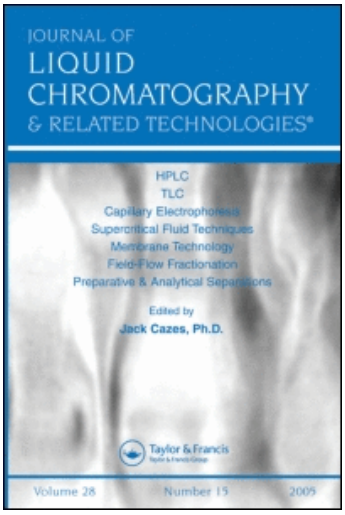

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chiral Polymers and Optical Resolution: Investigation of the Mechanism of Ligand Exchange for Resolution of DL-Amino Acids

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To cite this Article Jin, Renhua and He, Binglin(1989) 'Chiral Polymers and Optical Resolution: Investigation of the Mechanism of Ligand Exchange for Resolution of DL-Amino Acids', Journal of Liquid Chromatography & Related Technologies, 12: 4, 501 — 513
To link to this Article: DOI: 10.1080/01483918908051753
URL: http://dx.doi.org/10.1080/01483918908051753

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(4), 501-513 (1989)

CHIRAL POLYMERS AND OPTICAL RESOLUTION: INVESTIGATION OF THE MECHANISM OF LIGAND EXCHANGE FOR RESOLUTION OF DL-AMINO ACIDS

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ABSTRACT

Using novel class chiral ligand polymers which have different chemical environment in chiral cavity, we investigated their functional properties of chiral recognition for DL-amino acids and discussed ligand exchange chromatography of DL-amino acids on different chromatographic conditions in detail.

INTRODUCTION

17 years have passed since Davankov et al.[1] found a new way of resolving racemic compounds in ligand exchange chromatography. Although scientists had some knowledge and experience in this field, the problems involving resolution of DL-amino acids became more and more complicated. Therefore, their systematic investigation has been necessary[2]. Audebert et al. supplemented acrylamide[3] III and vinylpyridine[4] IV type polymers grafted with chiral moiety which was different from styrene type II polymer reported

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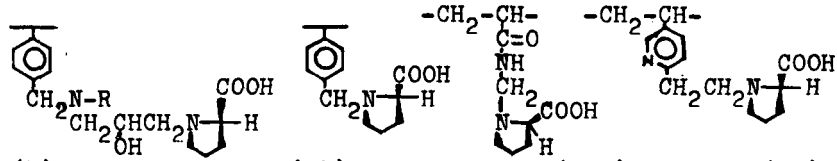
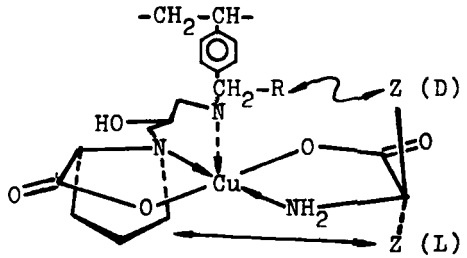
by Davankov et al. and effectively seperated racemic DL-amino acids utilizing the polymers as stationary phase. Recently, the authors prepared a series of styrenic type I polymers grafted with L-Pro or L-Hyp with different chemical environment in benzyl amine position[5,6] and tried to express mechanisms of chiral recognition of the polymers by means of far-IR, XPS, ESR and Verloop's stereo-substituent constant[7].



(I) (II) (III) (IV)

Of course, the aim of our work was to investigate the effect of the R group of polymer I on resolving power. On the other hand, the behaviours of ligand exchange under different chromatographic conditions also drew our interest.

In our previous studies, the following structure model was presented for polymer I system. Apparently, the L forms



(V)

preferably adsorbed onto the polymer more than D forms. However, it had to be redetermined if the model V is suitable for any R group (see Ref. (7)).

