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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

INFLUENCE OF THE DEGREE OF SATURATION OF THE ASYMMETRIC RESIN BY METAL IONS ON LIGAND EXCHANGE

V. A. DAVANKOV, S. V. ROGOZHIN, A. V. SEMECHKIN and T. P. SACHKOVA
Institute of Organo-Element Compounds, Academy of Sciences, Moscow (U.S.S.R.)

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SUMMARY

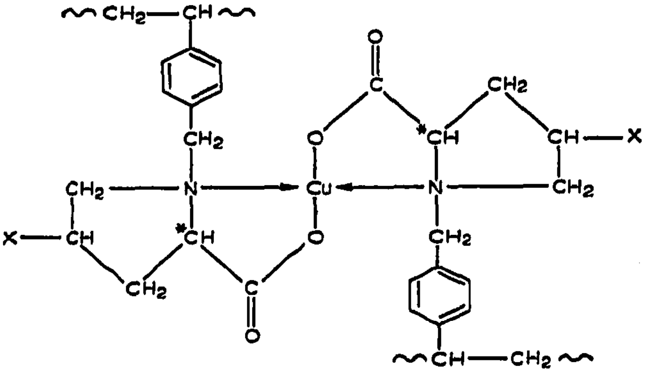
The application of an asymmetric resin containing optically active L-proline in the Cu^{2+} form for the effective resolution of racemates by means of ligand-exchange chromatography is reported. It was found in the static sorption of proline enantiomers that the difference in its sorption energy reaches 450 cal/mole and depends on the degree of saturation of the resin by copper ions. Chromatographic data such as elution volumes and the concentration of ammonia in the eluent also depend on this factor.

INTRODUCTION

The number of publications that describe the chromatographic resolution of racemic compounds by means of optically active stationary phases[1–3] totals almost 400. In addition to the well known advantages of chromatographic processes, the chromatographic resolution of racemates is superior to traditional enzymatic and crystallization methods in that it is in principle possible[4] to obtain quantitatively both enantiomers at 100% optical purity by using a resolving agent (asymmetric stationary phase) with an optical purity of less than 100%.

Exceptional progress has been made in the gas chromatographic resolution of racemates[3]. However, for resolutions on a larger scale, liquid ligand-exchange chromatography, recently reviewed by Davankov *et al.*[5], is much more promising. The ligand-exchange process differs from adsorption and ion-exchange chromatography in that the interaction between the stationary phase and the sorbates to be separated is due to the formation of coordination bonds inside the coordination sphere of the complex-forming metal ion, which thus ensures that sorbent–sorbate interaction occurs. The complex-forming metal ion itself may be bound to mobile ligands and in this case we deal with the ligand-exchange chromatography of several complexes formed by the metal ion with various ligands. However, the metal ion is more often retained by the fixed ligands of the stationary phase owing to the existence of coordination and/or ionic bonds, and in this case we deal with the chromatography of ligands[5].

The ligand chromatography of racemates on asymmetric complex-forming stationary phases was suggested by Rogozhin and Davankov in 1968[6,7], who described a series of asymmetric resins containing optically active bifunctional[4,8] and trifunctional[9] α-amino acids in a macroreticular isoporous polystyrene framework[10]. The methylene bridges between the aromatic rings of the framework and the NH_2-groups of the amino acids preserve the capability of the amino acids to form complexes with the transition metal ions. Copper ions, with a coordination number of 4, are able[11] to form stationary (fixed) complexes of the form $\bar{R}$–Cu–$\bar{R}$, with two bidentate fixed ligands, $\bar{R}$. The structure of the fixed complex $\bar{R}$–Cu–$\bar{R}$ in a resin containing L-proline (or L-hydroxyproline) as the fixed ligand $\bar{R}$ can be represented by formula I. In fact, the maximum amount of Cu(II) ions absorbed by the resin corresponds to half of their content of L-proline residues. Moreover, the analysis of the distribution of Cu(II) ions between the resin and a solution of $N(CH_2COOH)_3$ indicates the formation of fixed complexes containing two fixed ligands per metal ion in the resin phase. It was pointed out earlier by Hering and co-workers[11,12] that the formation of bis-complexes in the resin phase causes a strain in the polymer chains of the framework, which is shown by a decrease in the swelling capacity of the resin when the degree of saturation of the resin by Cu(II) ions increases. The increasing strain in the polymer chains results in the stability of the fixed complexes $\bar{R}$–Cu–$\bar{R}$ formed at low concentrations of Cu(II) ions being higher than that of the complexes formed at higher degrees of saturation of the resin when all of the favourably arranged pairs of fixed ligands have already been complexed.



