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Dually Stimuli-Responsive Hyperbranched Polyethylenimine with LCST Transition Based on Hydrophilic–Hydrophobic Balance

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ABSTRACT: Dually stimuli-responsive hyperbranched polyethylenimine derivatives (HPEI-star-PPOs) were successfully synthesized through Michael addition of commercial HPEI, poly(propylene oxide) dimethacrylate, and 2-mercaptoethanol. In aqueous solution, these HPEI-star-PPOs exhibited response to temperature and pH. The corresponding lower critical solution temperature (LCST) could be readily adjusted by changing the feed ratio of HPEI to PPO, which also presents the ratio of hydrophilicity to hydrophobicity, indicating that this LCST transition is based on hydrophilic–hydrophobic balance. Because of the tertiary amine as well as unreacted primary or secondary amine that can be protonated during pH decreases, HPEI-star-PPOs exhibited a pH-dependent thermoresponse. The 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay against COS-7 cells demonstrated that HPEI-star-PPO had relatively low cytotoxicity compared to HPEI. All these characteristic suggest that this stimuli-responsive polymer is a promising functional material for biomedical applications. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: hydrophilic–hydrophobic balance; hyperbranched polyethylenimine; temperature-; and pH-responsive

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INTRODUCTION

Because stimuli-responsive polymers respond to environmental stimuli, such as pH, temperature, light, redox, ionic strength, and so on,[1–5] they have attracted much attention and shown great potential in various biomedical applications, including smart drug/gene delivery, cell culture, tissue engineering, and biosensors.[6–15] Water-soluble thermoresponsive polymers are one of the most appealing stimuli-responsive species, which are absolutely soluble in water below their low-critical solution temperature (LCST), and a phase transition to insoluble state would occur at an increased temperature. Especially, the polymers whose LCST is around 37°C, close to body temperature, have gained much attention. It was well realized that temperature of target sites in the body can be changed sagely by hyperthermia.[16] Meanwhile, pH in some tissues and cellular compartments is lower than the normal physiological pH.[17,18] During the recent years, a large number of temperature- and pH-responsive polymers have been prepared, and the corresponding properties have also been well investigated.[19–26] Hudson[1] has summarized two important types of thermoresponsive polymers: type I is based on LCST and type II is based on the balance of hydrophobicity and hydrophilicity. Poly(*N*-isopropylacrylamide) (PNIPAM) is a representative thermoresponsive polymer of type I.[27–32] Because of the hydrogen-bonding interactions between the polymer and water molecules, the polymer chains are in random coil conformation and totally soluble in water when the temperature is below LCST. As the temperature increases, these interactions will be broken gradually, and the polymer chains collapse into globule conformation and precipitate from the solution. During this process, an intramolecular coil to globule transition occurs. Besides PNIPAM, PDMAEMA [poly(2-(dimethylamino)ethyl methacrylate)] and PDEAM [poly(*N*,*N*-diethylacrylamide)] also belong in this type.[33–37] Different from type I, the LCST transitions of type II polymers originate from intermolecular interactions, such as hydrogen bonding and hydrophobic interactions, instead of intramolecular interactions. During the LCST transition, the balance of hydrophobicity and hydrophilicity is broken, resulting in

Additional Supporting Information may be found in the online version of this article.

intermolecular aggregation. The type II thermoresponsive polymers are mostly amphiphilic copolymers, such as PEO-*b*-PPO [poly(ethylene oxide)-*block*-poly(propylene oxide)], PEO-*b*-PLA [poly(ethylene oxide)-*block*-polylactide], and other amphiphilic copolymers.[38–42]

