

Preparation of Copolymer of L-Aspartic Acid and L-Glutamic Acid

Zhang Rui, Tan Tianwei

College of Life Science and Technology, Beijing University of Chemical Technology,
Beijing 100029, People's Republic of China

Received 7 April 2005; accepted 1 September 2005

DOI 10.1002/app.23154

Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this article, a new copolymer of L-aspartic acid and L-glutamic acid, which may be a biodegradable high molecular polymer and can be used more widely in many areas, was synthesized. The conditions of preparation, such as catalyst, reaction time, reaction temperature, the amount of catalyst, the times of adding catalyst, and the molar ratio of L-aspartic acid to L-glutamic acid, were optimized. The copolymer was characterized by ^{13}C NMR, infrared spectroscopy, and X-ray diffractometer. The molecular weight was determined by GPC. The result indicated that production yield, purity of product, and molecular weight

of product increased with amount of catalyst and molar ratio of L-aspartic acid to L-glutamic acid increasing. The best condition of preparation was the following: reacting 2–4 h at the temperature of 180–200°C. The product yield with the molecular weight 13,000.00 reached 63.2% and the purity of product was 96.33% when the copolymerization was carried out at the temperature of 200°C under vacuum for 2 h. © 2006 Wiley Periodicals, Inc. *J Appl Polym Sci* 100: 3626–3633, 2006

Key words: copoly-(L-aspartic acid/L-glutamic acid) (PAG); product yield; purity; molecular weight; characterization

INTRODUCTION

Most polyamino-acids are high molecular polymers with wide applications in the fields of food, environment protection materials, and biomedical materials and drug delivery system.¹

Polyaspartic acid is one kind of amino acid polymer that has good biodegradability. It is environmental friendly and can be used widely to replace various chemicals, but may cause pollution to the environment¹ as well.

There are two methods for the synthesis of polyaspartic acid. (1) Thermal polymerization of aspartic acid.² The reaction conditions are the following: the temperature should be between 160 and 260°C, the time needs to be in the scale 1–4.5 h, and the catalyst is phosphoric acid.^{1–6} (2) A multi-step reaction from maleic acid anhydride. The route of the synthesis is as follows (Fig. 1).

The different synthetic pathways, such as the molecular weight or molecular geometry, determine different chemical structures of polyaspartic acid. Prop-

erties such as dispersing activity and biodegradability are also determined by different synthetic pathways.¹

Polyaspartic acid can be used in many different applications, such as home laundry detergents, automatic dishwasher detergents, water treatment chemicals, oil field treatment additives, or dispersants, for a variety of organic and inorganic solids and scales.²

Poly(γ -glutamic acid) is a kind of ideal biodegradable high molecular polymer, and can be produced by microbial methods⁷ and chemical methods (Fig. 2).⁸

Sometimes, poly(γ -glutamic acid) is used as the carrier of antineoplastic agents to control the drug's release and improve the curative effect.⁹ It can also be used as high water adsorption resin and its applications covered agriculture, pharmacy, and chemical industry⁷ very often.

As aforementioned, polyaspartic acid and poly(γ -glutamic acid) have many different properties as well as some common properties. So, this article reports a new copolymer of L-aspartic acid and L-glutamic acid, which may include both of their properties and can be used more widely.

Until now, some grafted copolymers of L-aspartic acid or L-glutamic acid were prepared by initiating the *N*-carboxy-anhydrides of α -amino acids with an immobilized primary amine.¹⁰ In 1995, Oosterling et al. studied (co)polyglutamates and (co)polyaspartates by initiating the *N*-carboxy anhydrides of α -amino acids with an immobilized primary amine^{11,12} and the conformation of (co)polyglutamates and (co)polyaspartates was grafted onto glass slides or silicon wafers.

Correspondence to: Z. Rui (stella1412@163.com).

Contract grant sponsor: National Science Foundation of China; contract grant numbers: 20136020, 20325622, 50373003 and 20576013. Contract grant sponsor: Beijing National Science Foundation; contract grant number: 2032013. Contract grant sponsor: Specialized Research Foundation for Doctoral Program; contract grant number: 20030010004.

