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HIGH-PERFORMANCE LIGAND-EXCHANGE CHROMATOGRAPHY OF ENANTIOMERS

STUDIES ON POLYSTYRENE-TYPE CHIRAL PHASES BONDED TO MICROPARTICULATE SILICAS

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SUMMARY

Highly efficient chiral ligand-exchange packings were prepared by bonding of polystyrene chains incorporating chiral amino acid ligands on to the surface of microparticulate silicas. The factors controlling retention were examined (type and content of organic modifier in the eluent, addition of ammonium and tetrabutylammonium acetate, temperature and pH of the eluent); of these, the eluent pH was shown to influence the resolution and enantioselectivity, whereas the organic modifier mainly affects the retention.

The presence of the silica surface in close proximity to the copper(II) ion chiral coordination site may result in changes in the magnitude and the sign of the enantioselectivity effects in ligand-exchange chromatography.

The efficiency and selectivity of the sorbents permit a rapid resolution of five to seven racemic amino acids into the corresponding enantiomers.

INTRODUCTION

Cross-linked polystyrene resins incorporating chiral α-amino acid groups were the first packings employed in the separation of racemic compounds by means of ligand-exchange chromatography (for a review, see ref. 1 and references cited therein). While showing high enantioselectivity and high exchange capacity, these resins suffer from low pressure resistance and slow mass transfer compared with rigid silica packings. An alternative approach using the addition of chiral complexes to the mobile phase led to highly efficient microparticulate silica packings in ligand-exchange chromatography (for a review, see ref. 2 and references cited therein), but involved unavoidable loss of the resolving chiral component of the system. This drawback was eliminated by steady adsorption of the chiral resolving ligand on to the surface of reversed-phase silica[3], which however limited the permissible set of eluents to aqueous media. However, a more promising method consists in the covalent binding of the asymmetrical chiral ligand to the surface of the silica support, yielding a stable and selective phase system[2,4–12].

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This paper describes the synthesis of silica-based sorbents with chemically bound polystyrene chains carrying residues of optically active L-proline or L-hydroxyproline, and their chromatographic properties using racemic α-amino acids as model solutes. With respect to the structure of the bound ligand, these sorbents resemble the well studied polystyrene-based sorbents; however, the fact that the ligands are bonded to a silica instead of a polystyrene matrix appears to cause changes in retention and enantioselectivity of the α-amino acids and also changes the efficiency of the columns.

EXPERIMENTAL

Synthesis of chiral sorbents

Sorbent 1. The starting materials were LiChrosorb Si 100, 10 μm (Merck, Darmstadt, G.F.R.) and a chloromethylated copolymer of styrene and methylvinyldimethoxysilane. The latter possessed a mean molecular weight of 25,000 and contained 23.6% of Cl and 0.28% of Si, corresponding to complete chloromethylation of all styrene units (at the *para*-position). A 10-g amount of silica gel was heated with 10 g of the copolymer in boiling chlorobenzene for 48 h. After reaction, the silica particles were separated and washed thoroughly with dioxane, acetone and finally with methanol. Then the chloromethyl groups of the grafted polystyrene were replaced by residues of L-proline methyl ester by heating the material for 24 h in boiling dioxane–methanol (6:1) containing 6.5 g of the ester and 0.5 g of sodium iodide. After washing the sorbent with methanol, water, 0.001 *M* hydrochloric acid and again with water, it was kept for 12 h in a copper(II) solution, in order to achieve hydrolysis of the ester groups. The sorbent was finally washed with 0.1 *M* acetic acid, water and acetone, and was then dried. Analytical data are presented in Table I.

Sorbent 2. Sorbent 2 was prepared similarly to sorbent 1, except that the initial copolymer (mean molecular weight 15,000) contained 17.2% of Cl and 1.7% of Si, corresponding to 79% chloromethylation. Instead of L-proline, L-hydroxyproline was used as the polymer-fixed chiral ligand (via the methyl ester, as above).

