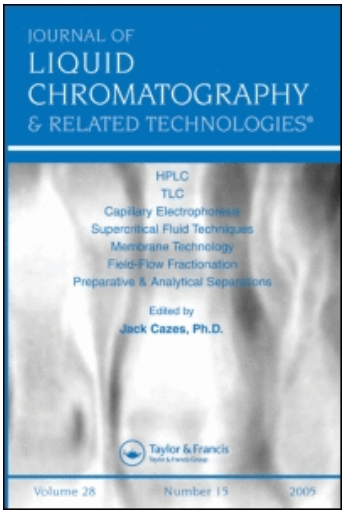

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Resolution of the Optical Isomers of Underivatisized Amino Acids on Chemically Bonded Chiral Phases by Ligand Exchange Chromatography

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RESOLUTION OF THE OPTICAL ISOMERS OF UNDERIVATISIZED AMINO ACIDS ON CHEMICALLY BONDED CHIRAL PHASES BY LIGAND EXCHANGE CHROMATOGRAPHY.

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ABSTRACT

A method for the direct separation of racemates by HPLC is described. A chiral stationary phase is synthesized, suitable for ligand exchange chromatography. L-proline is chemically bonded to silica gel via 3-glycidoxypropyltrimethoxysilane. The bonded support is loaded with Cu(II) ions as a complexing agent. Complete resolution of amino acid racemates can be obtained in less than ten minutes. α-values up to 3.5 are observed.

INTRODUCTION

Chromatographic resolution of optical isomers is based either on the formation of diastereomers or on application of chiral stationary or mobile phases. A number of reports appear in the literature, describing attempts to separate racemates using natural and later synthetic optically active polymeric sorbents (1-5).

Recently, ligand exchange chromatography was introduced for the separation of enantiomers. Davankov et al. (6-13), Snyder et al. (14), and later Josefonvicz et al. (15) used polystyrenedivinylbenzene resins, containing L-aminoacids as chiral groups. They described complete separations of DL-aminoacids with Cu(II) ions as a complexing agent. The time taken for separation, however, is about ten hours. Shorter separation times are obtained by Lefebvre et al. (16-18), using acrylamide polymers substituted by L-amino acids. The synthesis of such polymers in HPLC-quality, however, is very complicated and difficult to reproduce. Another approach, namely the addition of chiral metal chelates to the mobile phase was chosen by several authors (19-23).

In a previous paper, we reported the synthesis of a chemically bonded chiral phase for the separation of DL-amino acids by ligand exchange chromatography (24). The stationary phase consists of L-proline, chemically bonded to silica gel and loaded with Cu(II) ions. This paper deals with the optimation of this method and its application to the separation of a great number of racemic amino acids.

EXPERIMENTAL

Reagents:

Silica gel, LiChrosorb Si 100, 10 µm, was obtained from Merck (Darmstadt, Germany).

3-Glycidoxypropyltrimethoxysilane was purchased from Serva (Heidelberg, Germany).

Amino acids were obtained from Sigma chemical company (St.Louis Missouri, USA).

Potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Merck (Darmstadt, Germany).

All solvents were of reagent grade.

Instrumentation:

The liquid chromatograph consisted of a Perkin-Elmer Series 2 pump equipped with a Rheodyne 7105 injector, a Perin-Elmer LC 55 UV detector and a Perkin-Elmer 023 recorder.

Stainless steel columns of 5,10 and 25 cm length and 0.46 cm I.D. were used.

Volumes of 20 µl containing about 10 µg amino acid were injected. Detection was carried out at 220 nm.

Synthesis:

10 g Silica gel, 10 µm, dried for 2 hours at 150° are suspended in 50 ml dry benzene. After the addition of 5 ml 3-glycidoxypropyltrimethoxysilane, the suspension is refluxed for 6 hours. The reflux condenser is kept at 65° in order to remove the formed methanol from the mixture (25). After cooling, the benzene is removed by filtration and the product is suspended in methanol. 9 g sodium prolinate are added and the mixture is shaken for 48 hours at room temperature. The bonded support is then filtered, washed with methanol and dried.