Chemical synthesis of polyaspartates

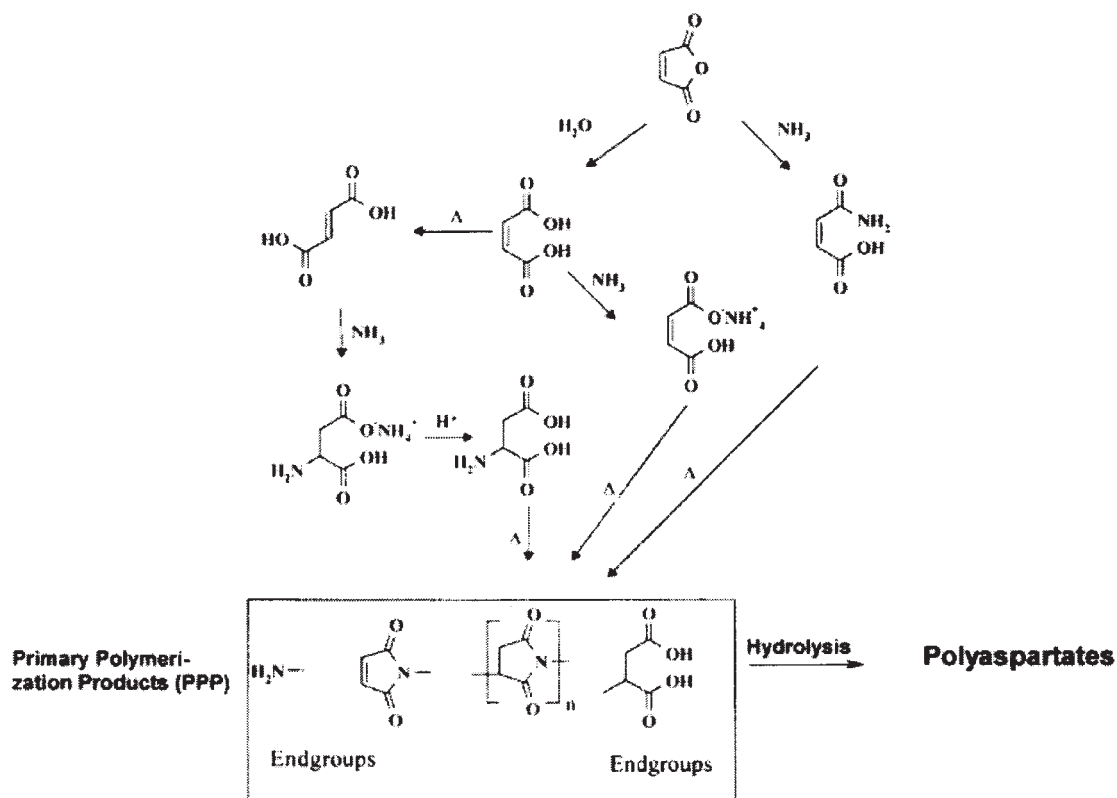


Figure 1 Chemical synthesis of poly-aspartates.

Yasuyosi et al. prepared copoly(*N*-hydroxyalkyl-D,L-glutamine) membranes in 1999,¹³ Peter et al. prepared fluorophore-labeled poly- α,β -poly(*N*-hydroxyethyl)-DL-aspartamide in 1997,¹⁴ and in 1997, Pang et al. prepared leucine-methyl glutamate-glutamic acid copolymer, which is kind of biomaterial and can be used as drug delivery.¹⁵ But, many poisonous reagents, such as phosgene, must be used in this synthetic pathway and it enhances the difficulty of operation.

In 2001, Tang et al. prepared a copolymer consisting of L-aspartic acid and L-glutamic acid (8 : 2 mol/mol) in a liquid phase system with rotating evaporator. 3-Hydroxyamine, as a side chain, was linked to the polymer, and poly-(3-hydroxypropyl)-L-aspartamide-L-glutamide was obtained. The product yield in the liquid phase synthesis was 56.83%.¹⁶ Poisonous DMF

must be used to deal with the product in the synthesis, and the operation is more complex.

In this article, copolymer of L-aspartic acid and L-glutamic acid was prepared in melt polymerization, which handled more easily and the product yield can reach 70%. The conditions of preparation, such as catalyst, reaction time, reaction temperature, the amount of catalyst, the times of adding catalyst, and the molar ratio of L-aspartic acid to L-glutamic acid, were investigated. The copolymer was characterized by ¹H NMR, ¹³C NMR, infrared (IR) spectroscopy, and X-ray diffractometer. The molecular weight was determined by GPC.

EXPERIMENTAL

Material

L-Aspartic acid used for the experiments is from Changmao Company (Changzhou, Jiangsu) of industry grade and L-glutamic acid used for the experiments is of food stuff grade. Phosphoric acid (85% w/w) from Hongxing Company (Bei) and sulfuric acid (98% w/w) are of analysis grade. DMF is from Beijing of analysis grade.

