Temperature and pH Sensitive Star-Shaped Material for the Controlled Release of Coenzyme A

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ABSTRACT: A novel star-shaped copolymer was prepared by tolulene diisocyanate (TDI) trimer, poly(ethylene oxide) (PEO), P(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), and 2-hydroxyethylacrylate (HEA) with TDI trimer as core and PEO, PDMAEMA as arm chains by addition of functional groups and copolymerization. The structure of the star-shaped copolymer was characterized by FTIR, ^{1}H NMR, and UV, and the molecular weight and polydispersity were determined by gel permeation chromatography (GPC). Their aqueous solution properties and controlled coenzyme A (Co A) delivery were also studied. The results showed that the copolymer had better temperature sensitivity and pH sensitivity. The release of Co A from the copolymer was dependent on the release medium such as concentration of the polymer, pH, and temperature in the aqueous solution. The higher concentration of the copolymer absorbed more Co A than the lower one. The increasing temperature accelerated Co A release from the copolymer. The pH of the solution had significant impact on the release of Co A from the copolymer. The results suggested that the novel copolymer could be used as a drug delivery carrier. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1279–1284, 2007

Key words: water-soluble polymers; biopolymers; star-shaped copolymer; stimulisensitive polymers; drug delivery systems

INTRODUCTION

Polymers and their complexes, which are sensitive to environmental stimulation, such as temperature,[1] pH,[2] ionic strength,[3] magnetic field,[4] and ultraviolet light[5] have promising potential applications in the fields of drug delivery system, separation, enzyme and cell immobilization, sensors, and so on.[6–11] However, biological applications need materials with good biocompatibility.[12,13]

Poly(ethylene oxide) (PEO) is a biocompatible nonionic water-soluble polymer. Polymer including PEO received growing attention for applications in various technologies including medical applications such as wound dressing, controlled release drug systems, and others.[14]

P(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) is a widely used polymer with thermo- and pH-sensitivity at the same time.[15,16] It can be used as drug delivery system, artificial skin, and contact lens in biomedical materials.[17]

In the past 20 years, drug delivery system has become an important research field of medicine. Self-correcting drug release system can control drug release by the information feedback from the body without the outside intervenor. The drug release rate was affected by the changes of pH or temperature, which is one of the important way.[6] So, the novel star-shaped copolymer with temperature sensitivity and pH sensitivity seems to be of great promise in drug delivery system.

In this article, a novel kind biodegradable star-shaped copolymer was synthesized with TDI trimer as core and methoxy capped PEG 2000 and PDMAEMA as arm chains. The structure, temperature, and pH sensitivity in aqueous solution and its controlled release on coenzyme A (Co A) were discussed.

EXPERIMENTAL

Materials

TDI trimer was purchased from Bayer Coating System, Shanghai. Methoxy-capped PEG2000 (MeO-PEG2000) was obtained from Shanghai Reagent Chemical, China. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), 2-hydroxyethylacrylate (HEA), and coenzyme A (Co A) were all purchased from Aldrich. AIBN was obtained from Peking chemical industry, China, and was recrystallized before use. All other chemicals used were of analytical grade, and were used without further purification.

Synthesis of the star-shaped copolymer

TDI trimer, MeO-PEG, and HEA were put into the dry flask with molar ratio NCO:MeO-PEG:HEA = 3 : 2 : 1 and

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2-butanone as solvent. After stirring for 6 h at 75°C, DMAEMA as monomer and AIBN as initiator were added into the flask. After stirring for 4 h at 80°C, the mixed solution was precipitated with 200 mL of ether, and then the product was centrifuged and dried under vacuum. The polymer was reprecipitated and dissolved for 3 times. Finally, the copolymer was dried in vacuum oven at 50°C.

Polyurethane (PU) has been found a wide range of application in medical aspect because of its good biological compatibility, excellent mechanical properties, easy processing, and controlled properties.[18–21] Here, to further confirm the safety of the star-shaped copolymer in biomaterials, we determine the content of TDI in the copolymer by the method of ion chromatography/DC ampere according to the literature.[22] Under the limit of detection, TDI was not detected.

