

CHIRAL CHELATING RESINS IN CHROMATOGRAPHY OF OPTICAL ISOMERS

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Abstract - The ways of binding optically active ligands, mainly of the amino acid type, to cross-linked polymers of styrene, acrylamide or glycidylmethacrylate are reviewed. When coordinated to transition metal ions, these polymers display a high discriminating ability in sorption of optical isomers of compounds capable of forming mixed-ligand complexes. The kinetic performance of the ligand-exchanging sorbents is considerably improved by applying a macronet isoporous polystyrene matrix. A mechanism of chiral recognition of amino acid enantiomers is discussed, which is based on structural studies of low-molecular-weight ligands and complexes simulating the active sites and the mixed-ligand sorption complexes in the resin phase.

INTRODUCTION

A great deal of the recent progress in designing selective polymeric sorbents has been due to advanced understanding of the nature of sorbent-solute interactions occurring in a chromatographic column. The selectivity of the column strongly depends on the selectivity of these interactions. The presence of groups capable of entering into desired selective interactions with the solutes to be separated is a first requirement on a selective sorbent.

Equally important is a high accessibility of the sorbent active sites to the solute molecules. It determines the rate of establishment of the equilibrium between the mobile and stationary phases and hence the efficiency of chromatography. From this point of view, the network structure of selective polymeric sorbents deserves special attention.

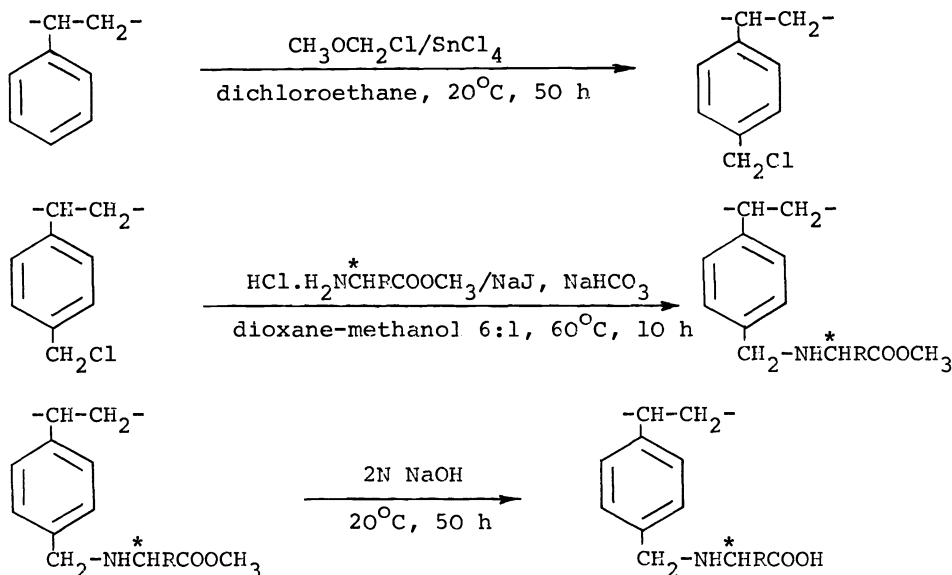
It is the aim of the present paper to survey the recent advances in enhancement of both the selectivity and efficiency of chiral chelating resins intended for the chromatographic resolution of racemic organic compounds. In this field of liquid chromatography a significant breakthrough was achieved in 1968 (Ref. 1 & 2) by resorting to ligand exchange as the basic mechanism of interactions between a racemic solute and an asymmetric sorbent. This mechanism provided an extremely high selectivity of sorbents towards optical isomers of solute molecules, which made it possible to resolve several racemates on a preparative scale. In addition to this, the recent trend has been the enhancement of the efficiency of chiral sorbents, which resulted in the development of rapid chromatographic analysis of the enantiomeric composition of chiral compounds.

SYNTHESIS AND APPLICATION OF CHIRAL LIGAND-EXCHANGING RESINS

The distinguishing feature of ligand-exchanging polymeric sorbents is the presence of chelating groups, viz, polymer-fixed ligands, capable of forming coordination compounds with transition metal ions. Typical metal ions suitable for ligand-exchange chromatography are bivalent cations of Cu, Ni, Zn, Cd. They readily bind electron donating O-, N- or S-atoms of the fixed ligand into their coordination sphere. The remaining vacant coordination positions of the central metal ion are responsible for a temporary binding of mobile ligands, i.e. organic molecules under separation. The fixed and mobile ligands enter into multi-point interactions with each other in the densely packed coordination sphere of the central metal ion, which is an important condition for the selective discrimination of the two mobile ligand enantiomers in the ligand-

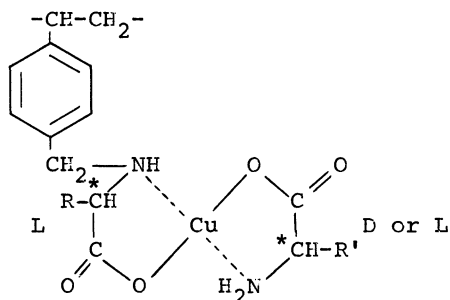
exchange chromatography.