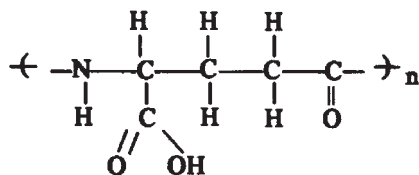


Figure 2 Structural formula of γ -PGA.

TABLE I
Relationship Between Product Yield, Purity of Product, and Catalyst^a

Condition	Product yield (%)	Purity of product (%)
Phosphoric acid (85%) under air condition	63.02	96.33
Phosphoric acid (85%) under vacuum condition	67.92	97.02
Sulfuric acid (98%) under air condition	42.38	93.22
Sulfuric acid (98%) under vacuum condition	54.01	93.67

^a L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g); reaction temperature: 200°C; reaction time: 2 h.

Methods

The choice of catalyst

All reactions were carried out at the same reaction temperature (200°C), the same reaction time (2 h), the same molar ratio of catalyst and reactants (1 : 2 mol/mol), and the same molar ratio of L-aspartic acid to L-glutamic acid (4 : 1 mol/mol).

L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g) were mixed in a flat pan. Phosphoric acid (8.647 g; 85% w/w) and sulfuric acid (12.252 g; 98% w/w), used as catalyst, were added into the mixture. The copolymerizations were carried out at the temperature of 200°C under vacuum conditions for 2 h. The products were taken out and cooled to the normal temperature. To remove catalyst and reactants in the product, the mixtures had to be washed several times with de-ionized water until their pH reach neutral, and then they were filtered. The copolymers obtained were dried at the temperature of 80°C for 4 h.

The choice of the concentration of catalyst

All reactions were carried out at the same concentrations of L-aspartic acid (10.64 g) and L-glutamic acid (11.76 g). Only 85% (w/w) phosphoric acids were at different concentration, ranging from 8.647, 4.325, 1.729, 0.865 to 0.576 g. Other conditions were the same as described earlier.

The choice of the molar ratio of L-aspartic acid to L-glutamic acid

All reactions were carried out at the same concentrations of 85% (w/w) phosphoric acid (8.647 g). Only the mixtures of the components were at different concentration, including 3.997 g L-aspartic acid and 17.609 g L-glutamic acid, 4.988 g L-aspartic acid and 16.544 g L-glutamic acid, 14.958 g L-aspartic acid and 5.508 g L-glutamic acid, 15.96 g L-aspartic acid and 4.41 g L-glutamic acid, 17.955 g L-aspartic acid and 2.209 g L-glutamic acid. Other conditions were the same as described earlier.

The choice of the reaction time

All reactions were carried out at the same concentration of 85% (w/w) phosphoric acid (8.647 g) and the same concentrations of L-aspartic acid (15.96 g) and L-glutamic acid (4.41 g). The reaction time ranged from 3, 4, to 5 h. Other conditions were the same as described earlier.

The choice of the reaction temperature

All reactions were carried out at the same concentration of 85% (w/w) phosphoric acid (8.647 g) and the same concentrations of L-aspartic acid (15.96 g) and L-glutamic acid (4.41 g). The reaction temperature

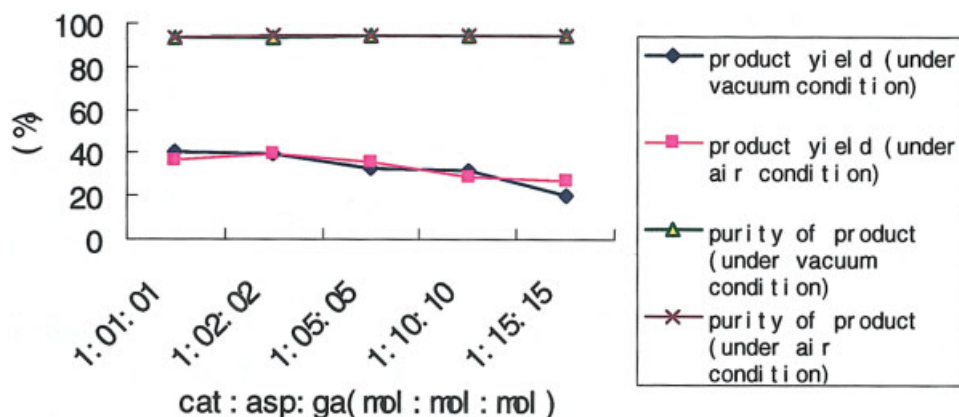


Figure 3 Relationship between product yield, purity of product, and the concentration of catalyst (L-aspartic acid: 10.64 g; L-glutamic acid: 11.76 g; reaction temperature: 200°C; reaction time: 2 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