EXPERIMENTAL

The novel class polymers I (bead, 400 mesh, cross liking 1%) was prepared by a previous method[5,6,7]. The polymers coordinated completely with copper ion. In the IR specturm, we could clearly see the moderately strong peak of carboxyl

TABLE 1. Elimental Analysis of Chiral Polymers

R-group	I(1) $-CH_3$	I(2) $-C_2H_5$	I(3) $n-C_3H_7$	I(4) $n-C_4H_9$	I(5) C_2H_4OH	I(6) $i-C_3H_7$	I(7) $c-C_6H_{11}$
% C	62.04	70.67	63.57	61.86	56.89	61.88	61.84
H	7.11	8.20	8.31	8.05	7.29	8.26	8.23
N	4.82	5.31	5.64	5.32	5.11	6.26	4.85
G*	40	48	58	56	48	61	55

*G shows the chiral units in the polymers

group of L-Pro moeity at 1630 cm^{-1} for the polymers. The properties of the polymers were listed in Table 1.

The beads containing L-Pro residues, treated with a solution of 0.5mol/l $Cu(OAc)_2$, were packed into the glass column by a slurry method. The column was then rinsed with the eluent until equilibrium was established. The sample was introduced from the top of column and the flow rate was controlled with a peristaltic pump. The eluent from the column was monitored with a UV detector at 254 nm. A copper containing (1 X 10^{-4}mol/l) solution was used as the eluent. The void volume was determined by the peak of solvent. Columns: I(1)-Cu packing (18 X 0.4 cm I.D.); I(2)-Cu packing (25 X 0.4 cm I.D.); I(3)-Cu packing (21 X 0.4 cm I.D.); I(4)-Cu packing (15 X 0.4 cm I.D.); I(5)-Cu packing (13 X 0.5 cm I.D.); I(6)-Cu packing (16 X 0.4 cm I.D.); I(7)-Cu packing (9 X 0.5 cm I.D.).

RESULTS AND DISCUSSION

Resolution

Taking DL-His as a probe compound, we investigated the resolving power of I(1)-Cu---I(7)-Cu complexes by carefully changing chromatographic conditions such as: eluent composition,flow rate, etc. Very interesting results were obtained: that I(1)-Cu, I(2)-Cu and I(4)-Cu could not seperate racemic compound DL-His, on the other hand, I(3)-Cu,I(6)

-Cu and I(7)-Cu excellently resolved DL-His into two parts and I(5)-Cu only partially resolved DL-His. The orders of elution are all D-His ahead of L form on columns I(3)-Cu, I(6)-Cu, I(7)-Cu and I(5)-Cu. Fig.1 shows elution curves for ligand exchange chromatography of DL-His. From Fig.1, we can realize that the retentions are apparently different between columns which could resolve DL-His and the other columns which could not resolve DL-His. It may be due to the difference of structure of the complexes on the polymers. We found, from the ESR studies, that the polymer-Cu complexes of I(3)-Cu, I(6)-Cu, I(7)-Cu and I(5)-Cu, $\overline{R}$-Cu-$\overline{R}$, could be greatly distorted from base planar to tetrahedral comformation, but polymer-Cu complexes of I(1)-Cu, I(2)-Cu and I(4)-Cu, $\overline{R}$-Cu-$\overline{R}$, might tend to keep a quasi-ideal planar conformation[7]. Perhaps, this means that the distorted conformation of the polymer-copper complexes have more activity for DL-amino acid substrate in ligand exchange reaction.

Influence of Eluent Composition

Using different amonium salt solutions as eluent, the retentions of DL-His showed very different results. The results on column I(3)-Cu and I(6)-Cu are listed in Table 2. The retentions of DL-amino acids are determined not only by pH values, but also by the kind of anion used. When Cl^- ion solution was used as eluent, the retentions of DL-amino acids became very long; thus, we can adjust the retentions through adding Cl^- ion to the eluent.

Influence of Temperature

Temperature effects on ligand exchange chromatography of DL-amino acids were not considered to be important in the previous investigation. Audebert et al. reported that the column temperature could improve resolution, but had no effect on retention[3]. Davankov et al. reported that retentions were not influenced by increasing column temperature over the range 25---70°C[8] and, in another system, the retentions decreased at higher temperature[9].

