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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

INFLUENCE OF TEMPERATURE AND CONCENTRATION OF ELUENT ON LIGAND-EXCHANGE CHROMATOGRAPHY

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SUMMARY

A decrease in the temperature or concentration of the displacing ligand (pyridine) in the eluent leads to an increase in the difference in the elution volumes of L- and D-proline enantiomers when ligand-exchange chromatography of racemic proline on a sorbent with L-proline stationary ligands in the Cu^{2+} form is employed. The high stereoselectivity of the sorption process permits a gradient elution to be used for the complete resolution of the racemate.

INTRODUCTION

The theory of ligand-exchange chromatography and its application to the resolution of different complex-forming compounds have recently been reviewed[1,2]. The great possibilities of ligand-exchange chromatography have been shown in the successful separation of compounds with similar physico-chemical properties such as isomers and homologues[3] and especially optical isomers[4–6].

In our experiments on the ligand-exchange resolution of racemates, asymmetric resins were used, the preparation of which included the reaction of natural α-amino acids with a chloromethylated macroreticular isoporous polystyrene matrix. Stationary complexes that are formed in the resin phase in the presence of metal ions contain two fixed amino acid ligands per ion of the metal[7,8]. This is shown by the strong influence of the degree of saturation of the resin by metal ions on the degree of separation of optical isomers and on the concentration of ammonia in the mobile phase that is necessary for the elution process to be satisfactory[9].

The influence of the temperature and concentration of eluent on the selectivity and efficiency of the ligand-exchange chromatography of racemic compounds was studied in the present work. There is only one paper, by De Hernander and Walton[10], that deals with the influence of temperature on the chromatographic separation (of amphetamine drugs) on copolymers of methacrylic acid (Bio-Rex 70) in their metal forms. It should be emphasized that the application of optical antipodes in the study of the influence of temperature or other factors on the ligand-exchange process eliminates all errors connected with differences in diffusion coefficients of competing ligands or with differences in their non-specific interactions with the polymer matrix.

EXPERIMENTAL

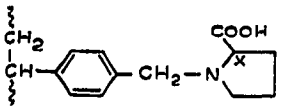
The asymmetric resin was produced from macroreticular isoporous polystyrene and L-proline[9]. The analytical capacity of the sorbent was 2.52 mequiv. of functional groups per gram of sorbent, the bead size was 100–150 mesh and the degree of saturation of the sorbent by copper ions was 35%.

For the chromatographic experiments, D-proline (Calbiochem, Los Angeles, Calif., U.S.A.), L-proline and D,L-proline (Reanal, Budapest, Hungary) were employed.

Chromatographic resolutions were carried out on a column 95 mm long and 9 mm I.D. using a standard amino acid analyzer with a flow-rate of eluent of 30 ml/h. EDTA was added to the ninhydrin reagent solution. The amount of amino acid to be resolved was 0.2 mg for D,L-proline and 0.1 mg for D- and L-prolines.

RESULTS AND DISCUSSION

The structure of the stationary (fixed) ligands ($\overline{R}$) in the resin phase can be represented by the formula



In the presence of Cu^{2+} ions, stationary complexes are formed that contain two fixed ligands per metal ion ($\overline{R}$–Cu–$\overline{R}$). The sorption of proline antipodes (L-Pro and D-Pro) on the resin in the Cu^{2+}-form takes place with the conversion of the stationary complexes into mixed sorption complexes:

$$\overline{R}\text{–Cu–}\overline{R} + \text{D-Pro} \overset{K_D}{\rightleftarrows} \overline{R}\text{–Cu–D-Pro} + \overline{R} \quad (1)$$

$$\overline{R}\text{–Cu–}\overline{R} + \text{L-Pro} \overset{K_L}{\rightleftarrows} \overline{R}\text{–Cu–L-Pro} + \overline{R} \quad (2)$$

The molecules of pyridine (Py) in the eluent replace the mobile amino acid ligands in the sorption complexes:

$$\overline{R}\text{–Cu–D-Pro} + 2\,\text{Py} \overset{K_{D'}}{\rightleftarrows} \overline{R}\text{–Cu–(Py)}_2 + \text{D-Pro} \quad (3)$$

$$\overline{R}\text{–Cu–L-Pro} + 2\,\text{Py} \overset{K_{L'}}{\rightleftarrows} \overline{R}\text{–Cu–(Py)}_2 + \text{L-Pro} \quad (4)$$

In order to study the influence of the temperature and concentration of pyridine on the efficiency and selectivity of ligand-exchange chromatographic separations, conditions were employed under which both of the proline antipodes are eluted simultaneously. Although these conditions are not optimal for complete resolution, they provide the opportunity for a simple determination of the chromatographic parameters of antipodes to be separated.