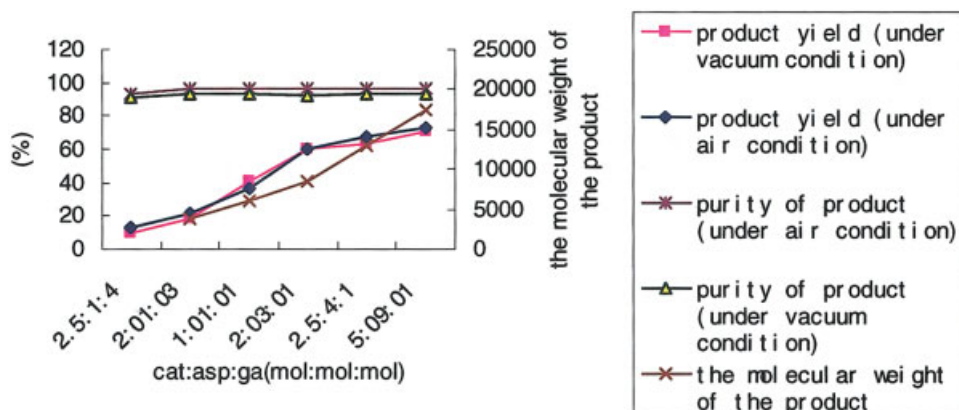


Figure 4 Relationship between product yield, purity of product, the molecular weight of the product, and the molar ratio of L-aspartic acid to L-glutamic acid (phosphoric acid: 8.647 g; reaction temperature: 200°C; reaction time: 2 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ranged from 150, 160, 170, 180, 190 to 210°C. Other conditions were the same as described earlier.

The choice of times of adding catalyst

All reactions were carried out at the same reaction temperature (180°C), reaction time (4 h), the same molar ratio of catalyst to reactants (1:1 mol/mol), and the same molar ratio of L-aspartic acid to L-glutamic acid (4:1 mol/mol).

L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g) were mixed in a flat pan. Phosphoric acid (10.000 g) was added into the mixture first, and the copolymerization was carried out at 180°C under vacuum conditions for 2 h. The product was taken out and cooled to the normal temperature following the earlier step. Then, the remaining 7.292 g catalyst was added into the pan and the reaction continued to react at the same condition for 2 h. Other conditions were the same as described earlier.

L-Aspartic acid (15.961 g) and L-glutamic acid (4.412 g) were mixed in a flat pan. Phosphoric acid (6.020 g) was added into the mixture first, and the copolymerization was carried out at 180°C under vacuum conditions for 1 h. After that, the product was taken out and cooled to the normal temperature. Then, 6.040 g of catalyst was added into the pan and the reaction reacted at the same condition for 1 h. Next, the product was taken out and cooled to the normal temperature for another time. At last, the left catalyst (5.234 g) was added into the pan and the reaction was carried out at the same condition for 2 h. Other conditions were the same as described earlier.

The determination of purity of product and the yield

The determination of purity of product

The copolymer can be dissolved in DMF, while the impurity cannot, so that a mass balance can obtain the purity of product.

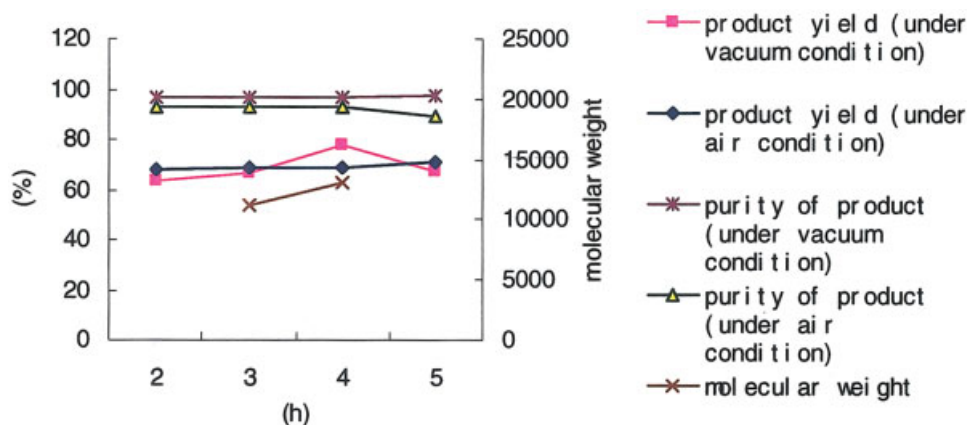


Figure 5 Relationship of product yield, purity of the product, the molecular weight of the product, and the reaction time (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction temperature: 200°C). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