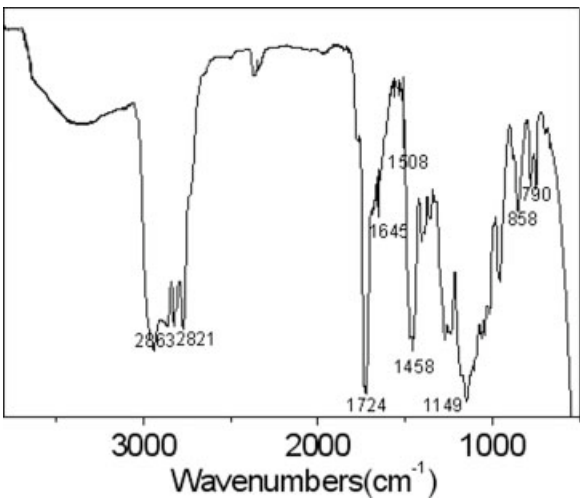

Figure 1 IR spectra of star-shaped copolymer.

Determinination of temperature and pH sensitivity of the polymer

The transmittance of the copolymer solution was measured by 721-spectrophotometer (Shanghai, China) switched to transmittance regime at $\lambda = 640$ nm using a thermostated holder at different temperature or pH. The transmittance of the solution changed with the increase in temperature. When the transmittance of the solution decreases violently, microphase separation occurs. The temperature corresponding to the turning point of the solution is the solution's lower critical solution temperature (LCST). According to the transmittance-temperature curve, we can determine the temperature and pH sensitivity of the polymer solution.

The delivery of Co A from the copolymer

The copolymer and Co A mixed solutions at different pH values or concentrations buffer were placed at room temperature for 24 h for the sufficient combination between the copolymer and Co A. Then, the UV spectra of the mixed solution were recorded with a UV–vis spectrophotometer (UV-540, US). The solvent of the sample solution and reference solution are the same, and so the effect of the solvent can be counteracted. According to the changes of height of characteristic absorption peak of Co A at 260 nm in the copolymer aqueous solution, we determine the release or control on Co A.

RESULTS AND DISCUSSION

Analysis of IR spectra of the star-shaped polymer

IR spectra were recorded using KBr pellets on AVATAR-360FTIR. The IR spectra of the star-shaped copolymer were shown in Figure 1. The curve showed signals at 1508 and 858 cm^{-1}, and 790 cm^{-1} in the fingerprint region for the phenyl stretching. The curve revealed a signal of C=O stretching at 1724 cm^{-1}, which is assigned to ester in HEA and DMAEMA. The peak around 1149 cm^{-1} was attributed to the characteristic absorption of $—CH_2—O—CH_2—$ on the arm chains. The spectra showed peaks assigned to $N—CH_3$ at 2821 cm^{-1} and the peak at 1645 cm^{-1} is attributed to amide. The IR spectra also showed the presence of $—CH_3$ at 1454 cm^{-1}. All the above-mentioned characterization showed that we synthesized the star-shaped copolymer. In addition, there is no peak between 2230 and 2280 cm^{-1} assigned to —NCO, which indicated that —NCO was completely consumed in the reaction. So, there is no need to worry about the safety of TDI in biomaterials.

Analysis of ^{1}H NMR spectra of the copolymer

To further determine the structure of star-shaped copolymer, the copolymer was analyzed by nuclear magnetic resonance spectrometer (EW360L, 400 MHz) by using D_2O as solvent. The ^{1}H NMR spectra of the star-shaped copolymer was shown in Figure 2. All chemical shifts are given in parts per million relative to the solvent signal. The chemical shift of H atom in the copolymer was as follows: $\delta_1 = 3.24$ ppm (3H, CH^*_3), $\delta_2 = 4.45$ ppm (2H, $CH^*_2—O—$), $\delta_3 = 8.06$ ppm (1H, NH*), $\delta_4 = 7$–7.8 ppm (3H, Ar—H*), $\delta_5 = 3.77$ ppm (2H, CH^*_2), $\delta_6 = 0.96$–1.21 ppm (3H, CH^*_3), $\delta_7 = 2.34$ ppm (2H, $N—CH^*_2$), and $\delta_8 = 2.22$ ppm (2H, CH^*_2). The chemical shift of H atom in the copolymer was well accordance with literature. All the above-mentioned data showed that the copolymer was the target star-shaped copolymer.

