Preparation and Characterization of Chemically Bound Tartrate-Silica Gel as Sorbent for Ligand Exchange Chromatography

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Tartrate-based sorbents of two kinds for ligand-exchange chromatography were prepared by covalent bonding of functional groups to silica gel through an amide bond. The structure and conversion of functional groups of the resin were confirmed with IR spectral and elemental analysis. The influence of pH on adsorption of metal ions to the resin was examined. The coordination behaviour of the sorbent was investigated by means of IR, EPR and electronic absorption spectroscopy. The results indicate that the affinity of metal ions toward the synthesized resins decreased in the order Cu(II) > Zn(II) > Cd(II). The conformation of the copper(II)-sorbent complexes may be distorted tetragonal, whereas those of zinc(II) and cadmium(II) may be tetrahedral. These complexes between metal ion and sorbent were investigated as a stationary phase for enantiomeric separation of underivatized amino acids. Factors affecting the retention and the sample selectivity were examined. The interactions between sorbent metal complexes and analytes in this ternary system were also investigated.

INTRODUCTION

Enantiomeric separations are vital in chiral synthesis, mechanistic experiments, catalysis, kinetics, geochronology, biology and biochemistry, pharmacology, medicine etc. Many methods are currently available for this purpose with extension of chiral separations to LC having begun about 1970.[1] Although the present interest in chiral separations was foreshadowed in reports of column-chromatographic separations, Davankov's work on ligand-exchange chromatography (LEC) was particularly significant.[2] Many new and improved chiral stationary phases or mobile-phase additives involving ligand exchange are introduced, among which tartaric acid is readily available in sufficient optical purity and at moderate cost. Therefore, synthesis of a chelating resin based on derivatives of tartaric acid as a stationary phase for LEC seems promising.[3] In reports related to this field, (R,R)-tartaric acid and (R,R)-tartaric acid monoamide derivatives were proved useful as covalently bonded chiral ligands by Kicinski et al.[4-5] and Aoki et al.,[6] and (R,R)-tartaric acid monooctylamide of copper and nickel complexes is selective as a mobile phase for enantiomeric separation.[7] However, no systematic investigation of coordination behavior of resin containing a tartrate derivative is reported.

In work concerned with chelating resins in this laboratory, our primary purpose is to investigate the coordination behavior of the synthesized resin applied as a sorbent of LEC. Hence we examined the chelating behavior of the sorbent and parameters or conditions that affect retention and selectivity of the separation of underivatized amino acids with sorbent-metal complexes via LEC. We also investigate interactions between sorbent metal complexes and analytes.

EXPERIMENTAL SECTION

Apparatus

Elemental analyses was performed on an elemental analyzer (Perkin-Elmer 240C). IR spectra of resin or metal-resin complex in KBr pellets were recorded on an infrared spectrophotometer (Perkin-Elmer 983). EPR spectra of metal-resin complexes in the solid state were recorded at room temperature on an EPR spectrometer (Bruker ESP 300). Diffuse-reflectance spectra were measured with an integrating sphere reflectance attachment (Hitachi). All metal-resin complexes were prepared as lightly packed powder pressed on filter paper. A freshly prepared resin provided the reference signal. Column packing was performed on a Chemco Econo-packer (model CPP-085, Scientific Co., Japan). Sieving was processed with a sonic sifter (Endecotts, UK) for a particle size smaller than 400 mesh and a conventional sieve for particle size larger than 400 mesh.

A high-performance liquid chromatograph (LDC/Milton Roy, Riviera Beach, FL, USA) consisted of pumps (Constametric I and III), Gradient Master (model 1601), a variable-wavelength detector (LDC, spectroMonitor D), a Rheodyne injection valve (Berkeley, CA, USA, model

Dedicated to Professor Sung-Mao Wang (王松茂) on the occasion of his seventieth birthday.

7125), a column heater (Eppendorf, model TC-45) and an integrator (Hitachi, D-2500).

A pH meter (Radiometer, Copenhagen, Denmark, PHM 61) was used to measure the pH of a solution.

Chemicals

Most chemicals were of analytical reagent grade (E. Merck, Darmstadt, Germany). 3-triethoxysilylpropylamine and N-[3-(trimethoxysilyl)propyl]ethylenediamine (Aldrich, Milwaukee, WI, USA), silica gel (particle size of 60 mesh and 15-40μm, E. Merck), and amino acids (Aldrich, or Sigma, St. Louis, MO, USA) were obtained from the indicated sources.

