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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

RESOLUTION OF α-AMINO ACIDS

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SUMMARY

Asymmetric resins containing optically active bi- or tridentate α-amino acids as fixed ligands coordinated with Cu^{2+}, Ni^{2+} or other transition metal ions can be used for the ligand-exchange chromatography of racemic α-amino acids. The degree of resolution of enantiomers depends both on the nature of the fixed and mobile ligands and on the metal ions employed.

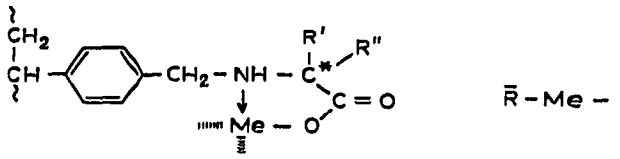
The separation of optical isomers is based on stereoselective effects and many types of stereoselective effects are used for the chromatographic resolution of racemates[1]. The first type is stereoselectivity of solvation of molecules of a racemic compound by optically active solvents. This effect is employed in gas–liquid partition chromatography on optically active liquid phases and also in countercurrent distribution with optically active extracting agents. The second stereoselective effect is ion-pair formation, which is used in all types of ion-exchange chromatography of racemates. Stereoselective effects of the formation of associates between the molecules of a racemic compound and the optically active neutral molecules of the resolving phase are the basis for all types of adsorption chromatography. It should be emphasized that these three kinds of stereoselective processes all exhibit an extremely low degree of stereoselectivity[1,2]. The fourth type of stereoselective effects is the formation of labile complexes. A relatively high degree of stereoselectivity of this type of process is an important prerequisite for the effective resolution of racemic complex-forming compounds. Therefore ligand-exchange chromatography[3] was suggested for the separation of optical isomers[4].

There are two other groups of investigators who have used ligand-exchange chromatography for the resolution of racemates. Angelici *et al.*[5] synthesized an asymmetric sorbent with N-carboxymethyl-L-valine as the fixed ligand. This sorbent in the Cu^{2+} form has been used for the partial resolution of isoleucine, valine and alanine. Bernauer and co-workers[6,7] suggested the use of the anionic optically active complexes of Fe^{3+} with β-hydroxyethyl-D-propylenediaminetriacetic acid or complexes of Cu^{2+}, Ni^{2+} or Zn^{2+} with D-(—)-propylene diaminetetraacetic acid bound to the anion exchanger Dowex 1-X2. Although the coordination sphere of the metal

ions in these complexes is saturated, one of the acetate groups of the stationary ligands can be replaced by a mobile ligand. The effect of stereoselectivity of the replacement process is not high, but is still sufficient for the partial resolution of acetyl and benzoyl derivatives of leucine, alanine, phenylalanine and methionine.

For the chromatographic resolution of racemates, we use special asymmetric complex-forming sorbents. These are produced from a macronet isoporous polystyrene matrix and an optically active bidentate or tridentate α-amino acid.

The unsaturated stationary complexes formed by treatment of the resin with a transition metal (Me) salt are the active centres of the sorbent:



The process of sorption of mobile complex-forming ligands (Lig) results in the formation of saturated mixed sorption complexes, R̄–Me–Lig. Desorption takes place by dissociation of the sorption complexes in the resin phase.

The different thermodynamic stabilities of sorption complexes containing L- or D-enantiomers of the mobile ligand are the basis of the separation of the latter. (The formation of fixed complexes, R̄–Me–R̄, with two stationary ligands per metal ion, somewhat changes, the mechanism of the sorption process[8,9].)

In most instances the best resolutions of different racemates were observed on resins containing L-proline and L-hydroxyproline as fixed ligands. The difference in free energy of formation of the two diastereomeric sorption complexes with L- and D-proline have been found to be 400–500 cal/mole for the L-proline (Cu^{2+}) resin . This value is 100-fold greater than the difference in the sorption energies of the α-methylbenzylamine enantiomers on a similar resin according to the ion-exchange mechanism. The large difference in sorption energies of proline antipodes leads to a high selectivity of the columns with L-proline (Cu^{2+}) and L-hydroxyproline (Cu^{2+}) resins. The ratio of the elution volumes of proline antipodes, for example, can be greater than 3.0. In some instances, it is even necessary to increase the temperature or the amount of the displacing ligand in the eluent in order to achieve the desorption of the second antipode.