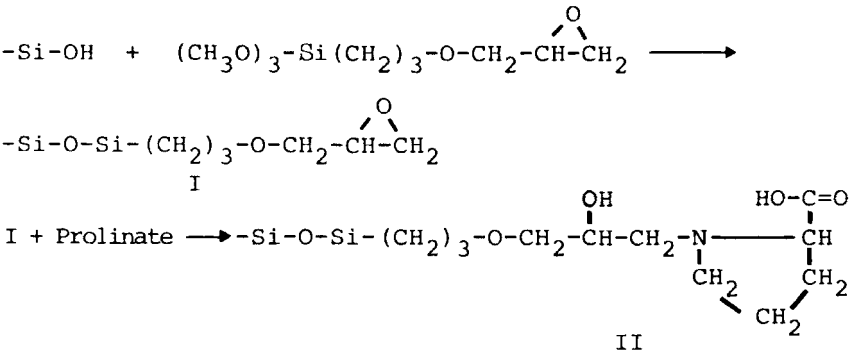
The complex formation is either subsequently carried out by treatment of the material with aquous copper nitrate solution or by passing copper nitrate solution through the column.

Columns are packed by the ascending slurry technique (26).

RESULTS AND DISCUSSION

Various silanes were tested for their suitability to form appropiate reaction intermediates with silica for the attachement of L-α-amino acids as chiral components. 3-Glycidoxypropyltrimethoxysilane, known as reagent in the synthesis of "diol"-phases (27), was found to be a suitable one.

The synthesis of the support is described in the following reaction scheme:



3-Glycidoxypropyltrimethoxysilane is bonded to silica gel in the first step. L-Proline (as sodium salt) is attached then to the epoxyd I in the second step to give product II. As a complexing agent Cu(II)ions were used. The loading of the support with metal ions can be carried out before packing and "in situ" after packing. Better results were achieved by "in situ" loading.

Repeated synthesis of the material resulted in products with well conformable elementary analysis and reproducible chromatographic properties. The elementary analysis of the final product showed 8.1% ± 1% C, 1.8% ± 0.2% H, 0.9% ± 0.1% N and 1.5% ± 0.2% Cu.

Per 2 mole L-proline 1 mole copper is bonded, indicating that a bidentate complex is formed (Fig. 1).

The material showed a good stability in the tested pH range from 4 to 8. The columns were used for several weeks without loss of selectivity. After longer use, they can be regenerated by passing a copper solution through the column. The number of theoretical plates, determined with D-valine, was about 2500/m. Fig. 2

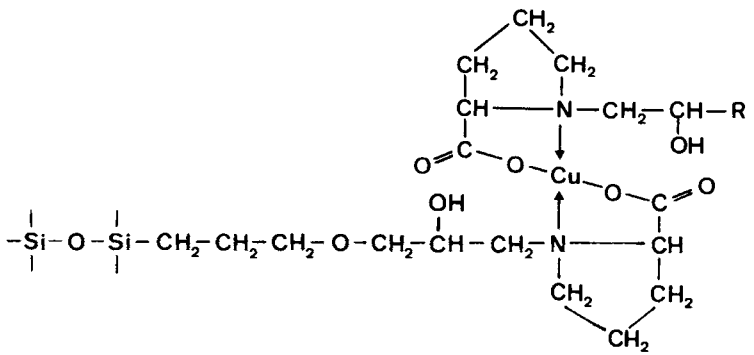


Figure 1 : Complex on the stationary phase

shows the dependence of the height equivalent of theoretical plates (HETP) on the linear flow.

