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LIGAND-EXCHANGE CHROMATOGRAPHY OF RACEMATES

XIII. MICROPREPARATIVE RESOLUTION OF L,D-LEUCINE

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SUMMARY

Data are presented on the enantioselectivity and efficiency of ligand-exchange chromatographic resolution of racemic α-amino acids using a polystyrene resin with L-hydroxyproline groups and saturated with Cu^{2+}. Micropreparative resolution of L,D-leucine is discussed with respect to the degree of sorbent saturation with Cu^{2+}, elution rate, eluent concentration, operating temperature, column loading and column parameters.

INTRODUCTION

Ligand-exchange chromatographic (LEC) resolution of racemates is based on the interaction between the enantiomers and an optically active metal complex fixed in the sorbent phase. This method appears to be highly efficient for the resolution of amino racemates, hydroxy acids, amino acids and their derivatives, and has recently been reviewed[1].

Rogozhin and Davankov[2] suggested that polystyrene sorbents containing L-α-amino acid group should be used as sorbents for LEC. Sorbents of this kind saturated by Cu^{2+}, Ni^{2+} and Zn^{2+} show a high enantioselectivity towards racemic α-amino acids.

Of special interest are sorbents containing cyclic amino acid groups[3]. In such sorbents L- and D-isomers of many amino acids have sorption energies differing by 200–1000 cal/mol. Such a high sorption enantioselectivity is a prerequisite for the qualitative chromatographic resolution of racemates.

Possible reasons for the high enantioselectivity of LEC using these sorbents have been studied with the help of data for corresponding model compounds[4]. The explanation evidently lies in the conformational and solvational effects of the diastereomeric sorption complexes.

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It was found in earlier studies of LEG using asymmetric sorbents that a high enantioselectivity could be combined with a low efficiency of chromatography, resulting in most cases in only a partial racemate resolution requiring several tens of hours.

LEC of such sorbents is described by intradiffusion kinetics[5,6]. The efficiency of chromatography rises markedly with an increase in sorbent capacity, swelling capacity and decrease in particle size. Racemate resolution time can be much shortened by appropriate selection of the degree of sorbent saturation by Cu^{2+} and of the ammonia concentration in the eluent[5].

A sorbent containing proline groups on a polystyrene matrix saturated by Cu^{2+} has been used for preparative resolution of proline racemates[7]. Data were presented showing the effect of elution rate and ammonia concentration on the degree of racemate resolution.

It should be noted that the attainable degree of racemate resolution depends on both the enantioselectivity and the LEC efficiency. Therefore we have made a systematic study of the efficiency and selectivity of LEC using L,D-leucine as the sample.

EXPERIMENTAL

An asymmetric sorbent with L-hydroxyproline groups was obtained by amination of the chloromethylated macronet polystyrene matrix with methyl L-hydroxyprolinate hydrochloride as described in ref. 8. The sorbent capacity was 3.86 mmol/g, its swelling capacity in water was 250% and the particle size was *ca.* 50 μm. A styrene copolymer with 0.7% divinylbenzene containing 5.3% of additional cross-links of the diphenylmethylene type[6] was used as a matrix.

Sorbent saturated to the required degree with Cu^{2+} (ref. 5) was suspended in 0.1 *N* NH_4OH and transferred into a column. Samples of L-leucine and L,D-leucine in the form of 5% solutions were introduced into the column by a microsyringe and eluted by 0.1–1.0 *N* NH_4OH at a flow-rate of 5–50 ml/h. A liquid-flow spectrophotometer was used as detector. Chromatographic studies were conducted with the aid of the LKB Vario Perpex peristaltic pump.

RESULTS AND DISCUSSION

The retention volumes of amino acid L- and D-isomers vary over a wide range with changes in copper saturation and eluent concentration. Fig. 1 shows the relationship between retention volumes (V) of L- and D-leucine isomers expressed in units of the column free volume and the degree of sorbent saturation with metal ions. A sorbent fully saturated with Cu^{2+} has all fixed amino acid groups in the form of bis-chelate copper complexes. It exhibits the highest affinity for enantiomers of the resolving amino acid. In the case of bidentate amino acids, the strongest sorption complexes are those containing the D-enantiomer. The enantioselectivity of the sorption, α, is characterized by the ratio of the retention volumes of the D- and L-enantiomers. A decrease in the degree of copper saturation from 100 to 59% causes a 4.5-fold reduction of V_L and V_D while α remains unchanged.

