



Preparation and Swelling Behavior of Poly(N-isopropylacrylamide-co-acrylic Acid Derivatived L-phenylalanine) Hydrogels

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Contract grant sponsor: National Natural Science Foundation of China (contract grant number: 20774064)

Abstract

A series of novel temperature-sensitive hydrogels with chiral group, poly(NIPA-co-AAc-L-Phe), were synthesized by copolymerization of *N*-isopropylacrylamide(NIPA) and acrylic acid derivatived L-phenylalanine (AAc-L-Phe) in ethanol with different feed ratios. Their swelling behaviors in response to temperature and deswelling/reswelling kinetics have been studied. The primary results showed that poly (NIPA-co-AAc-L-Phe) hydrogels are thermo-responsive and have potential applications in the biological chemistry.

Keywords: Hydrogels, Copolymerization, N-isopropylacrylamide, Temperature-sensitive, Swelling behavior

1. Introduction

The separation of chiral materials (Lu, H. 2007) has become the urgent need to solve the issue in the biochemistry and pharmaceutical industry (Huang, L. and Dai, L.X. 2002). In recent years, with the chiral materials (Izake, E. L. 2007) such as chiral stationary phase and chiral membrane have developed, a lot of techniques have been more application in Chiral drugs preparation (Dufresne, F. and Galanski, M. 2007). But these methods are weakly demand for large-scale industry produce which have high efficiency and low costs. Thus, it is very important that we further research chiral separation materials with high selective and handling ability.

Poly(N-isopropylacrylamide) (PNIPA) is a typical thermosensitive polymeric material and demonstrates a lower critical solution temperature (LCST) at $\sim 32^{\circ}\text{C}$ in aqueous solution (Hirokawa, Y and Tanaka, T. 1984). Below the LCST, PNIPA is hydrophilic and swells in water. In contrast, it becomes hydrophobic and shrinks dramatically above the LCST. Due to their unique properties, PNIPA hydrogels have been widely used in many fields (Zhang, X. Z. and Zhuo, R. X. 2000). For example, hydrogels have been used to recognize and capture a target molecule (Zhang, X. Z., Zhang J. T., Zhuo, R. X. and Chu, C. C. 2002) (Zhang, Z. L. and Wang, B.2008) (Kurisawa, M., Okano, T. and Yokoyama, M. 2000). By incorporating chiral recognizable groups, such as amino acids and their derivatives into the temperature sensitive PNIPA network, we speculated that PNIPA hydrogel could be used to extract enantiomeric molecules and applied to enantiomeric separation. Based on above considerations, we wish to synthesize a new hydrogel, which can combine the

temperature sensitive properties of PNIPA with the excellent capability of enantioselective separation by introducing amino acid groups into PNIPA hydrogel.

In this study, a series of new temperature-sensitive hydrogels containing acrylic acid derivatized L-phenylalanine (poly(NIPA-co-AAc-L-Phe)) were synthesized by copolymeration of AAc-L-Phe with NIPA in ethanol. The temperature-responsive properties and the effect of weight ratio r ($r = \text{AAc-L-Phe} / (\text{AAc-L-Phe} + \text{NIPA})$) on the swelling ratio (SR), deswelling kinetics and reswelling kinetics have been studied. The poly (NIPA-co-AAc-L-Phe) hydrogels have potential applications in the biological chemistry.

2. Experimental

2.1 Materials

N-isopropylacrylamide (NIPA) was purchased from Kohjin Co. Ltd., Japan and purified by recrystallization three times in the mixed solvent of benzene and n-hexane. D/L-phenylalanine (L-Phe/D-Phe) was obtained from Tianjin Kermel, China. Acryloyl chloride was purchased from Alfa Aesar, USA. All other reagents, including N, N-methylenebis (acrylamide) (MBAA), Azobisisobutyronitrile (AIBN), Ethanol, Triethylamine, N, N'-dimethylformamide (DMF), Dichloromethane, Thionyl chloride and Sodium chloride, etc. were analytical grade made in China, and used as received without further purification.

