, 2H), 4.00-4.23 (m, 2H), 4.42-4.70 (m, 2H), 6.70-6.90 (m, 1H), 8.10-8.25 (m, 1H), 9.00-9.10 (m, 3H), IR (KBr) $cm^{-1}$ 3250, 3080, 1740, 1640, 1550.

## Results and Discussion

The chromatographic separation of the stereoisomers of the N-(3,5-dinitrobenzoyl) derivatives of fifteen dipeptide methyl esters on 4.6×250 mm columns containing CSPs **2** and **3** was conducted using isopropyl alcohol-hexane (10 : 90) as the mobile phase with a flow rate of 2 m*l*/min. These results are summarized Table 1 and 2. Data obtained similarly using other alkyl esters of these derivatives and CSPs **1**, **2**, and **3** in order to see the effect of the direction of the connecting arm of CSPs are presented in Tables 3, 4 and 5. Representative chromatograms are shown in Figure 1. With few exceptions, the general elution orders for the four stereoisomers are (SS), (SR), (RR), (RS) on (R,S)-CSP **2** and (RR), (RS), (SS), (SR) on (S,S)-CSP **3**. In these sequences the absolute configuration of the N-(3,5-dinitrobenzoyl) amino acid portion of each stereoisomer is initially indicated as is the configuration of the α-arylalkylamine portion of CSPs. The elution orders for the four stereoisomers were determined by chromatographing dipeptide derivatives prepared from different combinations of racemic and enantiomerically pure amino acids as described in the previous study.[5] Since CSPs **2** and **3** were prepared from α-arylalkylamine of opposite absolute stereochemistry, the general elution orders of the stereoisomers from these CSPs, as shown in Table **1**, 2, 4 and 5, are those expected should both phases utilize the same mechanism of chiral recognition. In other words, it is the configuration of the α-arylalkylamine portion of these CSPs which principally determines the sense of chiral recognition. The usual elution order for the four stereoisomers of N-(3,5-dinitrobenzoyl) dipeptide alkyl esters from (S)-CSP **1** is (RR), (RS), (SR), (SS) as shown in Table 3 and this is consistent with that reported previously for the resoution of N-(3,5-dinitrobenzoyl) dipeptide methyl esters.[5]

From the observed elution orders, it is evident that the

**Table 2.** The Resolution of N-(3,5-Dinitrobenzoyl) Derivatives of Enantiomeric Dipeptide Methyl Esters on CSP **3**

| Entry | N-DNB-a-b-$OCH_3$ | Elution order | (RR)/(SS) Pair | | (RS)/(SR) Pair | |
|---|---|---|---|---|---|---|
| | a-b | | $\alpha^a$ | $k_1{}^b$ | $\alpha^a$ | $k_1{}^c$ |
| 1 | Ala-Val | (RR)(RS)(SS)(SR) | 2.10 | 3.19 | 2.10 | 4.53 |
| 2 | Ala-Leu | (RR)(RS)(SS)(SR) | 2.24 | 2.50 | 2.08 | 3.48 |
| 3 | Ala-Pheala | (RR)(RS)(SS)(SR) | 1.74 | 4.54 | 1.81 | 6.56 |
| 4 | Val-Val | (RR)(RS)(SS)(SR) | 2.83 | 1.33 | 2.59 | 1.98 |
| 5 | Val-Leu | (RR)(RS)(SS)(SR) | 2.49 | 1.10 | 2.07 | 1.50 |
| 6 | Val-Pheala | (RR)(RS)(SS)(SR) | 2.15 | 1.63 | 2.00 | 3.01 |
| 7 | Leu-Ala | (RR)(RS)(SS)(SR) | 3.11 | 2.19 | 2.18 | 3.13 |
| 8 | Leu-Val | (RR)(RS)(SS)(SR) | 2.53 | 1.69 | 2.34 | 2.51 |
| 9 | Leu-Leu | (RR)(RS)(SS)(SR) | 2.70 | 1.48 | 2.23 | 2.08 |
| 10 | Leu-Pheala | (RR)(RS)(SS)(SR) | 2.06 | 2.00 | 2.15 | 3.63 |
| 11 | Phegly-Ala | (RR)(RS)(SS)(SR) | 1.94 | 4.38 | 1.62 | 7.26 |
| 12 | Phegly-Val | (RR)(RS)(SS)(SR) | 1.90 | 3.41 | 1.65 | 5.95 |
| 13 | Phegly-Leu | (RR)(RS)(SS)(SR) | 1.98 | 2.88 | 1.48 | 5.11 |
| 14 | Phegly-Pheala | (RR)(RS)(SR)(SS) | 1.67 | 4.98 | 1.50 | 8.31 |
| 15 | Phegly-Phegly | (RS)(RS)(SS)(SR) | 2.08 | 5.65 | 1.59 | 7.38 |

[a,b,c] See footnotes in Table 1.