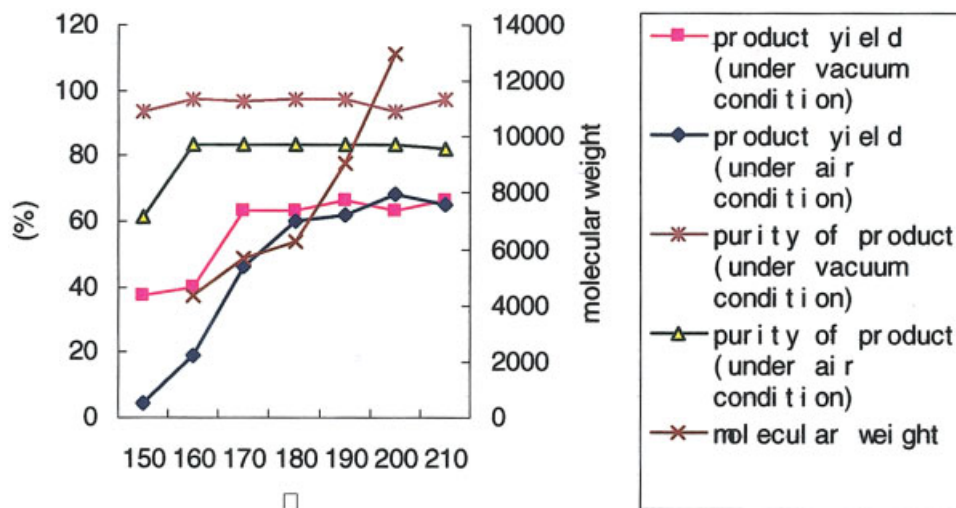


Figure 6 Relationship between product yield, purity of the product, the molecular weight of the product, and the reaction temperature (l-aspartic acid: 15.96 g; l-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 2 h). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

One gram of the product was dissolved in DMF (10 mL) and the mixture was allowed to stand for 24 h at room temperature. The mixture was filtrated by a filter paper and the filter paper was dried at the temperature of 40°C for 3 h to constant. The filter paper was weighted and the purity of product is expressed in eq. (1):

$$P_r = (A_0 - B) \div A_0 \times 100\% \quad (1)$$

where A_0 and B represent the weight of product and the filter paper and the weight of insoluble materials in filter paper, respectively; P_r is the purity of product.

The product yield

To remove catalyst and non reacting in the products, the products had to be washed several times with de-ionized water until their pH reach neutral, and pass to filtering process later. The copolymers obtained were dried at the temperature of 80°C for 4 h. The product yield can be calculated from eq. (2):

$$Y = A \times P_r \div W \times 100\% \quad (2)$$

where A and W stand for the weight of product and the weight of reactants respectively; P_r is the purity of product; Y is the product yield.

Characterization

^{13}C NMR high-resolution liquid-state spectra were obtained using a NMR spectrometer (AV600 MHz, Bruker, Germany.) operating at 600 MHz. IR spectra were obtained using a spectrophotometer (210.Nicolet, Waltham, MA) operating by potassium bromide (KBr) method. X-ray spectra were obtained using an X-ray diffractometer (D/Max2500, VB2t/PC, Rigaku, Japan) to operate at a 2θ range from 3° to 60°.

The molecular weight of copolymer

LS-GPC chromatogram was used to determine the molecular weight of copolymer. The column is Shodex Asahipak column No.7710074. Phosphate buffer was used as mobile phase at a flow rate of 0.4 mL/min. The standards for molecular weight determination were polyethylene glycol. Regression equation is $\log M = -0.3243 \text{tr} + 7.9251$, ($R^2 = 0.9971$, $n = 6$). According to the tr of copolymer, the molecular weight of copolymer can be calculated.

RESULTS AND DISCUSSION

Reaction condition optimization

The choice of catalyst

Table I shows the relationship between product yield, purity of product, and different catalysts. The product

TABLE II
Relationship Between Product Yield, Molecular Weight of Product, and the Times of Adding Catalyst^a

The times of adding catalyst	Product yield (%)	Molecular weight
One time	58.09	8732.683
Two times	72.50	9280.879
Three times	70.56	9320.456

^a L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g); phosphoric acid: 8.647 g; reaction time: 4 h; reaction temperature: 180°C, under vacuum condition.