The retention parameters of L- and D-amino acids can be varied by changing the ammonia concentration in the eluent; an increase in ammonia content results in

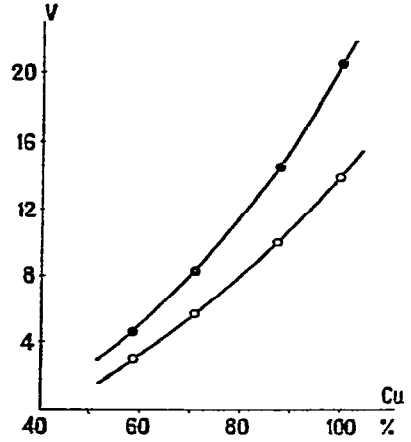
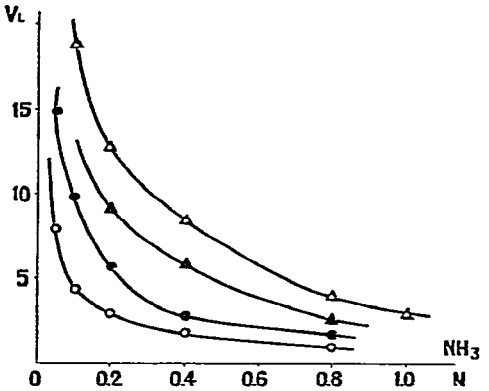


Fig. 1. Retention volumes of leucine enantiomers as a function of the degree of sorbent saturation by Cu^{2+}. ●—●, V_D; ○—○, V_L, Column: 140 × 9 mm. Eluent: 0.2 *N* NH_4OH; flow-rate 50 ml/h.

a reduction of V_L and V_D (Fig. 2). The dependence on ammonia concentration has the same features for sorbents with different degrees of saturation by Cu^{2+}.

Similar values of L-isomer retention for sorbents with different degrees of copper saturation may be obtained by using eluents of different ammonia content. However, the retention of the D-isomer will be different and decrease with increasing eluent concentration. Thus, the enantioselectivity of LEC of amino acid racemates decreases with increasing ammonia content in the eluent (Fig. 3) and is independent of the retention of the resolving amino acid and the degree of sorbent saturation by metal ions. With an increase in eluent concentration, ammonia replaces water in the axial positions of the amino acid sorption complexes, which apparently causes the reduction of α in the case of bidentate α-amino acids. To improve the selectivity of chromatography, eluents with a lower ammonia content should be used.



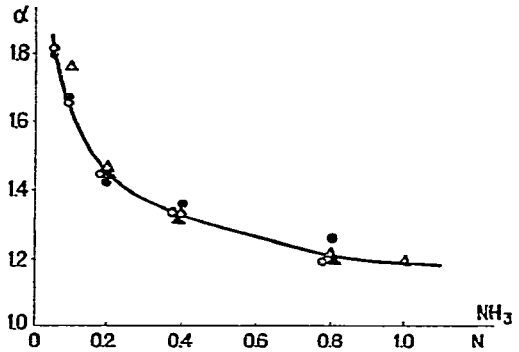


Fig. 2. Retention volumes of L-leucine as a function of ammonia concentration in the eluent. Degree of saturation of the L-hydroxyproline resin by Cu^{2+}: △, 100%; ▲, 88%; ●, 70%; ○, 59%. Sample: 1 mg of L,D-Leu. Chromatographic conditions as in Fig. 1.

Fig. 3. Dependence of enantioselectivity of sorption, α, for L,D-Leu upon ammonia concentration in in the eluent. Degree of sorbent saturation by Cu^{2+}: △, 100%; ▲, 88%; ●, 70%; ○, 59%.

The sorption rate on complex-forming sorbents tends to rise with increasing temperature for processes which can be described by both diffuse and chemical kinetics[9]. A significant increase in the efficiency of LEC of amino acids was observed with increasing temperature[10]. It was therefore of interest to study the effect of temperature on the efficiency of LEC of polystyrene sorbents containing fixed L-amino-acid groups. Fig. 4 shows the temperature dependence of chromatographic efficiency, selectivity and the retention volume of leucine enantiomers. The efficiency of chromatography is almost independent of temperature over a wide temperature range. Also, an increase in temperature has only slight affects on enantioselectivity and amino acid retention volume. Therefore an increase in LEC temperature cannot be used to improve the degree of resolution of racemic leucine.

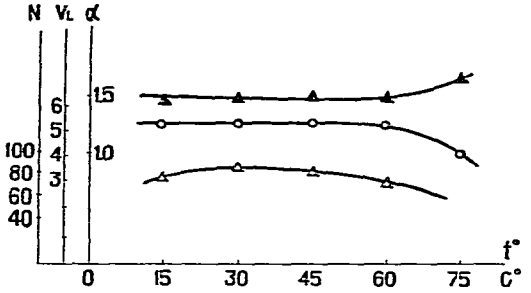


Fig. 4. Chromatography of 1.2 mg L,D-Leu showing temperature dependence of the number of effective plates, N (△) enantioselectivity, α (▲) and the retention volume of L-Leu, V_L (○). Column: 140 × 9 mm. Eluent: 0.2 N NH_4OH; flow-rate 35 ml/h.