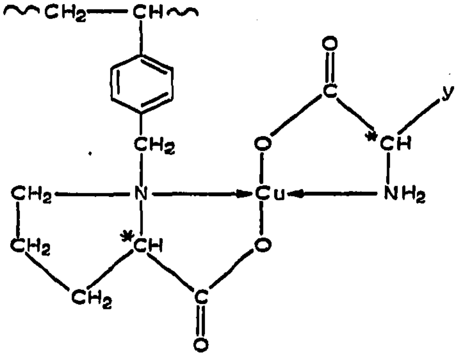
I: X = H
II: X = OH

The sorption of a molecule capable of forming complexes (mobile ligand A) in the ligand-exchange process according to the equilibrium

$$\bar{R}\text{–Cu–}\bar{R}+A \rightleftharpoons \bar{R}\text{–Cu–}A+\bar{R}$$

implies a higher free energy of the mixed sorption complex, $\bar{R}$–Cu–A, in comparison with that of the fixed $\bar{R}$–Cu–$\bar{R}$ complex. As the stability of the latter decreases with the increasing degree of saturation of the resin by Cu(II) ions, the adsorption capacity of the resin with respect to mobile ligands increases as the amount of metal ions in the system increases.

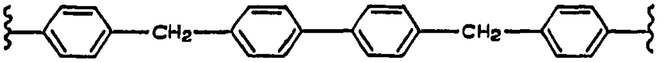
If the mobile ligand A is an amino acid, the mixed sorption complex, $\bar{R}$–Cu–A, formed in the ligand-exchange process can be represented by the formula:



We have found that the stability of mixed sorption complexes depends not only on the nature of the amino acid A, but also on its configuration. These stereoselectivity effects in the formation of mixed complexes $\bar{R}$–Me–A (ref. 13) permit the resolution of optical isomers during the ligand chromatography of the racemate.

EXPERIMENTAL

The asymmetric resin I contained 5 mole% of crosslinking bridges of the structure



between the polystyrene chains and 1.68 mequiv./g of L-proline residues. The diameter of the particles was 0.2–0.4 mm.

A 1.785-g amount of dry resin (containing 3 mequiv. of active groups) was treated with 25 ml of aqueous solution containing 0.345 g (3 mequiv.) of DL-proline, 0.28–4.19 mequiv. of $Cu(NO_3)_2$ and the appropriate amount of NaOH such that during equilibration of the mixture its pH was 10.3.

In order to prevent crystallization of $Cu(Pro)_2$, the total volumes of the last three of the eleven samples were increased to 30, 35 and 50 ml, respectively, the pH value of the final sample having decreased to 10.0 After shaking the mixtures for 70 h at 25°, the resin was filtered and washed with 5 ml of water. The liquid and resin phases, after extraction with 2 *N* HCl, were analyzed for their total copper content[14], total proline content[14,15] and optical activity (Roussel–Jouan Quick-polarimetre; $\lambda = 436$ nm; $l = 7$ cm; 1 *N* HCl; for the optically pure proline, $[\alpha]_{436}^{25} = 123°$).

For the chromatographic experiments, 10-g portions of resin I were treated with solutions of different amounts of $Cu(NO_3)_2$ in 1 *N* ammonia solution, washed with 0.5 *N* $CH_3COONa + NaOH$ solutions of pH 10.3 and loaded on to columns of I.D. 9 mm (thickness of resin layer *ca.* 200 mm). The chromatography of 0.100 g of DL-proline dissolved in 1 ml of 0.5 *N* $CH_3COONa + NaOH$ solution of pH 10.3

was carried out at a flow-rate of 6 ml/h of ammonia solutions prepared by using the above CH_3COONa buffer. Fractions of 5 ml were collected and their optical activities measured at 436 nm in a 2-cm cell.

The asymmetric resin II, containing 5 mole% of crosslinking bridges and 2.96 mequiv./g of L-hydroxyproline residues, had a particle diameter of 0.16–0.20 mm and was used in the Cu(II) form in a column 60 mm long and 9 mm I.D. for the chromatographic resolution of 1.5 mg of DL-proline in a 1.0 *M* pyridine solution at the rate of 15 ml/h and a temperature of 56°.

STATIC SORPTION

The ligand that is mainly sorbed in the case of competitive sorption between two mobile ligands (in our experiments, the L- and D-isomers of proline) is that which produces the stronger mixed complex $\bar{R}$–Cu–A and is able to destroy a greater number of the fixed complexes $\bar{R}$–Cu–$\bar{R}$. From the equilibrium distribution of L-proline (L-Pro) and D-proline (D-Pro) between the solution and the resin (Fig. 1), the difference in the thermodynamic stabilities of the mixed complexes $\bar{R}$–Cu–D-Pro and $\bar{R}$–Cu–L-Pro can be evaluated as follows:

$$\bar{R}\text{–Cu–}\bar{R} + \text{D-Pro} \overset{K_D}{\rightleftarrows} \bar{R}\text{–Cu–D-Pro} + \bar{R}$$