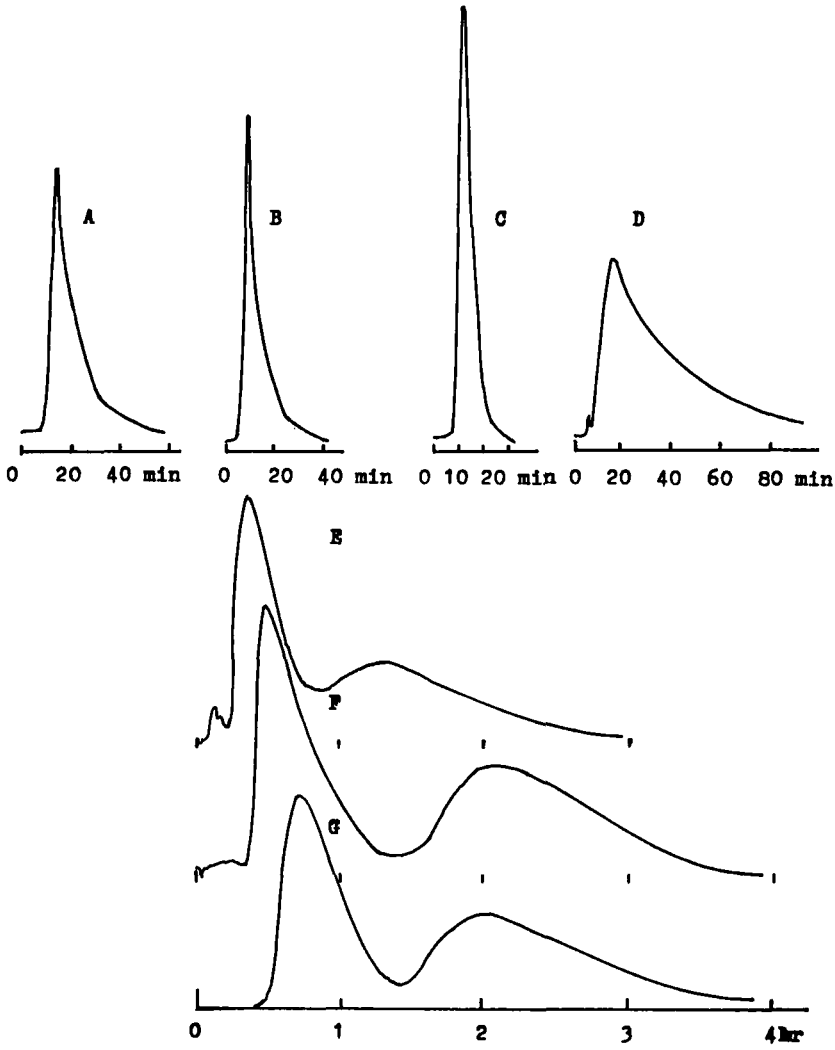


Figure 1: Chromatograms of DL-His on different columns. Column: A,I(2)-Cu; B,I(1)-Cu; C,I(4)-Cu; D,I(5)-Cu; E, I(3)-Cu; F,I(6)-Cu; G,I(7)-Cu. Conditions: A, 31°C, 24ml/hr, 0.1mol/l $(NH_4)_2CO_3$; B, room temp., 0.2mol/l NH_3/NH_4Cl (1/1,V/V), 24ml/hr; C, room temp., 8.5ml/hr, 0.1mol/l $(NH_4)_2CO_3$; D, room temp., 20ml/hr, 0.05mol/l $(NH_4)_2CO_3$; E, 40°C, 24ml/hr, 0.1mol/l $(NH_4)_2CO_3$, 1 mg DL-His; F, 35°C, 17ml/hr, 0.2mol/l $(NH_4)_2CO_3$, 1mg DL-His; G, 45°C, 7ml/hr, 0.15mol/l $(NH_4)_2CO_3$, 1mg DL-His.