TABLE I

CHARACTERIZATION OF CHIRAL SORBENTS

Parameter	*Sorbent*		
	1	*2*	*3*
Styrene-to-silane ratio	90:1	14:1	90:1
Degree of chloromethylation (%)	100	79	51
Bound chiral ligand	L-Pro	L-Hyp	L-Hyp
Elemental analysis:			
C	20.37	18.74	17.28
H	2.05	2.00	2.15
N	0.78	0.82	0.88
Extent of modification (mmol/g):			
Aromatic rings	1.60	1.46	1.40
Fixed ligands	0.56	0.59	0.63

Sorbent 3. Polystyrene of mean molecular weight 9000 was subjected to chloromethylation, yielding 11.7% of Cl, which corresponded to a degree of substitution of 51%. The polymer was then reacted with a small amount of 3-aminopropyltriethoxysilane to produce a ratio of styrene units to silane groups of about 90:1. A 10-g amount of LiChrosorb Si 100, 10 μm, was added to a solution of 10 g of the polymer in chlorobenzene, and boiled for 48 h, followed by treatment with L-hydroxyproline methyl ester as above. For analytical data of the packing obtained, see Table I.

Chromatographic measurements

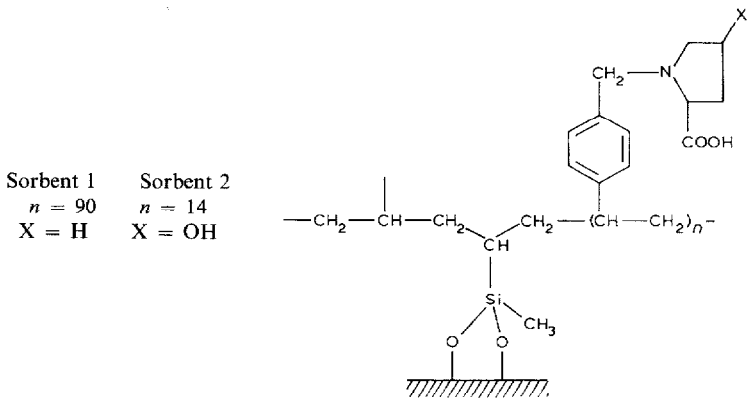
Chromatographic columns of 100 × 4.2 and 250 × 4.2 mm I.D. were packed by the balanced density slurry technique. The instrument used was a Hewlett-Packard 1084A, fitted with a UV detector (254 nm) and an automatic injection device. All eluents contained 10^{-4} *M* copper(II) acetate.

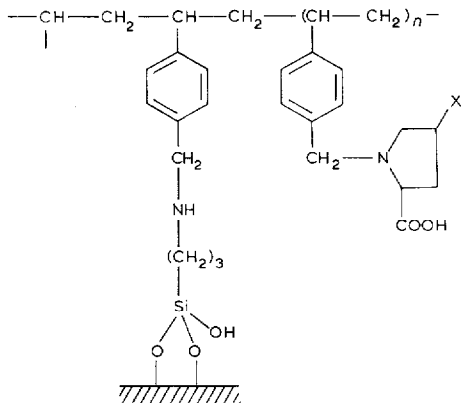
RESULTS AND DISCUSSION

Two methods were developed for chemical bonding of short polystyrene chains of 100–250 monomer units to the surface of LiChrosorb Si 100. As described in the first method, a copolymer of styrene with small amounts of methylvinyldimethoxysilane was used, which readily reacts with the silica silanol groups. In the second method, chloromethylated polystyrene was reacted with a small amount of 3-aminopropyltriethoxysilane and then bonded to the silica surface. Depending on the content of silane units, the average polystyrene chain length between two neighbouring links to the silica surface varied from 14 to 90.

Chiral chelating ligands (residues of L-proline or L-hydroxyproline) were introduced in a conventional manner via the reaction of chloromethyl groups with the amino acid methyl esters, followed by mild hydrolysis of the ester functions in the presence of copper(II) ions at pH 5.5.