$$\bar{R}\text{–Cu–}\bar{R} + \text{L-Pro} \overset{K_L}{\rightleftarrows} \bar{R}\text{–Cu–L-Pro} + \bar{R}$$

$$\begin{aligned}\delta\Delta G^0 &= \Delta G^0_{\bar{R}\text{–Cu–D-Pro}} - \Delta G^0_{\bar{R}\text{–Cu–L-Pro}}\\ &= -RT \ln \frac{K_D}{K_L}\\ &= -RT \ln \frac{[\bar{R}\text{–Cu–D-Pro}]}{[\bar{R}\text{–Cu–L-Pro}]}\cdot\frac{[\text{L-Pro}]}{[\text{D-Pro}]}\end{aligned}$$

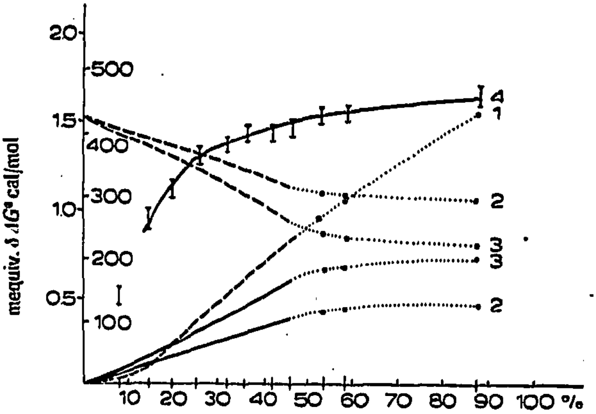


Fig. 1. The equilibrium distribution of Cu(II) ions (1), L-proline (2) and D-proline (3) between the solution phase (– – –) and the resin phase (——) and the difference in the free energies of formation of the two diastereomeric mixed adsorption complexes (4) as a function of the degree of saturation of the L-proline resin I by Cu(II) ions. The conditions of the last three experimental points differ from those of the other eight.

The decrease in the average stability of fixed $\bar{R}\text{–Cu–}\bar{R}$ complexes with the increase in the total concentration of Cu(II) ions results in a situation when the desorption of metal ions becomes more significant (Fig. 1):

$$\bar{R}\text{–Cu–L-Pro} + \text{L-Pro} \rightleftarrows \bar{R} + \text{Cu(L-Pro)}_2$$

$$\bar{R}\text{–Cu–L-Pro} + \text{D-Pro} \rightleftarrows \bar{R} + \text{Cu(L-Pro)(D-Pro)}$$

$$\bar{R}\text{–Cu–D-Pro} + \text{L-Pro} \rightleftarrows \bar{R} + \text{Cu(L-Pro)(D-Pro)}$$

$$\bar{R}\text{–Cu–D-Pro} + \text{D-Pro} \rightleftarrows \bar{R} + \text{Cu(D-Pro)}_2$$

Some mobile ligands in the solution are coordinated to the desorbed Cu(II) ions, which was taken into account in calculations of the concentration of the free mobile ligands in the solution.

From the results in Fig. 1, it is obvious that with an increase in the degree of saturation of the resin by Cu(II) ions, the amount of L-proline and D-proline adsorbed also increases. A rapid increase in the difference in the amounts of D-proline and L-proline adsorbed is reflected in the resulting tendency of the calculated $\delta\Delta G^0$ values to increase. This tendency, however, may be due to non-selective sorption of proline according to the Donnan distribution, which mainly affects the experimental points of low adsorption values of proline according to the ligand-exchange mechanism.

A large difference between the effective free energies of adsorption of D-proline and L-proline ($\delta\,\Delta G^0 \approx 400$ cal/mole) indicates a high sensitivity of ligand-exchange processes with respect to the steric structures of the isomers to be separated. In a similar experiment, with a cation-exchange resin containing L-proline residues, the difference in the sorption energies of the α-methylbenzylamine enantiomers (according to the ion-exchange mechanism) was found[16] to be *ca.* 5 cal/mole. A very high efficiency of the resolutions of racemates by means of ligand-exchange chromatography[17–19] is in good agreement with this result.