2.2 Synthesis of chiral monomer (AAc-L-Phe)

L-phenylalanine ethyl ester was synthesized based on as follow: ethanol (100ml) was put in a flask. In the ice bath, under the condition of stirring, thionyl chloride (64.5mmol) was added dropwise, the mixed solution was stirred for 1h. At room temperature (25°C), L-phenylalanine (50mmol) was added and stirred for 3h. Then the mixed solution was heated to reflux for 2h. The over ethanol and thionyl chloride were made by distillation; the mixed solution was precipitated to solid. The solid was filtered and dried by vacuum drying. Sodium carbonate solution (25wt %) was added dropwise in the solid, which was the solution's pH>12. The liquid was extracted by acetic ester (20ml) three times. L-phenylalanine ethyl ester was made by rotary evaporator. Then L-phenylalanine ethyl ester (10mmol) was dissolved and stirred in DMF, then added triethylamine (10ml) in a flask. In the ice bath, under the condition of stirring, acryloyl chloride (20mmol) was added dropwise. Then the mixed solution was stirred for 4h at room temperature, the result was filtered and the filtrate was washed distilled water (40ml). The synthetic scheme of chiral monomer is summarized in Figure 1. The liquid was extracted by dichloromethane (20ml) three times. The oily liquid was made by rotary evaporator. The product recrystallized in ethanol three times, and finally the obtained crystallized product.

2.3 Synthesis of poly (NIPA-co-AAc-L-Phe) hydrogels

For the synthesis of poly(NIPA-co-AAc-L-Phe) hydrogels, different quality ratios of precursors (NIPA and AAc-L-Phe) were dissolved in 1.2mL ethanol in existence of a crosslinker MBAA(3wt.% based on total precursors), according to r 0, 0.05 and 0.1, respectively. AIBN (1wt.%) were then added as initiators. After bubbling with nitrogen gas to remove oxygen, the copolymerization was carried out at 55°C for 24h and then the hydrogels obtained were first immersed in ethanol at room temperature to take out the unreacted chemicals. During this period, the ethanol was replaced with fresh ethanol every several hours. Then the hydrogels were further purified with distilled water at room temperature for at least 48h. Similarly, the distilled water was replaced every several hours to let the purified hydrogels reach equilibrium following characterization.

2.4 Measurements

Both AAc-L-Phe monomer and dried poly (NIPA-co-AAc-L-Phe) hydrogels were powdered with KBr, pressed into pellets under reduced pressure, and infrared spectrum analyses were taken on a TENSOR37-Fourier Transform Infrared Spectrometer (Bruker Corporation, Germany). ¹H-NMR measurements of AAc-L-Phe monomer were conducted at room temperature using CDCl₃ as solvent (Varian UNITY Plus-400 NMR, USA).

Swelling ratio (SR) of hydrogels was measured gravimetrically after wiping off the excess surface water with moistened filter paper in the temperature range from 20°C to 45°C. Gel samples were incubated in distilled water for at least 24h at every particular temperature. SR is defined as follows:

$$SR = W_s / W_d$$

Where W_s is the weight of water in a swollen hydrogels at the particular temperature, and W_d is the dry weight of hydrogels.

The deswelling kinetics of gels was measured gravimetrically at 45°C after wiping off the excess surface water with moistened filter paper. Before this measurement, the gel samples reached equilibrium in distilled water at 25°C. The weight changes of gels were recorded at the course of deswelling at regular time intervals. Water retention (WR) is defined as follows:

$$WR = 100(W_t - W_d) / W_s$$

Where W_t is the wet weight of hydrogels at regular time intervals and the other symbols are the same as defined earlier.

The reswelling kinetics of the gels was measured gravimetrically at 25°C, also after blotting the excess surface water with moistened filter paper. The weight changes of gels were recorded during this reswelling process at regular time intervals. The water uptake (WU) is defined as follows:

$$WU = 100(W_t - W_d)/W_s$$

Where W_s is the weight of water in swollen gel at 25°C and the other symbols are the same as defined above.

