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SEPARATION OF THE OPTICAL ISOMERS OF AMINO ACIDS BY LIGAND-EXCHANGE CHROMATOGRAPHY USING CHEMICALLY BONDED CHIRAL PHASES

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SUMMARY

This paper describes further studies of the separation of amino acid racemates on chemically bonded chiral phases by ligand exchange chromatography. The stationary phase consists of a silica support to which L-amino acids as chiral components are bonded via 3-glycidoxypropyltrimethoxysilane.

Important factors affecting the selectivity and efficiency of the separations are the pH and ionic strength of the mobile phase, as well as the temperature. Examples for the separation of mixtures of racemic amino acids are given.

INTRODUCTION

Ligand exchange chromatography was found to be a very useful method for separating α-amino acid racemates. It has been applied either by the use of chiral stationary phases[1–16], or by loading of chiral metal chelates to ion exchangers[17–20], or by adding them to the mobile phase[21–24].

We reported previously the synthesis of a chemically bonded chiral phase, carried out by bonding 3-glycidoxypropyltrimethoxysilane to silica, followed by the reaction with L-α-amino acids[14,15].

This paper deals with the study of the role of the bonded amino acid and the metal ion as well as the influence of the mobile phase on the separations. The method is applied to the optical resolution of mixtures of amino acids.

EXPERIMENTAL

Reagents

Silica gel, LiChrosorb Si-100, 10 μm and 5 μm, was obtained from E. Merck (Darmstadt, G.F.R.). 3-Glycidoxypropyltrimethoxysilane and chloropropyltrimethoxysilane were purchased from Serva (Heidelberg, G.F.R.). Amino acids were obtained from Sigma (St. Louis, MO, U.S.A.) and from Serva. Potassium dihydrogen phos-

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phate and disodium hydrogen phosphate were obtained from E. Merck. All solvents were of reagent grade.

Instrumentation

A Perkin-Elmer liquid chromatograph Series 2 equipped with a Rheodyne 7105 injector, a Perkin-Elmer LC 55 UV detector and a Perkin-Elmer 023 recorder, was used. Detection was carried out at 220 nm. As additional detector a Perkin-Elmer Model 241 polarimeter with a micro flow-cell of 100 cm path length and 0.65 cm I.D. was also used.

Packings

The synthesis of the stationary phase was carried out as previously described[14,15]. Stainless-steel columns of 5, 10 and 25 cm length and 0.46 cm I.D. were packed by the ascending slurry technique.

Mobile phases

Phosphate buffers of various pH and ionic strengths were used as mobile phases.

RESULTS AND DISCUSSION

Structural aspects influencing the retention

A bidentate complex is probably formed on the stationary phase (Fig. 1). One fixed ligand is exchanged by the D- or L-amino acid to give a mixed complex. The different complex stabilities between the mixed complexes with the D- and L-form respectively, lead to the separation of the enantiomers.

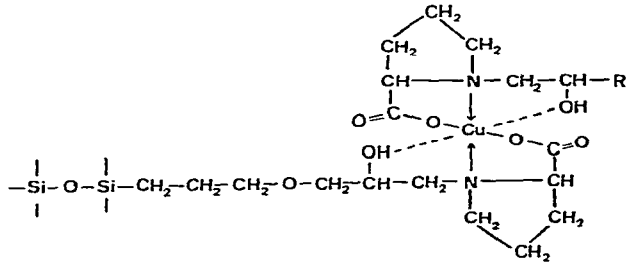

Fig. 1. Formula of the bidentate stationary complex.

An important factor is that the carboxyl group of the bonded amino acid is free for complex formation with the metal ion.

A second criterion is the hydroxyl group in the side-chain, formed by the opening of the epoxide. This hydroxyl group cause the hydrophilic properties of the material. Hydrogen bonds can possibly be formed with the amino acids. It is also possible that the hydroxyl group is involved in complex formation. The importance of the presence of the hydroxyl group is confirmed by comparing these experiments with those using a material synthesized with chlorpropyltrimethoxysilane, in which no separations could be obtained.