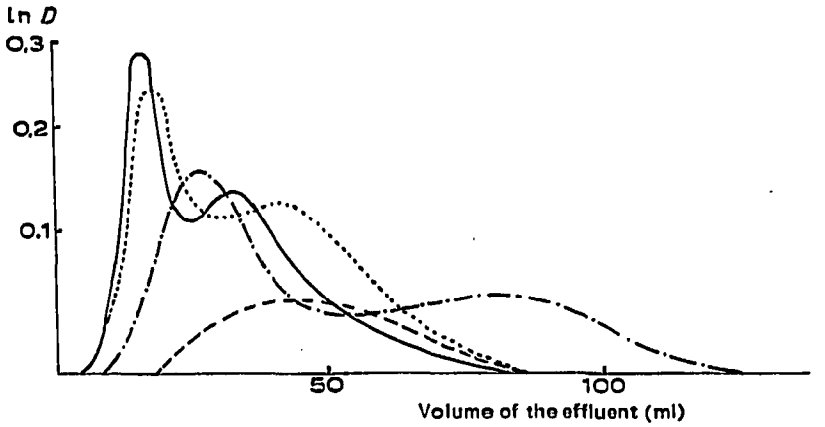


Fig. 1. Elution curves of proline isomers in 0.5 *N* aqueous pyridine solution at (———) 60°, (·····) 50°, (–·–·–) 40° and (– – – –) 20°. *D*, optical density of the effluent.

Influence of temperature

Fig. 1 illustrates the elution curves for the proline isomers in 0.5 *N* aqueous pyridine solution. It can be seen that when the temperature increases from 20° to 60°, the elution volumes of the antipodes decrease, which is the result of the increase in the degree of dissociation of the mixed sorption complexes $\overline{R}$–Cu–D-Pro and $\overline{R}$–Cu–L-Pro.

The difference in the free energies of formation of the two diastereomeric sorption complexes

$$\delta\Delta G^0 = \Delta G^0_{\overline{R}\text{–Cu–D-Pro}} - \Delta G^0_{\overline{R}\text{–Cu–L-Pro}} = -RT \ln \frac{K_D}{K_L}$$

reaches about 450 cal/mole in aqueous solution at pH 10.0 and 25°[8,9]. The $\delta\Delta G^0$ value decreases gradually with increase in temperature, as shown by the decreasing selectivity (a) of the column, which is determined by the ratio of the elution volumes of the two antipodes (V_D and V_L). The value of the selectivity (a) is related to the constants K_D and K_L in eqns. 1 and 2 by the equation

$$a = \frac{V_D}{V_L} = \frac{K_D}{K_L}$$

Table I summarizes the column selectivities that were obtained in experiments at different temperatures and using different concentrations of pyridine in the eluent.

As the selectivity decreases with increase in temperature, it is clear that the temperature dependence of the formation constant, K_D, of the more stable complex, $\overline{R}$–Cu–D-Pro, is greater than that of the less stable complex, $\overline{R}$–Cu–L-Pro.

The most important conclusion that can be drawn from the results in Table I is that the selectivities in the ligand-exchange chromatographic separations of optical isomers are extremely high. They can be compared with the best results observed in the ion-exchange resolutions (a=1.60) of racemic 3,4-dihydroxyphenylalanine[11] or in the widely used gas–liquid chromatography (a=1.10–1.30) of enantiomers[12].