Synthesis of Tartrate and Ethyl-tartrate Containing Resin

Silica gel was ground, sieved and refluxed in chloroform for 2 h to remove impurities, then washed successively with water and acetone before use. 3-triethoxysilylpropylamine (7 mL) was added to the solution of O,O′-diacetyl-L-tartaric anhydride (22 g) in dioxane (50 mL) and reacted in an ice bath under nitrogen for 3 h. The product was added to purified silica gel (5 g). After reaction at 90 °C under nitrogen with pyridine as solvent for 20 h, the resulting modified silica was hydrolyzed to diol with sodium hydrogen carbonate in methanol aqueous solution (50% V/V) at 50 °C for 24 h. The product, S-TAROH, was filtered and washed successively with methanol, acetone and pure water. The synthesis of ethyl-tartrate-containing resin was similar to the above procedure, but diethyl-L-tartrate (8 mL) was used instead of O,O′-diacetyl-L-tartaric anhydride, and no hydrolysis was performed; the final product is called as S-TAROEt. Both products were dried overnight under vacuum at 60 °C. A particle size 325-400 mesh was used for the experiments except the column study.

Potentiometric Titrations[8]

Acid dissociation constant

In this investigation, at least 14 samples of resin (0.2 g) were accurately weighed and placed respectively in each PE bottle (100 mL). Aqueous solutions containing various amounts of sodium hydroxide (0.1 M) were made to ionic strength (0.5 M) with potassium chloride and brought to total volume (25 mL). The solutions were added to the PE bottle containing the resin. The mixture was stirred for 4 h at 25 ± 0.1 °C. After equilibration, the pH was measured.

Stability constant

The apparatus and procedures of these experiments were identical to those described in the previous section with the exception that in each case metal and resin-ligand ratio either a 3:1 or 1:1 of metal salt were added to the resin solution. The total volume was 25 mL.

Modification of Langmuir Isotherm Equation[9]

Stability constant

This procedure assures a constant metal ion concentration (10^{-3} M) and variations of the amount of resin (0.5 g - 2.0 g). The total volume was 25 mL and the sample was placed respectively in each PE bottle (100 mL). Upon reaching equilibrium (24 h) aliquots were taken and the metal ion concentration was determined spectrophotometrically.

Analytical Application

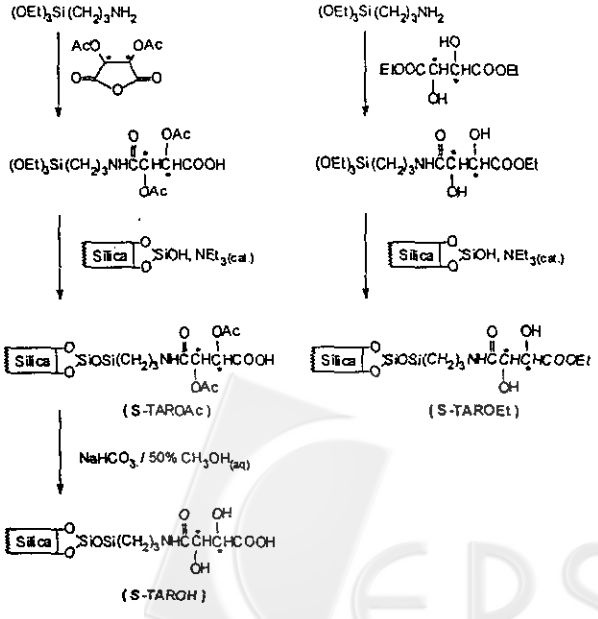
The ligand-exchange liquid chromatograph contained a stainless-steel column (10 cm × 4.6 mm I.D.) packed with metal-loaded resin phases. The column was conditioned for 3 h before measurement.

RESULTS AND DISCUSSION

Characterization of the sorbents

The synthesis of metal loaded sorbents is indicated in Scheme I. The composition and structure of the product at each step in the synthesis were characterized by elemental analysis and infrared spectra. The functionalities of tartaric acid and ethyl tartrate on silica gel were 1.27 and 1.37 mmol g^{-1} respectively.

Scheme I Syntheses of S-TAROH and S-TAROEt resins



The metal capacities as a function of pH are shown in Fig. 1; the capacity of the resin for each metal ion increased with pH of solution, but the capacity was less for a buffer system than for an unbuffered aqueous solution. The result is reasonable as acetate ion is a weakly bound ligand of most transition metal ions.

The pKa of S-TAROH and stability constants of both resin metal complexes were determined. For the equilibrium constants, two methods were used. Bjerrum's method was applied to the system of S-TAROH according to a detailed procedure.[8] The other method applied to the system of S-TAROEt was Smid's procedure.[9] The results are presented in Fig. 2 and Table 1. By comparison with the pK_a and stability constants of monomeric tartaric acid, the results are reasonable,[10] as there might be steric hindrance in the resin matrix.