TABLE 2. Influence of Eluent Composition on Retention

	DL-His*			DL-His**			DL-Pro**		
	V_D'	V_L'	α	V_D'	V_L'	α	V_D'	V_L'	α
0.1mol/l $(NH_4)_2CO_3$	6.0	30.4	5.07	7.0	35.6	5.09	8.7	3.7	2.35
0.2mol/l NH_4HCO_3	5.6	32.0	5.71	4.3	28.3	6.58	10.0	4.3	2.33
0.1mol/l $(NH_4)_2HPO_4$	9.1	62.8	6.90	17.3	73.3	4.24	18.9	8.5	2.22
0.2mol/l NH_3/NH_4Cl (1:1 V/V)	8.0	72.8	9.1	24.6	un-eluted		29.3	12.0	2.44

*Column I(3)-Cu, 35°C, 24ml/hr, 1.0mg DL-His, $\alpha=V_L'/V_D'$.
**Column I(6)-Cu, 35°C, 1.0mg DL-His, 1.0mg DL-Pro.

In our system, we found that retentions greatly depended upon the column temperature. As the temperature increased(25--45°C), the retentions effectively decreased. This means the ligand exchange reaction between polymer complexes and amino acids is very sensitive to the temperature. We think these phenomena relate to the distortion of polymer conformation in ligand exchange process. Fig.2 and Fig.3 show the relationships between ln k' and T^{-1}. From the results, we can easily obtain the linear form $\ln k' = a + b/T$. This equation shows, at least, there is kinetic action in the resolution of racemates. Perhaps, it is necessary to treat the chromatographic column as a reactor for the ligand exchange reaction. Hence, we can investigate the behaviour and properties of ligand exchange with retention parameters.

Influence of Eluent Concentration

Utilizing $(NH_4)_2CO_3$ solution as an eluent, we test the resolution of DL-His at different concentrations. When the

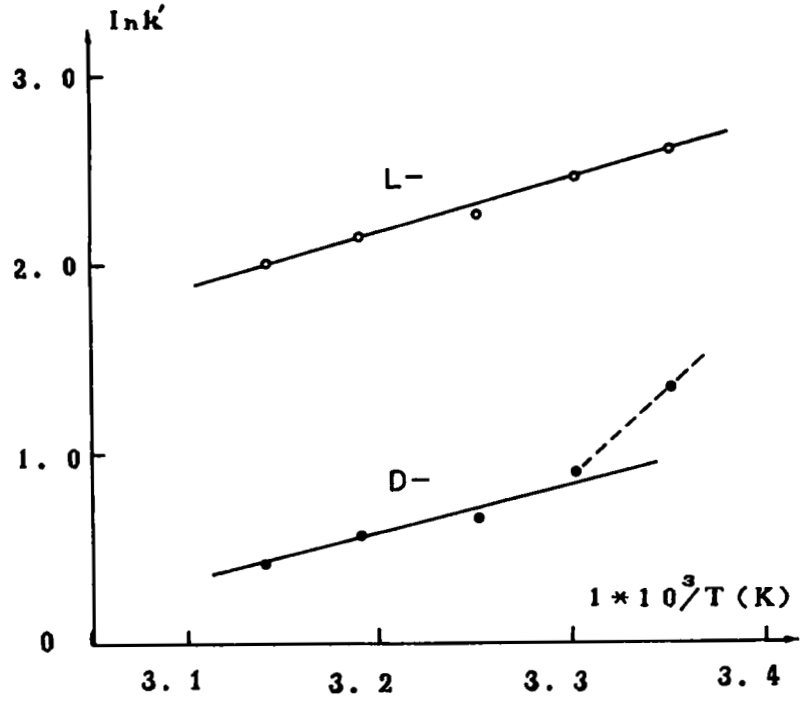


Figure 2: Variation of ln k' of DL-His versus 1/Temp. Column I(3)-Cu. Condition: 24ml/hr, 0.1mol/l$(NH_4)_2CO_3$, 1.0mg DL-His.