Thus, a strong differentiation in the length of the spacer between the polymer chain and the silica surface was achieved:





Sorbent 3
$n = 90$
X = OH

In spite of this, and the relatively small number of such spacers, adsorption phenomena seem to provide a dense covering of the silica surface with the polystyrene chains. All attempts to block the remaining surface silanol groups by treating the sorbent with trimethylchlorosilane (either before or after the substitution of the polystyrene chloromethyl groups by the amino acid residues), resulted in an increase in the total carbon content of less than 1%. The carbon content of the sorbents amounted to 17–20%, corresponding to 1.4–1.6 mmol styrene units per 1 g of sorbent. The concentration of the polymer-fixed amino acid ligands (*ca.* 0.6 mmol/g) cannot be evaluated precisely from elemental analysis data (Table I), because of the low nitrogen content of the sorbents (< 1%). The presence of a sufficient amount of fixed ligands can be easily made visible, however, by treating the sorbent with copper(II) acetate solution, which should result in colouring the material distinctly blue.

Owing to the high surface density of styrene units (which are partially unsubstituted), the above bonded phases display marked hydrophobic properties. This is the reason why the retention, k', of hydrophobic amino acids (Trp, Phe, Leu, Tyr) falls drastically on adding 20–30% of an organic modifier to the aqueous eluent in ligand exchange chromatography of amino acids (Fig. 1). In contrast, the retention of the hydrophilic amino acids (Glu, Ser, Ala) is small, both in the presence and in the absence of an organic component in the eluent.

The hydrophobicity of the bonded phases also explains the decrease in k' of hydrophobic amino acids on increasing the solvent strength of the organic modifiers, *i.e.*, methanol > acetonitrile > tetrahydrofuran (mixed in a constant proportion with water; see Fig. 2).

Increasing the pH of the eluent brings results in an increase in the retentions of the whole range of amino acids, which indicates the growing contribution of complexation reactions to the net retention of the solutes (Fig. 3). It is remarkable that the typical basic amino acid lysine was observed to remain the least retained solute in the pH range 4.6–5.0 investigated, where it certainly carries a positive charge, and should actively interact with the surface silanol groups if these were not sterically blocked by the interface polystyrene layer. It is appropriate to mention here that this situation contrasts completely with the behaviour of lysine on monomerically bonded chiral silica phases using methylene or propylene spacers between L-proline or L-hydroxyproline residues and the silica surface. In that case, owing to electrostatic interactions with accessible silanol groups, lysine and arginine became the species most strongly retained at pH 5 or higher.