Wavenumbers and assignments of lines in the IR spectra are given in Table 2. Specific evidence of the covalently bound ethyl tartrate is provided by infrared absorption due to the δ (C—O—C) mode of ester groups about 680 cm^{-1}. Coordination of tartrate caused the absorption of υ (C=O) to move towards smaller wavenumber and the maximum intensity decreased; a weak and sharp line appeared near 600 cm^{-1} as a consequence of the stretching vibration of coordinating bonds, υ (C—O—M), for most resin metal complexes except those of cadmium which may be the specific absorption overlapping resultant the disappearance of the stretching vibration of the coordinating bond.

In electronic spectra, the wavelengths of maximum absorption and characteristics of their resin metal complexes are presented in Table 3. The EPR parameters of the copper(II) loaded S-TAROH and S-TAROEt resin complexes were measured for media of varied acidity at room temperature (Table 3). All spectra had similar g values in the perpendicular direction, with $g_\perp = 2.076 \pm 0.005$ and no resolved hyperfine splitting, but the spectra differed significantly in the parallel direction. The values of $g_\perp$, $g_{//}$ and $A_{//}$ in Table III are typical of copper complexes with a distorted tetragonal conformation[11] and indicate an ionic character of

Table 1. Characteristics of Synthesized Resins

	S-TAROH	S-TAROEt	tartaric acid[a]
Functionality[b] /mmol g^{-1}	1.21-1.32	1.29-1.45	
Metal capacity[c]	/mmol g^{-1}		
Cu(II)	0.76	0.59	
Zn(II)	0.41	0.32	
Cd(II)	0.28	0.17	
pK_a[d]	5.72		4.37
log K_{f1}[d]			
Cu(II)	2.83	2.63	3.39
Zn(II)	2.57	2.47	2.68
Cd(II)	2.28	2.31	
log K_{f2}[d]			
Cu(II)	2.18		
Zn(II)	2.13		
Cd(II)	1.93		

[a] Ref. 10.
[b] Data from elemental analysis.
[c] In acetate buffer (0.1 M, pH 4.87); temperature: 25 ± 0.1 °C.
[d] Ionic strength: 0.5 M KCl; temperature: 25 ± 0.1 °C.

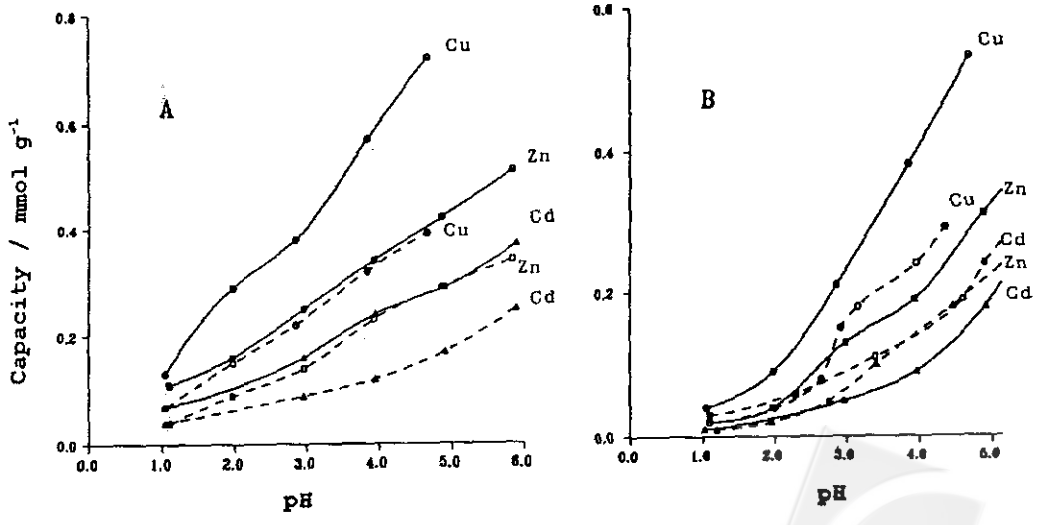


Fig. 1. Metal capacity as a function of pH; solid line pertains nonbuffer system, dashed line to acetate buffer solution (0.1 M). (o): Cu(II), (□): Zn(II), (Δ): Cd(II). metal ion (0.01 mmol); solution (25 mL). A. S-TAROH resin (0.2 g); B. S-TAROEt resin (0.2 g).

Table 2. Wavenumber/cm^{-1} of Principal IR Lines of S-TAROH (R1) and S-TAROEt (R2) Resins and Their Metal Complexes

Resin	υ(C=O)[a]	υ(C=O)[b]	υ(C-N-H)	υ(C-O-C)	υ(C-O-M)
R1	1717	1635	1542		
R1-Cu(II)	1708	1631	1541		620
R1-Zn(II)	1714	1632	1541		617
R1-Cd(II)	1715	1541	1543		
R2	1653	1563	1544	685	
R2-Cu(II)	1641	1564	1542	686	622
R2-Zn(II)	1647	1561	1543	685	620
R2-Cd(II)	1651	1562	1543	684	

[a] Free acid group.
[b] Amide group.

the metal-ligand bond.[12] By comparison with the EPR parameters of Cu(II) complexes in polyacrylamide gels, we conclude that there might be vacant coordination sites of each copper ion,[13] assumed to be occupied by water molecules. As unsaturated coordinating sites arise in copper(II), the sorbent-metal complexes are appropriate as stationary phases for LEC.