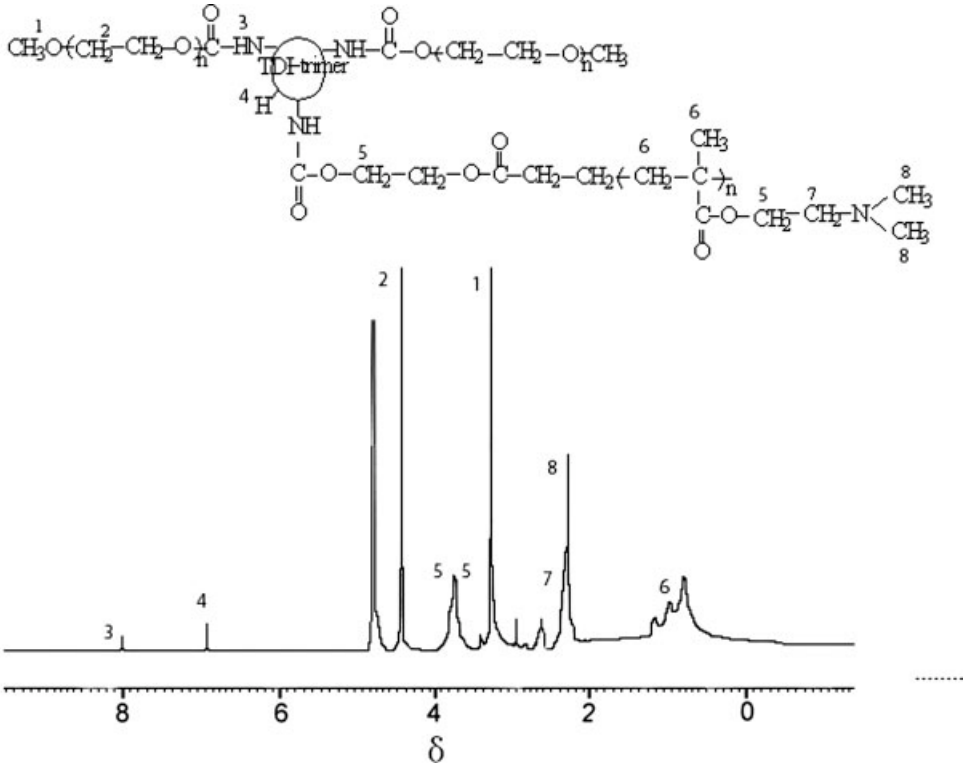

Figure 2 The ^{1}H NMR spectra and schematic structure of the star-shaped copolymer.

Molecular weight and polydispersity of the star-shaped copolymer

The molecular weight and polydispersity (PDI) were determined by gel permeation chromatography (GPC; Wyatt Technology). The mobile phase was THF at flow rate of 1.0 mL/min. Calibration was monodisperse PS standards. The GPC diagrams of the copolymer are shown in Figure 3. It was found that molecular weight of the star-shaped copolymer ($\overline{M}_n$) was 3.66×10^4, and PDI of the star-shaped copolymer was 1.54.

Temperature sensitivity of the star-shaped copolymer solution

The copolymer was dissolved in water with 0.5, 1, 1.5, and 2 wt %, respectively. The transmittance-temperature curves of the solution were shown in Figure 4. In Figure 4, curves a, b, c, and d revealed turning point between 42 and 54°C. The temperature corresponding to the turning point is the solution's LCST. This may be because of the copolymer containing a lot of $—N(CH_3)_2$ and $(CH_2—CH_2—O)_n$ groups. As a result, the hydrophilicity of the copolymer increased, microphase separation occurs at higher temperature.

We also found that different concentration of the copolymer solution has different LCST and the sensitivity of the phase separation is also varied. The 0.5 wt % solution changed most sensitively. The rule

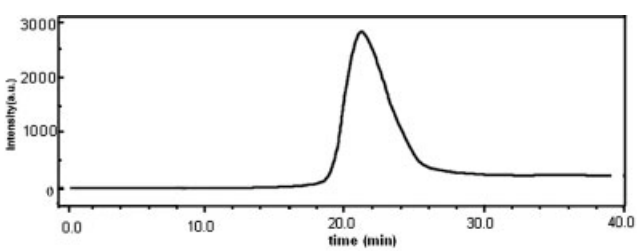

Figure 3 GPC diagram of the star-shaped copolymer.