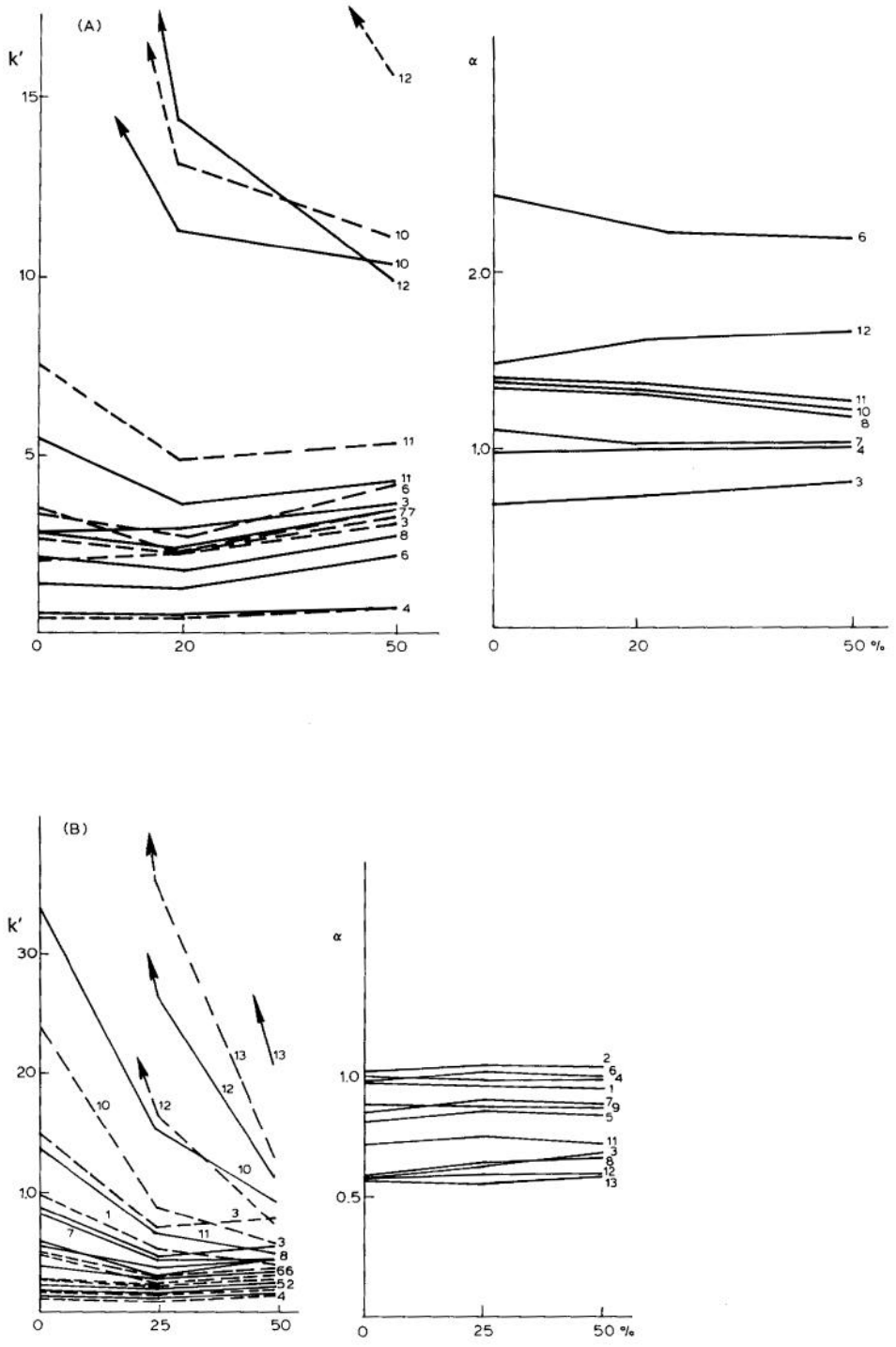


Fig. 1.

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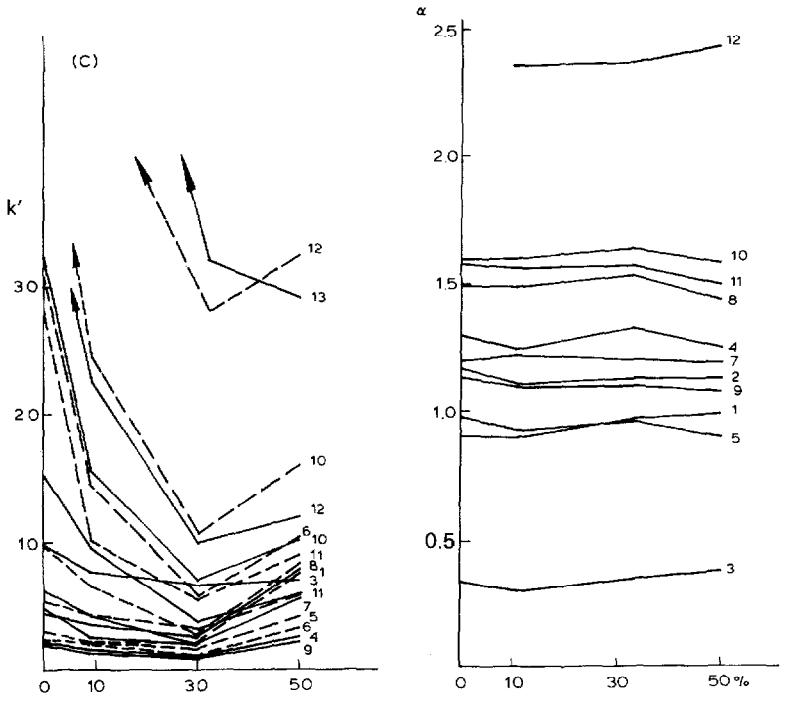


Fig. 1. Dependence of retention k'_L (——) and k'_D (– – –) and enantioselectivity ($\alpha = k'_D/k'_L$) of amino acids on the content of organic component (%, v/v) in the aqueous–organic eluent. Column, 100 × 4.2 mm I.D.; d_p, 10 μm; flow-rate, 2 ml/min; temperature, 50°C; copper(II) content in the eluent, 10^{-4} *M*. 1 = Asp; 2 = Glu; 3 = His; 4 = Ala; 5 = Ser; 6 = Pro; 7 = Thr; 8 = Val; 9 = Lys; 10 = Tyr; 11 = Leu; 12 = Phe; 13 = Trp. (A) Sorbent 1, water–methanol, 0.1 *M* ammonium acetate, pH 4.8. (B) Sorbent 2, water–acetonitrile, 0.1 *M* ammonium acetate, pH 5.0. (C) Sorbent 3, water–methanol, 0.01 *M* ammonium acetate, pH 5.0.