Analytical Application

In order to elucidate the structure necessary for enantiomeric separation via LEC and to survey the selectivity of the various metal systems, we synthesized some sorbents for comparison, i.e. tartaric acid or ethyl tartrate with 3-triethoxysilylpropylamine or N-[3-(trimethoxysilyl)-propyl]ethylenediamine as linking agent to the silica gel, respectively. The preliminary test showed that both tartaric acid and ethyl tartrate with 3-triethoxysilylpropylamine as linking agent exhibit better resolution than the others. The tested analytes were alanine, asparagine, leucine, methionine, phenylalanine, proline, serine and valine etc. They were assumed to be retarded on the stationary phase mostly via formation of a ternary complex. Other interactions, e.g. hydrogen bonding and van der Waals force etc., may be involved and the retention mechanism may be more complicated than that assumed. However, comparison of retention periods, elution order, selectivity and resolution of the solutes on all columns provides insight into possible retention and chiral recognition mechanisms.

The pH, ionic strength of the eluent, concentration of the organic modifier etc. also control the retention period and enantioselectivity of the separation (Table 4). Among these parameters, the pH of the eluent is the most important quantity, because the stability and concentraion of mixed-metal complexes depend strongly on the acidity of the solution. Fig. 3 shows the effect of the eluent pH on the capacity factor, k′. These factors of solutes increased with increasing

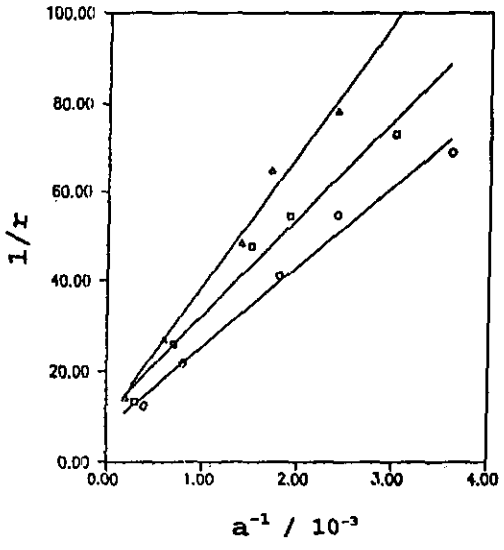


Fig. 2. Plots of 1/r vs. 1/a for metal ions on S-TAROEt resin. (o): Cu(II), (□): Zn(II), (Δ): Cd(II). 1/r denotes the ratio of total resin functionality to the complexed metal ion concentration; a is the free metal ion concentration; n refers to the number of binding sites per mole of resin ligand units; hence, 1/n represents the minimum number of functional group units required to bind a metal ion.

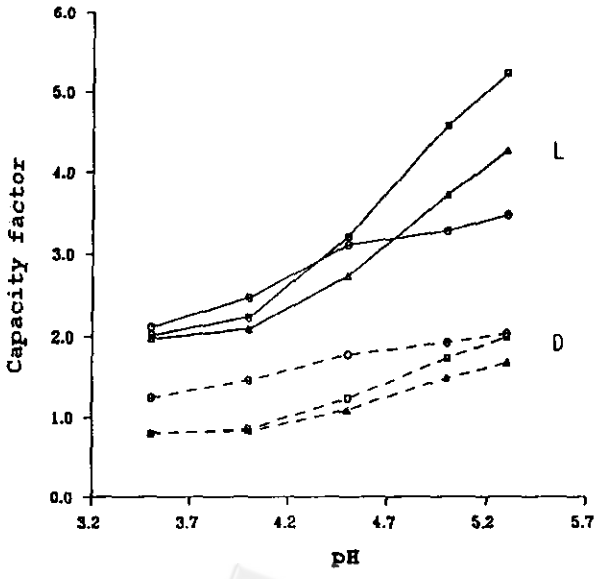


Fig. 3. Capacity factor as a function of acidity of eluent. S-TAROEt-Cu column (100 cm × 4.6 mm I.D.); Mobile phase: KH_2PO_4 (0.025 M)-$Cu(OAc)_2$-(10^{-4} M); Temp. 30 °C; Flow rate: 0.5 mL min^{-1}. (o): Proline, (□): Phenylalanine, (Δ): Alprenolol.

