Synthesis and Characterization of a Thermo-sensitive Poly(*N*-propionyl-aspartic acid-*co*-dimethyl tartrate) with no cytotoxicity

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Abstract: In this paper, we used *L*-aspartic acid, dimethyl tartrate and propionic anhydride as raw materials to prepare a new thermo-sensitive poly(*N*-propionyl-*L*-aspartic acid-*co*-dimethyl tartrate) (PPADT). The chemical structures of the intermediates, monomer and polymer have been confirmed by FTIR and ^{1}H NMR measurements. UV spectrum data showed the thermo-sensitivity of PPADT and its UCST was tunable via changing the ratio of methanol and water. It was consistent with the phenomenon of reentrant phase separation. Besides, we found that PPADT had no cytotoxicity. The survival rate of HeLa cells was more than 85% in all cases. The results suggested that poly(*N*-propionyl-*L*-aspartic acid-*co*-dimethyl tartrate) was a new promising material with good biocompatibility and thermo-sensitivity.

Introduction

Due to its academic value and potential applications, the temperature-sensitive polymer has attracted more and more attention. As an important member of the temperature-sensitive materials, polymers which have an upper critical solution temperature (UCST) can be completely dissolved in a solvent above the UCST, while below the UCST, the polymers are insoluble in the solvent. Some temperature-sensitive polymers with UCST have been reported, such as poly[*3*-dimethyl (methacrylamide) propanesulfonate] [1] in pure water. Additionally, poly(*N*-acryloyl glycinamide) (PNAGA) (in pure water) [2] demonstrats a significant phenomenon of UCST. Temperature-sensitive materials can be used in smart bioactive surface, immunoassays, protein and drugs trafficking, tissue engineering and other fields [3-5]. Thus, there should be no rejection in body when these materials are applied. Namely, the temperature-sensitive polymers with no cytotoxicity have a great potential application in the biomedical fields.

Experiment

Materials. *L*-aspartic acid, propionic anhydride, anhydrous methanol and sodium hydroxide were purchased from Kermel chemical reagent (Tianjin, China). Dimethyl tartrate was supplied from TCI(Shanghai)Development Co., Ltd. Oxalyl chloride from Tianjin Huadong reagent factory was distilled before used. Concentrated hydrochloric acid was purchased from Tianjin Guangfu Fine Chemical Research Institute. *N*,*N*-dimethylformamide, acetone, ether and petroleum ether were purchased from Kermel chemical reagent (Tianjin, China) and purified before used. Moreover, HeLa cells were purchased from Peking Union Medical College Hospital.

Preparation of *N*-propionyl-aspartic acid. Firstly, 13.31 g aspartic acid (0.1 mol) and 50 mL NaOH solution (4 mol/L) were added into a 250 mL three-neck round bottom flask. The reaction needed to be carried out at 0 ℃ in ice-water bath. After stirring 1 hour, aspartic acid completely dissolved in the NaOH solution. Then, propionic anhydride (16.918 g, 0.13 mol) and NaOH solution (4 mol/L, 40 mL) were simultaneously added into the system slowly to control the reaction system pH between 8 and 10. The dropping continued for 2 hours. After dropping, stirring for 2 hours was performed in the ice bath. Finally concentrated hydrochloric acid was added into the solution to adjust the pH (1~3). Then, the flask with the reaction solution was cooled for crystallization. After filtrating and drying, we got white titled powder, *N*-propionyl-aspartic acid.

Preparation of *N*-propionyl-aspartic acid chloride. We put 1.2 g *N*-propionyl-aspartic acid (6.35 mmol) and 8 mL acetone in a 100 mL single-necked flask. The reaction system was placed in ice bath (0 ℃) for cooling. After few minutes, 8 mL (84.14 mmol) oxalyl chloride and a drop of DMF as catalyst were added. After 4 hours, 70 mL dried petroleum ether was added drop wise into the flask in ice bath. The precipitated supernatant was poured and then washed for 3 times with dried petroleum ether. Finally, the powder, *N*-propionyl-aspartic acid chloride, was dried under reduced pressure.

Preparation of PPADT. In this study, melting polycondensation was performed to synthesize poly(*N*-propionyl-*L*-aspartic acid-*co*-dimethyl tartrate) (PPADT). 0.4523 g *N*-propionyl-aspartic acid chloride (2 mmol) and 0.3563 g dimethyl tartrate (2 mmol) were weighed accurately and added into a large test tube with a rubber stopper and a syringe needle. The condition of polymerization was listed in Tab.1.