Polyethylenimine (PEI), a polycation with high-positive charge density, allows the condensation of DNA into very small particles, which facilitates the endocytosis as well as preventing the DNA from endosomal disruption due to its high-protonation capacity, and has recently been investigated and used as a nonviral gene therapy delivery agent.[43–46] Both linear and hyperbranched PEI (LPEI and HPEI) have been reported to be used successfully to transfect a variety of cells including cell lines and primary cells *in vitro* and *in vivo* and shown high-transfection efficiency.[47–49] To endow PEI with the stimuli-responsive property, several groups have made great efforts. Pişkin and coworkers[50] incorporated carboxyl-ended PNIPAM and HPEI into a copolymer, which is temperature- and pH-responsive. Chen and coworkers[51,52] described another approach to stimuli-responsive hyperbranched PEI by *N*-acylation of its terminal amine groups. Because terminal group had similar molecular structure with NIPAM and HPEI contained multiple tertiary amine groups inside, *N*-acylated HPEI gained temperature- and pH-responsive. And the thermoresponse can be controlled by degree of acylation. In both cases, the modified HPEIs are all type I polymer whose thermoresponsive transition originates from intramolecular coil-to-globule transition of polymer chains.[1] However, type II thermoresponsive HPEI has not been reported and is still very attractive to investigate its feasibility and responsive behavior.

In the present work, double stimuli-response hyperbranched polyethylenimine (HPEI) derivative was synthesized successfully by introducing hydrophobic poly(propylene oxide) chains into hydrophilic polyethylenimine via Michael addition. The resulting polymer, HPEI-star-PPO, shows thermoresponsive behavior, which is based on the balance of hydrophobicity and hydrophilicity, and belongs to type II thermoresponsive polymer mentioned previously. By changing the molar ratio of HPEI and PPO, the LCST value can be well adjusted. Because this polymer contains multiple tertiary amine groups, it maintains the pH response. The dependence of the thermoresponse on pH is also discussed in detail in this work.

EXPERIMENTAL

Materials

HPEI [M_n = 10,000, PD (M_w/M_n) = 2.5, and degree of branching (DB) = 0.61[43,53]; Supporting Information), poly(propylene oxide) dimethacrylate (PPO-DM, M_n = 560), and 2-mercaptoethanol were all purchased from Sigma-Aldrich. Anhydrous ethanol was purchased from Pharmco-aaper. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich. Clear polystyrene tissue culture treated 96-well plates was obtained from Coring Costar. All reagents were used without further purification.

Synthesis of HPEI-Star-PPOs

HPEI-star-PPOs were synthesized by Michael addition reaction in one-pot two-step process. In a typical reaction procedure, PPO-DM (M_n = 560) and 2-mercaptoethanol were dissolved in 50 mL of anhydrous ethanol, respectively. The solutions of PPO-DM were added to a 250-mL three-necked flask equipped with a nitrogen inlet tube. In the first-step reaction, the 2-mercaptoethanol/ethanol solution was added into the PPO-DM/ethanol solution by dropwise, under an equal molar ratio of 2-mercaptoethanol to PPO-DM. The mixture was stirred at room temperature for 24 h. Then the ethyl alcohol solution of HPEI (50 mL) was added into the flask for the second-step reaction.

After the mixture was stirred for 48 h, most of the solvent was removed by reduced pressure distillation, and the mixture was enclosed in dialysis bag (MWCO = 10 kDa) and purified by dialyzing in 2-L cold deionized water for 96 h in order to remove the low-molecular weight impurities. The deionized water was exchanged for several times. After dialysis, the polymer/water mixture was dried with reduced pressure distillation, lyophilization, and vacuum oven at 50°C for 2 days, successively. Finally, transparent and viscous HPEI-star-PPOs were obtained. By controlling the ratio of HPEI and PPO, three HPEI-star-PPO samples were prepared, which were named as HPEI-star-PPO-1, -2, and -3, respectively.