$(NH_4)_2CO_3$ concentration was increased (from 0.05 to 0.2mol/l) the retentions decreased. Particularly, another linear form, k' = c + d/con. was obtained(Fig.4 and Fig.5). This kind of linear form was also presented by Audebert et al.[4]. However, we are interested in expressing the difference that the slope of line for L enatiomer is larger than that for the D enantiomer. Generally, there is the following equilibrium in a ligand exchange system,

$$\overline{R}\text{-Cu-}\overline{R} + AA \rightleftharpoons \overline{R}\text{-Cu-}AA + \overline{R} \qquad (1)$$

$$\overline{R}\text{-Cu-}AA + S \rightleftharpoons \overline{R}\text{-Cu-}S + AA \qquad (2)$$

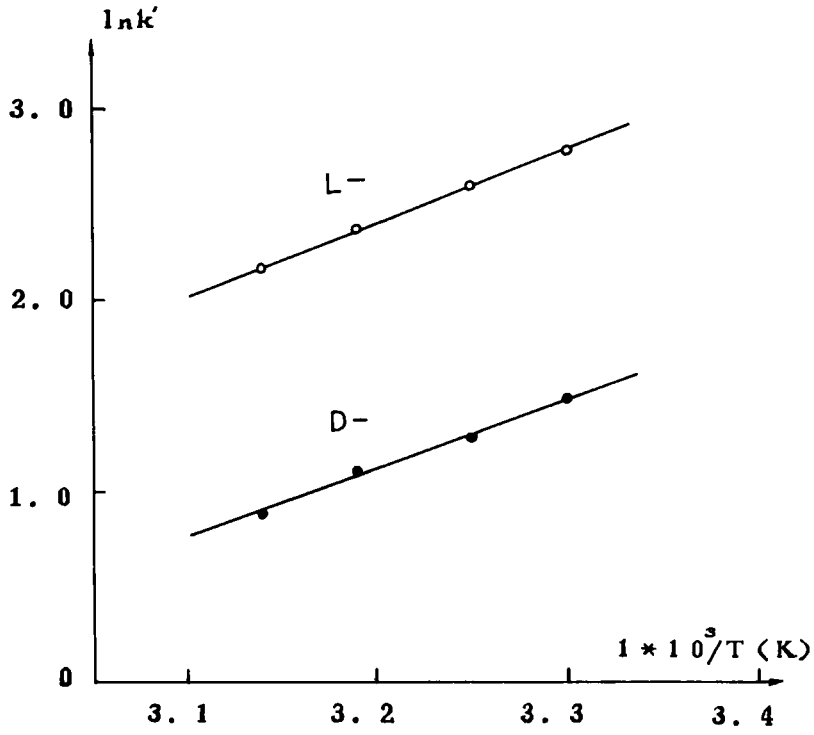


Figure 3: Variation of ln k' of DL-His versus 1/Temp. Column I(7)-Cu. Condition: 18.5ml/hr, 0.2mol/l$(NH_4)_2CO_3$, 1.0mg DL-His.

in which, $\overline{R}$, AA and S represent polymer phase, amino acid and the ligand in elution, respectively. When the concentration of S (e.g. elution concentration) increased, the two complexes, $\overline{R}$-Cu-D-AA and $\overline{R}$-Cu-L-AA, resulted in a different slope. It may be due to a difference of mechanism (S_N1 or S_N2) of the ligand exchange reaction for D and L forms. The more difficulty with which the AA enantiomer removes the ternary complex $\overline{R}$-Cu-AA, the more critically the ligand exchange depends on the eluent concentration. As a result, we consider