Changing the temperature of the column in the range 25–70°C does not significantly influence the retention of amino acids on the grafted polymeric chiral phases.

As follows from Figs. 1–3, neither the type nor the amount of organic modifiers in the eluent can systematically and strongly influence the enantioselectivity of the columns, expressed by the ratio of the capacity factors of two enantiomeric forms of the corresponding amino acids, $\alpha = k'_D/k'_L$. Only raising the pH of the eluent results in a distinct enhancement of the resolution selectivity. This fact emphasizes the importance of the formation of the mixed-ligand sorption complexes [involving copper(II) ions, polymer-fixed ligands and mobile amino acid ligands] for the chiral recognition of the latter. It is this process that is responsible for the enantioselectivity of the chromatographic system discussed, whereas the retention of the solutes is a superposition of contributions from several different types of interactions with the stationary phase.

However, the stereochemical interactions between the chiral fixed and mobile ligands within the mixed-ligand sorption complex can depend on several factors, including conformation of polystyrene chains, which in the system discussed form a dense interphase layer between the eluent and the silica matrix. One can easily imagine conformations where the active sorption sites of the grafted chiral phase appear in the close vicinity of the matrix surface. This would unavoidably affect the interligand interactions within the sorption complex and, correspondingly, the value or even sign of the enantioselectivity.

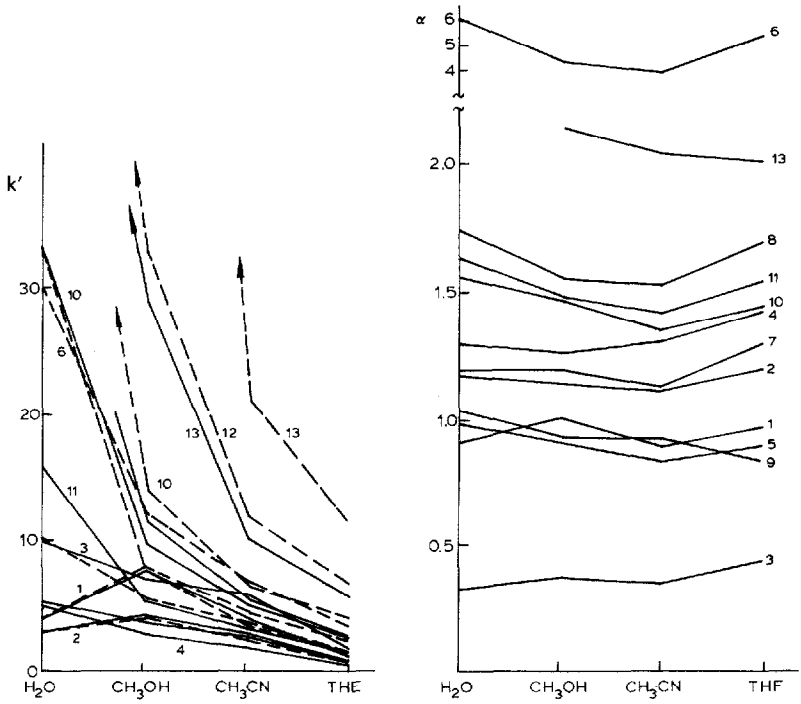


Fig. 2. Dependence of retention and enantioselectivity of amino acids on the type of organic component (1 part to 3 parts of water). Conditions and numbers as in Fig. 1. Sorbent 3, 0.01 *M* ammonium acetate, pH 5.0.