**Table 3.** The Resolution of N-(3,5-Dinitrobenzoyl) Derivatives of Enantiomeric Dipeptide Alkyl Esters on CSP **1**

| Entry | N-DNB-a-b-OR | Elution order | (RR)/(SS) Pair | | (RS)/(SR) Pair | |
|---|---|---|---|---|---|---|
| | a-b-OR | | $\alpha^a$ | $k_1{}^b$ | $\alpha^a$ | $k_1{}^c$ |
| 1 | Val-Val-$OCH_3$ | (RR)(RS)(SR)(SS) | 2.21 | 2.60 | 1.46 | 3.93 |
| 2 | Val-Val-O-*n*-Butyl | (RR)(RS)(SR)(SS) | 2.64 | 1.45 | 1.56 | 2.17 |
| 3 | Val-Val-O-*n*-Hexyl | (RR)(RS)(SR)(SS) | 2.80 | 1.07 | 1.73 | 1.73 |
| 4 | Val-Val-O-*n*-Octyl | (RR)(RS)(SR)(SS) | 2.88 | 0.97 | 1.89 | 1.40 |
| 5 | Leu-Val-$OCH_3$ | (RR)(RS)(SR)(SS) | 1.87 | 3.77 | 1.35 | 5.29 |
| 6 | Leu-Val-O-*n*-Butyl | (RR)(RS)(SR)(SS) | 2.26 | 2.47 | 1.45 | 3.45 |
| 7 | Leu-Val-O-*n*-Hexyl | (RR)(RS)(SR)(SS) | 2.40 | 1.93 | 1.61 | 2.73 |
| 8 | Leu-Val-O-*n*-Octyl | (RR)(RS)(SR)(SS) | 2.52 | 1.67 | 1.80 | 2.33 |
| 9 | Phegly-Val-$OCH_3$ | (RR)(RS)(SR)(SS) | 1.70 | 7.45 | 1.10 | 12.67 |
| 10 | Phegly-Val-O-*n*-Butyl | (RR)(RS)(SS)(SR) | 1.99 | 4.51 | 1.19 | 7.52 |
| 11 | Phegly-Val-O-*n*-Hexyl | (RR)(RS)(SS)(SR) | 2.15 | 3.52 | 1.30 | 5.80 |
| 12 | Phegly-Val-O-*n*-Octyl | (RR)(RS)(SS)(SR) | 2.26 | 3.00 | 1.42 | 5.09 |

[a,b,c] See footnotes in Table 1.

**Table 4.** The Resolution of N-(3,5-Dinitrobenzoyl) Derivatives of Enantiomeric Dipeptide Alkyl Esters on CSP **2**

| Entry | N-DNB-a-b-OR | Elution order | (SS)/(RR) Pair | | (SR)/(RS) Pair | |
|---|---|---|---|---|---|---|
| | a-b-OR | | $\alpha^a$ | $k_1{}^b$ | $\alpha^a$ | $k_1{}^c$ |
| 1 | Val-Val-$OCH_3$ | (SS)(SR)(RR)(RS) | 2.93 | 1.88 | 2.66 | 2.99 |
| 2 | Val-Val-O-*n*-Butyl | (SS)(SR)(RR)(RS) | 2.75 | 1.40 | 2.36 | 2.07 |
| 3 | Val-Val-O-*n*-Hexyl | (SS)(SR)(RR)(RS) | 2.61 | 1.27 | 2.17 | 2.04 |
| 4 | Val-Val-O-*n*-Octyl | (SS)(SR)(RR)(RS) | 2.35 | 1.11 | 2.07 | 1.73 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5 | Leu-Val-$OCH_3$ | (SS)(SR)(RR)(RS) | 2.10 | 2.50 | 1.70 | 4.11 |
| 6 | Leu-Val-O-*n*-Butyl | (SS)(SR)(RR)(RS) | 1.87 | 2.32 | 1.46 | 3.60 |
| 7 | Leu-Val-O-*n*-Hexyl | (SS)(SR)(RS)(RR) | 1.66 | 1.97 | 1.32 | 3.27 |
| 8 | Leu-Val-O-*n*-Octyl | (SS)(SR)(RS)(RR) | 1.62 | 1.81 | 1.25 | 2.93 |
| 9 | Phegly-Val-$OCH_3$ | (SS)(RR)(RS)(SR) | 1.21 | 6.61 | −1.07[d] | 12.74 |
| 10 | Phegly-Val-O-*n*-Butyl | (SS)(RR)(RS)(SR) | 1.13 | 4.55 | −1.19 | 7.40 |
| 11 | Phegly-Val-O-*n*-Hexyl | (SS)(RR)(RS)(SR) | 1.07 | 3.80 | −1.29 | 5.95 |
| 12 | Phegly-Val-O-*n*-Octyl | (SS)(RS)(SR)(RR) | 1.00 | 3.45 | −1.38 | 5.17 |

