Synthesis of Crosslinked Poly(vinyl alcohol) with L-Proline Pendant as the Chiral Stationary Phase for Resolution of Amino Acid Enantiomers

JIANBIAO MA,* LI CHEN, and BINGLIN HE

The State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Institute of Polymer Chemistry, Nankai University, Tianjin 300071, People's Republic of China

SYNOPSIS

The porous crosslinked poly(vinyl alcohol) beads with the L-proline pendant was synthesized as the chiral stationary phase (CSP) for ligand-exchange chromatography of amino acid racemates via suspension polymerization of vinyl acetate and triallyl isocyanurate as a crosslinker, methanolysis of the copolymer, glycidylation of the formed crosslinked poly(vinyl alcohol), and final functionalization with L-proline. After the polymer with the chiral ligand was complexed with copper(II) cations, it was employed as the CSP to resolve the enantiomers of 15 amino acids. The results showed that the CSP possessed powerful enantioselectivity and all the tried amino acid racemates were completely separated. The large loadability (up to 60 mg per injection) permitted its application on a large scale. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Many types of polymers with chiral pendants capable of forming chelates with doubly charged transition-metal cations such as Cu and Ni have been widely studied as chiral stationary phases (CSP) for ligand-exchange chromatography (LEC) of racemates of amino acids and other bi- or multidentate chiral ligands since early 1970s.[1,2] The first attempt by Rogozhin and Davankov[3,4] was made to anchor L-proline onto crosslinked polystyrene. After being complexed with copper(II) ions, the formed polymer chelate could be employed as the CSP to result in a complete and reliable separation of amino acid enantiomers via a ligand-exchange mechanism. This idea was then developed by several groups[5-12] and in our laboratory[13-17] because the enantioselectivity of chiral LEC system was found to be excellent. In general, a CSP for LEC was prepared by incorporation of a bi- or multidentate chiral ligand (commonly L-proline or L-hydroxy proline) on a beaded support through a reasonable spacer. A CSP with hydrophilic support often affords better chromatographic kinetics than that with hydrophobic support because the hydrophilic support adsorbs less solute in an aqueous eluent, so that silica gel becomes a useful CSP support for high-performance ligand exchange chromatography. However, the CSPs based on silica gel usually had low loadability and could not be applied for a preparative resolution of enantiomers. Recently, a polystyrene-type support modified with hydrophilic spacers[13-15] or hydrophilic supports was prepared[16,17] to synthesize the new CSPs of preparative LEC. In this article, the synthesis of crosslinked poly(vinyl alcohol) with L-proline pendants (as shown in Scheme 1) and its enantioselectivity to amino acid racemates in LEC are reported.

EXPERIMENTAL

Reagents and Instruments

Vinyl acetate, azobisisobutyronitrile, epichlorohydrin, and common solvents were purchased from Tianjin Chemical Co. Triallyl isocyanurate, L-pro-

* To whom correspondence should be addressed.

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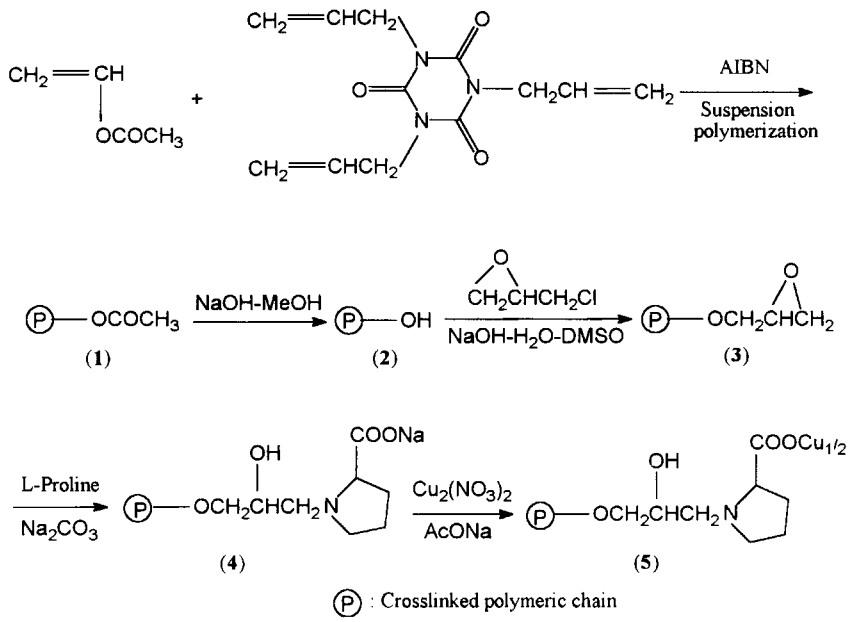


Scheme 1 The route for the synthesis of crosslinked poly(vinyl alcohol) with L-proline pendant and its copper chelate.

line, and all DL-amino acids were obtained from Aldrich Chemical Co.