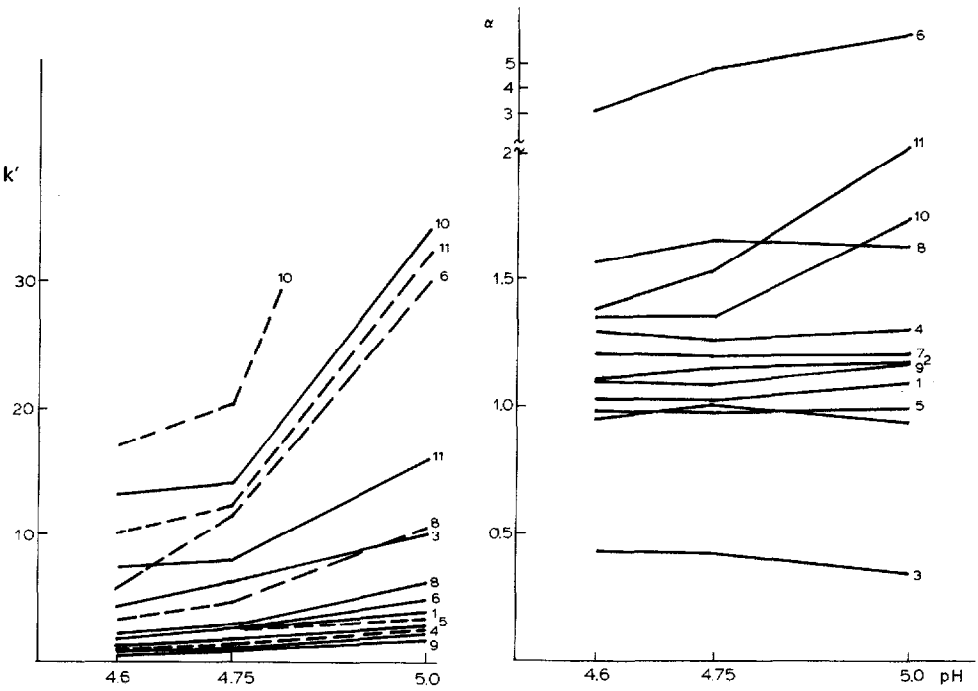
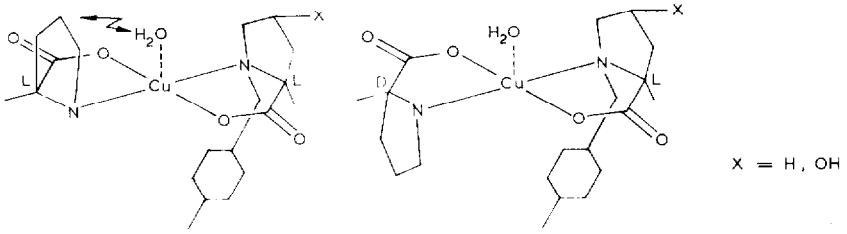


Fig. 3. Dependence of retention and enantioselectivity of amino acids on pH of the eluent. Sorbent 3; eluent 0.01 *M* ammonium acetate in water; flow-rate, 3 ml/min. Other conditions and numbers as in Fig. 1.

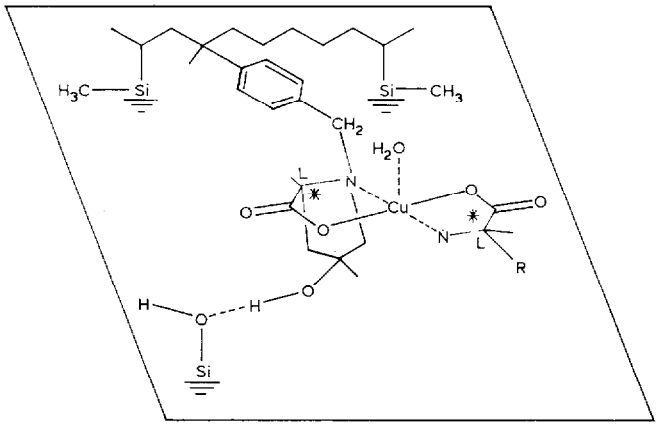
The nature of the chiral recognition of amino acid enantiomers by the copper(II) form of polystyrene resins incorporating residues of L-proline and L-hydroxyproline has been discussed in our earlier work. The main feature of the mechanism suggested[13] is that the coordination of the L-enantiomer of a bidentate amino acid suffers steric hindrance from a water molecule coordinated in one of two axial positions of the Cu(II) ion coordination sphere. In contrast, the sorption complex with a bidentate D-enantiomer should be additionally stabilized by hydrophobic interactions between the α-substituent of the mobile ligand and the N-benzyl radical of the resin-fixed ligand:



According to the above scheme, bidentate amino acid ligands having the D-configuration are more strongly retained ($\alpha = k'_D/k'_L > 1$), provided L-proline or L-hydroxyproline are the resin-fixed ligands. This rule is valid for both alkaline and acidic eluents (Table II).