^{1}H-NMR (400 MHz, DMSO-d_6, D_2O, 298 K) δ ppm: 0.90–1.25 (m, —OCH(CH_3)CH_2—), 1.88 (s, —CH_2(CH_3)COOCH_2NH—), 2.40–2.90 (m, —CH_2NH_2, —NHCH_2—, >NCH_2—), 3.18–3.95 (m, —OC*H* (CH_3)CH_2—), 5.64, and 6.02 (s, —OCOC(CH_3)=CH_2).

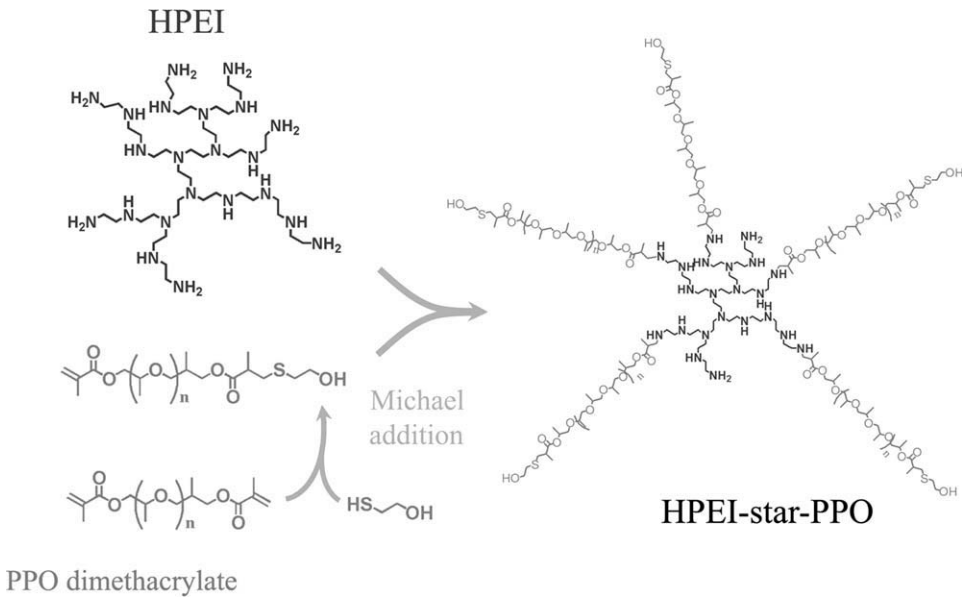
^{13}C-NMR (400 MHz, DMSO-d_6, D_2O, 298 K) δ ppm: 16.35, 17.05, 17.90 (—*C*H_3), 34.45 (—OCOCH(CH_3)*C*H_2SCH_2 CH_2OH), 35.01 (—OCOCH(CH_3)CH_2S*C*H_2CH_2OH), 38.50–58.49 (carbon atoms in HPEI, details is presented in Supporting Information), 61.05 (—SCH_2*C*H_2OH), 69.89, 70.96, 72.45 (—OCH_2*C*H<), 74.63 (—O*C*H_2CH<), 125.29 (—OCOC(CH_3)= *C*H_2), 136.22 (—OCO*C*(CH_3)=CH_2), 166.10 (—O*C*OC(CH_3)= CH_2), and 174.02 (—O*C*OC(CH_3)CH_2CH_2—).

IR (cm^{-1}): 3452 ($\nu_{as\ OH}$), 3350 ($\nu_{as\ NH}$), 3283 ($\nu_{s\ NH}$), 2974 ($\nu_{as\ CH3}$), 2933 ($\nu_{as\ CH2}$), 2899 ($\nu_{s\ CH3}$), 2873 ($\nu_{s\ CH2}$), 1730 ($\nu_{C=O}$), 1638 ($\nu_{C=C}$), 1573 (δ_{NH}),1469 ($\delta_{s\ CH2}$), 1455 ($\delta_{s\ CH3}$), 1375 ($\delta_{as\ CH3}$), 1255 ($\nu_{as\ C—O—C}$), 1120 ($\nu_{C—N}$), and 1106 ($\nu_{s\ C—O—C}$).