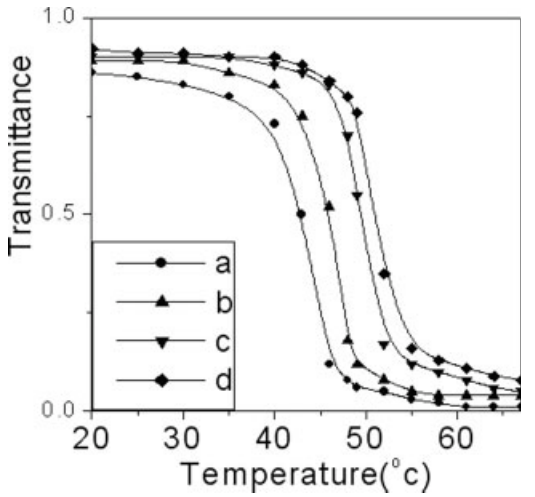

Figure 4 Effect of temperature on transmittance of star-shaped copolymer in aqueous solution, pH = 7; concentration (wt %): (a) 2; (b) 1.5; (c) 1; (d) 0. 5.

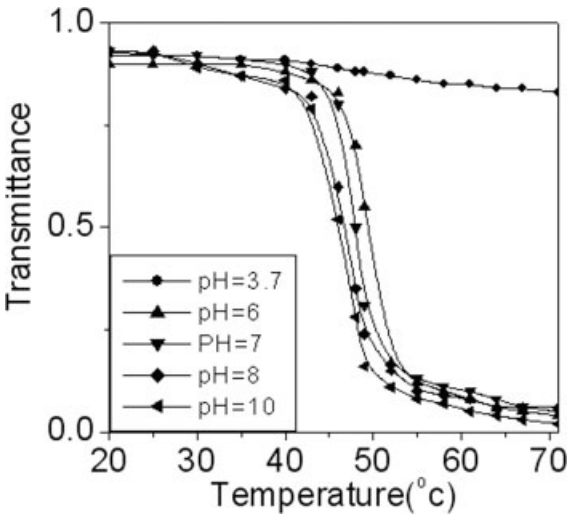

Figure 5 pH dependence of transmittance for the different pH values copolymer solution, C: 1 wt %.

is that the lower the concentration, the more sensitive the solution. This may be because with the copolymer concentration increasing, the concentration of segment is also increasing. The free movement of segment is restricted, and hydrophobic association between macromolecules was strengthened to a certain extent. The micelle formed at the lower temperature, and so the sensitiveness of phase change declined.

pH sensitivity of star-shaped copolymer solution

To determine the effect of pH on the LCST of the copolymer, buffers with the same ionic strength ($I = 0.5$) and various pH values were used in this work. The 1 wt % solution at different pH buffers was to be tested. The charge of the investigated polymer system in aqueous solution is determined by the pK_a value of the polymer blocks. The pK_a value of the PDMAEMA block was reported to be approximately pK_a (PDMAEMA) $\approx$ 8.[23,24] Depending on the pK_a value, the investigated pH range can be divided into two different regimes. Under condition below pH < 8, PDMAEMA was positively charged, but with pH value changing, the degree of ionization of PDMAEMA was also changing. Under alkaline condition with pH values higher than pH > 8, PDMAEMA was nearly uncharged. Figure 5 shows that with the increasing of pH, the LCST of polymer solution varied. Under acid condition at pH = 3.7, $—N(CH_3)_2$ on the arm chains formed amine salt, and the solubility of the copolymer greatly increases so as to no phase separation occurs. At pH = 6 slight acid solution, the degree of ionization of $—N(CH_3)_2$ decreased and the hydrophilicity of the macromolecule became weaker than strong acid solution (pH = 3.7). At pH = 7 neutral solution, the degree of ionization of PDMAEMA further decreased. At pH = 8 value solution, the copolymer is nearly uncharged. All the function groups in the macromolecule could form H bond between intramolecule and intermolecule, and so the hydrophobic association between macromolecules greatly enhanced. Under alkaline condition (pH = 9), the hydrophilicity of the macromolecule is almost the same for the case of pH 8 solutions. As discussed earlier, with the increasing of pH, the hydrophilicity of the macromolecule decreased, and at pH > 8 values, the hydrophilicity of the macromolecule was almost unchangeable. So, LCST of the copolymer solution could be understandable.