Although the selectivity of ligand-exchange chromatography is excellent, the efficiency is not so satisfactory. Table II summarizes the heights equivalent to a

TABLE I

SELECTIVITY (V_D/V_L) OF THE COLUMN AT DIFFERENT TEMPERATURES AND DIFFERENT CONCENTRATIONS OF PYRIDINE IN THE ELUENT

Temperature (°C)	*Concentration of pyridine (M)*			
	0.4	*0.5*	*0.7*	*1.0*
20	High	High	High	2.10
40	3.50	3.15	2.28	1.94
50	2.85	2.45	2.08	1.74
60	—	2.10	1.95	1.67

TABLE II

HEIGHTS EQUIVALENT TO A THEORETICAL PLATE IN THE CHROMATOGRAPHIC RESOLUTION OF D,L-PROLINE IN 0.5 *N* AQUEOUS PYRIDINE

Temperature (°C)	*HETP (mm)*	
	L-*Pro*	D-*Pro*
20	13.5	—
40	11.2	16.8
50	9.2	13.9
60	7 3	11.2

theoretical plate (HETP) calculated according to Glueckauf[13] (pure enantiomers were used in cases of insufficient resolution of racemic proline).

Influence of pyridine concentration in the eluent

When the concentration of pyridine in the eluent is sufficiently high, the equilibria in eqns. 3 and 4 are shifted to the right, thus decreasing the elution volumes of the proline enantiomers. This tendency is evident from Fig. 2, which shows the influence of the concentration of pyridine on the shape of the elution curves of D,L-proline.

The selectivity should be independent of the concentration of the displacing ligand as it is determined by the constants of the equilibria in eqns. 3 and 4:

$$a = \frac{V_D}{V_L} = \frac{K_{L'}}{K_{D'}}$$

However, from the results in Table I, a marked increase in selectivity at low pyridine concentrations is obvious. The most probable explanation is that the equilibria in eqns. 1–4 cannot be characterized by any true thermodynamic constants, as the starting complexes for all the equilibria, namely the stationary complexes $\overline{R}$–Cu–$\overline{R}$, are energetically non-equivalent[8,9]. Any changes in the positions of the equilibria that are connected with a change in the extent of the formation of the stationary complexes $\overline{R}$–Cu–$\overline{R}$ must affect the mutual stabilities of all the components of the equilibria. The dependence of the difference in the sorption energies of the proline enantiomers on the degree of saturation of the resin by copper ions found earlier[8,9], and the observed influence of the pyridine concentration on the selectivity, can

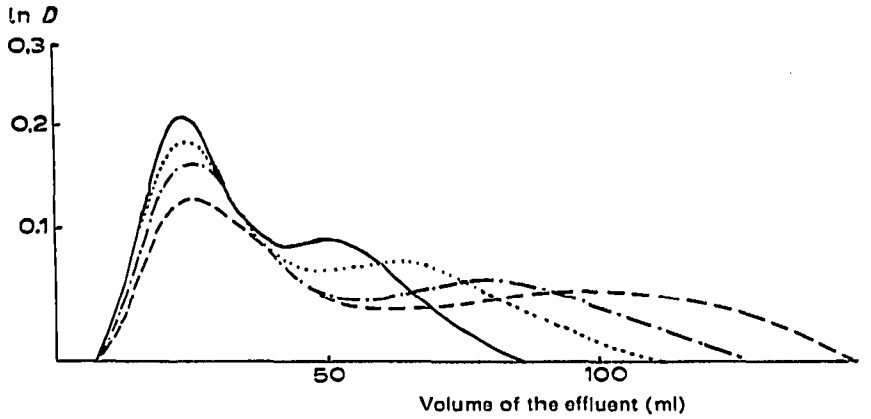


Fig. 2. Elution curves of proline isomers in (———) 1.0 *M*, (·····) 0.714 *M*, (–·–·–) 0.5 *M* and (– – – –) 0.4 *M* aqueous pyridine solutions at 40°. *D*, optical density of the effluent.

easily be explained in terms of the thermodynamic non-equivalence of the fixed complexes $\overline{R}$–Cu–$\overline{R}$. This might be the reason for the low efficiency, *i.e.*, for the broad elution peaks during the ligand-exchange chromatography on the resin. (The heights equivalent to a theoretical plate become smaller with an increase in pyridine concentration, but this might be simply the effect of smaller retention times.)

In spite of the low efficiency, it is very easy to achieve the complete resolution of proline antipodes by the suggested ligand-exchange procedure owing to its extremely high selectivity. The results in Table I provide the opportunity of selecting the most convenient conditions for the stepwise elution of enantiomers: L-proline can be eluted with 0.4–0.7 *M* pyridine solution at room temperature, while for the elution of D-proline a higher temperature or a stronger eluent is required. There is no other chromatographic process which could compete with ligand-exchange chromatography in the separation of enantiomers on a preparative scale.

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