Table 3. Electronic and EPR Spectra of Metal-Resin Complexes

Complex	pH	Electronic spectra Wavelength/nm	$d_{xz} \rightarrow d_{z}2$	CT[a](1) /1000 cm^{-1}	CT(2)	EPR spectra $g_{\perp}$	$g_{//}$	$A_{//}$ /G
R1-Cu(II)	1.10					2.08	2.35	140
	2.50					2.08	2.35	141
	3.00					2.07	2.35	144
	4.87	401.6(broad)	24.90			2.08	2.35	140
R1-Zn(II)	4.87	281.2 ; 389.4		35.56	25.68			
R1-Cd(II)	4.87	283.6 ; 400.1		35.26	25.00			
R2-Cu(II)	1.20					2.08	2.36	154
	2.35					2.08	2.36	156
	3.48					2.07	2.36	155
	4.87	378.4;282.0;311.2	26.43	35.46	32.13	2.07	2.36	152
R2-Zn(II)	4.87	318.8 ; 445.0		31.37	22.47			
R2-Cd(II)	4.87	364.4 ; 471.0		27.44	21.23			
R3-Cu(II)$(H_2O)_3$[b]						2.08	2.41	119
R3-Cu(II)$(H_2O)_4$						2.08	2.37	138
R3-Cu(II)$(H_2O)_5$						2.08	2.32	155
Cu(II)$(H_2O)_6$						2.07	2.29	173

[a] Charge transfer transition.
[b] Ref.13.
R1: S-TAROH; R2: S-TAROEt; R3: polyacrylamide gel

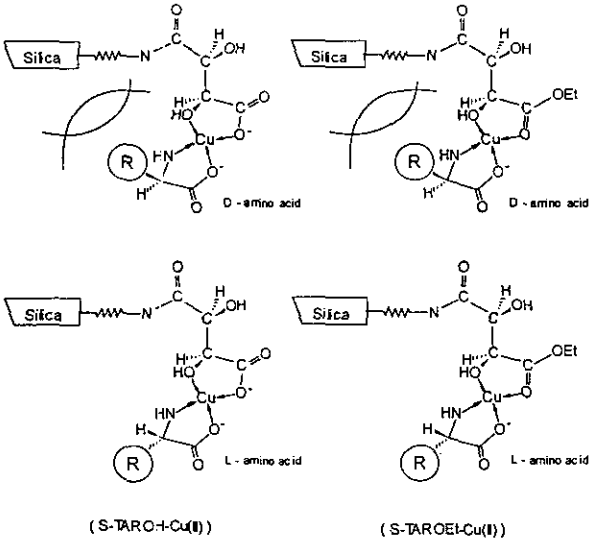
pH and enantioseparation was improved at greater pH. The D-isomer of each amino acid is eluted more quickly than the L-isomer, which indicates that the stability of the ternary complex involving the D-isomer is less than that with the L-isomer as shown in Scheme II. Various buffer systems-sodium acetate, ammonium acetate and phosphate buffer-were

Table 4. Retention Behavior of Enantiomers for Various Mobile Phases

Mobile phase Buffer	Additive	pH	k_L	k_D	α
KH_2PO_4 (10^{-2}M)	(a)	5.3	5.23	1.99	2.63
KH_2PO_4 (10^{-2}M)	(a)	4.5	3.21	1.23	2.61
KH_2PO_4 (10^{-2}M)	(a)	4.0	2.23	0.86	2.59
KH_2PO_4 (10^{-2}M)	(a)	3.5	2.02	0.79	2.56
KH_2PO_4 (10^{-2}M)*	(a)	4.5	1.44	0.76	1.89
HOAc-NaOAc (10^{-2}M)	(a)	4.0	1.86	0.89	2.09
HOAc-NaOAc (10^{-2}M)	(a)	4.0	1.45	0.76	1.91
KH_2PO_4 (10^{-2}M)	(a)- CH_3CN(30%)	4.5	3.01	1.94	1.55
KH_2PO_4 (10^{-2}M)	(a)- CH_3OH(10%)	4.5	3.52	2.04	1.72
KH_2PO_4 (10^{-2}M)	(a)- CH_3OH(30%)	4.5	3.07	1.83	1.67
KH_2PO_4 (10^{-2}M)	(a)- CH_3OH(50%)	4.5	2.77	1.72	1.61
KH_2PO_4 (10^{-2}M)	(a)- NaCl(0.01M)	4.5	3.02	1.18	2.56
KH_2PO_4 (10^{-2}M)	(a)- NaCl(0.05M)	4.5	2.97	1.17	2.54
KH_2PO_4 (10^{-2}M)	(a)- NH_4Cl(0.05M)	4.5	2.45	1.03	2.38
KH_2PO_4 (10^{-2}M)	(b)	4.5	2.72	1.19	2.29