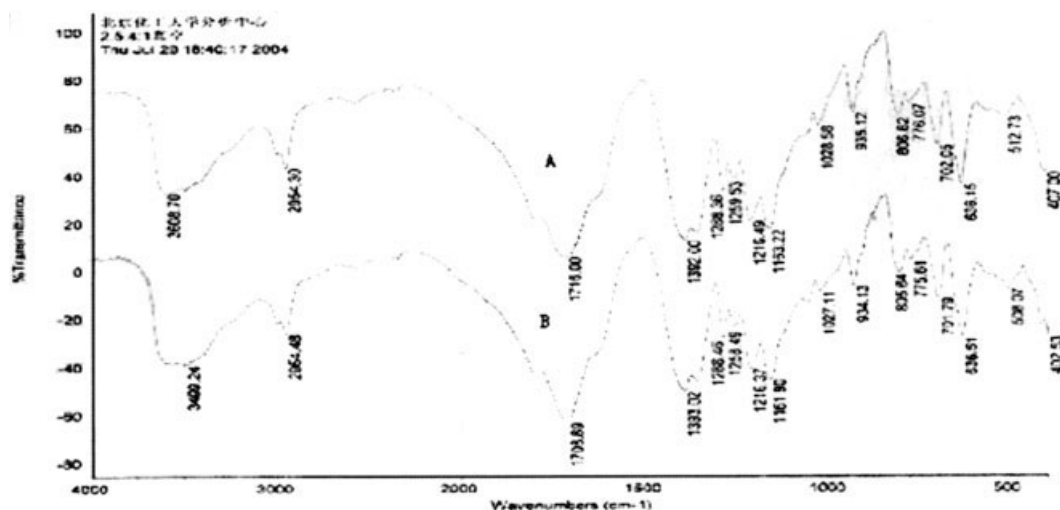


Figure 7 IR spectroscopy. A: Copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 200°C, under vacuum condition). B: PSI (113000).

yield and the purity were high when phosphoric acid was used, because sulfuric acid (98%) was a dehydrated acid that caused some products to carbon or by-product.

The influence of the concentration of catalyst

As the illustration shows, the product yield increased with the concentration of catalyst increasing, while the concentration of catalyst did not influence the purity (Fig. 3). The reason is that the catalyst induced the reaction and it was hardly induced when the concentration of the catalyst was low.

The influence of the molar ratio of L-aspartic acid to L-glutamic acid

The product yield, as well as the purity of product and the molecular weight of product, increased with the amount of catalyst and molar ratio of L-aspartic acid to

L-glutamic acid increasing (Fig. 4). Another conclusion can be obtained that the copolymer has high L-aspartic content (Fig. 4).

The influence of the reaction time

Figure 5 shows the relationship between product yield, purity of product, molecular weight of product, and the reaction time. It is obvious that the product yield and the molecular weight of product increased with the increasing of reaction time. From Figure 5, the purity of product (under air condition) decreased with increasing reaction time, while this phenomenon was not present under vacuum condition. The reason was that the reactants were oxygenated by oxygen in the air.

The influence of the reaction temperature

As seen in Figure 6, product yield, as well as purity of product and molecular weight of product, increased with the reaction temperature increasing. But, if the reaction temperature were too high, there would be some by-products; and on the other side, if the reaction temperature were too low, the degree of reaction would be very low.

The influence of times of adding catalyst

The product yield, as well as the molecular weight of product, increased when the catalyst was added twice (Table II). The explanation here is that the catalyst may become deactivated after a certain time, so adding catalyst many times would increase the degree of polymerization. When the time of adding catalyst was increased more, the production yield, as well as the

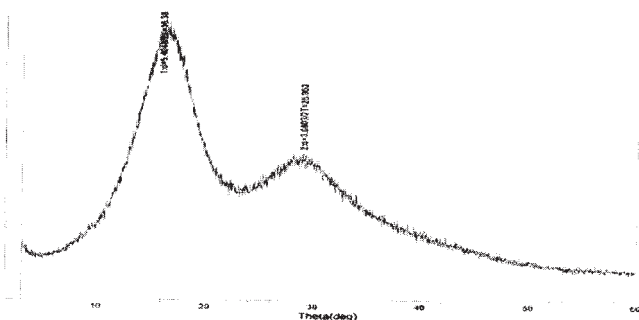


Figure 8 Copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 200°C, under vacuum condition).

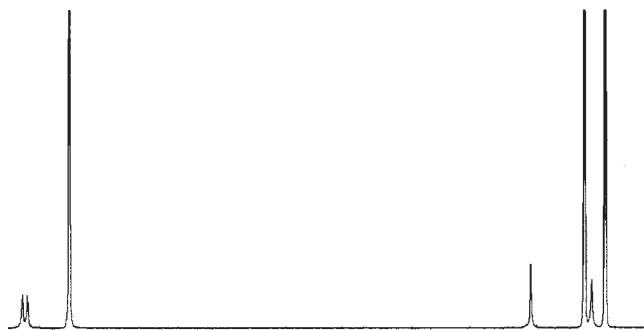


Figure 9 ^{13}C NMR PSI (113000).

molecular weight of product, would not change further (Table II).