Star-shaped copolymer for controlled release of coenzyme A

To assess the polymer's use in biomedical application, we use the copolymer to control the release of Co A at different experimental conditions.

Effect of concentration

The UV spectrum of Co A in buffer solution at pH 7 under room temperature was shown in Figure 6. The concentration of Co A was 0.5 wt % and the concentration of copolymer was 0.05, 0.025, 0.01, 0.001, and 0 wt % as shown in Figure 6 as curves a, b, c, d, and e, respectively. In Figure 6, the characteristic absorption peak of Co A in the copolymer aqueous solution is higher than that in water without the copolymer, and the absorbance of Co A increased with the increasing concentration of the star-shaped copolymer. This indicated that the copolymer could adsorb Co A at different concentrations. This is probably because of $(CH_2—CH_2—O)_n$ and

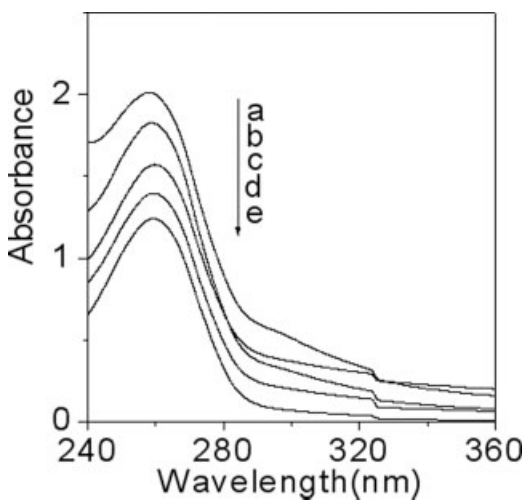

Figure 6 UV absorbance of coenzyme A controlled delivery at different concentration of copolymer solution. pH = 7, [Co A] = 0.5%, concentration of copolymer (wt %): (a) 0.05; (b) 0.025; (c) 0.01; (d) 0.001; (e) 0.0.

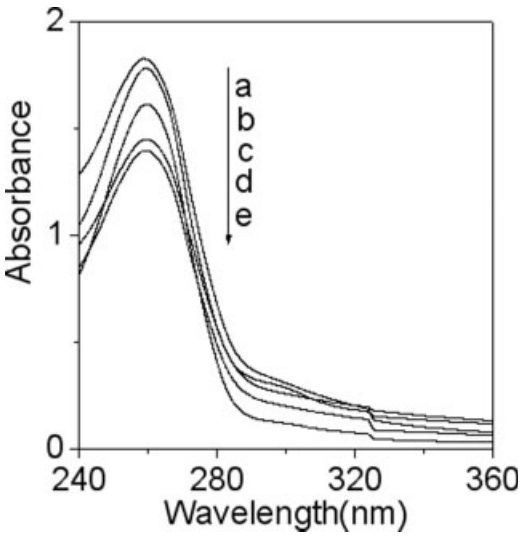

Figure 7 UV absorbance of coenzyme A controlled delivery in the copolymer solution at different temperature. pH = 7, [Co A] = 0.5%, [copolymer] = 0.025%, temperature (°C): (a) 25; (b) 35; (c) 45; (d) 55; (e) 65.

$—N(CH_3)_2$ groups in the copolymer combined with the phosphoric group of Co A, which reduced the phosphoric group combining with the N atom in pyridine. The electron supplying capacity of pyridine increased, and so the characteristic absorbance peak of Co A increased. We can determine proper concentration according to the height of the peak when the copolymer was used as drug carrier.