Thermoresponsive Behavior of HPEI-Star-PPOs

Lower Critical Solution Temperature Measurements. HPEI-star-PPOs were dissolved in ice-cold deionized water to form transparent solutions (2 mg mL^{-1}). As the temperature increased, the solution turned turbid, indicating the occurrence of phase transition. And the thermoresponsive phase-transition behaviors were recorded by an EV300 UV–visible spectrophotometer by monitoring the transmittance of a 500-nm light beam. The lower critical solution temperatures (LCSTs) were defined as the temperature corresponding to 90% transmittance of aqueous solution during the heating process.

The Relative Cytotoxicity of HPEI-Star-PPOs. The relative cytotoxicity of HPEI-star-PPOs was estimated with MTT viability assay. COS-7 cells (a cell line derived from kidney cells of the African green monkey) were seeded in 96-well plates with an initial density of 1×10^4 cells/well in 200-μL cell-culture medium and incubated for 24 h to reach 80% confluency. Then,



Scheme 1. Synthesis of HPEI-star-PPO.

50-μL culture medium containing various amount of HPEI-star-PPO was injected into the growth medium. After the cells were incubated for another 24 h, 20 μL of 5 mg/mL MTT assays stock solution in phosphate-buffered saline was added to each well. Four hours later, the medium containing unreacted MTT was removed gingerly. Blue formazan crystals were obtained and dissolved in 200 μL dimethylsulfoxide (DMSO) in each well. The absorbance was measured with a Perkin Elmer 1420 Multi-label counter at a wavelength of 490 nm.

Characterization. The molecular structure was analyzed with nuclear magnetic resonance (NMR) and Fourier transform infrared (FTIR) spectrometer. NMR results were recorded on a Varian Mercury Plus 400 MHz spectrometer with d_6-DMSO and deuterated water (D_2O) as solvents, and FTIR spectra were recorded on a Paragon 1000 instrument with KBr method. Dynamic light scattering (DLS) measurements at various temperatures were performed on a Malvern Zetasizer Nano S apparatus equipped with a 4.0 mV laser operating at $\lambda = 633$ nm at various temperature and at a scattering angle of 173°.

RESULTS AND DISCUSSION

Synthesis and Characterization of HPEI-Star-PPOs

HPEI-star-PPO can be obtained through one-pot two-step Michael addition reaction of commercial HPEI, PPO-DM, and 2-mercaptoethanol, and the process of synthesis was shown in Scheme 1. Because PPO-DM had two functional groups, it is possible to act as a cross-linker when it reacted with primary and secondary amines on HPEI. To inhibit the cross-linking reaction, PPO-DM reacted with 2-mercaptoethanol in equimolar ratio via thiolene click reaction, turning most PPO-dimethacrylate into PPO-methacrylate (PPO with single methacrylate, S-PPO-M). After that, S-PPO-M was grafted on HPEI via nucleophilic Michael addition reaction between amino group and methacrylate. After purification, the lyophilized HPEI-star-

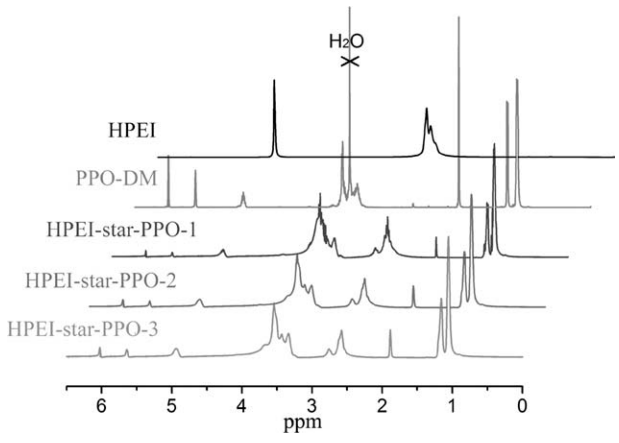


Figure 1. ^{1}H-NMR spectra of HPEI (in D_2O), PPO-DM, and HPEI-star-PPOs (in deuterated DMSO).