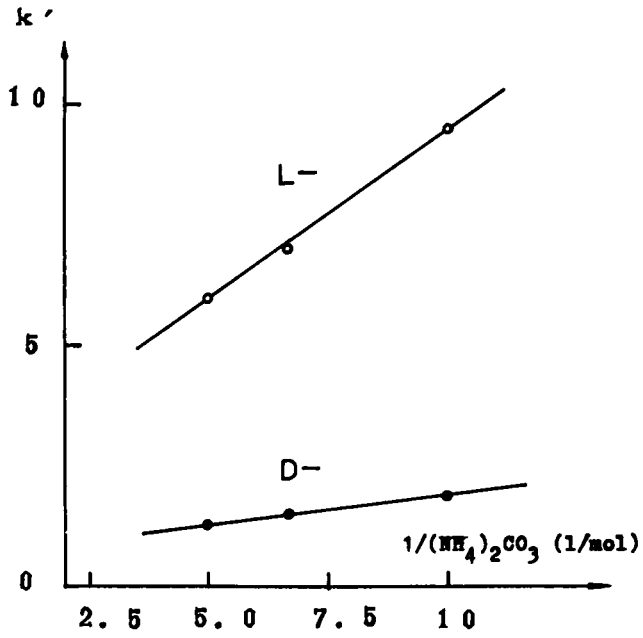


Figure 4: Variation of k' of DL-His versus $1/[(NH_4)_2CO_3]$. Column I(3)Cu . Condition: 24ml/hr, 35°C, 1.0mg DL-His.

that the slope of line in the equation reflects the property of the ligand exchange process for enantiomer.

Influence of Amount of Sample

When the amount of DL-His introduced into the column was increased, the retention was quickly decreased. The value α (V'_L/V'_D) was increased with increasing of amount of DL-His within the allowed capacity of column. In Fig.6 and Fig.7 , the retention of D form was linearly related to 1/amount, but the L form was not.

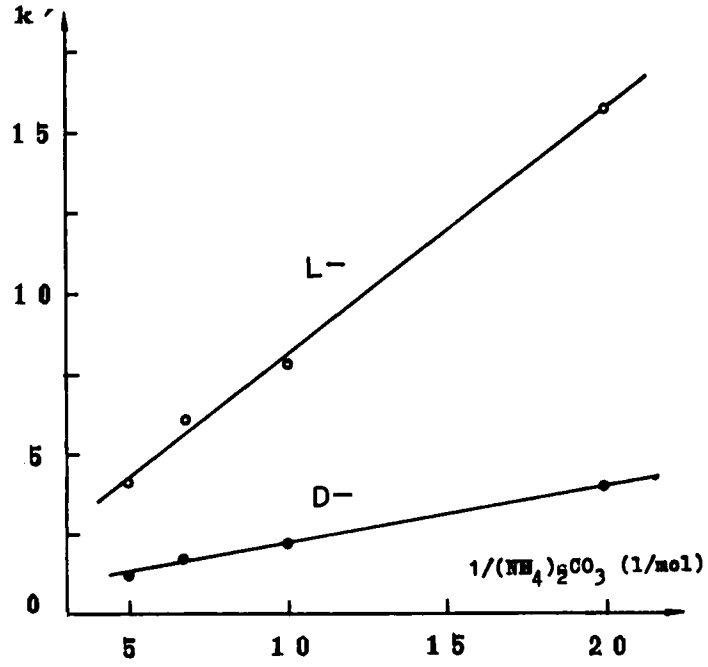


Figure 5: Variation of k' of DL-His versus $1/[(NH_4)_2CO_3]$. Column I(7)-Cu. Condition: 18.5ml/hr, 45°C, 1.0mg DL-His.

TABLE 3. Influence of Flow Rate on Retention

Flow rate (ml/hr)	0.05mol/l $(NH_4)_2CO_3$			0.15mol/l $(NH_4)_2CO_3$		
	V'_D	V'_L	α	V'_D	V'_L	α
7.2				3.28	12.88	3.92
18.6	8.23	31.79	3.86	3.58	12.26	3.42
24.0	8.0	27.60	3.45	4.40	11.60	2.64
34.2	7.69	21.94	2.85	4.84	- overlapped	