The time required for LEC was investigated by studying the effects of elution rate and sample volume upon the efficiency of chromatography. In 140 × 9 mm columns the LEC efficiency was not affected by sample volume over the range 5–200 μl. However, an increase in the elution rate from 30 to 100 ml/h resulted in a slow reduction of efficiency (Fig. 5). The degree of racemate resolution and the chromatography efficiency can be raised without changing the chromatography time by simultaneous increase of the column length and elution rate.

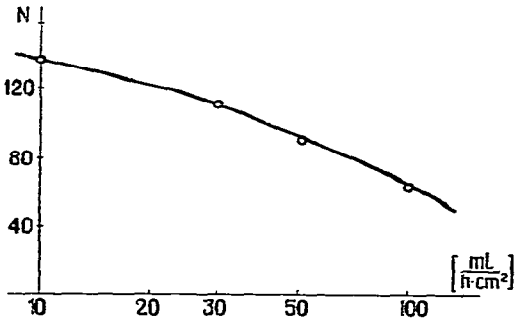


Fig. 5. Chromatography of 2.0 mg L-Leu showing the number of effective plates, N, as a function of elution rate. Column: 140 × 9 mm.

There is a pronounced dependence of the LEC efficiency upon the column loading (Fig. 6). A high efficiency for resolving small quantities of racemate can be achieved on a sorbent with *ca.* 50-μm particles. However, the efficiency is drastically reduced and the chromatographic zone croded in short (14 cm) columns under high-load conditions. On changing to longer (34 cm) columns with high loadings only the upper part of the column is overloaded while the larger part is active at low amino acid concentrations in a zone with efficiency approaching that of a low-load process.

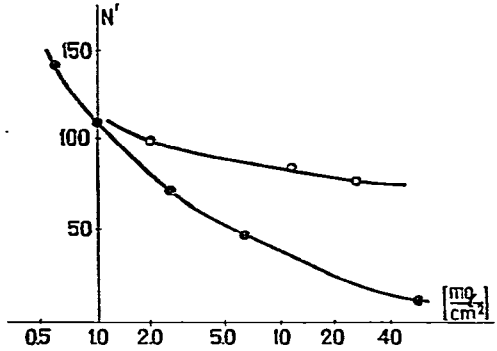


Fig. 6. Chromatography of L-leucine showing the number of effective plates per 10 cm of column length, N', as a function of the amount of L-Leu per cm² of the column cross-section. ○—○, Column 340 × 10 mm; eluent 0.2 *N* NH_4OH, flow-rate 60 ml/h. ●—●, Column 140 × 9 mm, flow-rate 50 ml/h.

The number of effective chromatographic plates, N, increases with the column length. At low loads this increase is proportional to the increase in length, while on the micropreparative scale it is several times higher. Increase of the column length appears to be more effective in micropreparative LEC resolution than reduction of the load at the expense of increase in cross-section. One can readily resolve 20 mg of L,D-leucine on a 34-cm column (Fig. 7).

The amount of racemate separated can be raised by further increase of the column length.

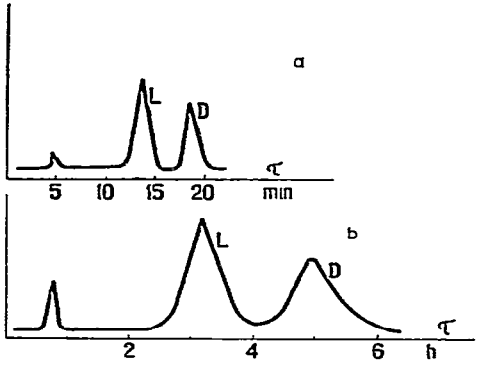


Fig. 7. Chromatography of L,D-leucine on a sorbent with L-hydroxyproline groups, satureted by Cu^{2+}. a, Particle size 10 μm, column 100 × 2 mm, sample 0.01 mg L,D-leucine, eluent 0.2 *N* NH_4OH, flow-rate 5 ml/h. b, Particle size 50 μm, column 340 × 10 mm, sample 20 mg L,D-leucine, eluent 0.1 *N* NH_4OH, flow-rate 60 ml/h.

CONCLUSION

Six hours were required to effect a quantitative resolution of 20 mg of L,D-leucine using L-hydroxyproline as sorbent (particle size *ca.* 50 μm), 70% saturated by Cu^{2+}, with 0.1 *N* NH_4OH as an eluent on a 340 × 10 mm column. The enantioselectivity of this process, α, is 1.6, whereas in the resolution of proline on L-proline resin[7] it is as high as 4 (ref. 3).

However, ways exist for further improving the efficiency and reducing the time of chromatography. Thus, the use of a sorbent with particle size of 10 μm on a 10-cm column enables qualitative resolution of leucine in minutes.

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