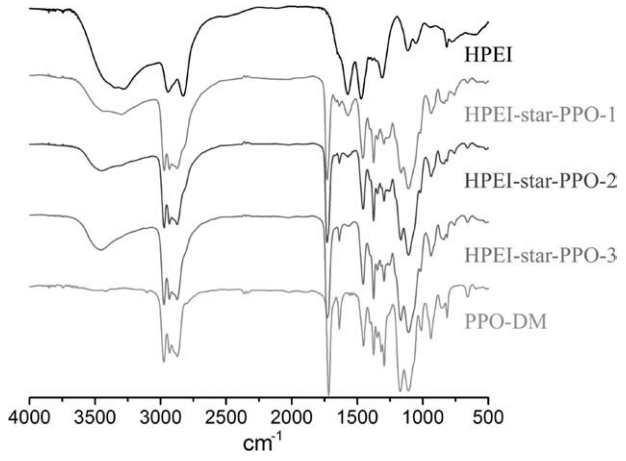


Figure 2. FTIR spectra of HPEI, PPO-dimethacrylate, and HPEI-star-PPOs.

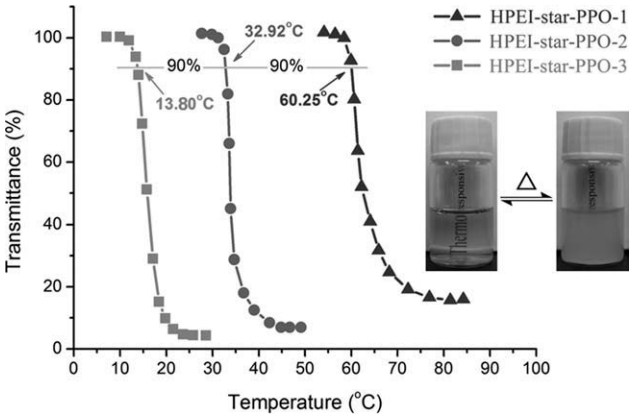


Figure 3. Thermoresponsive behavior of various HPEI-star-PPOs aqueous solution at pH 7.4.

PPOs were transparent ointment-like solid with light yellow color and able to dissolve in cold water.

^{1}H-NMR spectra of HPEI, PPO-DM, and HPEI-star-PPOs were shown in Figure 1 and analyzed in detail. Compared to PPO-DM, the intensity of peaks, which were assigned to methylene in methacrylate (at 5.64 and 6.02 ppm), decreased apparently after reactions, indicating the Michael addition reactions between amine groups of PEI and methacrylate and thiol groups of 2-mercaptoethanol and methacrylate. It was also confirmed by the reduction of peak intensity at 1.88 ppm, which indicated the —OCOC(CH_3)=CH_2 turned into —OCOCH(CH_3)CH_2—. The whole reaction process can be described as follows. In the first-step reaction, PPO-DM and 2-mercaptoethanol were fed in the same molar ratio, and because of high-reaction activity of thiol and methacrylate, it is highly possible that a small portion of PPO-DM reacted with 2-mercaptoethanol in 1/2 molar ratio to form PPO. Correspondingly, a few PPO-DM remained after the first step reaction and reacted with HPEI during the second-step reaction. Parts of these PPO-DM chains only reacted amine with one methacrylate, resulting double-bond residual in the final products. Molar ratios of HPEI to PPO in HPEI-star-PPOs were calculated from the integral area ratio of methylene peak at 2.40–2.90 ppm (—$NHCH_2$—) to methylene and methyne at 3.18–3.95 ppm (—OCH_2CH—) and were 1 : 2.1, 1 : 2.91, and 1 : 3.71, very similar to the feed molar ratios of HPEI to PPO-DM (1 : 2, 1 : 3, and 1 : 4).

