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Resolution of DL-Valine by Countercurrent Solvent Extraction with Continuous Sample Feeding

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Abstract

DL-Valine was resolved completely by a solvent extraction system with a continuous sample feeding process called continuous countercurrent fractional extraction (CFE). The two-phase system contained *n*-butanol and water with a copper(II) complex of *N*-*n*-dodecyl-L-hydroxyproline as a resolving reagent. The upper (alcohol-rich) phase and the lower (water-rich) phase proceeded in the segmented extraction columns countercurrently; the D-isomer was recovered in the upper phase, while the L-isomer was recovered in the lower phase. The optical purity of the enantiomers obtained was consistently 99.5% or higher. The D-isomer extracted in the upper phase was recovered completely by using a backextraction column in series after the extraction column, allowing the upper phase to be used repeatedly for the resolution. The efficiency of the CFE system was estimated at more than 24 theoretical stages.

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INTRODUCTION

The resolution of enantiomers is attracting interest in a variety of fields, such as organic chemistry, pharmaceutical chemistry, and related disciplines. Liquid chromatography with chiral stationary phases or with chiral additives is an effective technique for this purpose, and many analytical separations have been achieved with various classes of compounds (*1*). Enantiomeric separations on a preparative scale are also in progress (*2*).

The characteristic feature of the resolution of enantiomers as a separation problem is that the mixture is separated into only two fractions, whereas chromatography is generally best suited to the separation of more complicated mixtures. In fact, fractional crystallization is the usual procedure applied to the preparation of enantiomers. In this case the separation is achieved by the selective distribution of enantiomers between solutions and crystals. In some instances, repeated recrystallization is required for complete resolution when the selectivity is not high enough to obtain pure compounds by a single crystallization. The two phases which selectively distribute enantiomers need not necessarily be solid and liquid. Two immissible liquid phases may also be used, if good selectivity and a simple method for fractional extraction are available.

In a previous paper we reported an enantioselective solvent extraction system which consisted of *n*-butanol and water containing *N*-*n*-alkyl-L-proline or *N*-*n*-alkyl-L-hydroxyproline and copper(II) ions (*3*), and resolved DL-isoleucine completely by droplet countercurrent chromatography with this two-phase system (*4*). Theoretical calculations based on the countercurrent extraction process (*5*) indicate that a complete resolution of several neutral DL-amino acids should be possible with this system if a fractional extraction procedure of approximately 20 to 30 theoretical stages is achieved.

In this paper a laboratory-scale fractional extraction procedure is devised and the efficiency of the procedure is demonstrated by the resolution of DL-valine.

EXPERIMENTAL

Reagents

N-*n*-Dodecyl-L-hydroxyproline (C_{12}-Hyp) was prepared from L-hydroxyproline and *n*-dodecylaldehyde as described in a previous paper (*3*).

The other reagents were purchased from commercial sources and used without further purification.

Determination of the Distribution Ratio of Amino Acid Enantiomers

The two-phase system was prepared as follows. *n*-Butanol containing 5 m*M* copper(II) acetate and 10 m*M* C_{12}-Hyp was mixed with an equal volume of 100 m*M* acetate (Na) buffer (pH 6.5), and the mixture was shaken vigorously in a separatory funnel for 20 min at 25°C. The solution separated into two clear and stable phases after standing for 5 min. C_{12}-−Hyp was distributed almost exclusively in the upper (alcohol-rich) phase; the distribution ratio was estimated as ~8000 (*3*). The concentration of copper(II) ions in the lower (water-rich) phase was 0.2 m*M* when determined by chelometric titration using EDTA and PAN. The pH of the lower phase was 6.18. When a large volume of the lower phase was required in the countercurrent extraction experiments, 0.2 m*M* copper(II) acetate solution in 100 m*M* acetate buffer (pH 6.18) saturated with *n*-butanol was used in place of the lower phase, since an extremely small amount of C_{12}=Hyp is dissolved in the lower phase. Two-phases in various molar ratios of copper(II) ions to C_{12}-Hyp were prepared by changing the concentration of the copper(II) acetate.

To determine the distribution ratio of amino acid enantiomers, 2 mL of the lower phase containing 0.1 m*M* DL-amino acid was mixed with an equal volume of the upper phase by vortex mixing in a test tube for 2 min. After phase separation the concentration of each enantiomer in thelower phase was determined by reversed-phase chromatography by employing a chiral copper(II)-L-proline mobile phase (*6*). The distribution ratio (*D*) was expressed as $D = (C_1 - C_e)/C_e$, where C_1 is the initial concentration of an enantiomer in the lower phase and C_e is the concentration in the lower phase at distribution equilibrium. A separation factor, i.e., the ratio of *D* for the DL-isomer to that of L-isomer, was taken as a measure of the enantioselectivity of the two-phase system.