Tab.1 The condition of preparation of PPADT

Item	Temperature/℃	Time/h
1	75	4-6
2	85	5-6
3	95	7-8
4	105	12
5	115	12

After the polymerization, 5 mL anhydrous methanol was added into the test tube and the reaction system was heated to 70 ℃. After the polymer fully dissolved, the solution was added drop wise into anhydrous ethyl ether. The precipitate was dried for 24 h. The synthetic route of PPADT was shown in Fig. 1.

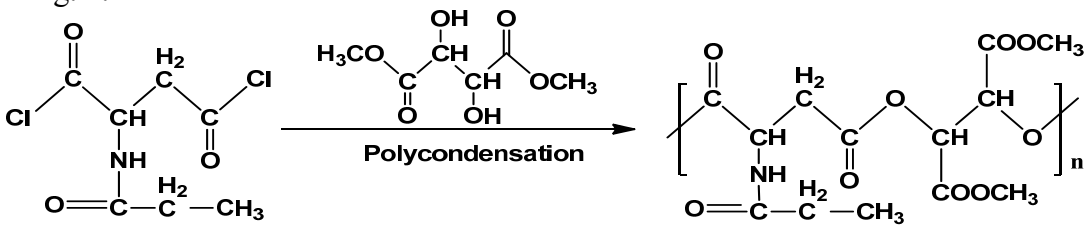

Fig. 1 The synthetic route of PPADT

Results and Discussion

The FTIR spectrum of the PPADT. The FTIR spectrum of the PPADT was shown in Fig. 2. The peaks appearing at 3364 cm^{-1} and 1534 cm^{-1} could be attributed to the amide group (-NH-) stretching vibration and bending vibration, respectively. The stretching vibration of methylene was observed at 2963 cm^{-1}. The peak appearing at 1752 cm^{-1} was owned to the stretching vibration of ester group (C=O). The peaks corresponding to absorption vibration of the C-N in the outer surface appeared at 1284 cm^{-1}.

The ^{1}H NMR spectrum of the PPADT. The ^{1}H NMR spectrum of PPADT in $DMSO_{-6d}$ was shown in Fig. 3. The signals for methyl (a) and methylene (b) in the structural units of *N*-propionyl-aspartic acid were observed at 1.02 ppm (a) and 2.12 ppm, 2.41 ppm (b), respectively. The signals at 2.85 ppm and 4.67 ppm were found to be H atoms from the methylene and methine in the main chain of *N*-propionyl-aspartic acid units. Moreover, the peaks at 3.70 ppm and 5.67 ppm demonstrated methyl and methine from dimethyl tartrate units, respectively. The peak ascribing to H atom from amide group was observed at 8.46 ppm. Additionally, the ratio of hydrogen atoms was in good agreement with the structure of PPADT. The above-mentioned spectral data showed that we had prepared the titled PPADT.