3. Results and discussion

3.1 Structural analysis of chiral monomer (AAc-L-Phe)

In this experiment, the IR analysis was applied to the characterization of samples' structure and the certification of functional groups existing in molecules. The synthesis route in this experiment is shown in Figure 1, simultaneously, the infrared spectrum of samples is shown in Figure 2. The structures of chiral monomer AAc-L-Phe were determined by IR. There is a broad band in the range of 3600–3200 cm^{-1} (\sim , 3312 cm^{-1}) which belongs to N-H stretching vibration. This can be proved secondary amine group (RNH-R'). The typical amide I band (\sim , 1679 cm^{-1}), consisting of C=O stretch. At the same time, it is saw by the presence of the typical band of unsymmetrical vibration (\sim , 1210 cm^{-1}), consisting of C-O-C stretch. There is which belongs to C-H stretching vibration of the aromatic hydrocarbon (\sim , 3033 cm^{-1}). There is a peak band which belongs to C-H of olefin (RHC=CH₂) out-of-plane vibration (\sim , 910 cm^{-1}); The typical Benzene ring skeleton I band (\sim , 1527, 1449 cm^{-1}), consisting of C=C stretching vibration and Benzene ring II band (\sim , 743 cm^{-1}), including C-H out-of-plane morph vibration were evident by spectrum.

The structures of chiral monomer AAc-L-Phe were determined ¹H-NMR measurement (CDCl₃) which is shown in Figure 3. The peaks at 3.26 and 5.07ppm can be assigned to -CH=C and methyl protons from AA, respectively. And the peaks at 1.28, 4.21ppm can be assigned to -CH₂- and methyl protons of ester group. The signals of 7.73, 7.29 and between 7.40 and 7.53ppm were assigned to the protons of benzene ring. The peak at 7.15ppm was attributed to the protons of -CH₂- linked with benzene ring. The signals of 6.60ppm corresponded to the chiral proton.

3.2 Structural analysis of hydrogels

IR spectra show that the FT-IR spectra of hydrogels in Figure 4, which curve A means PNIPA hydrogels and curve B represents poly(NIPA-co-AAc-L-Phe) hydrogels. Even though the spectrum of each hydrogels showed some changes, it is found that the FT-IR spectra were similar. Every spectrum showed a broad band in the range of 3600–3200 cm^{-1} , which belongs to N-H stretching vibration of the PNIPA. The typical amide I band (\sim , 1641 cm^{-1}), consisting of C=O stretch of PNIPA and amide II band (\sim , 1549 cm^{-1}), including N-H vibration were evident in curve A. The existence of chiral monomer was evident by the presence of the typical band of unsymmetrical ester group (\sim , 1211 cm^{-1}), consisting of C-O-C stretch, although this band was weak due to the low chiral monomer content in hydrogels in curve B.

3.3 Effect of temperature on SR of poly(NIPA-co-AAc-L-Phe) hydrogels

The swelling behaviors of poly(NIPA-co-AAc-L-Phe) hydrogels with different r values were investigated at various temperatures, and the results are shown in Figure 5. We can see that compared with PNIPA hydrogel, the SRs of the hydrogels decreased with the increasing contents of chiral units (AAc-L-Phe) at temperatures below the LCST. This result indicates that poly(NIPA-co-AAc-L-Phe) hydrogels maintain the temperature-sensitive characteristics of PNIPA hydrogel, which is attributed to the temperature sensitive component PNIPA. However, incorporating the hydrophobic group (benzene ring) from L-phenylalanine ethyl ester to the hydrogel increases the hydrophobic nature and exerted negative an effect on the extensibility of the hydrogel network.

The deswelling kinetics of hydrogels after a temperature jumping from the equilibrated swollen state at 25°C to the hot water at 45°C is showed in Figure 6. The most important observation is an abrupt shrinkage with all the hydrogels which lost more than 80% of their original water contents within 80min and quickly reached their stable WRs within 100min. The deswelling speed and the WR increased with the increase of r; it might be associated with the increase of the amount of the hydrophobic units (AAc-L-Phe) and thus the force that forces the PNIPA chain into globule conformation was strengthened with the increase of r.