Column: S-TAROEt resin (Particle size: 325-400 mesh;
Dimensions: 10 cm × 4.6 mm I.D.)
Temperature: 30 °C
Flow rate: 0.5 mL min^{-1}(*: 0.7 mL min^{-1})
Sample: D,L-proline (10^{-3} M)
Detection: UV, λ: 254 nm
(a): $Cu(OAc)_2$ (10^{-4}M) (b): $Cu(OAc)_2$ (10^{-5}M)

Scheme II *Proposed structures of ternary complex formation for separation of amino acids*



tested regarding their capacity to elute the analyte molecules. The phosphate buffer produced the best results. Other situations may be complicated by complexation of acetate or ammonium ion with the central metal ion. For convenience of detection with a UV-visible spectrophotometer and to surpress loss of metal ion from the aqueous phase, copper ion was added to the mobile phase. Fig. 4 illustrates the effect of concentration of copper(II) in the eluent on retention of amino acids. The capacity factor increased with increasing copper(II) concentration at a small concentration, but the separation coefficient α decreased at a large concentration. Experimental results showed that the capacity factor decreased with increasing temperature of eluent; however, the system performed at 30 °C for the best separation. The effect of organic modifier revealed that methanol was more effective than acetonitrile; the retention periods of samples decreased with increasing content of methanol. The results varied from those of Kettrup;[5] the reason might be that their samples were more hydrophobic than ours. The chromatogram in Fig. 5 indicates that the performance of the S-TAROH-Cu column is better than that of the S-TAROEt-Cu column for enantiomeric separation of amino acids and pharmaceuticals. However, the resolution of the latter seems better than that of the former.

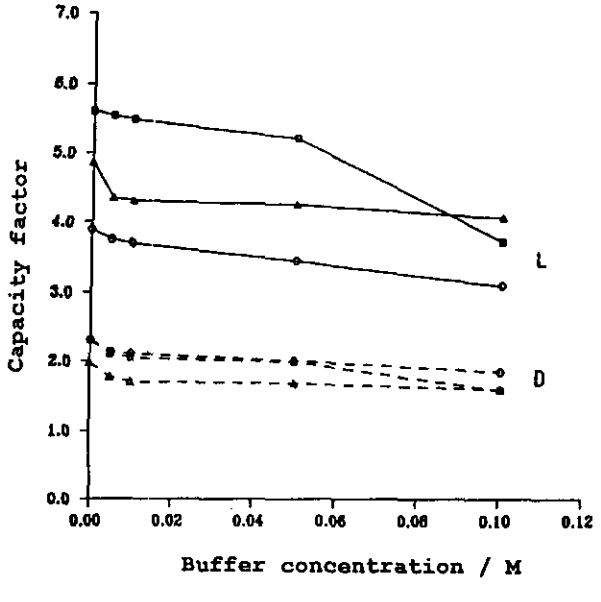


Fig. 4. Capacity factor as a function of copper concentration of eluent. (o): Proline, (□): Phenylalanine, (Δ): Alprenolol. Conditions as for Fig. 2.

Formation Constants for Amino Acids on S-TAROH-Cu(II) and S-TAROEt-Cu(II) Complex

The determination of formation constants for amino acids on the metal resin complex column facilitates understanding of the mechanism of enantioseparation. The ternary formation constants for amino acids in these systems were determined with the chromatographic system according to Takayanagi's method.[14] The results listed in Table 5 are conditional constants. To obtain the ternary formation constant, K_{ft}, we substitute βC_T for [a.a.] in the formation constant expression and calculate it from the conditional constant, K'_{ft},

$$K_{ft} = \frac{[\text{R-Cu-a.a.}]}{[\text{a.a.}][\text{R-Cu}]} = \frac{[\text{R-Cu-a.a.}]}{\beta C_T[\text{R-Cu}]} = \frac{K_{ft}'}{\beta}$$

Here, R is S-TAROH or S-TAROEt, and C_T is the sum of the concentration of species containing the amino acid exclusive of that combined with metal-resin complex (S-TAROH-Cu or S-TAROEt-Cu). For solutions containing amino acid and additional copper ion,

$$C_T = [\text{a.a.}] + [\text{a.a.-Cu}] + [(\text{a.a.})_2\text{Cu}]$$

in which for simplicity charges are omitted.

$$\text{As} \quad K_{f1} = \frac{[\text{a.a.-Cu}]}{[\text{a.a.}][\text{Cu}]}$$

$$\text{and} \quad K_{f2} = \frac{[(\text{a.a.})_2\text{-Cu}]}{[\text{a.a.}][\text{a.a.-Cu}]}$$

$$\text{hence} \quad \beta = \frac{1}{1 + K_{f1}[\text{Cu}] + K_{f1}K_{f2}[\text{Cu}][\text{a.a.}]}$$