Continuous Countercurrent Fractional Extraction (CFE)

The CFE system consisted of two columns and five pumps (Fig. 1). Each column was a glass tube (100 cm × 2 cm i.d.) segmented at 1 cm intervals by glass disks having a central hole of 5 mm i.d.; the number of cells formed in each column was over 80. The appearance of the columns

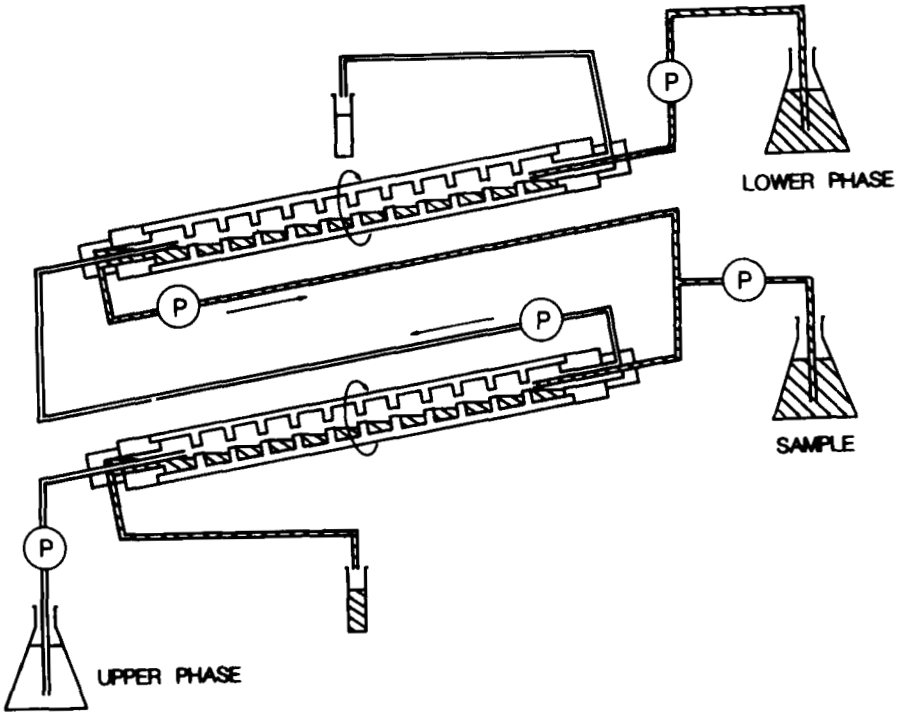

FIG. 1. Schematic diagram of CFE. P = reciprocating pump.

was similar to those of rotation locular countercurrent chromatography (7). Rotation devices (Fig. 2) were attached to both ends of each column. The upper phase or the lower phase entered the column at the Teflon tube inlet (F) and exited at I-a through a space between the glass tube (E) and the Teflon tube inlet (F). The Diflon block (G) was fixed to the column holder and inclined at 10° while the other part was rotated by a motor. The Teflon rotating seal (K) prevents a leak of the solution. Parts F, G, I, and K were not rotated during the operation.

Before each experiment, each column was held upright and the outlet for the lower phase was closed. The upper phase was fed at the lower inlet and, after the column was filled completely with the upper phase, the column was set in the operating position. The lower phase was fed at the upper inlet of the column until it reached the bottom end. The flow rates

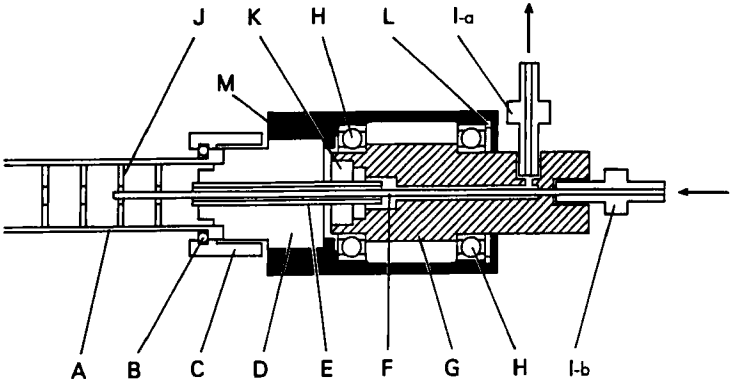

FIG. 2. Schematic drawing of the rotation device. (A) Glass column; (B) O-ring (Teflon); (C) cap nut (Diflon); (D) glass tube holder (Diflon); (E) glass tube; (F) Teflon tube: (G) bearing shaft (Diflon); (H) ball bearing; (I-a) outlet joint (Diflon); (I-b) inlet joint (Diflon); (J) glass disk: (K) rotating seal (Teflon); (L) stop ring; (M) roller.