[a,b,c,d] See footnotes in Table 1.

**Table 5.** The Resolution of N-(3,5-Dinitrobenzoyl) Derivatives of Enantiomeric Dipeptide Alkyl Esters on CSP 3

| Entry | N-DNB-a-b-OR | Elution order | (RR)/(SS) Pair | | (RS)/(SR) Pair | |
|---|---|---|---|---|---|---|
| | a-b | | $\alpha^a$ | $k_1{}^b$ | $\alpha^a$ | $k_1{}^c$ |
| 1 | Val-Val-$OCH_3$ | (RR)(RS)(SS)(SR) | 2.83 | 1.33 | 2.59 | 1.98 |
| 2 | Val-Val-O-*n*-Butyl | (RR)(RS)(SS)(SR) | 2.64 | 0.97 | 2.12 | 1.41 |
| 3 | Val-Val-O-*n*-Hexyl | (RR)(RS)(SS)(SR) | 2.54 | 0.87 | 2.01 | 1.29 |
| 4 | Val-Val-O-*n*-Octyl | (RR)(RS)(SS)(SR) | 2.42 | 0.76 | 1.83 | 1.17 |
| 5 | Leu-Val-$OCH_3$ | (RR)(RS)(SS)(SR) | 2.53 | 1.69 | 2.34 | 2.51 |
| 6 | Leu-Val-O-*n*-Butyl | (RR)(RS)(SS)(SR) | 2.45 | 1.36 | 1.91 | 2.04 |
| 7 | Leu-Val-O-*n*-Hexyl | (RR)(RS)(SS)(SR) | 2.28 | 1.20 | 1.70 | 1.84 |
| 8 | Leu-Val-O-*n*-Octyl | (RR)(RS)(SS)(SR) | 2.06 | 1.12 | 1.59 | 1.67 |
| 9 | Phegly-Val-$OCH_3$ | (RR)(RS)(SS)(SR) | 1.90 | 3.41 | 1.65 | 5.95 |
| 10 | Phegly-Val-O-*n*-Butyl | (RR)(RS)(SS)(SR) | 1.79 | 2.59 | 1.43 | 4.33 |
| 11 | Phegly-Val-O-*n*-Hexyl | (RR)(RS)(SR)(SS) | 1.68 | 2.27 | 1.31 | 3.80 |
| 12 | Phegly-Val-O-*n*-Octyl | (RR)(RS)(SR)(SS) | 1.60 | 2.00 | 1.22 | 3.41 |

[a,b,c] See footnotes in Table 1.