Table II shows that sorbents 1 and 3 display the same kind of enantioselectivity as do their polystyrene analogues. Unexpectedly, sorbent 2 demonstrates an inversed order of enantiomer elution ($\alpha < 1$). As shown above, sorbent 2 differs from sorbent 3 in that the polystyrene chains of the former are more tightly bound to the silica surface. In addition, sorbent 2 differs from sorbent 1 in that the latter does not possess a hydroxy function in the active sorption site.

An explanation of the unusual behaviour of sorbent 2 can be found in the assumption that the normal structures of sorption complexes are strongly distorted, owing to lack of flexibility of the short polystyrene segments between the adjacent grafting points and also because of hydrogen bonding with the surface silanol groups. Additional interactions with the silica surface become possible for the α-substituent of the L-mobile ligand. These interactions must be responsible for the stronger retention of the L-enantiomers, where the resolution selectivity increases the larger the size of the α-substituent:



Trifunctional amino acids such as lysine, aspartic acid, glutamic acid and serine were observed to resolve on all chiral grafted sorbents with a comparatively small selectivity. Depending on the elution conditions, the sign of enantioselectivity for these solutes sometimes inverts.

Nevertheless, these amino acids can often be completely resolved owing to the high efficiency of the bound polymeric chiral phases (Figs. 4 and 5). With the 10-μm particles of the initial silica matrix, the plate number for a 250 mm column amounts on average to 2500, which permits a resolution of mixtures of five to seven racemic amino acids to be carried out in 30–50 min. Increasing the column temperature was observed to improve the chromatographic performance.

The addition of organic modifiers to the eluent is advisable in order to shorten

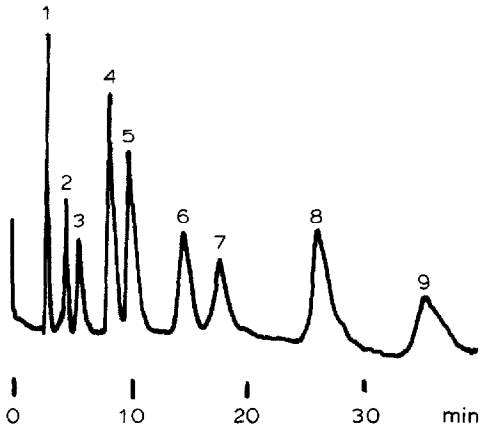


Fig. 4. Resolution of racemic amino acids on sorbent 1. Column, 250 × 4.2 mm I.D.; eluent, water–0.05 *M* tetrabutylammonium acetate–10^{-4} *M* copper(II) acetate, pH 4.2; temperature, 60°C; flow-rate, 1 ml/min; d_p, 10 μm. 1 = D,L-Arg; 2 = L-Pro; 3 = D-Pro; 4 = L-Leu; 5 = D-Leu; 6 = L-Tyr; 7 = D-Tyr; 8 = L-Phe; 9 = D-Phe.