CHROMATOGRAPHY

The increase in the affinity of the resin for the mobile ligands with the increasing degree of saturation of the former by Cu(II) ions is observed in chromatographic experiments through a rapid increase in the retention times of the mobile ligands (Fig. 2). If small amounts of Cu(II) ions are present in the resin, their fixed complexes $\bar{R}\text{–Cu–}\bar{R}$ are stable so that both enantiomers of proline are readily eluted from the column by water, only a small resolution being achieved. When larger amounts of metal ions are present in the resin, it becomes necessary to use 0.1–0.2 N ammonia solution in order to elute L-proline, whereas for the elution of the D-isomer, whose adsorption is higher by 300–400 cal/mole, 1.0–2.0 N ammonia solution is required. In these elution processes, the NH_3 molecules compete with the proline molecules for the formation of mixed adsorption complexes:

$$\bar{R}\text{–Cu–Pro} + 2NH_3 \rightleftarrows \bar{R}\text{–Cu–}(NH_3)_2 + \text{Pro}$$

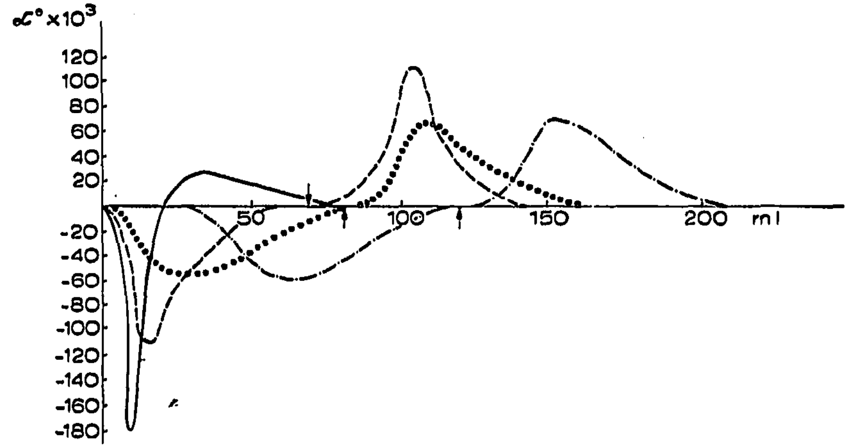


Fig. 2. Optical activity of the eluate during the chromatography of 0.1 g of DL-proline on 10 g of L-proline resin I saturated to 10.1% (——), 21.3% (– – – –), 54.4% (○○○) and 71.0% (–·–·–·–) of the theoretical amount of Cu(II) ions. The NH_3 contents of starting eluent are 0, 0, 0.1 and 0.2 *N*, respectively. At the points marked with arrows, the starting eluent was changed to 0, 1.0, 1.0 and 1.5 *N* ammonia solution, respectively.

If the degree of saturation of the resin by copper (II) ions does not exceed 50%, desorption of metal from the column by the proline solution passing through is negligible. It is noteworthy that the D-isomer desorbs Cu(II) ions from the resin saturated by the metal to higher degrees than does the L-isomer. This phenomenon is due to the difference in the conditions of elution. A high total concentration of the mobile ligands required for the elution of D-proline facilitates the desorption of copper. Because in the absence of proline a 1 *N* ammonia solution loses its desorption properties (when the saturation of the resin is less than 70%), it may be suggested that copper (II) ions are desorbed in the form of $Cu(D\text{-}Pro)(NH_3)_2$ complexes.

Fig. 3 shows an example of the complete resolution of DL-proline on a small

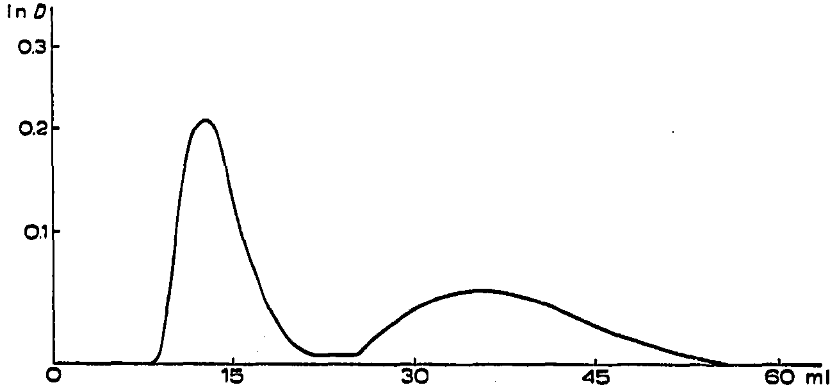


Fig. 3. Resolution of 1.5 mg of DL-proline on the asymmetric resin II containing L-hydroxyproline–Cu(II) fixed complexes.

column of L-hydroxyproline resin (of the above-mentioned formula II) as recorded with a standard amino acid analyzer. The resolving factor of 2.8 far exceeds the results obtained by any other chromatographic technique.

The possibility of greatly changing the sorbent–sorbate affinity by varying the degree of saturation of the resin by metal ions extends significantly the application of the ligand-chromatography technique. Substances with both weak and strong complexing abilities can be successfully separated on the same resin. The amount of metal ions present in the resin for second separations should be relatively small.

As the results of the chromatographic resolution of a racemate depend on the degree of saturation of the resin by metal ions, the optimum conditions for the resolution must be strictly controlled.

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