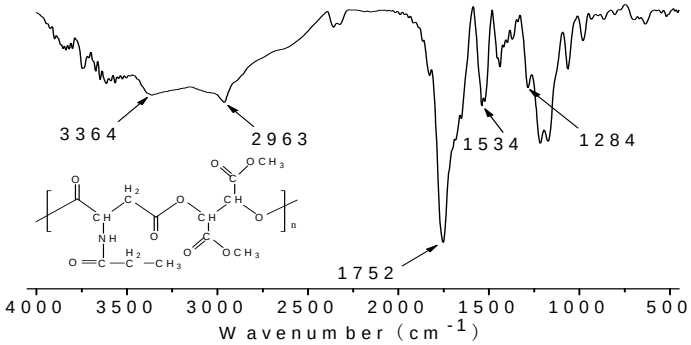

Fig. 2 The FTIR spectrum of the PPADT

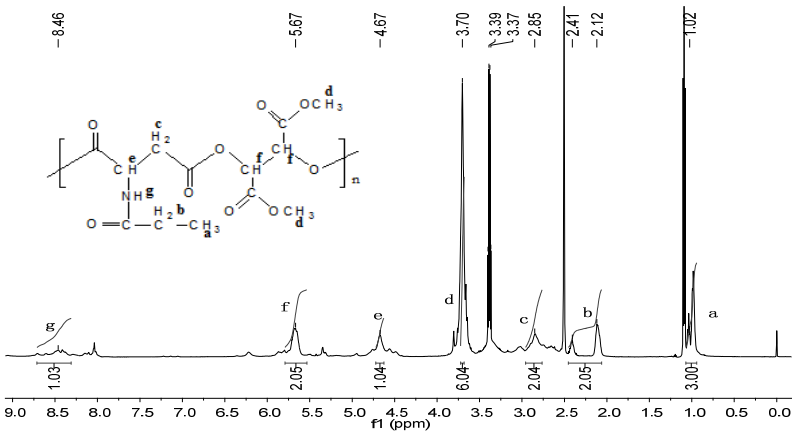

Fig. 3 The ^{1}H NMR spectrum of the PPADT

Thermo-sensitive Study. Fig. 4 showed the phase transition curves of PPADT. The UCST of PPADT (0.6 wt. %) in methanol-water mixture with methanol content 0%, 10%, 30% and 50% were 6.9 ℃, 35.6 ℃, 26.1 ℃ and 6.1 ℃, respectively. The UCST increased before reducing which coincided with the phenomenon of reentrant phase separation. Some scholars believe that methanol molecules have effect directly on the hydrophobic group from temperature-sensitive molecules with the increasing of methanol content. The environment between the polymer chains and solvent molecules is changed which results in that the temperature-sensitive polymer is dissolved in the methanol-water mixture.

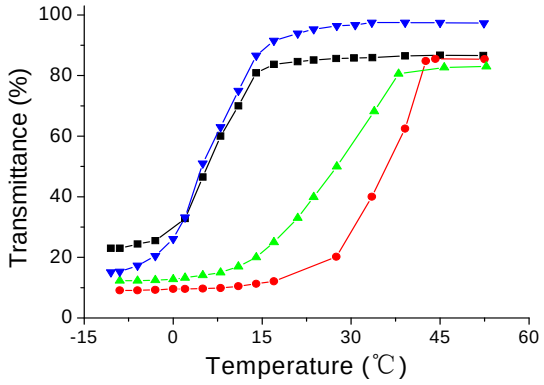

Fig.4 The phase transition curves of PPADT in methanol-water mixture (methanol content: ■ 0%, ● 10%, ▲ 30%, ▼ 50%).

Cytotoxicity Evaluation for PPADT. The MTT assay was used to detect the cytotoxicity. The result was shown in Fig. 5. The concentrations of PPADT were 0.01, 0.1, 1, 10, 100 μg/mL and the

cultivation continued 24 h, 48 h and 72 h. When the concentration of PPADT was controlled as 0.01 μg/mL, the cell viability remained growth in the state. The lowest viability was still about 85% even in the 100 μg/mL solution for 48 h incubation. The data showed that the PPADT was no cytotoxicity.

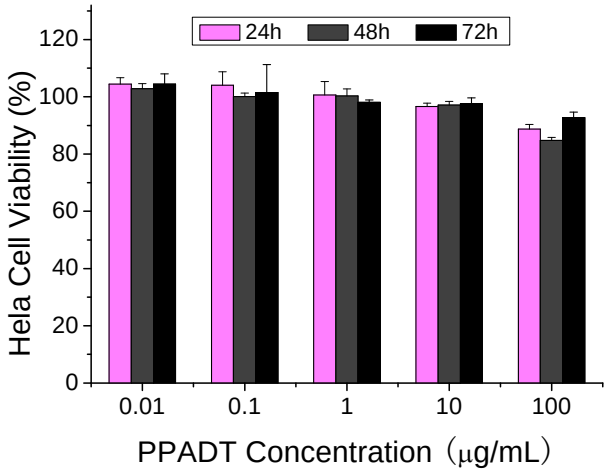

Fig.5 Viability of HeLa cells after 24, 48, and 72 h incubation

Conclusions

In this investigation, we synthesized a new temperature-sensitive PPADT using *L*-aspartic acid and dimethyl tartrate as raw materials. UCST of PPADT was regulated by changing the ratio of methanol and water simply. When the methanol content was 30%, the UCST of the polymer was at about 36 ℃ which was close to body temperature. The data from MTT assay showed PPADT was no cytotoxicity. Due to the good temperature-sensitivity and no cytotoxicity, PPADT will be a promising polymer in the biomedical application fields.

Acknowledgements

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