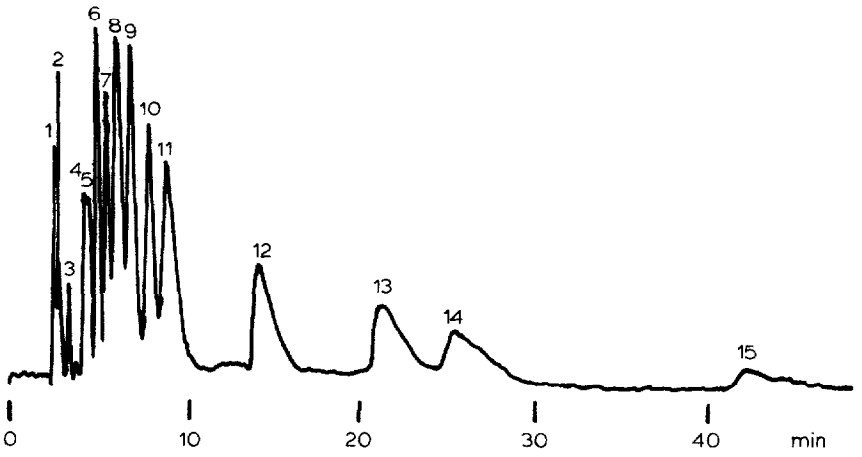


Fig. 5. Resolution of racemic amino acids on sorbent 2. Eluent, water–0.05 *M* tetrabutylammonium acetate–10^{-4} *M* copper(II) acetate, pH 4.6; temperature, 65°C; other conditions as in Fig. 4. 1 = D,L-Lys; 2 = D,L-Arg; 3 = unknown; 4 = D-Ala; 5 = L-Ala; 6 = D-Ser; 7 = L-Ser; 8 = D-His; 9 = D-Thr; 10 = L-Thr; 11 = L-His; 12 = D-Met; 13 = L-Met; 14 = D-Ethionine; 15 = L-Ethionine.

TABLE II

INFLUENCE OF THE ARRANGEMENT OF POLYSTYRENE CHAINS CONTAINING COPPER(II) CHELATES OF L-PRO AND L-HYP ON THE ENANTIOSELECTIVITY ($\alpha = k'_D/k'_L$) OF THE RESOLUTION OF AMINO ACIDS

Copper content of the eluents: 10^{-4} *M*.

Amino acid	*Polystyrene resin*			*Sorbent 1:* L-*Pro*, H_2O–CH_3OH*, *pH 5.0*	*Sorbent 2:* L-*Hyp*		*Sorbent 3:* L-*Hyp*, H_2O–CH_3OH*, *pH 5.0*
	L-*Pro*, *pH 11*	L-*Hyp*			H_2O, *pH 4.7*	H_2O–CH_3CN*, *pH 4.7*	
		pH 11	*pH 4.5*				
Alanine	1.08	1.04	1.28	1.00	0.92	1.01	1.26
Serine	1.09	1.29	1.18	1.00	0.81	0.86	0.90
Threonine	1.38	1.52	1.49	1.00	0.86	0.91	1.20
Valine	1.29	1.61	1.64	1.18	0.59	0.69	1.45
Leucine	1.27	1.70	–	–	0.62	0.65	1.53
Isoleucine	1.50	1.89	–	1.26	0.58	0.74	1.48
Proline	4.05	3.95	6.00	2.01	0.97	1.02	4.30
Methionine	1.04	1.22	1.01	1.00	0.63	0.93	1.34
Phenylalanine	1.60	2.89	–	1.60	0.50	0.63	2.69
Thyrosine	2.46	2.23	–	1.08	0.51	0.62	1.47
Tryptophan	1.40	1.77	–**	–	–**	0.59	2.49
Lysine	1.10	1.22	1.05	–	1.18	1.24	0.93
Histidine	0.37	0.36	0.20	0.81	0.58	0.70	0.39
Aspartic acid	0.91	1.00	1.10	1.00	0.97	0.87	1.02
Glutamic acid	0.62	0.82	1.13	–	0.99	1.00	1.14

* Content of the organic modifier in the eluent: 50% (v/v).
** Retention of amino acids is too large.

the elution times of hydrophobic solutes. Tetrabutylammonium acetate was found to produce the same effect as the organic modifier. The use of the salt is sometimes preferable because of the higher resolution selectivities in aqueous tetrabutylammonium solutions than in aqueous–organic eluents.

Although the hydrolytic stability of the bound chiral silica phases has not been systematically examined to date, no change in column performance could be observed during their use over several weeks. Owing to the multi-point binding of polystyrene chains to the matrix, combined with the distinct adsorption of polystyrene on silica, the chiral sorbents described here are expected to be stable. The simplicity of preparation of these sorbents, their high efficiency and enantioselectivity and the variety of applications under different chromatographic conditions should make the bound chiral polystyrene phases increasingly popular.

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