Infrared spectra were recorded on Nicolet 5DX FTIR spectrometer using a KBr disc. The LEC of amino acid racemates on the synthesized CSP was performed on a YZS-3 medium-pressure chromatography system with a UV detector at 254 nm.

Suspension Polymerization of Vinyl Acetate and Triallyl Isocyanurate[18]

An organic solution composed of vinyl acetate (75 parts in weight), triallyl isocyanurate (25 parts), *n*-butyl acetate (50 parts), gasoline (50 parts), and azobisisobutyronitrile (1%) was mixed with the aqueous solution (about 1000 parts) of poly(vinyl alcohol) (0.1% w/w) and NaCl (3%) in a three-necked round-bottomed flask equipped with a mechanical stirrer, a thermometer, and a reflux condenser. The mixture was stirred to give a suspension of oil beads with a suitable size in an aqueous solution and then heated at 65°C for 5 h, 69°C for 3 h, and 75°C for 4 h. The crosslinked poly(vinyl acetate) beads (**1**) were filtered out, washed with a large amount of hot water, and extracted with acetone in a Soxhlet extractor. The polymer beads were dried in the air and then in a vacuum at 50°C in the presence of phosphorus pentoxide to a constant weight.

Methanolysis of Crosslinked Poly(vinyl acetate) Beads

The crosslinked poly(vinyl acetate) beads were hydrolyzed by 5% NaOH in methanol at 25°C for 40 h to afford crosslinked poly(vinyl alcohol) beads (**2**). After being washed successively with distilled water three times and with acetone three times, the product was dried as above. The hydroxy content in the crosslinked poly(vinyl alcohol) was determined by acetylation with acetic anhydride and the methanolysis percentage was calculated.

Glycidylation of Crosslinked Poly(vinyl alcohol)

The crosslinked poly(vinyl alcohol) beads were soaked in the solution containing NaOH (0.4*M*), epichlorohydrin (18%, v/v), dimethyl sulfoxide (75%, v/v), and water. The mixture was stirred at 45°C for 4 h. The glycidylated poly(vinyl alcohol) (**3**) was treated as described above. The content of epoxy groups was measured from the amount of hydrogen chloride consumed in epoxy-opening reaction of the produced polymer.

The Reaction of Glycidylated Polymer with L-Proline

L-Proline was transferred its sodium salt with equal moles of sodium carbonate in water and then the

glycidylated polymer was added. The mixture was stirred at 80°C for 24 h and treated as above to afford a chiral chelating polymer (**4**). The elemental analysis of the sample gave 53.99% of C, 8.09% of H, and 8.58% of N totally from the L-proline pendant and the crosslinker in the polymer.

Complexion of the Chiral Chelating Polymer with Copper(II) Ions

The chiral chelating polymer was soaked in an aqueous solution of $Cu(NO_3)_2$ (0.5*M*) and NaOAc (0.1*M*) for 1 h and stirred at room temperature for 48 h. The formed polymeric chelate (**5**) was collected and washed with pure water until no copper cations were found in the eluate using an aqueous solution of $K_4Fe(CN)_6$ as a detective reagent. After the polymeric chelate was dried in a vacuum, its copper content was determined as 1.06 mmol/g with the procedure as follows: A small amount of dry polymer chelate was accurately weighed and packed in a small glass column. Dilute hydrochloric acid was passed through the column to wash off the copper in the chelate. The eluate was collected and neutralized with a dilute sodium hydroxide solution to about pH 4. Sodium acetate buffer and several drops of α-pyridyl-β-azonaphthol solution in ethanol (as indicator) were added. The mixed copper solution was titrated by a 0.02*M* ethylenediaminetetraacetic acid tetrasodium salt solution and then the copper content was calculated.

The Chromatographic Resolution of DL-Amino Acid on Polymeric L-Proline/Cu(II) Chelate

The chiral polymeric chelate (14 g, 150–300 mesh) was packed in a stainless-steel column (250 × 10 mm i.d.) using a slurry method. The column was equipped on the chromatographic system. The resolution of amino acid racemates dissolved in water was performed on this system.