The principle of ligand exchange chromatography was discussed by Davankov et al. (5).

$$R - Me - R + D(L)R' \overset{K_{D(L)}}{\rightleftharpoons} R - Me - D(L)R'$$

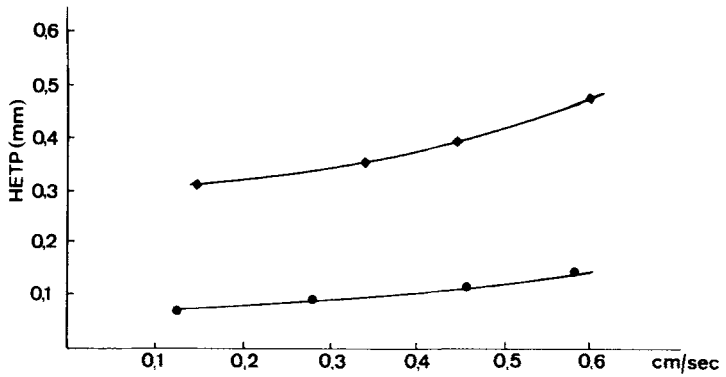


Figure 2 : Dependence of HETP on the linear flow.
D-Valine (□□□), $NaNO_3$ (ooo)
Column: 25 x 0.46 cm; mobile phase: 0.05 M KH_2PO_4, pH 4.6;
Temperature: 50°

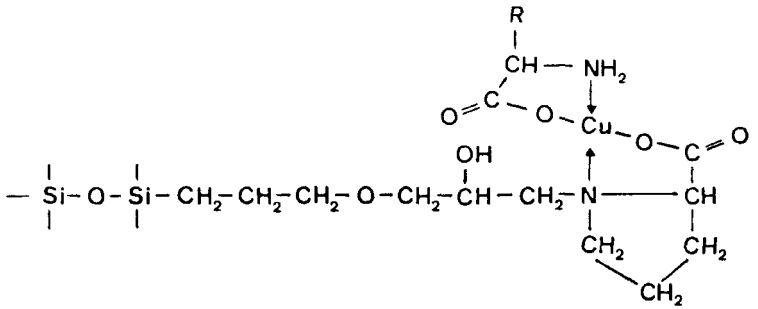


Figure 3 : Structure of the mixed complex between the fixed ligand and D- or L-amino acid

A ligand (R = L-proline), fixed on the stationary phase is replaced by a mobile ligand (R' = D and L-amino acid, respectively) to give the mixed complex, shown in Fig. 3.

The stereoselectivity depends greatly on the structure of the stationary phase.

For optimal resolution it seems to be necessary for the carboxy group to be free for complex forming with the metal ion. These observations have been confirmed by the results of Foucault et al., who bonded L-proline to silica via an amide bonding, using 3-triethoxysilylpropylamine as the coupling component. They described only partial resolution with this sorbens (28).

For the optimation of the conditions of separation, the influence of temperature, pH and ionic strength of the mobile phase have been thoroughly investigated. The increase in temperature results in a significant reduction of HETP.

A significant improvement of selectivity can be observed. Using a 0.05 M KH_2PO_4 solution, pH 4.6, at 50° a complete resolution of a great number of amino acids can be obtained within 2 - 15 minutes. Typical results of separation are shown in Fig. 4 and 5.

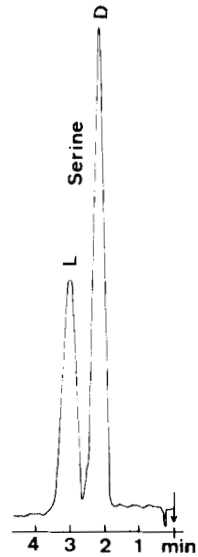


Figure 4 : Separation of D,L-serine
Column: 25 x 0.46 cm
Mobile phase: 0.05 M KH_2PO_4-buffer, pH 4.6
Temperature: 50°
Flow rate: 5 ml/min