We further studied the reswelling kinetics of the dry poly(NIPA-co-AAc-L-Phe) hydrogels in distilled water at 25°C (Figure 7). It was found that all the hydrogels reswelled and reached to equilibrium within 12h, and the water uptake decreased with the increase of r, which is also attributed to the increasing hydrophobic nature of AAc-L-Phe.

4. Conclusions

Acrylic acid derivatized L-phenylalanine (AAc-L-Phe) was successfully synthesized and incorporated into the backbone of the PNIPA hydrogel by copolymerizing in ethanol. Both monomer and poly(NIPA-co-AAc-L-Phe) gels were characterized by using IR spectra and ¹H-NMR. The swelling and deswelling behaviors of hydrogels responding to external stimuli were studied, and poly(NIPA-co-AAc-L-Phe) hydrogels showed evident temperature-sensitivity.

Compared with PNIPA hydrogel, poly(NIPA-co-AAc-*L*-Phe) hydrogel exhibits better absorption property and enantioselectivity for D/L-phenylalanine. The thermosensitive poly(NIPA-co-AAc-*L*-Phe) hydrogel have potential applications the biological chemistry.

Acknowledgements

I am grateful to my respectful supervisor, Professor Chen for her encouragement and support, at the same time, thanks the National Natural Science Foundation of China for the financial support.

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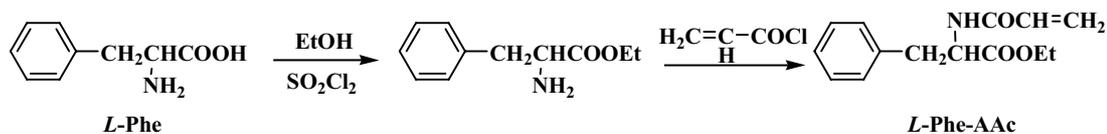


Figure 1. The synthetic scheme of AAc-*L*-Phe monomer

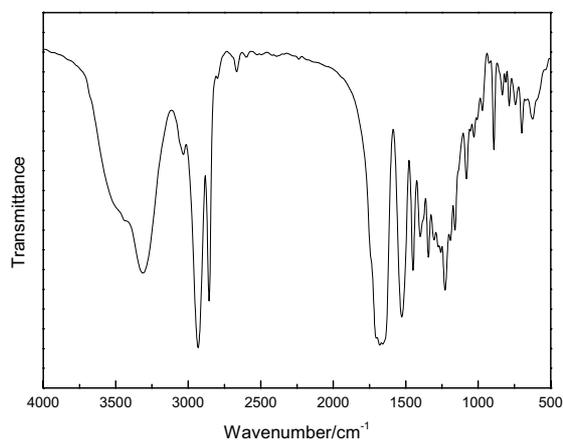


Figure 2. Infrared spectra of AAc-L-Phe monomer

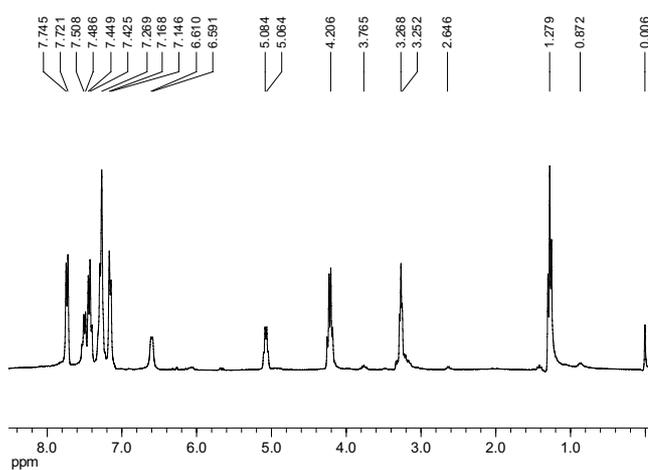
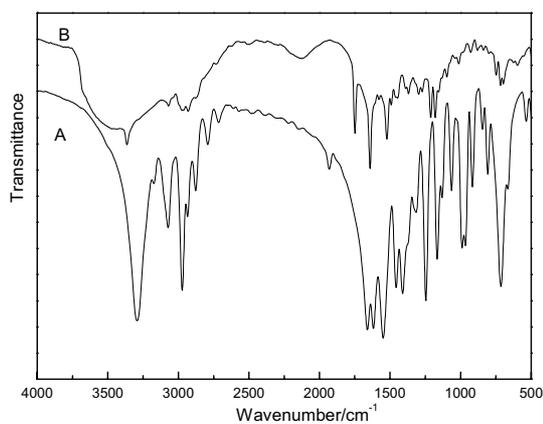
Figure 3. ¹H-NMR of AAc-L-Phe monomer