RESULTS AND DISCUSSION

Synthesis of Crosslinked Poly(vinyl alcohol) with L-Proline Pendant

The crosslinked poly(vinyl alcohol) with the L-proline pendant and its copper chelate were synthesized via suspension of vinyl acetate and triallyl isocyanurate and several steps of functionalizations as shown in Scheme 1. The suspension polymerization was carried out using *n*-butyl acetate and gasoline as the mixed porogent to obtain macroporous polymeric beads (**1**) with a perfect appearance and a narrow dispersion in size. The crosslinking networks in the copolymer would be homogeneous since vinyl acetate and triallyl isocyanurate possessed quite similar reaction ratios, respectively, as $r_1 = 0.70$ and $r_2 = 0.95$.[19] The research[18] on the specific surface area, pore structure, and pore stability in different solvents showed that 25% (w/w) crosslinked poly(vinyl acetate), formed from 25 triallyl isocyanurate and 75 vinyl acetate in weight, was the best one among those, respectively, with 8, 15, 20, 25, 30, and 40% crosslinking percentages. Hence, the 25% (w/w) crosslinked poly(vinyl acetate) was further functionalized.

The influence of NaOH concentration on the methanolysis of crosslinked poly(vinyl acetate) beads to form crosslinked poly(vinyl alcohol) (**2**) was investigated. The results are demonstrated in Figure 1. The curve shows that the methanolysis percentage of poly(vinyl acetate) increased with NaOH concentration in the methanol. But the use of 5% NaOH in the methanol could give a quite high methanolysis percentage above 80%. As we know, the reactions on polymer chains are very difficult to reach completion because of the entanglement of polymeric chains. As to the crosslinked poly(vinyl acetate) in this research, it is impossible for methanolysis to complete since the base (OH^- and/or

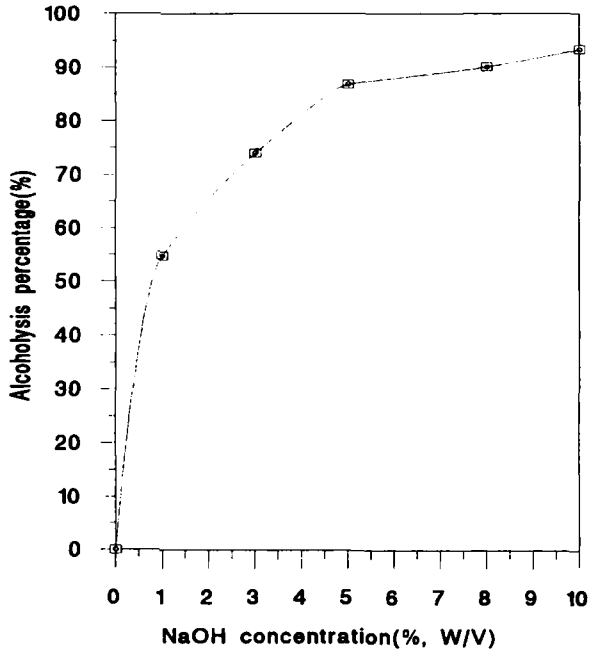


Figure 1 The effect of NaOH concentration on the methanolysis of crosslinked poly(vinyl acetate) (25°C for 40 h).

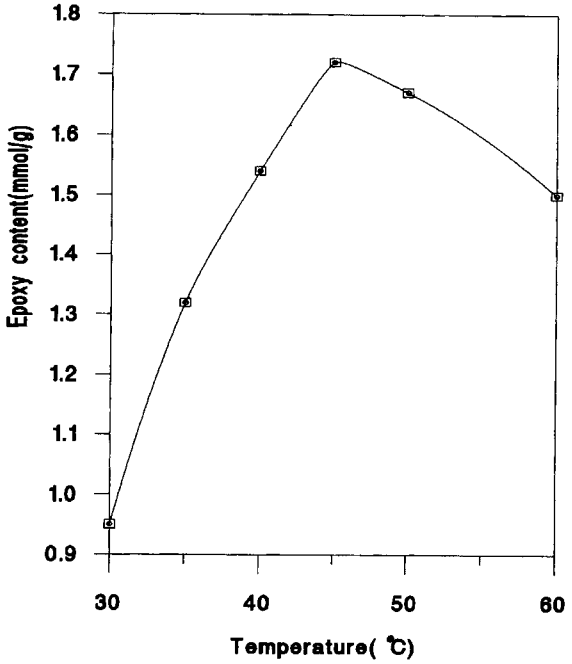


Figure 2 The effect of reaction temperature on glycidylation of crosslinked poly(vinyl alcohol) (reaction time 4 h).