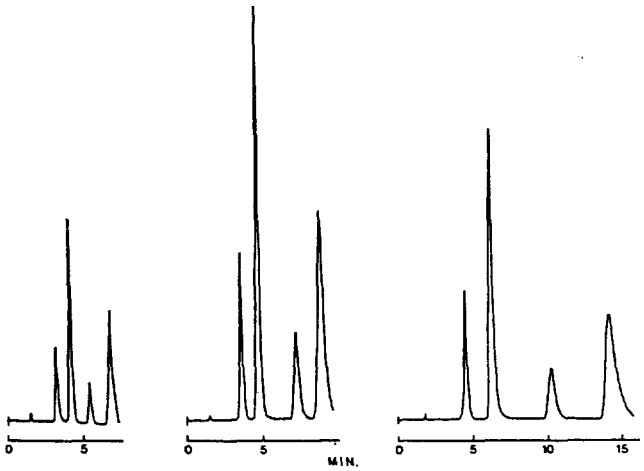
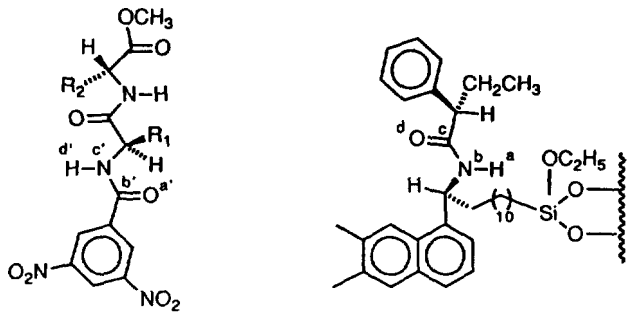



**Figure 1.** The resolution of N-(3,5-dinitrobenzoyl)-Val-Val-OR on CSP 2. Left : R=*n*-Octyl, Middle : R=*n*-Butyl, Right : R =Methyl. For the chromatographic conditions, see text.

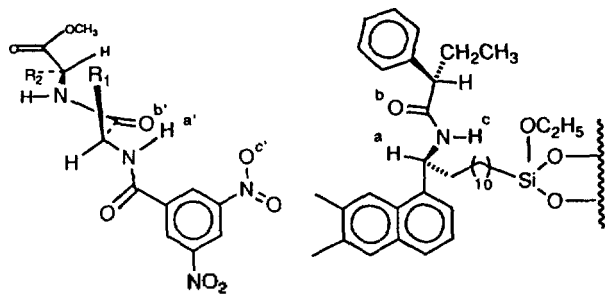
absolute stereochemistry of the α-arylalkylamine portion of α-arylalkylamine based CSPs controls elution orders. The chiral acyl group of CSPs 2 and 3 seem to contribute second order effects only, influencing the degree but not the sense of enantioselectivity. It was noted earlier that the (R)-enantiomers of N-(3,5-dinitrobenzoyl) amino acid esters elute first from (S)-CSP 1.[7a] In consequence, from the observed elution orders, it is also evident that the absolute configration of the N-(3,5-dinitrobenzoyl) amino acid portion of dipeptide derivatives is the principal factor in determining the elution order of a pair of enantiomers. This is not surprising considering the importance of the π-π interaction between the analyte's 3,5-dinitrobenzoyl group and the 6,7-dimethylnaphthyl group of the CSPs.[7]

The elution orders of the enantiomers of N-(3,5-dinitrobenzoyl) dipeptide methyl esters from (S)-CSP 1 has been explained on the basis of two competing opposite-sense chiral recognition processes, termed the "dipole-stacking process" and the "hydrogen-bonding process".[5] These two competing chiral recognition processes appear to be operative on CSPs 2 and 3 for the dipeptide alkyl ester derivatives whose chromatographic behaviors are summarized in Table 1 and 2 and the proposed two processes are drawn in Figure 2 and 3. From the known conformational preferences of amides in general and amides of α-(1-naphthyl) alkylamine in particular [7] and those of the acyl part of amides,[8] the conformation



(RR)

**Figure 2.** Overlapping the (RR)-enantiomer on CSP 3 with the $\pi$-$\pi$ interaction bwtween the 3,5-dinitrobenzoyl and the 6,7-dimethylnaphthyl group and the dipole-stacking between the dipoles marked as a-b-c-d and a'-b'-c'-d' shows the dipole-stacking process. This process is non-intercalative.



(SS)

**Figure 3.** Overlapping the (SS)-enantiomer on CSP 3 with the $\pi$-$\pi$ interaction between the 3,5-dinitrobenzoyl and the 6,7-dimethylnaphthyl group and the hydrogen-bondings (a'-b, b'-a and c'-c) shows the hydrogen-bonding process. In this process, the ester alkyl group of the (SS)-enantiomer is intercalating between the strands of bonded phase.