The β functions of ternary complexes for formation constants obtained are presented in Table 6. The reason for weaker chemical bonding of analytes on the metal-resin

Table 5. Determination of Ternary Conditional Constants (K_{fi}') of Amino Acids on Copper (II) Resin Complexes

Sample	S-TAROH-Cu(II) Linear equation[a] (k′ vs. 1/L)	K_{fi}'	S-TAROEt-Cu(II) Linear equation[a] (k′ vs. 1/L)	K_{fi}'
Alanine			y = 6.62X + 1.98	7.59
D-Asparagine	y = 7.41X + 1.82	7.72		
L-Asparagine	y = 9.05X + 2.38	9.43	y = 8.65X + 1.63	9.02
Leucine			y = 5.25X + 1.52	6.02
Methionine			y = 3.64X + 1.20	4.17
D-Phenylalanine	y = 6.49X + 2.06	7.45	y = 6.18X + 2.02	7.09
L-Phenylalanine	y = 9.80X + 2.59	11.2	y = 12.5X + 3.61	14.3
D-Proline	y = 7.08X + 1.78	8.12	y = 8.24X + 2.03	9.45
L-Proline	y = 11.1X + 2.42	12.8	y = 15.0X + 5.27	17.2
Serine			y = 8.09X + 2.04	9.28
D-Tyrosine	y = 6.21X + 1.95	7.12		
L-Tyrosine	y = 9.36X + 2.24	9.76		
Valine			y = 14.1X + 5.50	16.2

[a] by Takayanagi's method.

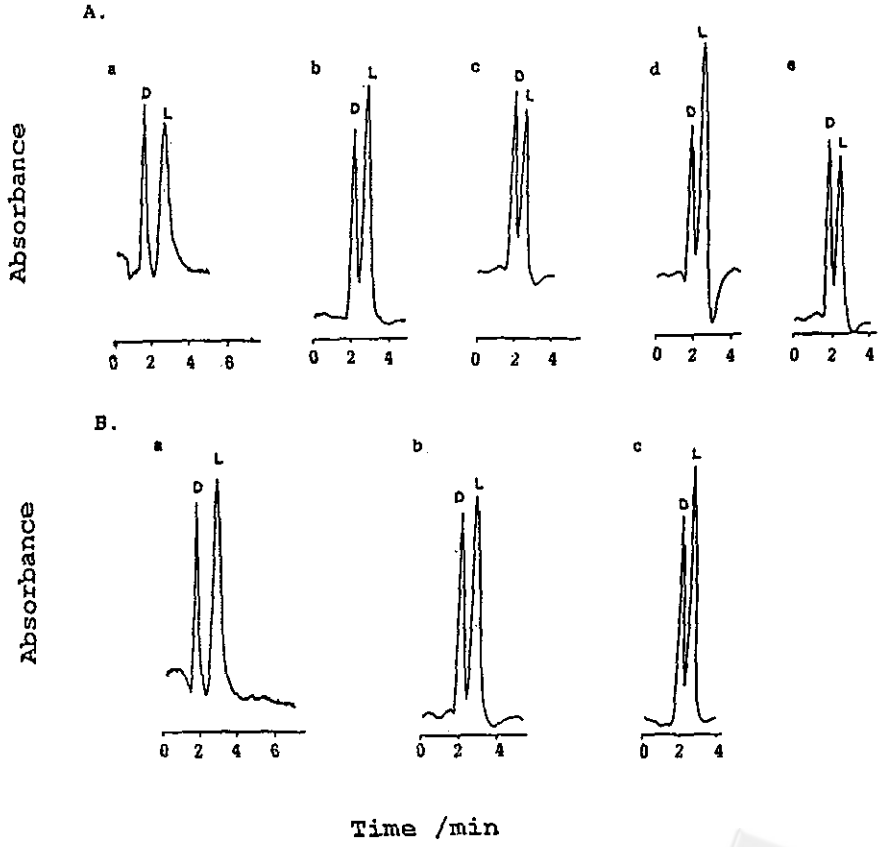


Fig. 5. Chromatogram of unmodified amino acids and pharmaceuticals. A. S-TAROH-Cu column (150 cm × 4.6 mm I.D.); B. S-TAROEt-Cu column (150 cm × 4.6 mm I.D.); Particle size: 10 μm. Mobile phase: KH_2PO_4 (0.01 M, pH 5.3)-$Cu(OAc)_2$(5×10^{-5} M); Temp. 30 °C; Flow rate: 1.0 mL min^{-1}; Detection: UV λ = 254 nm; Sample concentration: 5×10^{-4} M; Sample volume: 20 μL. Peak: A. a: Alprenolol; b: Asparagine; c: Phenylalanine; d: Proline; e: Tyrosine. B. a: Alprenolol; b: Phenylalanine; c: Proline.