MeO^-) cannot access the ester groups near the crosslinking points.

The effect of reaction temperature on the glycidylation of crosslinked poly(vinyl alcohol) was detected and the result is shown in the Figure 2. The curve implied that the suitable temperature (45°C) existed for the formation of glycidylated poly(vinyl alcohol) (**3**). When reaction temperature was lower than 45°C, the reaction was slow and a lower epoxy content of the product was observed. But if the temperature was higher than that, a part of the anchored epoxy groups would react with another hydroxy group in poly(vinyl alcohol), resulting in a decrease of epoxy content in the final product.

The reaction of the glycidylated poly(vinyl alcohol) with the sodium salt of L-proline was easily fulfilled in the described condition. In the infrared spectrum of the formed chiral chelating polymer (**4**), the absorption at 1105.8 cm^{-1} allocated for C—N stretches appeared instead of that at 1093.7 cm^{-1} for epoxy groups in the reactant polymer. Also, a new absorption peak at 1639.5 cm^{-1} was originated from the asymmetrical stretches of COO^- groups. The spectra data indicated that it was the N atom in the sodium salt of L-proline that reacted with the epoxy group in the glycidylated poly(vinyl alcohol). When the chiral chelating polymer with the L-proline pendant complexed with the copper cation as described in the Experimental part, saturated polymeric chelate (**5**) composed of two units of L-proline pendants and one copper ion would be formed because uncomplexed and instably complexed copper cations had been washed off. In this case, it was imagined that some L-proline pendants located at positions where they could not match each other would be kept free in the polymeric chelate.

Enantioselectivity of the Crosslinked Poly(vinyl alcohol) with the L-Proline Ligand

The enantioselectivity of the synthesized polymer with the L-proline ligand was evaluated using its copper chelate as the CSP of LEC. DL-Histidine was selected as a probe to screen the suitable eluent. From the results in Table I, it was concluded that (1) a monodentate complexant of copper which competed with the solutes was necessary in the eluent for accelerating the ligand exchange of the solutes; (2) a slightly basic condition was helpful for the resolution of DL-histidine, indicating that ammonia played an important role; and (3) a small amount of copper cations had to be added into an eluent to compensate for the consumption of copper on the CSP in the chromatographic process. The best eluent condition for LEC of DL-histidine proved to be 0.05 *M* $(NH_4)_2CO_3$—0.1 m*M* $Cu(NO_3)_2$.

Table I Influence of Various Eluents on the Resolution of DL-Histidine on the CSP

Eluent	0.2*M* NH_4Cl	0.2*M* NH_4OAc	0.2*M* NH_4HCO_3	0.2*M* $(NH_4)_2CO_3$	0.1*M* $(NH_4)_2CO_3$	0.05*M* $(NH_4)_2CO_3$
K'_D	7.80	4.12	4.64	4.08	4.72	5.20
K'_L	7.80	6.20	7.00	6.60	7.56	8.80
α^b	1.00	1.50	1.51	1.62	1.60	1.69
Rs		1.5	1.5	1.5	1.6	2.0

0.1 m*M* $Cu(NO_3)_2$ was contained in the eluents; flow rate = 1.5 mL/min. $\alpha = K'_L/K'_D$. Column: 25 × 1 cm i.d. stainless-steel column; temperature: 15°C.

Table II The Resolution of DL-Amino Acids on Polymeric Chiral Stationary Phase

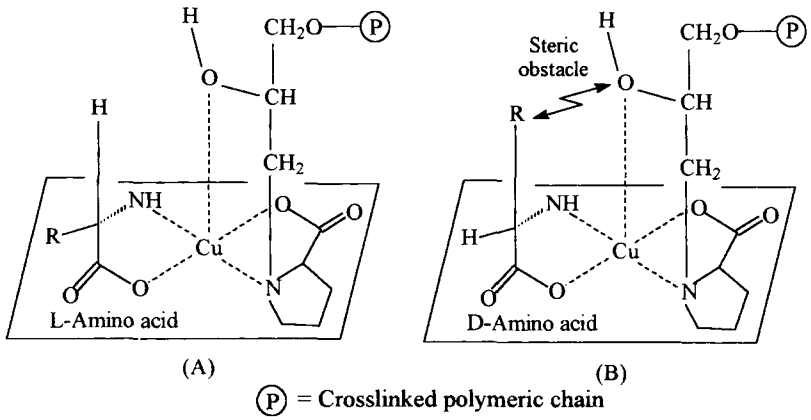
DL-Amino Acid	K'_D	K'_L	α	Rs	Loadability (mg)
DL-Alanine	1.60	2.44	1.53	1.3	—
DL-Valine	2.80	4.00	1.43	1.5	20
DL-Leucine	2.96	4.96	1.68	1.5	30
DL-Isoleucine	3.28	5.60	1.71	1.5	30
DL-Phenylalanine	2.00	3.40	1.70	1.4	20
DL-Traptophan	3.44	5.44	1.58	1.6	30
DL-Serine	2.84	5.24	1.85	1.6	35
DL-Threonine	2.44	3.32	1.36	1.2	—
DL-Methionine	2.72	4.92	1.81	1.6	20
DL-Asparagine	3.52	6.12	1.74	1.7	30
DL-Aspartic acid	2.20	3.00	1.36	1.3	—
DL-Glutamic acid	1.40	2.12	1.51	1.2	—
DL-Histidine	5.20	8.80	1.69	2.0	50
DL-Arginine	6.20	11.0	1.77	2.1	60
DL-Proline	5.20	2.60	0.50	2.0	40