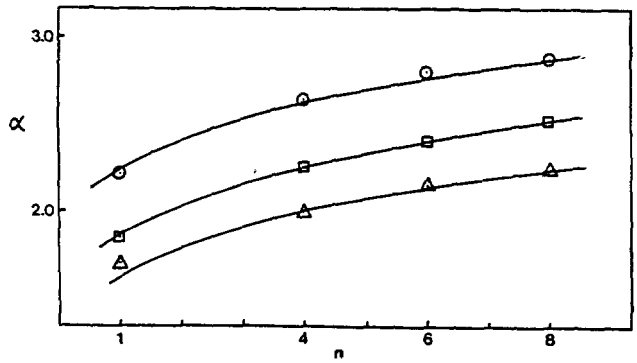



**Figure 4.** The chromatographic resolution of the (RR)/(SS) pairs of N-(3,5-dinitrobenzoyl)-amino acid-amino acid-O-$(CH_2)_n$-H on CSP 1. Circle = Val-Val, Square = Leu-Val, Triangle = Phegly-Val. For chromatographic conditions, see text.

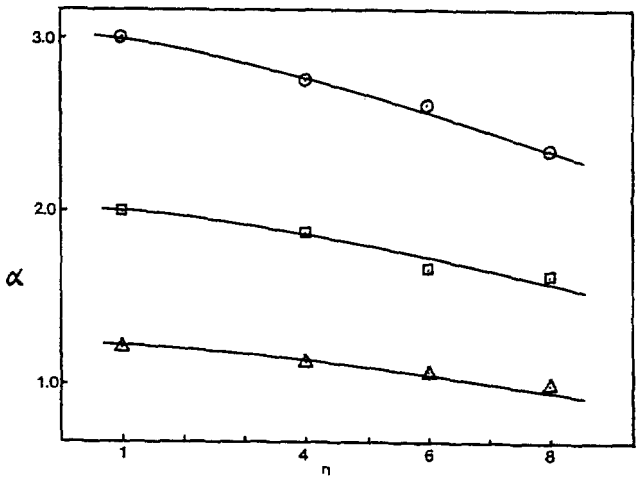



**Figure 5.** The chromatographic resolution of the (SS)/(RR) pairs of N-(3,5-dinitrobenzoyl)-amino acid-amino acid-O-$(CH_2)_n$-H on CSP 2. Circle = Val-Val, Square = Leu-Val, Triangle = Phegly-Val. For Chromatographic conditions, see text.

of the CSP shown in the drawings are thought to be heavily populated. When face to face interaction occur, analytes preferentially occupy a position "above" the CSP in order to avoid the steric repulsion from the back side interaction. Since ethyl is effectively smaller than phenyl, (R,S)-CSP 2 will typically allow closer approach of the enantiomers which are retained by the dipole-stacking process than does (S,S)-CSP 3. In consequence, the capacity factors ($k_1$) for the enantiomers which are retained by the dipole-stacking process should be larger on CSP 2 than on CSP 3 and the separation factors ($\alpha$ values) on CSP 2 are expected to be smaller than on CSP 3 as shown in Table 1 and 2 with few exceptions.

Similarly, the separation factors and the capacity factors on CSPs 2 and 3 shown in Table 1 and 2 are larger and smaller respectively than those reported previously on (S)-CSP 1[5] because the large acyl group of CSPs 2 and 3 suppress the dipole-stacking process more effectively than does the acyl group (the acyl connecting arm in this case) of CSP 1.

The data for the resolution of N-(3,5-dinitrobenzoyl) dipeptide alkyl esters with varying length of ester alkyl functionality on CSPs 1, 2 and 3 which are summarized in Table 3, 4 and 5 more clearly indicates that the two competing chiral recognition processes are operative. As the ester alkyl functionalities of the N-(3,5-dinitrobenzoyl) dipeptide alkyl esters increase in length, the capacity factors decrease in every instance and these are considered as the normal chromatographic results with the normal phase separation. The separation factors for the (RR)/(SS) enantiomeric pairs and for the (RS)/(SR) enantiomeric pairs on (S)-CSP 1 increase as the ester alkyl groups of analytes increase in length as shown in Table 3. However, on CSPs 2 and 3, the separation factors for the two enantiomeric pairs decrease as the length of the ester alkyl group is increased, as shown in Tables 4 and 5. These trends shown graphically in Figures 4-6 are indicative of a great degree of intercalation of the ester alkyl group of the least retained enantiomers between the strands of CSP 1 and of the most retained enantiomers on CSPs 2 and 3. This is consistent with a preference of the most retained enantiomers for the hydrogen-bonding process. Conversely, the least retained enantiomers have a preference for the dipole-stacking process. The basic arguments for this were presented earlier in a previous study of the resolution