The first and still the most important generation of chiral ligand-exchanging sorbents is the series of cross-linked styrene copolymers containing different optically active amino acid type fixed ligands. The synthesis of these resins starts with the chloromethylation (in the p-position) of cross-linked styrene-divinylbenzene copolymers. The second step involves two reactions: (i) substitution of the rather inert chlorine atom in the chloromethyl group by the much more reactive iodine atom and (ii) substitution of the latter by the nitrogen of an optically active α -amino ester. This combined step was made possible by finding conditions (Ref. 3) in which the two reactions proceeded at a sufficiently high rate. Simultaneously, reduction of the required amounts of NaI to a catalytic level was achieved. Another improvement of this stage was possible due to the finding that, in the presence of NaHCO_3 , the rather unstable free amino esters could be replaced by their hydrochlorides. Finally, the last step of the synthesis of the chiral resins is the hydrolysis of the fixed ligand ester group:



Due to the mild conditions of all above steps, asymmetric α -carbon atoms of the starting amino acids preserve their asymmetry. Moreover, it was found that a benzyl type substituent at the amino acid nitrogen atom enhanced substantially the configurational stability of the amino acid (Ref. 4). No evidence for a possible partial racemization of the chiral resins was observed even after using them in alkaline media for several years.

The mechanism of sorption of a mobile ligand of the α -amino acid type by a chiral amino acid incorporating polystyrene resin, saturated with Cu(II) ions, can be described in terms of formation of a mixed-ligand sorption complex:

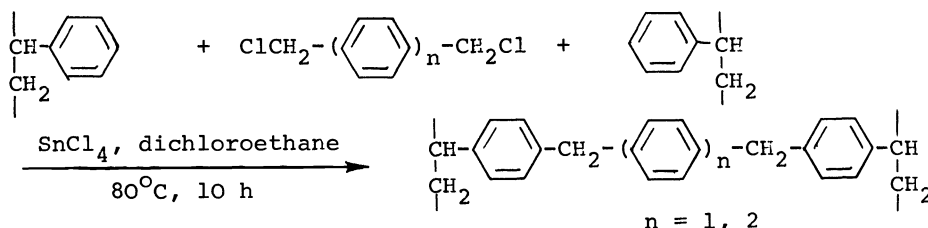


This complex is kinetically labile, which means that the bound mobile ligand is quickly released again.

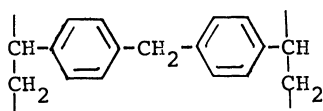
Considering the large volume of the above mixed-ligand sorption complex, one can easily realize that the fast progress of the ligand exchange reaction requires a sufficient free space in the sorbent matrix. However, the swellabi-

lity of resins based on the hydrophobic polystyrene matrix and having their amino acid ligands in the zwitterionic form or in the form of copper complexes proved rather low. Enhancing the resin swellability in aqueous media by introducing additional polar groupings was considered undesirable because of unavoidable interference of these polar groupings with the process of metal coordination.

To solve the problem of low water swellability of the polystyrene type matrix, macronet isoporous styrene polymers have been developed (Ref. 5). The initial idea was to preserve the favorable loose structure of a slightly cross-linked styrene-divinylbenzene copolymer strongly swollen with dichloroethane by introducing a large number of long and rigid cross-bridges between the polystyrene chains by using bifunctional cross-linking reagents, like p-xylylene dichloride, in the Friedel-Crafts reaction:



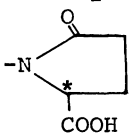
Similarly, by using specified amounts of monochlorodimethyl ether or formaldehyde (or its derivatives), it was possible to obtain macronet structures with long and rigid bridges



uniformly distributed through the matrix.

When large amounts of cross-bridges are introduced, hypercrosslinked styrene polymers result displaying unusual properties (Ref. 6), for instance the ability to swell (i.e. to increase their volume) in any liquid medium, including hexane, methanol, water, toluene, etc. In this manner, contrary to the generally accepted opinion that an additional cross-linking must reduce swellability, we succeeded in enhancing the solvent uptake by many chiral ligand exchangers. Table I shows how the introduction of 4,4'-bis(chloromethyl)biphenyl (CMB) into a swollen with dichloroethane styrene-divinylbenzene copolymer affects the swelling of ligand exchangers prepared from the initial and modified polystyrene matrix (Ref. 7).