of the two phases at two inlets and one outlet of a column were controlled by pumps, while the second outlet was left open in order to prevent a pressure build-up. The column was rotated at 90 rpm to promote the partitioning of solutes.

In the countercurrent process, an optimum ratio of the flow rate of the lower phase to that of the upper phase (r) is calculated by

$$r = (D_1 D_2)^{1/2} \quad (1)$$

where D_1 and D_2 are the distribution ratios of the components to be separated. Under the conditions, the same amount of products can be obtained per unit time (*5*). When the pH in the lower phase was 6.18, the r value for the valine racemate was close to unity (Table 1), and the flow rate magnitudes for the upper and lower phases were arbitrarily set equal to 0.5 mL/min. DL-Valine was dissolved in the lower phase to yield 2.4 mM and was fed continuously into the middle between the two columns at 0.05 mL/min. All experiments were carried out at 25°C.

The enantiomers in the effluent were determined at appropriate time intervals by the same HPLC system used for the determination of the distribution ratio. The lower phase was injected into the HPLC column directly. The upper phase was extracted with the same volume of lower

TABLE 1
Distribution Ratios (D) and Separation Factors (α) of Various DL-Amino Acids[a]

Amino acid		D	α	Amino acid		D	α
Alanine	D	0.24	1.41	Isoleucine	D	4.96	3.54
	L	0.17			L	1.40	
Valine	D	1.66	2.86	Methionine	D	1.67	1.62
	L	0.58			L	1.03	
Norvaline	D	1.94	2.40	Tyrosine	D	2.65	1.25
	L	0.81			L	2.12	
Leucine	D	4.03	2.04	Phenylalanine	D	7.33	1.83
	L	1.98			L	4.01	
Norleucine	D	5.88	2.50				
	L	2.35					

[a]The two-phase system was prepared by mixing 100 mM acetate buffer (pH 6.5) with an equal volume of n-butanol containing 5 mM copper(II) acetate and 10 mM C_{12}-Hyp. DL-Amino acids were dissolved in the lower phase at 0.1 mM.

phase, and its lower phase was injected into the column. An optical purity (%) was expressed as $100|C_{D\text{-Val}} - C_{L\text{-Val}}|/(C_{D\text{-Val}} + C_{L\text{-Val}})$, where $C_{D\text{-Val}}$ is the concentration of D-valine in the effluent and $C_{L\text{-Val}}$ is that of L-valine.

Recovery of D-Valine in the Upper Phase by Continuous Backextraction

The system consisted of two pumps and one backextraction column which was the same as the extraction columns and was rotated at 90 rpm. The upper phase containing D-valine was fed at the lower inlet of the 10° inclined column at 0.5 mL/min, while the fresh lower phase was fed at the upper inlet at 1.5 mL/min.

RESULTS

Distribution Behavior of DL-Amino Acids in the Two-Phase System

The separation factors found for isoleucine and valine were higher than those found for the rest of neutral amino acids which had a value of ap-