Effect of temperature

Curves a, b, c, d, and e in Figure 7 were the absorbance curves of Co A in the copolymer solution (pH = 7, concentration is 0.025 wt %, [Co A] = 0.5%) under different temperature. It was found that the absorbance peak decreased with the increase in temperature. When the temperature increased from 35 to 45°C and from 45 to 55°C, the peak decreased greatly. The Co A was largely released. Two essential reasons seem to regulate the release behavior. One is that DMAEMA on the arm chains violently shrink when the temperature is above LCST of the solution. The conformation of the macromolecule changed and $(CH_2—CH_2—O)_n$ chain was shielded from shrinking arm chain DMAEMA, and so the combination of $(CH_2—CH_2—O)_n$ with Co A reduced. The other reason is that the H bond between macromolecular and Co A was broken with the increase in temperature. The combination of macromolecular and Co A decreased. At pH = 7 solution, Co A was negatively charged. The ionic complex of the negative charge of Co A and $—NH^+(CH_3)_2$ also becomes weaker with the increase in temperature. As a result of the above-mentioned reason, the Co A was largely released from the copolymer. The copolymer is potentially used to control the release of biomacromolecule by changing the temperature.

Effect of pH

In Figure 8, curves a, b, and c were the absorbance of Co A in the copolymer solution at different pH values [(a) pH = 7; (b) pH = 9; and (c) pH = 3.7, the copolymer concentration was 0.01 wt %, and [Co A] = 0.5%]. Curve d was the absorbance of Co A in water without the copolymer. According to Figure 8, the characteristic absorption peak of Co A in the copolymer solution is higher than that in solution without copolymer. That is to say that the copolymer can adsorb Co A in all pH values. Among curves a, b, and c, the absorbance peak of Co A is the lowest when pH is 3.7, and when pH is 7, the absorbance peak is the highest. That is to say that the effect of pH of the copolymer solution on the controlled release of Co A is considerable. We also found that the peak difference between pH 7 and pH 9 is notable. That is to say, when pH of solution was changed from 7 to 9, the Co A is largely released. We can use the copolymer to control the release of Co A at different pH values. This can be explained by the different conformation of the macromolecular at different experimental conditions and the effect between the macromolecular and Co A. At pH = 7 solution, Co A was negatively charged. The copolymer can absorb a large amount of Co A by the electrostatic attraction between the negative charge of Co A and $—NH^+(CH_3)_2$ groups in the copolymer. As what was discussed earlier, the copolymer was nearly

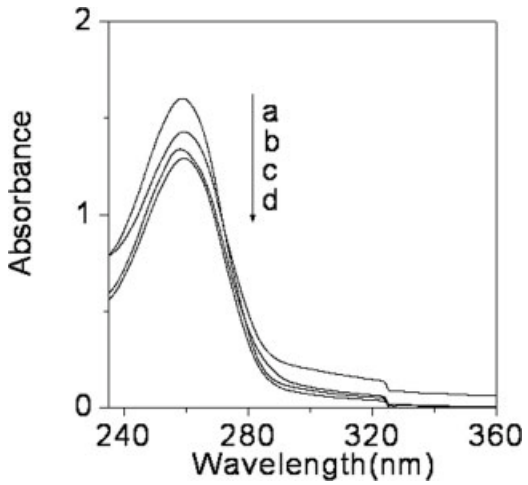

Figure 8 UV absorbance of coenzyme A controlled release in the copolymer solution at different pH values with [Co A] = 0.5% and [copolymer] = 0.01%, pH: (a) pH = 7; (b) pH = 9; (c) pH = 3.7; (d) UV absorbance in water without copolymer.

uncharged at pH = 9 solution. Co A was absorbed by the copolymer though H bond between $(CH_2—CH_2—O)_n$, $—N(CH_3)_2$ and phosphoric groups. At pH = 3.7 solution, there are two effects between the copolymer and Co A. On one hand, the electrostatic repulsion between $—NH_3^+$ and $—NH^+(CH_3)_2$ exist in the system. On the other hand, there are H bond between $(CH_2—CH_2—O)_n$ and phosphoric groups. But the electrostatic repulsion was dominant in this system. So, at pH = 3.7 solution, the amount of the absorbed Co A was the least, and the UV absorbance was the lowest.

CONCLUSIONS

In this article, we used functional monomer DMAEMA, PEG, and TDI trimer to prepare novel star-shaped copolymer. The structure of the star-shaped copolymer was characterized by FTIR, ^{1}H NMR, and UV, and the molecular weight and PDI were determined by GPC. It was found that the star-shaped polymer had temperature sensitivity and pH sensitivity. It can be used to control the release of Co A at different temperature and at different pH. So, it seems to be of great promise in drug delivery systems.

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