Figure 4. Infrared spectra of hydrogels

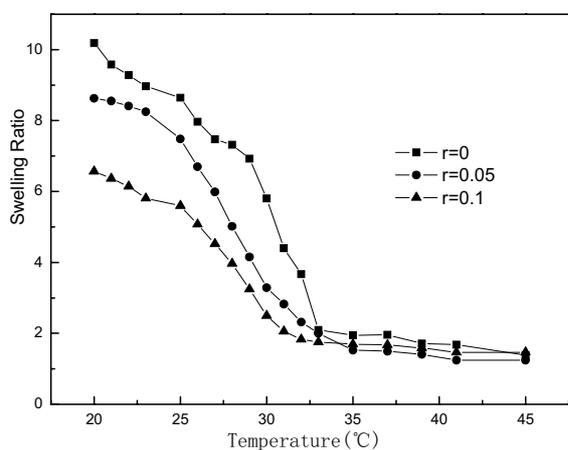


Figure 5. Temperature dependence of equilibrium SR at the temperature range from 20 to 45°C

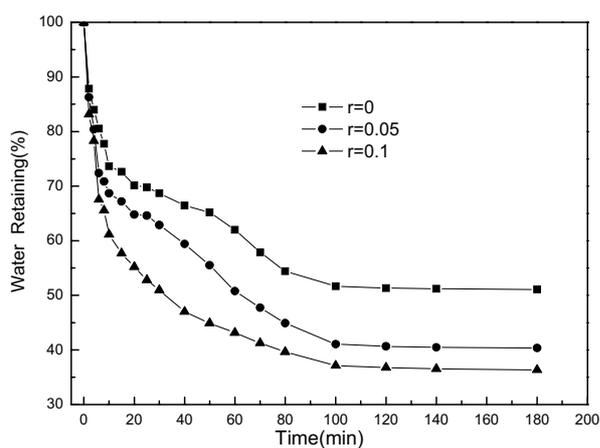


Figure 6. Deswelling kinetics of hydrogels in distilled water

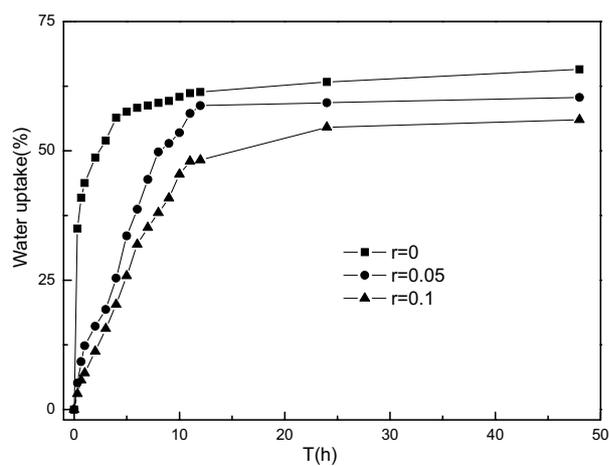


Figure 7. Reswelling kinetics of hydrogels at 25°C in distilled water