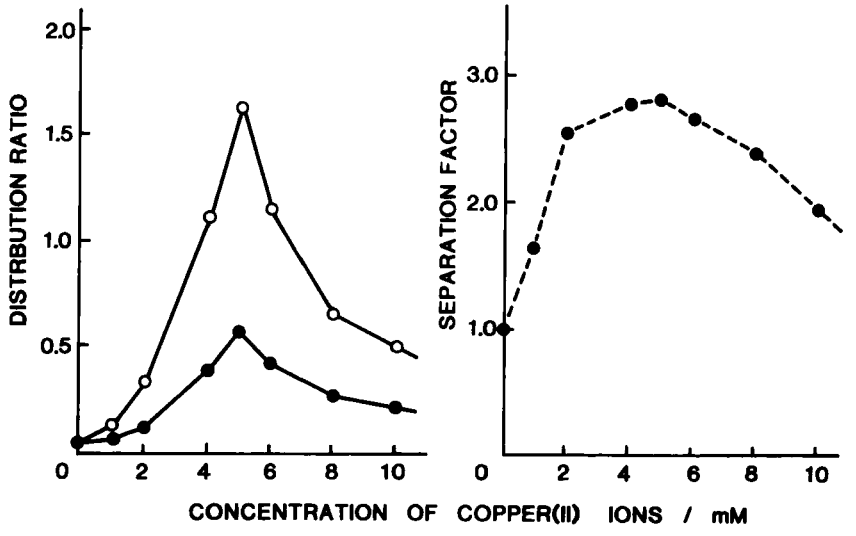

FIG. 3. Effect of copper(II) ions on the distribution ratio and separation factor of valine enantiomers. (——) Distribution ratio of D-valine (O) and L-valine (●); (- -) separation factor; C_{12}–Hyp: 10 m*M*; DL-valine: 0.1 m*M* in the lower phase.

proximately 2 (Table 1). Both the separation factor and the distribution ratios showed a maximum value when the molar ratio of copper(II) ion to C_{12}-Hyp was 1:2 (Fig. 3). The initial concentration of racemic amino acid in the lower phase had no noticeable effect on the distribution ratio between the values of 0.05 to 2 m*M*. However, if the concentration of the racemic amino acid was increased beyond 2 m*M* and came closer to the concentration of the C_{12}-Hyp copper(II) complex, the distribution ratio rapidly decreased. The separation factor for the valine enantiomers was not significantly affected by pH, although their distribution ratios increased almost five times from pH 4.4 to 6.18.

Continuous Resolution of DL-Valine by CFE

The resolution of racemic valine was attempted with the CFE device, and the concentration of enantiomers in both eluates were monitored by HPLC (Fig. 4). About 5 to 10 h after the flow of the 2.4 m*M* DL-valine solution was started, D- and L-valine eluted in the upper and lower phases, re-

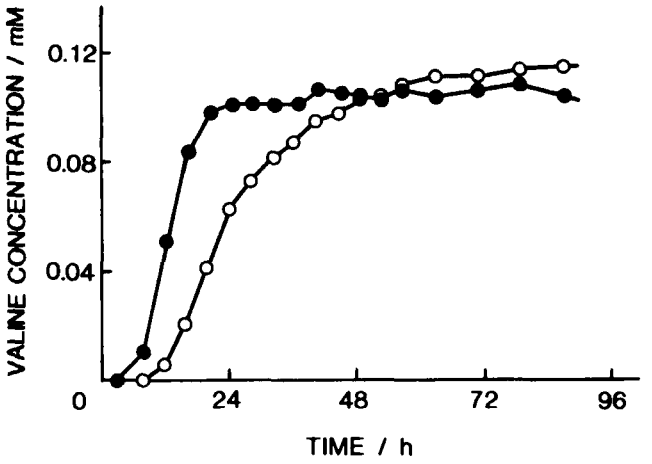

FIG. 4. Concentration of valine enantiomers in the effluent of CFE. (○) D-Valine in the upper phase; (●) L-valine in the lower phase. The concentration of valine enantiomers in the upper phase was estimated from the corresponding concentration of those backextracted with an equal volume of the lower phase and the distribution ratio.

spectively. The concentration increased gradually and reached a plateau at 0.118 m*M* for D-valine and 0.109 m*M* for L-valine in the steady state. Throughout the opertion, neither D-isomer in the lower phase nor L-isomer in the upper phase was detected with the HPLC system used; thus the concentrations were less than 0.25 μ*M* and the optical purity of both enantiomers was 99.5% or more.

Efficiency of Extraction Column

To calculate the efficiency of the extraction columns, the method of calculation used in countercurrent extraction was applied (*5*). The assumptions are made that the system consists of equivalent theoretical stages connected in series (Fig. 5) and that the partitioning of solutes in each state is in complete equilibrium. The right side of the sample feed stage is called the extraction section and the left side is the wash section. The sample feed stage is included in both sections, and the total number of theoretical stages is expressed as $m + n - 1$. The fraction eluted in the upper phase (ψ) is expressed by