Table I. Solvent uptake of standard and macronet isoporous ligand exchange resins

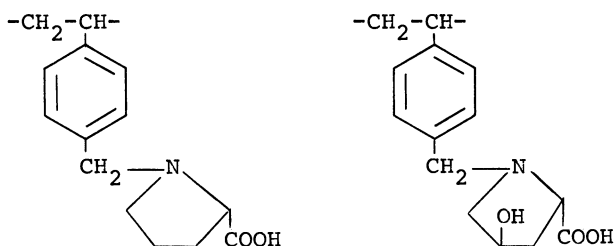
Fixed ligand	Cross-linking of the matrix	Exchange capacity (mmol/g)	Solvent uptake (g/g)		
			water	dioxane	methanol
$-\text{NH}-\overset{*}{\text{C}}\text{H}-\text{COOH}$	0.8% DVB	2.4	0.16	0.42	0.31
$\begin{array}{c} \\ \text{CH}_2-\text{COOH} \end{array}$	0.8% DVB + 5% CMB	2.1	1.39	1.10	1.05
	0.8% DVB	1.6	0.19	0.70	0.40
	0.8% DVB + 5% CMB	2.0	0.95	1.00	0.83

The use of macronet isoporous polystyrene as the starting matrix made it also possible to enhance the exchange capacity of the final ligand exchanging resins, since the chemical transformations of the functional groups in polystyrene were all favored by the macronet isoporous structure of the matrix.

Altogether, about 40 chiral chelating resins have been studied containing

different α -amino carboxylic and α -amino phosphonic acids as well as their derivatives in the macronet isoporous polystyrene matrix. Some special aspects of the synthesis of these resins and of their use in ligand exchange chromatography of optical isomers have been summarised in (Ref. 8).

In general, the highest resolving power and the widest range of application were exhibited by chiral resins containing cyclic fixed ligands of the type of N-benzyl-L-proline and N-benzyl-L-hydroxyproline:



When saturated with copper(II) ions, these resins resolve racemates of almost all α -amino acids (Fig. 1) as well as of many β -amino acids, α -hydroxy acids, amino alcohols, amino amides, 1,2-diamines etc. (Ref. 9).

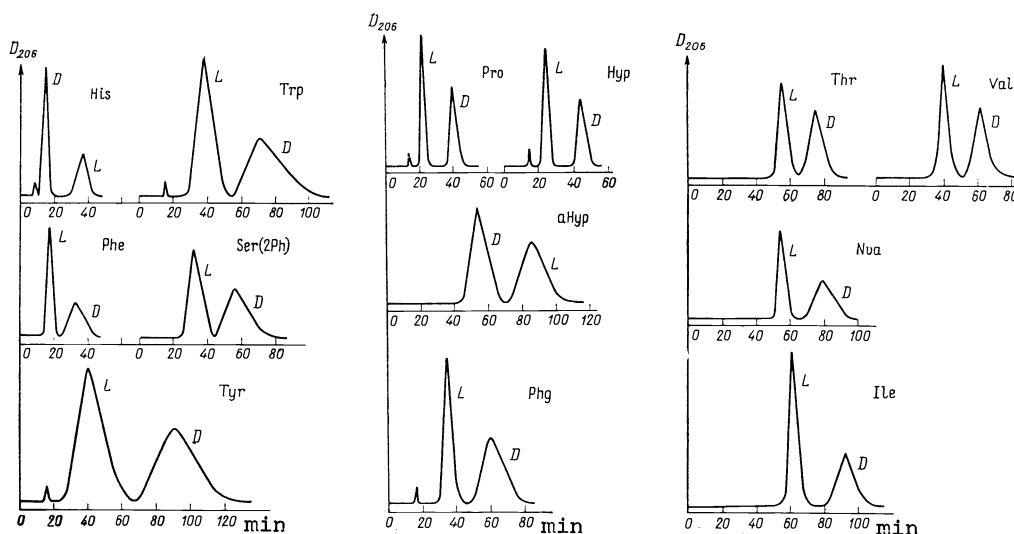


Fig. 1. Chromatography of racemic amino acids (histidine, tryptophane, phenylalanine, phenylserine, tyrosine, proline, hydroxyproline, allo-hydroxyproline, phenylglycine, threonine, valine, norvaline and isoleucine) on the L-hydroxyproline containing polystyrene resin.