Characterization

Infrared spectroscopy

PSI and copolymer have same peaks, but the appearance of peaks of C[dbonds]O and N—H were obviously different. The N—H stretch assignment of PSI was showed by the absorptions at 3499.24 cm^{-1} , and the C=O stretch assignment of PSI was showed by the absorptions at 1708.69 cm^{-1} . The N—H stretch assignment of copolymer was showed by the absorptions at 3608.70 cm^{-1} , and the C=O stretch assignment of copolymer was showed by the absorptions at 1716.00 cm^{-1} .

The peaks of C=O and N—H were related with the copolymerization, and so according to the difference of the two curves in Figure 7, copolymerization was taken place and the structure of molecule was influenced.

X-ray diffractometer

Figure 8 is the X-ray diffraction figure of copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid

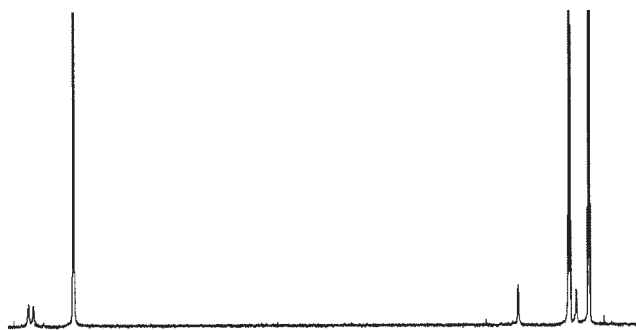


Figure 11 ^{13}C NMR copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 180°C , under vacuum condition).

(15.96 g) and L-glutamic acid (4.41 g); phosphoric acid (8.647 g); reaction time: 2 h; reaction temperature: 200°C , under vacuum condition). X-ray diffractometer can speculate the crystallization of materials, and so according to Figure 8, the peak was dispersion, and in result that the copolymer is random copolymer.

^{13}C NMR

Figure 9 is the ^{13}C NMR figure of PSI and Figure 10 is the ^{13}C NMR figure of copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid (15.96 g) and L-glutamic acid (4.41 g); phosphoric acid (8.647 g); reaction time: 2 h; reaction temperature: 200°C (under vacuum condition)). In the ^{13}C NMR figure of PSI, there were two peaks of C=O and the data were $\delta = 173.69$ and 172.54 ppm , and there was the peak of —CH— ($\delta = 47.93\text{ ppm}$) and the peak of —CH₂— ($\delta = 32.81\text{ ppm}$). In the ^{13}C NMR figure of copolymer, there were both peaks of PSI and polyglutamic acid. There were four peaks of C=O ($\delta = 173.69, 172.54, 169.71,$ and 169.21 ppm), two peaks of —CH— (δ

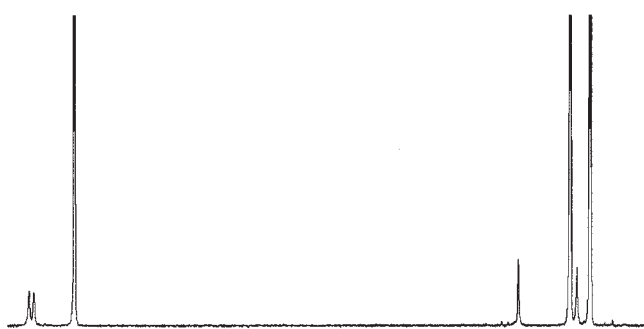


Figure 10 ^{13}C NMR copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 200°C , under vacuum condition).

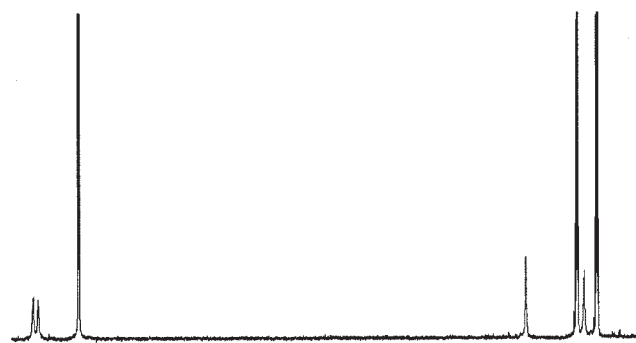


Figure 12 ^{13}C NMR copolymer of L-aspartic acid and L-glutamic acid (L-aspartic acid: 15.96 g; L-glutamic acid: 4.41 g; phosphoric acid: 8.647 g; reaction time: 4 h; reaction temperature: 180°C , under vacuum condition).