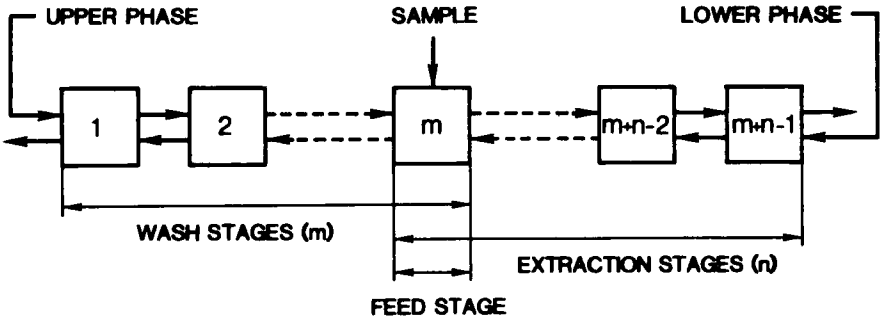

FIG. 5. Schematic diagram of two-solvent countercurrent extraction.

$$\psi = \frac{\text{amount obtained in the upper phase (mol/min)}}{\text{feed amount (mol/min)}} \tag{2}$$

$$= \frac{(\varepsilon_1 - 1)(\varepsilon_2^m - 1)}{(\varepsilon_1^{n+1} - 1)(\varepsilon_2 - 1)\varepsilon_2^{m-1} + (\varepsilon_2^{m-1} - 1)(\varepsilon_1 - 1)} \tag{3}$$

where m and n are the number of stages in the extraction section and the wash section, respectively; ε is the extraction factor expressed as r/D; and suffixes 1 and 2 represent the extraction section and the wash section, respectively. Since the sample solution was fed into the lower phase, the flow rate ratios in the extraction section (r_1) and the wash section (r_2) were 1.0 and 1.1, respectively. According to the experimental results, the ψ values of D- and L-valine obtained should be more than 0.998 and less than 0.002 (that means more than 99.6% optical purity). Then m and n are estimated as approximately 14 and 11 by using Eq. (3). Consequently, the total number of theoretical stages was at least 24, that is, less than seven cells corresponded to a theoretical stage.

Continuous Backextraction of D-Valine in the Eluted Upper Phase

The D-valine recovered in the upper phase was extracted continuously by passing it through the backextraction column. The flow rate of the lower phase in the backextraction column was adjusted to remove D-

valine completely from the upper phase. Since the distribution ratio of C_{12}-Hyp is extremely large, little C_{12}-Hyp was removed from the upper phase in the backextraction process. Consequently the D-valine-free upper phase can be repeatedly used for the resolution. The distribution ratio observed with the regenerated upper phase and the 0.2 m*M* copper(II) acetate solution of 100 m*M* acetate buffer (pH 6.18) saturated with *n*-butanol was essentially the same as the value obtained with the original two-phase system.

DISCUSSION

Lengthening a column is a common approach used to obtain high separation efficiencies; however, too long a column is unsuitable for practical use because this leads to the accumulation of solutes in the column and decreases the throughput. In this experiment the concentration of each solute in the feed stage was about 4 times that in the final stage, and it took a day or two to reach a constant concentration level (Fig. 4). Therefore, it would not always be advantageous to use a longer column. The selection of column length is a compromise between purity, operation time, and throughput. If column efficiency in the current system is 30 theoretical stages, it is estimated by calculation (*5*) that the separation factors would be required to be about 1.6 or more to achieve 95% resolution.

The resolution of enantiomers for which both distribution ratios are more than or less than unity (Table 1) can also be achieved by changing the flow rate ratio according to Eq. (1), although one of two phases will be consumed at a greater rate. For example, DL-leucine can be resolved at a flow rate ratio of about 2.8, while for DL-alanine this value is about 0.2.

Besides our ligand-exchange system, some enantioselective solvent extraction systems were reported previously (*8–13*). Among them, such systems as a chloroform–methanol–water system containing a chiral 2-aminobutanol for carboxylic acids (*8*) and a chloroform–acetonitrile–water system containing a chiral crown ether for amino acids and their esters (*9*) would be expected to have good separation factors to allow enantiomers with high optical purity to be obtained by CFE.

In conclusion, the advantageous feature of our CFE system is that both the inflow and the outflow of the two phases can be performed simultaneously with column rotation. The development of the rotation device (Fig. 2) makes a complicated continuous countercurrent procedure feasible. DL-Valine was completely resolved by CFE, and the results indicate that CFE could be applied to the preparation of optically active com-

pounds. Actually, the 100-fold concentrated DL-valine was resolved under the present conditions (*14*). In addition, the use of the closed system, which consists of the separation section and the backextraction section, minimizes the loss of the resolving reagent. Besides DL-amino acids, various compounds which have different distribution ratios would also be separated by CFE. Separation of a single component from mixtures of various components is expected to be possible by two operations.

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