exchange capacity: 3.86 mmol/g; copper content 0.6-1.5 mmol/g
degree of cross-linking: 0.8 % DVB + 5.0 % monochlorodimethyl ether
particles size: ca 50 μ m

column: 140 mm x 7.8 mm I.D.

detection: UV 206 nm

temperature: 25^o C

eluent: 0.5 M NH₄OH for His, Hyp, aHyp

0.4 M NH₄OH for Trp

0.1 M NH₄OH for Phe, Tyr, Phg, Val, Ile

0.05 M NH₄OH for Ser(2Ph), Thr, Nva

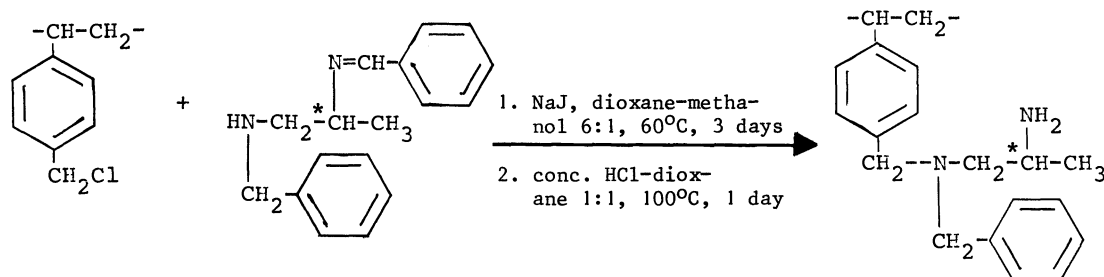
1.0 M NH₄OH for Pro

flow rate: 13-25 ml/h

The L-hydroxyproline containing polystyrene resin was used since 1977 in the commercial production of tritium-labelled amino acids in the optically and radiochemically pure state. Because of the high ligand exchange capacity (up to 3.9 mmol/g depending on the nature of the fixed ligand), the above chiral re-

sins are especially suitable for the preparative scale resolution. Under favorable conditions, i.e. at high resolution enantioselectivity, as much as 20 g of a racemate can be quantitatively resolved into optically pure components in a 1 liter big column containing 300 g of the resin.

Several other types of chiral fixed ligands, aside from the α -amino acid residues, can be introduced into the macronet isoporous polystyrene matrix to give chiral ligand exchanging resins. Thus a sorbent of very high resolving power was obtained according to the following scheme by aminating chloromethylated polystyrene matrix with derivatives of chiral propylenediamine:



Both the primary and tertiary amino groups of the sorbent fixed ligand are involved in coordination to copper(II) ion to result in the normal bidentate binding of mobile amino acid ligands. The remarkable high enantioselectivity of the sorbent allows resolution of several difficult-to-resolve amino acids like alanine, amino butyric acid, leucine (Fig. 2).

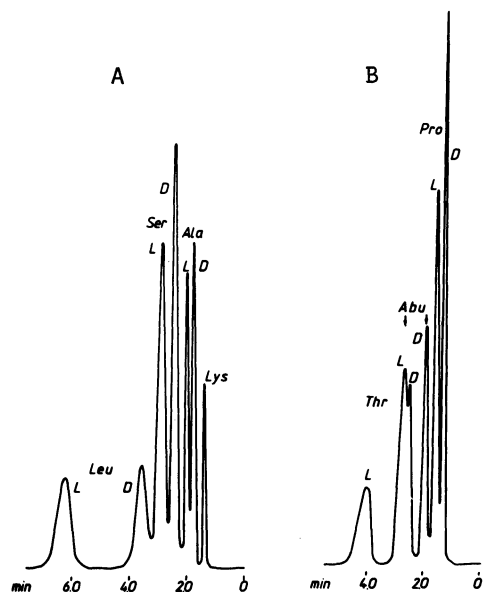
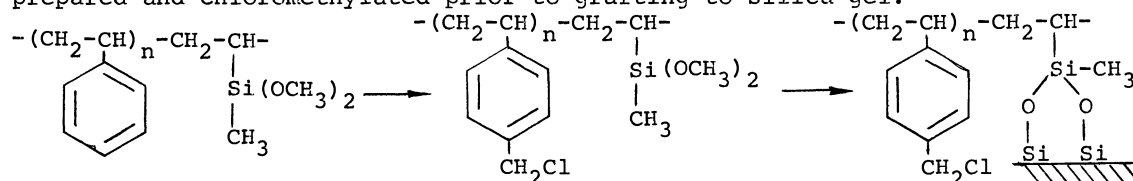


Fig. 2. Chromatograms of mixtures of racemic lysine, alanine, serine and leucine (A) and proline, aminobutyric acid and threonine (B) in a glass microcolumn (100 mm x 1 mm I.D.) packed with the chiral diamine resin. Eluent: 0.25 M sodium acetate + 0.0015 M copper acetate (Ref.10)