A possible structure of a mixed complex between L-proline as fixed ligand and an amino acid is given in Fig. 2.

The retention of the amino acids seems to depend on their hydrophobity. In particular amino acids with aromatic substituents show high k' values and extremely high α-values. Additionally, existing polar groups like hydroxyl, which can form hydrogen bonds, also cause an increase in k'.

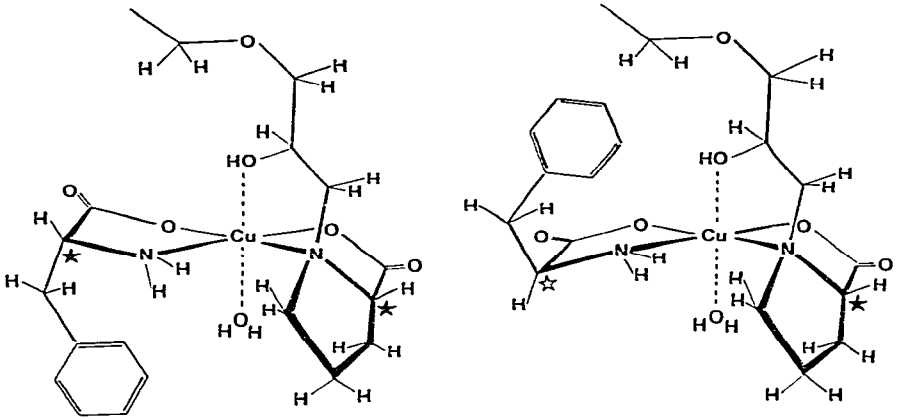

Fig. 2. Possible structure of the mixed complex between L-proline as fixed ligand and D- and L-phenylalanine.

Role of the bonded amino acid and the metal ion

Various amino acids were bonded as chiral components to the stationary phase. As can be seen from the α-values in Table I, the best separations were observed with L-hydroxyproline and L-proline as fixed ligands.

TABLE I

INFLUENCE OF THE FIXED LIGAND ON THE RELATIVE RETENTION OF SOME AMINO ACID ENANTIOMERS

Column, 10 × 0.46 cm I.D.; mobile phase, 0.05 *M* KH_2PO_4, pH 4.6; flow-rate, 1 ml/min.

Mobile ligand	*Stationary ligand*				
	L-*Proline* k_L/k_D	D-*Proline* k_L/k_D	L-*OH-Proline* k_L/k_D	L-*Valine* k_L/k_D	L-*Histidine* k_L/k_D
DL-Tryptophan	3.5	0.36	4.2	1.85	0.76
DL-Phenylalanine	2.9	0.4	3.3	1.7	0.8
DL-Tyrosine	3.1	0.38	4.3	1.8	0.65
DL-Valine	1.5	0.66	1	1.45	1
DL-Proline	0.6	2.3	0.54	1	1
DL-Histidine	1.8	0.53	2.1	2.0	1
DL-Threonine	1.6	0.6	1.5	1.25	1

As indicated by the polarimeter detector, the L-enantiomers always appeared with higher k' values. An exception is proline, for which a reversed sequence was observed on columns containing L-proline or L-hydroxyproline as a fixed ligand. If the fixed ligand is D-proline, the opposite elution order is observed (Table I).

More work needs to be done in order to clarify the theoretical background of these observations.

Copper-(II), cobalt(II), nickel(II) and zinc(II) were tested as complexing ions. Acceptable results, however, were only obtained with copper(II).

Influence of pH and ionic strength of the mobile phase

The optimum pH range for complex formation is in an alkaline medium. The retention using alkaline buffers as mobile phase is much higher than with neutral or acid ones. High resolutions are obtained, but separation times are long and a marked band-broadening is observed. The lifetime of the columns is also reduced when the pH value in the mobile phase is high.