The nature of the central complexing metal ion has a great influence on the chromatographic process. The resolution of enantiomers is more effective when the mixed sorption complexes are more stable. Therefore, it is better to use sorbents in the Ni^{2+} form, but not in the Zn^{2+} form as the Zn^{2+}–amino acid complexes are less stable. Also, the sum of the dentations of the stationary and the mobile ligands should be equal to the coordination number of the central metal ion. The best resolutions of bifunctional α-amino acids are obtained on sorbents with bifunctional stationary ligands in the Cu^{2+} form and not in the Ni^{2+} or Zn^{2+} form. Most probably, complementary sorption of the antipodes to be resolved takes place under these conditions.

The chromatographic resolution of various racemic α-amino acids was carried out on different sorbents containing different transition metal ions (Table 1). The con-

TABLE I

EXTENT OF RESOLUTION (%) OF RACEMIC α-AMINO ACIDS

Column, *ca.* 30 ml; resin beads, 0.1–0.2 mm; rate of elution: 10–15 ml/h; 0.1–0.2 g of racemate.

Stationary ligand	Me^{2+}	*Mobile ligand*			
		Proline	*Valine*	*Threonine*	*Aspartic acid*
L-Valine	Cu	17.0	31.0	20.0	15.0
	Ni	11.0	5.0	24.0	10.0
L-Hydroxyproline	Cu	96.0	90.0	100.0	0
	Ni	15.0	21.0	10.0	6.0
L-Proline	Cu	100.0	53.0	0	0
L-Histidine	Cu	94.0	7.0	10.0	35.0
	Ni	47.0	4.0	14.0	36.0
L-Cysteic acid	Cu	33.0	15.0	9.0	0
	Ni	14.0	5.0	2.0	0
L-Aspartic acid	Cu	90.0	—	16.0	12.0
	Ni	6.0	—	2.0	12.0

centration of the eluent (aqueous ammonia) was increased to 1 *N* after the elution of the first antipode.

The degrees of resolution summarized in Table I can be substantially increased by optimization of the temperature, dimensions of the column or resin beads, flow-rate the eluent and other parameters. The concentration of the outer ligand in the mobile phase used at the beginning of elution is especially important. Because of the lack of data on the thermodynamic stability of mixed complexes, the empirical selection of the type and concentration of the eluent is rather difficult. Nevertheless, in several instances we were able to obtain the total resolution by changing the concentration of ammonia in the mobile phase only.

There are several ways of achieving desorption in ligand-exchange chromatography. Elution with solutions of ammonia, pyridine and amines is usually employed. These compounds compete with the mobile ligands and replace them in the sorption complex:

$$\bar{R}\text{–Me–Lig} + n\text{NH}_3 \rightleftarrows \bar{R}\text{–Me–}(\text{NH}_3)_n + \text{Lig}$$

Mobile ligands can be desorbed by means of transition metal ions. In this case, the ligands leave the column in form of complexes:

$$\bar{R}\text{–Me–Lig} + \text{Me} \rightleftarrows \bar{R}\text{–Me} + \text{Lig–Me}$$

Finally, changing the pH of the eluent or increasing the temperature of the column can lead to dissociation of the sorption complexes and to elution of the mobile ligands. However, both increasing the temperature and increasing the concentration of the replacing ligands in the eluent decreases the stereoselectivity[10].

Sorbents with L-proline and L-hydroxyproline in the Cu^{2+} form always retain the D-isomers of bifunctional α-amino acids more strongly than the L-enantiomers, which gives the possibility of determining the absolute steric configuration of α-amino acids by a simple chromatographic method. However, the L- and D-isomers may leave the columns containing other sorbents or other metal ions in the opposite sequence. Ligand-exchange chromatography is also useful for resolving racemic diamines, hydroxyamines, hydroxy acids, their derivatives, etc.[11].

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