By using small resin particles ($d_p = 7.5 \pm 2.5 \mu\text{m}$), a very high column efficiency can be achieved, such that the resolution of a mixture of 3 to 4 racemic amino acids on a 10 cm long microbore column takes no more than 10 minutes (Ref. 10). Shorter times would require a very high pressure of the eluent. Due to the relatively high cross-linking degree, the pressure resistance of macronet isoporous resins is higher than that of the majority of polystyrene-based resins. It is nevertheless lower than the pressure resistance of silica gel-based sorbents used in modern high pressure chromatography.

In order to enhance the mechanical strength of chiral ligand exchangers, which is especially important for developing rapid chromatographic analysis, we decided to graft the polystyrene chains onto the surface of porous microparticulate silica gel. A copolymer of styrene with methylvinyltrimethoxysilane was prepared and chloromethylated prior to grafting to silica gel:



The chloromethyl groups were then substituted by the residues of optically active proline and hydroxyproline in a usual manner. The chiral sorbents obtained behave similarly to the polystyrene matrix sorbents and possess the advantage of high pressure stability. Their efficiency is comparable to that of other types of packings used in modern high performance liquid chromatography.

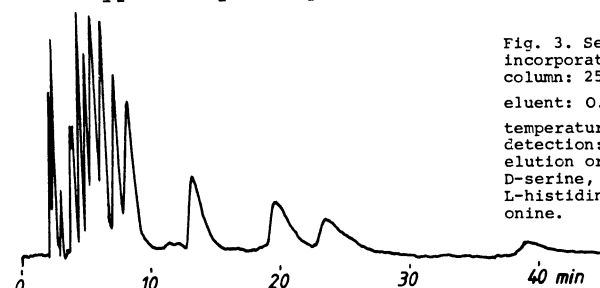
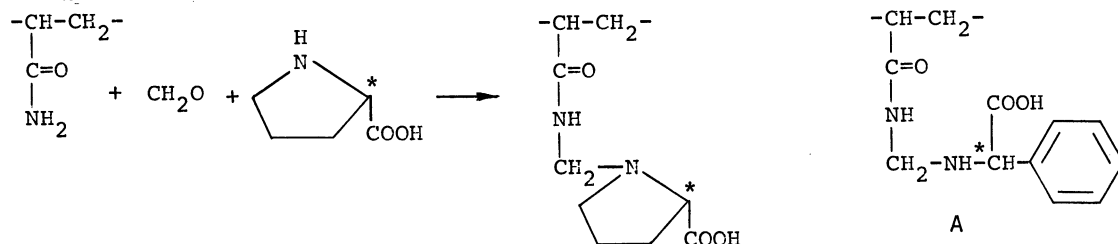


Fig. 3. Separation of racemic amino acids on L-hydroxyproline incorporated polystyrene bonded to LiChrosorb Si 100 column: 250 mm x 4.2 mm I.D.; $d_p = 10 \mu$ eluent: 0.05 M $\text{Bu}_4\text{N}^+\text{CH}_3\text{COO}^-$, 10^{-4} M $\text{Cu}(\text{CH}_3\text{COO})_2$; pH = 4.2 temperature: 65°C detection: UV 254 nm elution order: DL-lysine, DL-arginine, D-alanine, L-alanine, D-serine, L-serine, D-histidine, D-threonine, L-threonine, L-histidine, D-methionine, L-methionine, D-ethionine, L-ethionine.

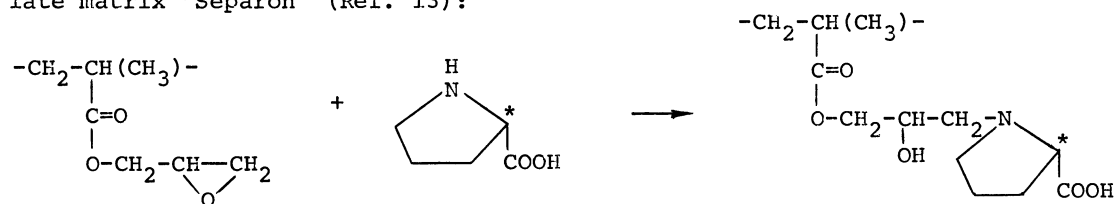
A new series of chiral ligand exchanging polymers starts from grafting L-proline to a hydrophilic polyacrylamide gel as suggested by Lefebvre, Audebert and Quivoron (Ref. 11):



When saturated with Cu(II) ions, this polymer was found to resolve many racemic amino acids into their enantiomers. About a dozen other complexing metal ions have been tested with the result that Cu(II) appears to be one of the most favored ones. This result coincides with that of earlier studies on polystyrene type resins. Similarly, the residues of cyclic amino acids, proline and hydroxyproline, were found to operate with the highest enantioselectivity both in the polystyrene and polyacrylamide series of chiral resins. However, when bound to these two different matrixes, they yield the opposite elution order for the amino acid enantiomers.