The use of neutral or even acid buffers as a mobile phase results in a significant reduction of the k' values with a simultaneous improvement of the peak forms, while maintaining complete resolutions for nearly all amino acids.

The increase in ionic strength also leads to a reduction of the k' values, but is limited in order to avoid crystallization of the buffer in the column. Fig. 3 shows the dependence of the k' values on pH and the ionic strength of the mobile phase.

The addition of organic solvents to the mobile phase leads to a decrease in the resolution.

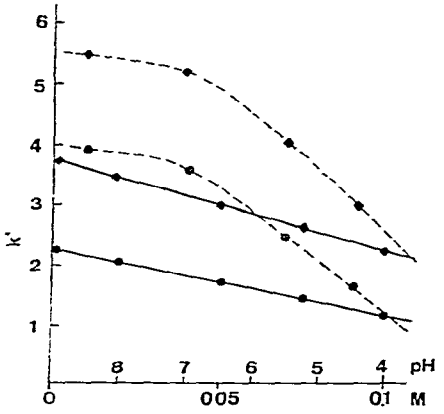

Fig. 3. Dependence of k' values on ionic strength and pH. D-Serine (●), L-serine (■), ionic strength (———), pH (– – –). Column, 25 × 0.46 cm I.D.; silica, 10 μm, with L-proline as fixed ligand, loaded with copper(II); mobile phase, phosphate buffer of different composition; flow-rate, 2 ml/min; temperature, 50°C.

Influence of the temperature

The selectivity can be greatly improved by increasing the temperature. Fig. 4 shows the change in height equivalent to theoretical plate and resolution, using DL-arginine as an example, by increasing the temperature from 0 to 60°C.

Using 0.05 *M* KH_2PO_4, pH 4.5, at 50°C, a complete optical resolution of a large number of amino acid racemates can be obtained with separation times of 2–15 min. Some amino acids, which could not be resolved at this temperature, were resolved at 80°C. The lifetime of the columns is reduced, however, by longer use at this temperature.

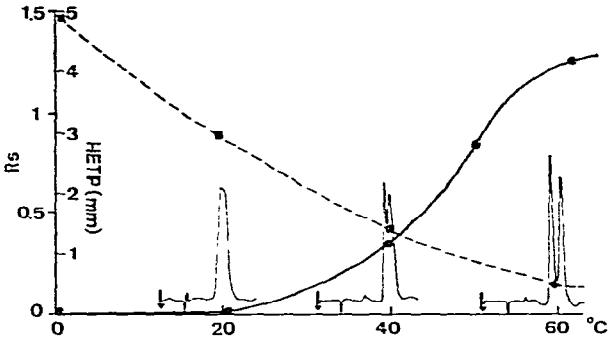

Fig. 4. Influence of temperature on the resolution R_s (●) of DL-arginine and HETP (■) (D-arginine). Column, 25 × 0.46 cm I.D.; silica, 10 μm, with L-proline as fixed ligand, loaded with copper(II); flow-rates, 2 ml/min.

DL-Alanine, DL-leucine, DL-cystine and DL-glutamic acid could not be separated even at 80°C. If a polarimeter detector is used, an enrichment of one enantiomer at the front of the band and an other at the back is observed (Fig. 5).

The chromatographic data of a series of α-amino acids are given in Table II.

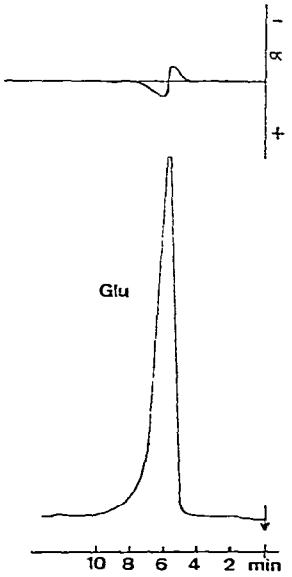

Fig. 5. Partial separation of DL-glutamic acid. Lower curve, UV detector; upper curve, polarimeter detector. Column, 25 × 0.46 cm I.D.; silica, 10 μm, with L-proline as fixed ligand, loaded with copper(II); mobile phase, 0.05 *M* phosphate buffer, pH 4.6; flow-rate, 1 ml/min.