$\alpha = K'_L/K'_D$. Loadability is the sample amount of an DL-amino acid injected into the column when Rs reaches 1. Other chromatographic conditions are same as in the Table I.

Using 0.05M $(NH_4)_2CO_3$—0.1 mM $Cu(NO_3)_2$ as the eluent, all 15 tried DL-amino acids were separated completely on the polymeric chiral stationary phase and the resolutions (Rs) were larger than 1 (Table II). Separation factors (α) are quite high as expected. That indicated that the synthesized polymeric CSP possessed excellent enantioselectivity for amino acid racemates. The enantiomers of amino acids with a bulky and/or rigid side chain or with a third complexing group on the side chain had larger relative retention times, separation factors, and sometimes larger Rs than those of the others. D-Isomers were flown out first for most amino acids except proline. All these results could be explained by the ligand-exchange mechanism.

Enantioselectivity Mechanism of the Polymeric CSP for Amino Acid Racemates

The enantioselectively molecular recognition on a natural or artificial polymer could be explained using a three-point attachment model.[20,21] In the modern interpretation, three interactions may be attractions and repulsions including steric and static ones. The ligand-exchange mechanism of DL-amino acids on the used polymeric CSP could be described as follows: When amino acid enantiomers were introduced into the column, one of both ligand units of the polymeric chelate was exchanged by D-amino acid or L-amino acid to give two discriminative ternary sorption complexes as shown in Scheme 2. In **B** of



Scheme 2 The model of the ternary sorption complexes formed from L- and D-amino acid, L-proline pendant on the polymer, and copper ion after ligand exchange.

Scheme 2, the side chain of D-amino acid was directed above the complexing plane and met a steric obstacle with the hydroxy group on the spacer which complexed to the central cation so that the ternary sorption complex (**B**) formed from the D-enantiomer was thermodynamically less stable than that from the L-isomer, and as a result, the D-enantiomer was first flown out. If the side chain of an amino acid was bulky such as leucine, isoleucine, phenylalanine, and traptophan, the difference of thermodynamic stability between two ternary sorption complexes would be larger and, hence, a higher separation factor would be observed. If a complexing group existed on the side chain of an amino acid such as serine, methionine, asparagine, histidine, and arginine, it would complexed with the central ion beneath the original complexing plane in complex **A**, resulting in a much more stable complex **A**. So, these amino acids displayed the larger α values as well as larger retention times. As to DL-proline, the L-isomer was passed through the column first because the D-enantiomer could form a more stable ternary sorption complex (**B**) in which no obvious steric hindrance took place owing to the rigidity of its ring. But in the complex (**B**) formed from L-proline, two proline rings were located at same direction (i.e., under the complexing plane), making the complexing plane deformed so as to obtain a less stable **A**.

Preparative Resolution of Some DL-Amino Acids

The direct resolution of enantiomers in a large scale is of much importance in scientific research and in the pharmaceutical industry and has been attracted many scientists in different fields. Ligand-exchange chromatography on the CSPs proved powerful and hopeful to achieve the lofty goal. We tried the preparative resolution of some amino acid racemates which demonstrated the larger α values. The results in Table II showed that the loadability of the column was up to 60 mg per injection when *Rs* was not less than 1. Both amino acid enantiomers in the eluate could be separately collected and recovered by removal of copper ions. The evaluation of the synthesized L-proline-incorporating poly(vinyl alcohol) beads in a larger scale will be carried out in the near future to make sure of the possibility for its application in industry.

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