Of the polyacrylamide type sorbents, that incorporating L-phenylalanine (Formula A) was found to display the widest range of application (Ref. 12). Enantioselectivity values, α , of at least 1.25 - 1.30 were observed in resolutions of all the 20 common amino acids including basic (lysine, ornithine), acidic (aspartic and glutamic acids) and the most difficult-to-resolve neutral amino acids (alanine, methionine, serine, leucine). This resin was used in the preparation of tritium labelled alanine and glutamic acid.

Finally, L-hydroxyproline and L-proline were also fixed onto the polymethacrylate matrix "Separon" (Ref. 13):



The resins obtained behave similarly to the products of modification of the polyacrylamide matrix.

More recently, several ways of modification of microparticulate silica gel by chiral amino acid ligands have been suggested. This approach is directed toward the synthesis of efficient and pressure-resistant ligand exchangers for the rapid analysis of the enantiomeric composition of chiral compounds and mixtures thereof. First advances achieved along these lines have been reviewed in Ref. 14. However, having some distinct advantages, these silica-based sorbents are inferior to above chiral polymers in the exchange capacity and chemical stability, which makes polymeric sorbents especially suitable for preparative scale resolutions.

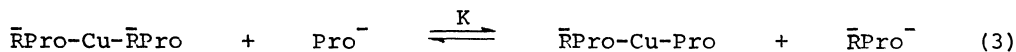
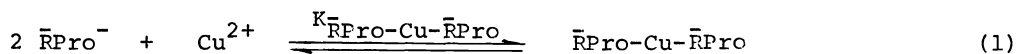
MECHANISM OF LIGAND EXCHANGE AND NATURE OF ENANTIOSELECTIVITY

Of great theoretical and practical importance is the question whether the sorption properties of a polymeric sorbent can be qualitatively and quantitatively investigated using appropriate low-molecular-weight model compounds. Summarizing our experience in this field, we would answer this question positively but add simultaneously that in some cases it is extremely difficult, if not impossible at all, to find adequate low-molecular-weight models. The difficulty is that the model should simulate all important interactions responsible for retention and discrimination of solute molecules in the polymeric resin phase.

We have found that an N-substituted amino acid can satisfactorily simulate the structure and properties of the respective polymer-fixed ligand. Thus, N-benzyl-L-proline should be regarded as a model for the active site of the L-proline incorporated polystyrene resin ($\bar{R}Pro$). The potentiometrically determined dissociation constants of the carboxylic and amino groups of the polymer were found to agree reasonably well with those for its model (Ref. 15):

	pK_{a1}	n	pK_{a2}	n
BzlPro	2.12	-	9.77	-
$\bar{R}Pro$	2.2	1.8	9.5	1.6

A more involved situation occurs with the coordination of copper(II) ions and the binding of mobile ligands. Each metal ion tends to bind two negatively charged ligands:



Scheme (1) shows the formation of the stationary complexes in the resin phase, Scheme (2) that of the mixed-ligand sorption complex and Scheme (3) represents the actual ligand exchange process responsible for the binding of the mobile ligand (in this case, an L-proline anion). The stability constants of the stationary and sorption complexes can be written as follows:

$$K_{\bar{R}Pro-Cu-\bar{R}Pro} = \frac{[\bar{R}Pro-Cu-\bar{R}Pro]}{[\bar{R}Pro^-]^2 \cdot [Cu^{2+}]}$$

$$K_{\bar{R}Pro-Cu-Pro} = \frac{[\bar{R}Pro-Cu-Pro]}{[\bar{R}Pro^-] \cdot [Cu^{2+}] \cdot [Pro^-]}$$

When calculating these constants from the equilibrium distribution of copper and L-proline between the resin phase and solution, one should take into account the following important circumstances:

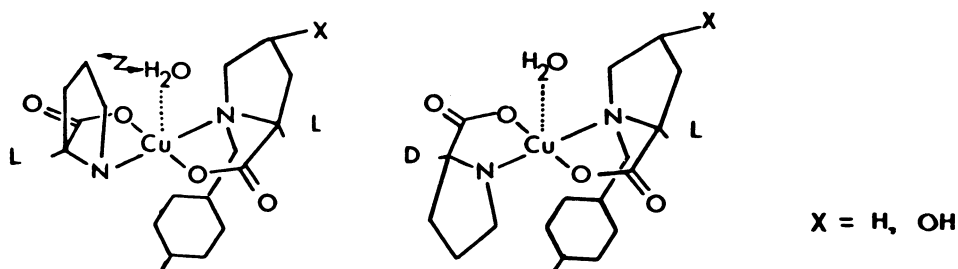
- (i) the fraction of anionic forms of both the fixed and mobile ligands ($\bar{R}Pro^-$ and Pro^-) drastically changes with pH of the equilibrium solution;
- (ii) to evaluate the concentrations of all the polymer-fixed species, for instance that of $\bar{R}Pro^-$, one has to know the water uptake of the resin phase at equilibrium;
- (iii) the real concentration of the charged Cu^{2+} and Pro^- ions in the charged resin phase strongly depends on the pH and ionic strength of the equilibrium solution and in any case differs from the concentration of these ions in the aqueous phase.

Only after a careful examination of these partial equilibria one has the right to compare the calculated values of the complex stability constants in the resin phase to those of model complexes. The following example (Ref. 16) shows a good agreement between the stability constants of polymeric and monomeric complexes, evaluated with due allowance for the above factors:

complex	$(BzlPro)_2Cu$	$(\bar{R}Pro)_2Cu$	BzlPro-Cu-Pro	$\bar{R}Pro-Cu-Pro$
log K	12.4 ± 0.1	12.5	14.9 ± 0.2	15.0

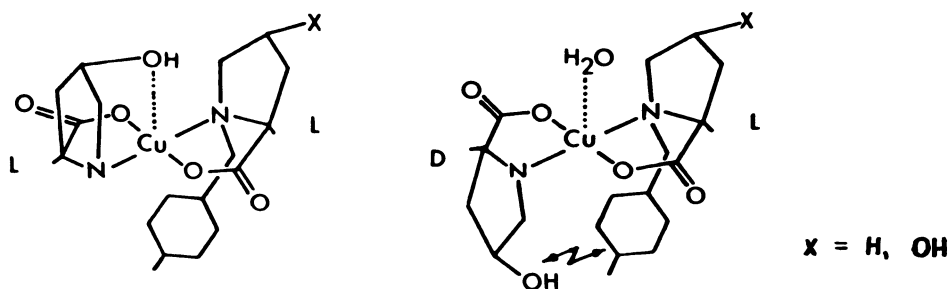
It should be however emphasized that for the polymeric stationary complex, $(RPro)_2Cu$, no real stability constant can be obtained, as its value steadily falls with increasing saturation of the resin with copper ions.

The understanding of the discriminating mechanisms of sorption of amino acid enantiomers on the L-proline incorporated polymer is based on a systematic study of its chromatographic behavior and on a thorough structural investigation of complexes of N-benzyl-L-proline. The main feature of this ligand is that its N-benzyl radical precludes coordination of the water molecule in one of the two axial coordination positions of the Cu(II) ion coordination sphere. We believe this feature to be retained in the p-substituted benzyl group which is the repeating unit in the polymeric sorbent. When mobile ligands of the L-configuration form the sorption complex, destabilizing sterical interactions arise between the alkyl radical at the asymmetric α -carbon atom and the water molecule coordinated in the axial position. Contrary to this, no hindrance occurs in the case of the binding of the mobile ligand of the D-configuration. Moreover, this structure can be additionally stabilized by possible hydrophobic interactions between the D-amino acid alkyl group and the benzyl radical of the fixed ligand.



The higher stability of the second sorption complex explains the longer retention of D-amino acids in the chromatographic column as compared to the L-enantiomers (Ref. 9).

However, this order of enantiomer elution is inverted when tridentate amino acid ligands such as allo-hydroxyproline are chromatographed. In this case the ligands of the L-configuration are the longer retained ones because of additional coordination of the side radical donating group in the axial position of the coordination sphere. The ligands of the D-configuration cannot display their third functionality because of the interaction with the benzyl group:



The above mechanism of chiral recognition of amino acid isomers well explains the chromatographic behavior of all amino acids on polystyrene resins incorporating cyclic fixed ligands, proline, hydroxyproline, allo-hydroxyproline and azetidine carboxylic acid. The same is true for the L-proline-substituted polystyrene chains grafted on the silica surface. On the contrary, some unusual effects were observed for the L-hydroxyproline-substituted polystyrene chains on the silica support. Table 2 shows the differences in the elution order of amino acid enantiomers between a column with the macronet polystyrene resin and that with silica-grafted polystyrene, both bearing L-hydroxyproline as the fixed ligand.