Table 6. β Function Correction for Formation Constants of Amino Acids on Copper (II) Resin Complexes

Sample	(β)	S-TAROH-Cu(II)		S-TAROEt-Cu(II)	
		$\log K_{ft}'$ (conditional)	$\log K_{ft}$ (corrected)	$\log K_{ft}'$ (conditional)	$\log K_{ft}$ (corrected)
Alanine	1.20×10^{-7}			0.88	7.80
D-Asparagine	3.80×10^{-7}	0.89	6.37		
L-Asparagine	3.80×10^{-7}	0.97	6.41	0.96	7.38
Leucine	1.26×10^{-7}			0.78	7.68
Methionine	3.91×10^{-7}			0.62	7.34
D-Phenylalanine	1.70×10^{-7}	0.87	6.71	0.85	7.62
L-Phenylalanine	1.70×10^{-7}	1.05	6.79	1.16	7.93
D-Proline	3.98×10^{-7}	0.91	8.36	0.98	9.38
L-Proline	3.98×10^{-7}	1.11	8.45	1.23	9.64
Serine	3.31×10^{-7}			0.97	7.45
D-Tyrosine	3.85×10^{-7}	0.85	6.34		
L-Tyrosine	3.85×10^{-7}	0.99	6.41		
Valine	1.25×10^{-7}			1.21	8.11

Table 7. Thermodynamic Parameters for Reaction of Amino Acids with Copper (II) Resin Complexes

Sample	S-TAROH-Cu(II)			S-TAROEt-Cu(II)		
	ΔH /kJ mol^{-1}	ΔS /J mol^{-1}	ΔG[a] /kJ mol^{-1}	ΔH /kJ mol^{-1}	ΔS /J mol^{-1}	ΔG[a] /kJ mol^{-1}
D-Phenylalanine	- 8.24	- 9.84	-5.26	- 8.96	-10.95	- 5.64
L-Phenylalanine	-11.09	-15.21	-6.48	-14.22	-23.16	- 7.20
D-Proline	- 7.79	- 9.12	-5.03	- 7.28	- 7.83	- 4.91
L-Proline	- 9.60	-10.38	-6.45	-13.37	-21.85	- 6.75

[a]temperature: 30 ± 0.1 °C

complex compared with that of analytes on free copper ion might be the heterogeneous system and steric hindrance of the resin matrix.

Thermodynamic Considerations of Enantiomeric Separation of Amino Acids

Separation of enantiomers on a chiral stationary phase requires that the diastereomeric adsorbates have unequal Gibbs energies. In this work, all formation constants were determined in phosphate buffer over the range of temperature 30-55 °C. A plot of lnK vs 1/T has a slope -ΔH/R and ordinate intercept ΔS/R. The thermodynamic parameters for reactions of amino acids with S-TAROH-Cu or S-TAROEt-Cu(II) complex are summarized in Table 7. The Gibbs energy change difference Δ(ΔG) is expressed as -RTln$_\alpha$, with α the selectivity factor for enantiomers.[15] The Δ(ΔG) (30 °C) observed for proline and phenylalanine is larger in S-TAROEt-Cu than that in S-TAROH-Cu. The selectivity factors calculated for both systems are consistent with the experimental values (Table 8).

CONCLUSION

The work reported here concerns the coordination behavior of tartrate sorbent in LEC. In this investigation we showed that, by virtue of the fixed attachment to the silica surface, both tartaric acid and ethyl tartrate provide exceptional possibilities for enantiomeric separation. Moreover ethyl tartrate exhibits better resolution for the separation of underivatized amino acids with more hydrophobic character. Both the ternary formation constant and the Gibbs en-

Table 8. Δ(ΔG) Values and Selectivity Factors of Enantiomers on Copper (II) Resin Complexes

Sample	S-TAROH-Cu(II)		S-TAROEt-Cu(II)	
	Δ(ΔG)/kJ mol^{-1}	α	Δ(ΔG)/kJ mol^{-1}	α
Phenylalanine	1.22	1.62(1.25)[a]	1.56	1.86(1.46)[a]
Proline	1.42	1.76(1.31)[a]	1.84	2.08(1.49)[a]

[a] Experimental value.

ergy change difference give evidence for these phenomena. To explain these experimental results, we propose that for chiral separation in LEC, the most important property is the key functionalities (asymmetric center) not dispersed by a multiple site capable of donating electron pairs. Despite the variation of complexing ability and selectivity factor of these two sorbents, both have great potential for separation of anilines, phenols and phthalate esters or biomolecules.

ACKNOWLEDGEMENT

We thank the National Science Council of the Republic of China for financial support (Grant No. NSC 82-0208-M-002-063).

Received September 22, 1994.

Key Words

Tartrate resin; Coordination behavior; Ligand-exchange chromatography; Underivatized amino acid.

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