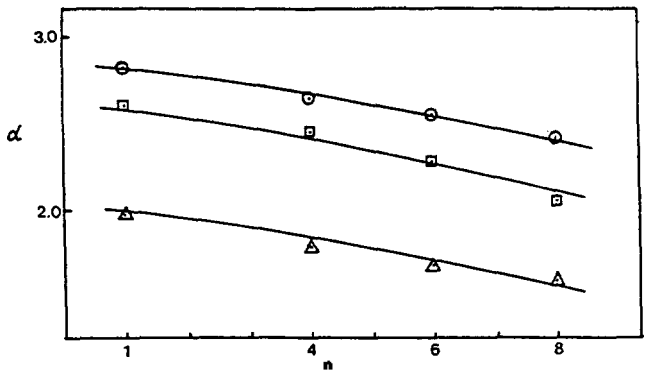



**Figure 6.** The chromatographic resolution of the (RR)/(SS) pairs of N-(3,5-dinitrobenzoyl)-amino acid-amino acid-O-$(CH_2)_n$-H on CSP **3**. Circle = Val-Val, Square = Leu-Val, Triangle = Phegly-Val. For chromatographic conditions, see text.

of N-(3,5-dinitrobenzoyl) dipeptide methyl esters on (S)-CSP **1**.[5] In that study, we proposed that the (SS) and (SR)-enantiomers are retained principally by the hydrogen-bonding process while the (RR) and (RS)-enantiomers are principally retained by the dipole-stacking process. On (S)-CSP **1**, the dipole-stacking process retaining the (RR) and (RS)-enantiomers requires the intercalation of the second amino acid unit between the strands of bonded phase. Such intercalation is not required by the hydrogen-bonding process. In a normal phase eluent, intercalation is sterically demanding. Consequently, as the length of the second amino ester is increased by increasing the length of the ester alkyl group, the intercalative dipole-stacking process is progressively suppressed. As the (RR) and (RS)-enantiomers elute relatively sooner, the separation factors for the (RR)/(SS) and for the (RS)/(SR) enantiomeric pairs increase. However, on (S,S)-CSP **3**, it is the hydrogen-bonding process which intercalates the ester alkyl group of the (SS) and (SR)-enantiomers. As the length of the ester alkyl group is increased, the (SS) and (SR)-enantiomers elute relatively sooner. Since these normally elute after the (RR) and (RS)-enantiomers, the separation factors for the (RR)/(SS) enantiomeric pairs and for the (RS)/(SR) enantiomeric pairs decrease. The same type of arguments apply for (R,S)-CSP **2**, bearing in mind its stereochemical difference. However, on (R,S)-CSP **2**, the elution order of the (RS)/(SR) enantiomeric pairs for the phenylglycine-containing dipeptides is "inverted" and the separation factors increase (or decrease in a sence of enantioselectivity) as the length of the ester alkyl group is increased as shown in Table 4. One infers that the phenyl groups of enantiomers ((SR)-enantiomers in this particular case) which are retaind by the dipole-stacking process may provide an additional interaction site to interact with CSP. For example, a phenyl group may serve as a basic site for hydrogen bonding[9] or may serve as an interaction site for the face to edge π-π interaction.[10] In consequence, the (SR)-enantiomers retained by the dipole-stacking process on CSP **2** elute later than the (RS)-enantiomers retained by the hydrogen-bonding process, leading to the inversion of elution order. However, the detailed additional interation is not known yet. After the inversion of elution order, the separation factors increase continuously because the (RS)-enantiomers elute sooner and sooner as the ester alkyl group is increased in length.

Even though the two competing chiral recognition processes proposed can possibly explain the chromatographic resolution behaviors for the enantiomeric pairs of N-(3,5-dinitrobenzoyl) dipeptide esters on CSPs **2** and **3**, we do not have any physical evidence such as X-ray crystallographic data or NMR NOE data to support the chiral recognition mechanisms. Therefore we do not insist that the two competing chiral recognition mechanisms proposed are perfect and we do not rule out the possibility of other chiral recognition mechanisms which can explain the data summarized in Table 1-5. As the other physical evidences come out, the two competing chiral recognition mechanisms proposed may be modified. However, we believe that the two competing chiral recognition processes proposed can be successfully used as a guidance for understanding the chromatographic resolution behaviors of the stereoisomers of dipeptide derivatives.