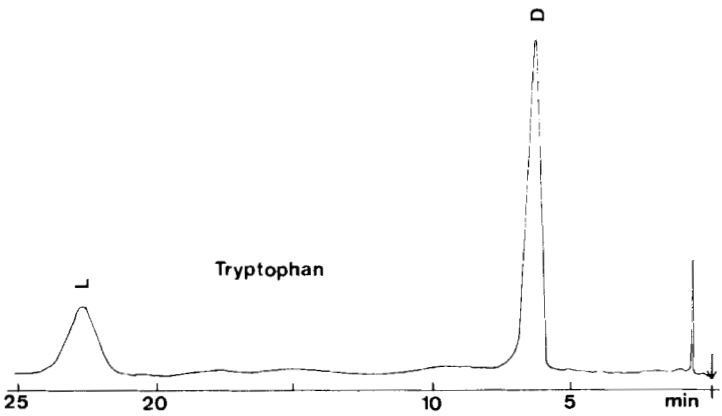


Figure 5 : Separation of D,L-tryptophan
Conditions as in figure 4

Amino acids with aromatic substituents are more strongly retarded and show high α-values. The α-value for DL-tryptophan, for example, is 3.5. By using a short column (4 cm) and a high flow rate, a rapid resolution of DL-tryptophan is achieved within 3 minutes. The K' and α-values of some DL-amino acids are given in Table 1.

The identification of the separated enantiomers was carried out by comparing their retention times with those of reference substances. Furthermore, fractions were collected and the optical rotation was measured.

An enzymatic method was also used as proof. L-Tyrosinedecarboxylase, for example, destroys only the L-form of tyrosine and forms tyramine (Fig. 6).

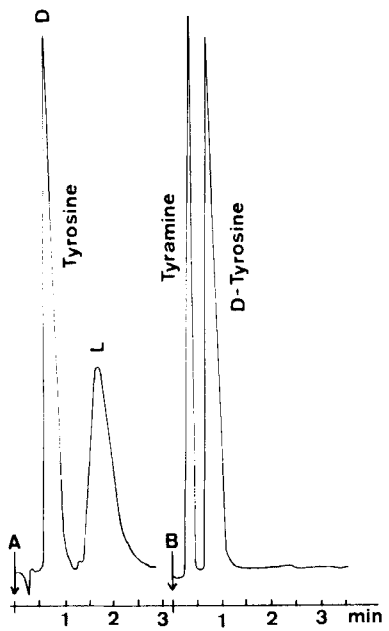


Figure 6 : Enzymatic cleavage of D,L-tyrosine with L-tyrosinedecarboxylase
A : Injection of the racemate before treatment
B : After treatment

TABLE 1

K'-values and relative retention ($\alpha = K'_{(L)}/K'_{(D)}$) for DL-amino acids.

Column: 25 x 0.46 cm; mobile phase: 0.05 M KH_2PO_4, pH 4.6; flow rate: 2 ml/min.

DL-amino acid	$K'_{(D)}$	$K'_{(L)}$	α
Valine	2.5	3.8	1.5
Serine	2.0	3.2	1.6
Proline	2.4	1.4	0.6
Histidine	6.7	12.1	1.8
Phenylalanine	3.2	9.4	2.9
Tyrosine	3.3	10.2	3.1
Tryptophan	7.8	27.4	3.5

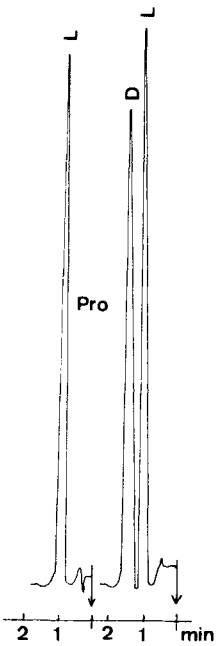


Figure 7 : Separation of D,L-proline
Conditions as in figure 4

On columns containing L-proline as a fixed ligand, the L-enantiomers constantly appeared with higher K' values. An exception was proline, where a reversed sequence was observed (Fig. 7).

An application of this method on a preparative scale is also possible. On an analytic column of 25 cm length and 0.46 cm I.D. up to one and a half mg amino acid racemate can be separated without significant change in K'-values and resolution.

CONCLUSION

Ligand exchange chromatography on chemically bonded chiral phases offers new possibilities for rapid separation of racemic compounds on an analytical or preparative scale. The use of a special bonded phase combines the selectivity of ligand exchange chromatography and the efficiency of HPLC. Further work needs to be done in order to clear some theoretical aspects. The influence of various bonded amino acids and also of the fixed metal ions, on the stereoselectivity, is now beeing studied.

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This work is dedicated to Univ.-Prof.Dr.G.Zigeuner on his 60th birthday.

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