Separation of α-amino acid enantiomers

The method has been applied to the optical resolution of mixtures of amino acid racemates. In Figs. 6–8 typical examples of the separation of some mixtures of

TABLE II

k' VALUES, RELATIVE RETENTION ($\alpha = k'_L/k'_D$) AND CHROMATOGRAPHIC RESOLUTION ($R_s = 1.177\,(t_2 - t_1\,/b_{0.5(2)} + b_{0.5(1)})$ FOR DL-AMINO ACIDS

Column, 25 × 0.46 cm I.D.; mobile phase, 0.05 *M* KH_2PO_4, pH 4.6; flow-rate, 2 ml/min.

DL-Amino acid	k_D	k_L	α	R_s
Alanine	1.3	1.3	1.0	0
Norvaline*	2.0	2.2	1.1	0.2
Norleucine*	3.1	3.7	1.2	1.1
Valine	2.5	3.8	1.5	1.9
Leucine	3.1	3.1	1.0	0
Asparagine	3.0	4.1	1.4	2.0
Glutamine	1.0	1.0	1	0
Aspartic acid	3.2	4.1	1.3	1.2
Glutamic acid	2.0	2.0	1.0	0
Arginine	2.4	3.0	1.25	1.15
Isoleucine*	2.7	3.6	1.3	1.5
Serine	2.0	3.2	1.6	3.3
Threonine	2.0	3.2	1.6	1.5
Phenylalanine	3.2	9.4	2.9	6.9
Phenylserine	6.2	10.2	1.7	3.5
p-Tyrosine	3.3	10.2	3.1	7.2
o-Tyrosine	2.8	9.0	3.2	8.5
m-Tyrosine	2.2	6	2.7	6.4
DOPA	3.4	11.2	3.2	7.3
Tryptophan	7.8	27.4	3.5	7.1
5-Hydroxytrytophan	7.4	25.2	3.4	7.0
Proline	2.4	1.4	0.6	2.1
Histidine	6.7	12.1	1.8	3.8
Lysine*	1.8	2.2	1.2	1.2
Ornithine	0.6	0.75	1.2	1.1
Citrulline*	2.1	1.6	1.25	1.25
Cystine	5.4	5.4	1.0	0
Methionine*	3.0	3.4	1.15	0.6
Ethionine*	4.0	4.6	1.15	1.1

*Temperature 80°C.

amino acids are presented. For the identification of the enantiomers in the mixtures a polarimeter detector was used in addition to the UV detector.

The separation of more complex mixtures would necessitate the use of pre-columns for the preseparation into fractions. The method of column switching could probably solve such problems.

CONCLUSION

Our experiments have shown that the use of chemically bonded chiral phases, loaded with copper ions, is a very successful and simple way of separating amino acid racemates.

More work needs to be done to study the mechanism of separation. As preliminary experiments have shown, the method also seems to be applicable to the optical resolution of racemic dipeptides. The extension of this method to the separation of diverse racemic complex-forming drugs is the subject of further investigations.