Column I(7)-Cu, 45°C, 1.0 mg DL-His

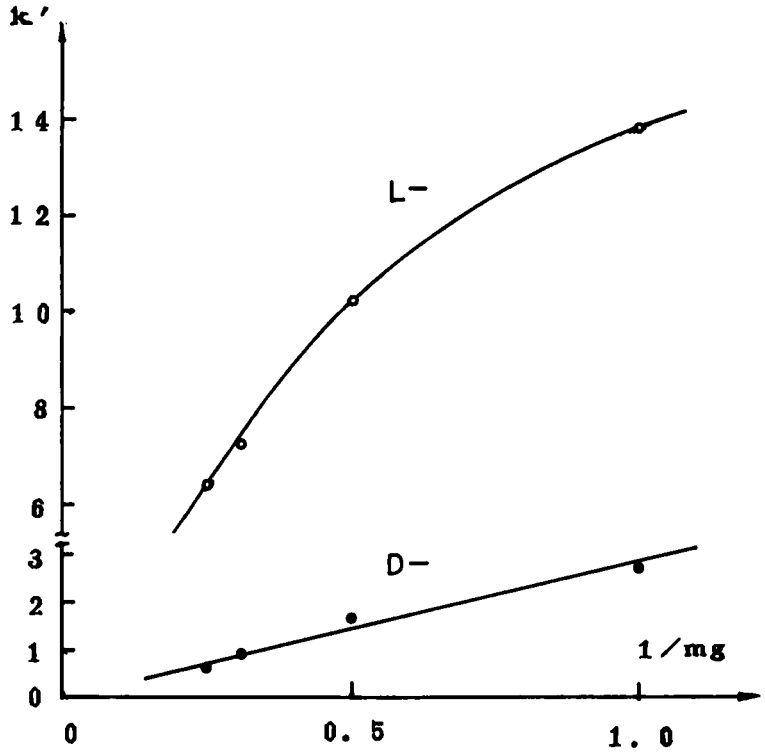


Figure 6: Variation of k^a of DL-His versus 1/Sample Amt. Column I(6)-Cu. Condition: 20ml/hr, 35°C, 0.1mol/l $(NH_4)_2CO_3$.

Influence of Flow Rate

Flow rate may also affect retention (Table 3). Generally, the retention and α value decreased with increasing flow rate. However, this effect mainly occurs with the L form which elute last. Under suitable conditions, we can effectively diminish the times for resolving DL-amino acids by increasing flow rate.

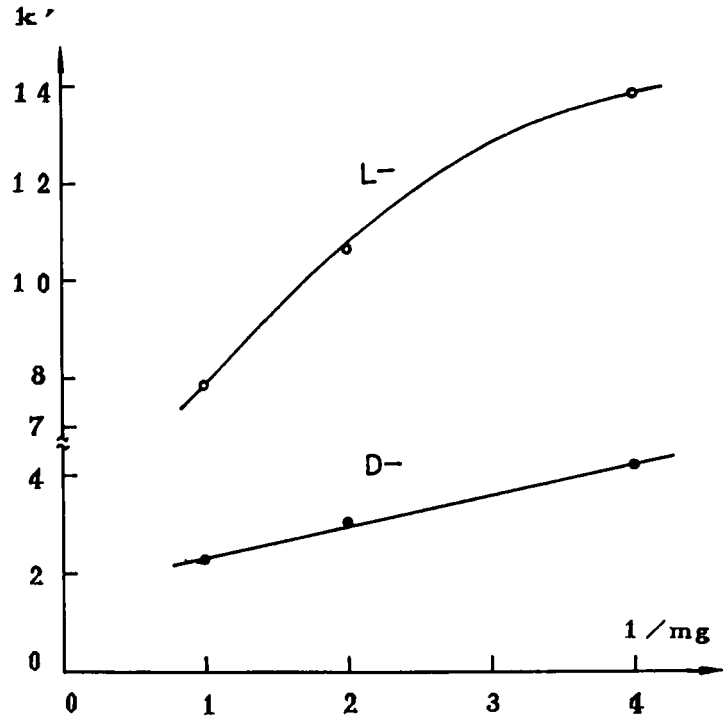


Figure 7: Variation of k' of DL-His versus 1/Sample Amt. Column I(7)-Cu. Condition: 18.5ml/h,45°C,0.1mol/l$(NH_4)_2CO_3$.

The polymers I(3), I(6) and I(7) coordinated with Cu(II) ion could be utilized to seperate several DL-amino acids , His, Pro, Val, Thr, Tyr, Mpa*, Hpa** etc, and usually D forms eluted ahead of L forms, except Pro.

*Mpa = HN⟨⟩N-CH_2-⟨⟩-CH_2CHCOOH
NH_2

Hpa = ⟨⟩N-CH_2CHCOOH
NH_2

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