TABLE III
The Data of Copolymers' Peaks

Copolymer	C=O (δ ; ppm)	—CH ₂ — (δ ; ppm)	—CH— (δ ; ppm)
A	173.69	32.81	47.93
	172.54	24.02	52.20
	169.71	23.76	
	169.21		
B	173.67	32.82	47.91
	172.54	25.62	56.24
	171.90	23.76	
	169.82		
C	173.67	32.79	47.88
	172.53	25.26	52.16
	171.54	25.09	
	169.83		

A: L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g); phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 200°C, under vacuum condition. B: L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g); phosphoric acid: 8.647 g; reaction time: 2 h; reaction temperature: 180°C, under vacuum condition. C: L-Aspartic acid (15.96 g) and L-glutamic acid (4.41 g); phosphoric acid: 8.647 g; reaction time: 4 h; reaction temperature: 180°C, under vacuum condition.

= 47.93 and 52.20 ppm), and three peaks of —CH₂— (δ = 32.81, 24.02, and 23.76 ppm).

According to Figures 9 and 10, the copolymer has both the peak of L-aspartic acid and L-glutamic acid, so the conclusion here is that the copolymer consisted of L-aspartic acid and L-glutamic acid. In this copolymer, the content ratio of L-aspartic acid to L-glutamic acid can be seen as the altitude ratio of the C=O peak of PSI to copolymer is 9.5 : 1.2.

Figures 11 and 12 are the ¹³C NMR figures of two more samples of copolymer. Both of them can prove that the copolymer was composed of L-aspartic acid and L-glutamic acid. The data of their peaks are presented in Table III (Fig. 13).

CONCLUSIONS

The structure of copolymer of L-aspartic acid and L-glutamic acid was characterized by ¹³C NMR, IR spectroscopy, and X-ray diffractometer. The molecular weight was determined by GPC. As a result of this study, it can be concluded that the production yield, purity of product, and molecular weight of the product increased with amount of catalyst and molar ratio of L-aspartic acid to L-glutamic acid increasing. The best condition of preparation was reacting 2–4 h at the temperature of 180–200°C. The product yield with the molecular weight 13,000.00 reached 63.2% and the purity of product was 96.33% when the copolymerization was carried out at the temperature of 200°C under vacuum conditions for 2 h.

References

- Li, F.; Tan, T. *Dev Appl Chem Ind* 2001, 3, 24.
- Schwamborn, M. *Polym Degrad Stab* 1998, 59, 39.
- Tomida, M.; Nakato, T. *Polymer* 1997, 38, 4733.
- Tomida, M.; Yabe, M.; Arakawa, Y. *Polymer* 1997, 38, 2790.
- Kroner, M.; Schornick, G. U.S. Pat. 5,830,985 (1998).
- Chang, C. J.; Swift, G. *Polym Mater Sci Eng* 1998, 79, 232.
- You, Q.-H.; Zhang, X.-M.; Chen, G.-G.; Xu, H.; Ou-Yang, P.-K. *Mod Chem Ind* 2002, 22, 56.
- Sanda, F.; Fujiyama, T.; Endo, T. *J Polym Sci Part A: Polym Phys* 2001, 39, 732.
- Peng, Y.-X.; Xu, H.; Chen, G.-G.; Wei, P.; Ouyang, P.-K.; Zhang, J.-S. *Chin J New Drugs* 2002, 11, 515.
- Ciardelli, F.; Carlini, C.; Solaro, R.; Altomare, A.; Pieroni, O.; Houben, J. L.; Fissi, A. *Pure Appl Chem* 1984, 56, 329.
- Oosterling, M. L. C. M.; Willems, E.; Schouten, A. J. *Polymer* 1995, 36, 4463.
- Oosterling, M. L. C. M.; Willems, E.; Schouten, A. J. *Polymer* 1995, 36, 4485.
- Yasuyosi, M.; Kazu uyuki, J.; Masahito, O.; Toshio, H. *Eur Polym J* 1999, 35, 767.
- Peter, R. B.; Hirokazu, K.; Zhuuang, S.; Frantisek, R. *Pharm Res* 1994, 11, 221.
- Pan, S.; Shi, F.; Huang, L.; Zhou, Q.; Lin, Z.; Wu, Y. *Biomed Eng* 1997, 14, 101.
- Tang, G.; Zhu, Y.; Xie, X.; Wu, Q. *J Boimed Eng* 2001, 18, 337.