The resultant polymers and their HPEI and PPO-DM precursors were also characterized by the FTIR (Figure 2). In all curves, the bands at 2974 and 2899 cm^{-1} correspond to asymmetric and symmetric —CH_3 stretching vibrations, respectively. The absorption peaks at 2933 and 2873 cm^{-1} came from asymmetric and symmetric —CH_2— stretching vibration, and the asymmetric and symmetric bands of C—O—C stretching vibration appeared at 1255 and 1106 cm^{-1}. For HPEI, the bands at 3350 and 3283 cm^{-1} were ascribed to NH-stretching vibration. After reaction with 2-mercaptoethanol and PPO-DM, the absorbance of amine groups at 3350 and 3283 cm^{-1} decreased greatly. Meanwhile, the characteristic absorption of C=O stretching appeared at 1730 cm^{-1}. All these results were consistent with the NMR analysis very well, indicating the successful Michael addition reaction.

Thermo and pH-Responsive Behaviors of HPEI-Star-PPOs

HPEI-star-PPOs consisted of hydrophobic PPO chains and hydrophilic HPEI chains, and all HPEI-star-PPOs were highly soluble and stable in their cold aqueous solution. Interestingly, their aqueous solutions became opaque at a specific temperature as the temperature increased and became transparent again when the temperature decreased. The opaque solutions were stable, and no precipitation was observed. Over the experimental temperature range, the solution of unmodified HPEI kept transparent and no thermoreliant behavior was observed, indicating that HPEI was not thermoresponsive in aqueous media. These phenomena indicated that HPEI-star-PPOs aqueous solution possessed a reversible thermoresponse. The thermoresponsive behavior could be attributed to the combination of the hydrophilic HPEI chains and hydrophobic PPO chains in the

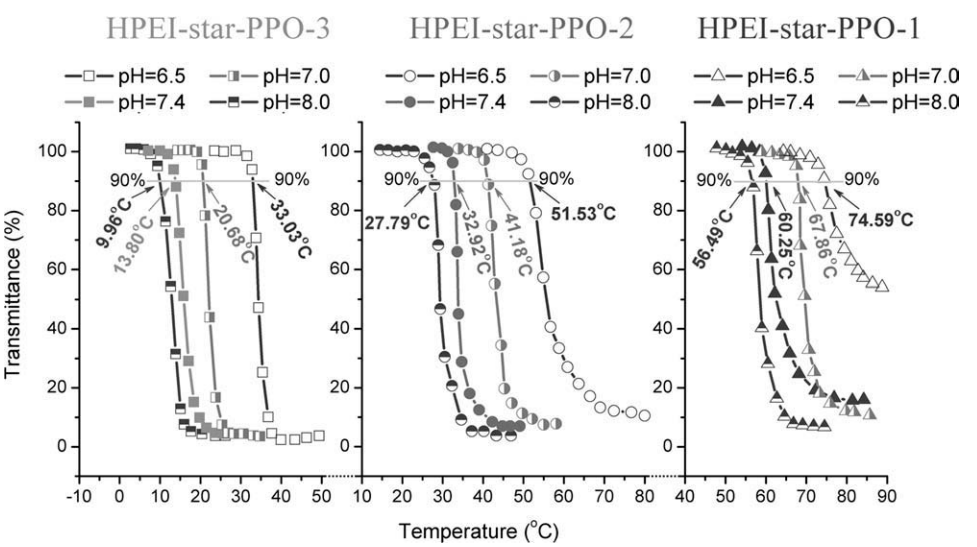


Figure 4. pH dependence of optical transmittance at 500 nm for HPEI-star-PPO-1, -2, and -3 aqueous solution.