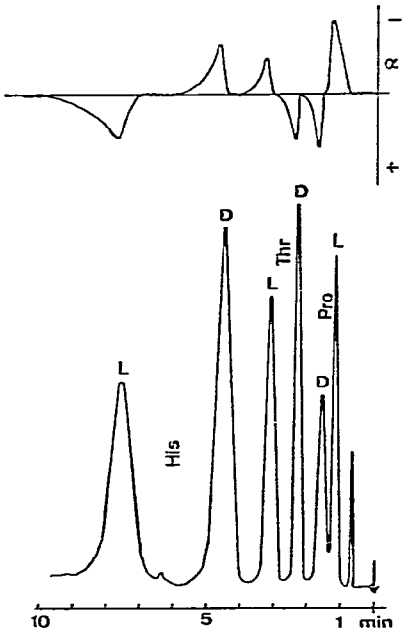

Fig. 6. Separation of DL-proline, DL-threonine and DL-histidine. Lower curve, UV detector; upper curve: polarimeter detector. Column, 25 × 0.46 cm I.D.; silica, 10 μm, with L-proline as fixed ligand loaded with copper(II), mobile phase 0.05 *M* KH_2PO_4, pH 4.6; temperature, 50°C; flow-rate, 5 ml/min.

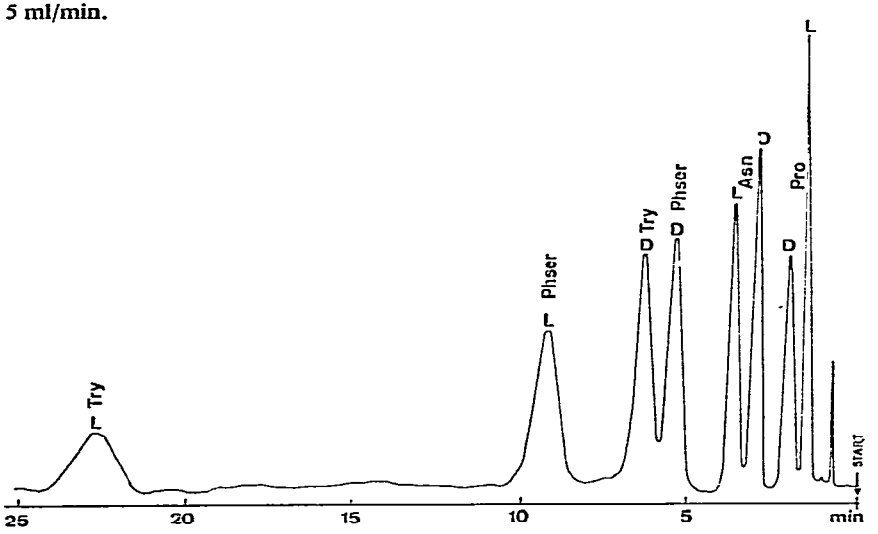

Fig. 7. Separation of DL-proline, DL-asparagine, DL-phenylserine and DL-tryptophan. Conditions as as in Fig. 6.

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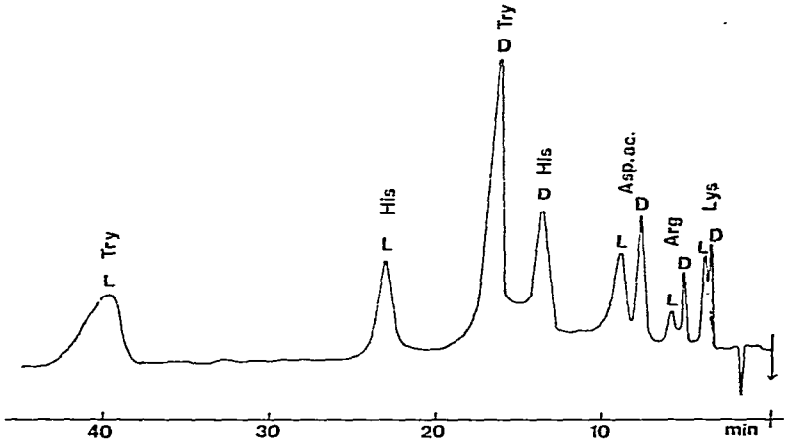

Fig. 8. Separation of DL-lysine, DL-arginine, DL-aspartic acid, DL-histidine and DL-tryptophan. Column, 25 × 0.46 cm I.D.; silica, 5 μm; mobile phase, 0.05 M KH_2PO_4, pH 4.5; temperature, 50°C; flow-rate, 5 ml/min; detection, UV at 220 nm.

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