In conclusion, we were able to show that CSPs **2** and **3** with two stereogenic centers derived from α-arylalkylamine can be employed for the resolution of the stereoisomers of N-(3,5-dinitrobenzoyl) dipeptide esters. The secondary stereogenic center of CSPs **2** and **3** were proposed to contribute second order effects only, influencing the degree but not the sense of enantioselectivity. From the elution orders and the trend of separation factors for the separation of N-(3,5-dinitrobenzoyl) peptide esters with varying length of the ester alkyl group on CSPs **2** and **3**, it was proposed that the intercalation of the ester alkyl group of dipeptide derivatives between the connecting arms of CSPs is responsible for the chromatographic resolution behaviors.

**Acknowledgement.** This work was supported by the Professor Training Program (1990), the Ministry of Education, Korea.

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# The Rheological and Mechanical Model for Relaxation Spectra of Polydisperse Polymers

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The theoretical equation for the relaxation spectrum of nonlinear viscoelastic polymeric material was derived from the Ree-Eyring and Maxwell non-Newtonian model. This model consists of infinite number of hyperbolic sine law Maxwell elements coupled in parallel plus a spring without a dashpot. Infinite number of nonlinear viscoelastic Maxwell elements can be used by specifying distribution of relaxation times, hole volumes, molecular weights, crystallite size and conformational size, etc. The experimentals of stress relaxation were carried out using the tensile tester with the solvent chamber. The relaxation spectra of nylon 6 filament fibers in various electrolytic solutions were obtained by applying the experimental stress relaxation curves to the theoretical equation of relaxation spectrum. The determination of relaxation spectra was performed from computer calculation.

## Introduction

The relaxation spectra for the linear viscoelastic polymeric materials have been reported[1-4]. However, the theoretical and phenomenological analysis for the relaxation spectrum of nonlinear viscoelasticity has not been performed sufficiently, due to the theoretical treatments involving more complicated function. The viscoelastic behavior of polymeric materials at modest to large deformation is complicated by material nonlinearities. These nonlinearities are thought to arise from the distribution in molecular weights, crystallite size, conformational size and relaxation times of flow segments.

Viscous interfaces also play a significant role in determining the time-dependent properties of bone[5], for which a logarithmic spectrum has been used successfully in constructing a constitutive equation[6]. The logarithmic relaxation spectrum may be of use in describing system in which viscoelastic behavior arisen from motion along viscous interfaces in an elastic matrix. Although it is relatively easy to obtain the viscoelastic functions from the relaxation spectra, the reverse process is difficult, and usually involves successive approximations of some kind. For these reasons, a variety of approximation methods[7,8] have been developed for performing such calculation. Generally, the approximation methods have an analytical foundation based on the properties of the integrands of the corresponding exact equation. Such an integrand is usually the product of the viscoelastic function initially known and an additional dimensionless intensity functions. If, within a particular zone of viscoelastic behavior, an empirical equation can be used to fit a viscoelastic function, an exact expression for the corresponding spectrum and sometimes be derived as shown by Smith[9].

In this work, the theoretical equation for the relaxation spectrum of nonlinear viscoelastic materials was derived from the Ree-Eyring and Maxwell non-Newtonian model. This model consists of infinite number of hyperbolic sine law Maxwell elements coupled in paralled plus a spring without a dashpot. Each non-Newtonian Maxwell element can be specified by relaxation time and intrinsic strain modulus, and in the general case infinite number of nonlinear viscoelastic Maxwell elements can be used by specifying a distribution of relaxation times, hole volumes and molecalar weights. The relaxation spectra of the solid polymers were obtained by applying the experimental stress relaxation curves to the theoretical relaxation spectrum equation. It is observed that the relaxation spectra of these samples are directly related to the distribution of viscosities, activation free energies, relaxation times and molecalar weights.

## Theory

**The Ree-Eyring and Maxwell Non-Newtonian Model.** The Ree-Eyring and Maxwell non-Newtonian model put forward by Ree, Hahn and his colleagues, shown in Figure 1. This model consists of a spring and infinite number of non-Newtonian Maxwell elements coupled in parallel. As can be seen, it necessarily possesses many relaxation times and shear moduluses. For real viscoelastic polymeric materials, we postulate the existence of a continuous spectrum of relaxation times. The concept that a continuous distribution of relaxation times should be required to represent the