With the polystyrene type resin, an improvement in resolution selectivity is generally observed on replacing an alkaline eluent by a weakly acidic one (Table 2). Also, the elution order of aspartic acid and glutamic acid is inverted,

TABLE 2. Enantioselectivity (ratio of capacity factors k'_D/k'_L) of the polystyrene- and silica-based sorbents containing L-hydroxyproline as the fixed ligand

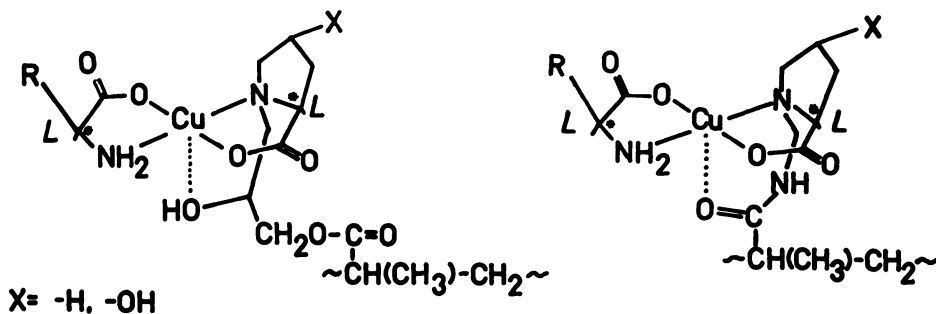
Racemate		Polystyrene resin 0.1M NH ₄ OH pH 4.5	Silica pH 4.7
HOOCCH ₂ CH(NH ₂)COOH	Aspartic acid	0.99	1.10
HOOC(CH ₂)CH(NH ₂)COOH	Glutamic acid	0.82	1.13
CH ₃ CH(NH ₂)COOH	Alanine	1.04	1.28
HOCH ₂ CH(NH ₂)COOH	Serine	1.29	1.18
CH ₃ CH(OH)CH(NH ₂)COOH	Threonine	1.52	1.49
(CH ₃) ₂ CHCH(NH ₂)COOH	Valine	1.61	1.64
CH ₃ S(CH ₂) ₂ CH(NH ₂)COOH	Methionine	1.22	1.01
$ \begin{array}{c} \text{CH}_2 - \text{CH}_2 \\ \quad \quad \\ \text{CH}_2 \quad \quad \text{CH-COOH} \\ \quad \quad \quad \\ \quad \quad \quad \text{NH} \end{array} $	Proline	3.95	6.0
$ \begin{array}{c} \text{HN} \quad \text{CH}=\text{C}-\text{CH}_2-\text{CH}-\text{COOH} \\ \quad \quad \quad \quad \\ \quad \quad \text{CH}=\text{N} \quad \quad \text{NH}_2 \end{array} $	Histidine	0.36	0.20
H ₂ N(CH ₂) ₄ CH(NH ₂)COOH	Lysine	1.22	1.00

which may indicate that these amino acids operate as bidentate ligands in acidic media and as tridentate ligands under basic conditions.

In the case of the silica grafted polymer, unexpected inversions in the elution order of enantiomers are observed for bifunctional ligands. This probably indicates that, due to the presence of hydroxy groups in the structure of fixed ligands, the polystyrene chains are adsorbed on the silica surface in such a manner that some hindering groupings appear in close proximity to the coordination center. Therefore, the above discussed structures of the sorption complex do not simulate any longer the important interactions between the fixed and mobile ligands. The responsibility of the fixed ligand hydroxy group for the specific interactions with the silica surface follows from the facts that the anomalies reported disappear on adding certain organic modifiers to the eluent or increasing the distance between the polystyrene chains and the silica surface and that no anomalies are observed for an analogous sorbent containing proline groupings instead of hydroxyproline.

The peculiarities in the behaviour of the silica-grafted sorbents do not preclude their efficient use in the analytical chromatography of racemic amino acids as well as other classes of complex forming organic compounds.

The binding of L-proline or L-hydroxyproline to the polyacrylamide or polyacrylate matrix results in a very different structure of fixed ligands which most probably offer three donating atoms to the Cu(II) ion coordination sphere:



It is quite natural that the enantioselectivity of formation of the mixed-ligand sorption complexes is now governed by other factors, and other model ligands should be used to simulate the stereochemical situation in the polyacrylic resins, as compared to those for polystyrene type sorbents.

CONCLUSION

Separation of optical isomers is one of the most difficult tasks in chromatography. Ligand exchange developed in this direction especially fruitfully, afforded a series of highly efficient enantioselective polymeric sorbents and continues to attract the rapidly rising interest in chromatographic circles.

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