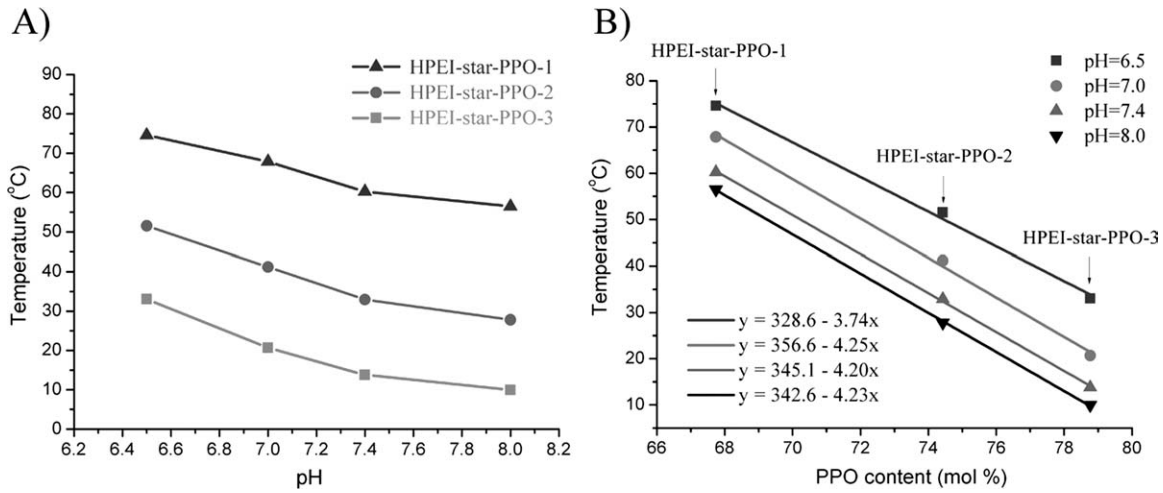


Figure 5. A: The effect of pH on the LCST of HPEI-star-PPOs aqueous solution. B: The linear fitting of the LCST of HPEI-star-PPOs aqueous solution versus PPO content at various pH.

polymer. At the temperature above LCST, the hydrophobic interaction drove the polymer chains to aggregate and separate from the water. A temperature-controlled UV–vis spectrophotometer was used to characterize the thermoresponsive transition of HPEI-star-PPOs by measuring the transmittance of the solution as a function of temperature. Figure 3 shows the transmittance change of HPEI-star-PPO-1, -2, and -3 in aqueous solutions at 500 nm. All the polymers exhibited phase transition in water. The LCST was defined as the temperature corresponding to 90% transmittance of aqueous solution during the heating process. As can be seen from Figure 3, the transmittances of all HPEI-star-PPOs exhibited a sharp decrease above their LCST. With increasing of PPO content in HPEI-star-PPOs, LCST decreased from 60.3 to 13.8°C. These results clearly demonstrated that the composition of HPEI-star-PPOs had direct effect on LCST. And because of the higher hydrophobicity of PPO chain compared to HPEI core, the increase of PPO content could make HPEI-star-PPOs more hydrophobic, resulting in lower LCST.

As the amino groups in the HPEI core can be protonated and deprotonated while the pH value changes, HPEI-star-PPOs aqueous solutions were expected to exhibit a pH-dependent thermoresponse. To test the effect of pH on thermoresponse, temperature dependence of transmittance for HPEI-star-PPOs aqueous solutions at different pH was measured. Figure 4 shows the transmittance versus temperature at different pH for HPEI-star-PPO-1, -2, and -3, indicating that LCST of HPEI-star-PPOs decreases as increasing of pH value from 6.5 to 8.0. Figure 5(A) shows the effect of pH on LCST of HEIP-star-PPOs aqueous solution, indicating that when pH value increased, LCST became lower. This phenomenon was caused by the fact that the deprotonation of amino groups at high pH reduced the hydrophilicity of HPEI-star-PPOs, resulting in aggregation at a lower temperature. According to the LCST data of HPEI-star-

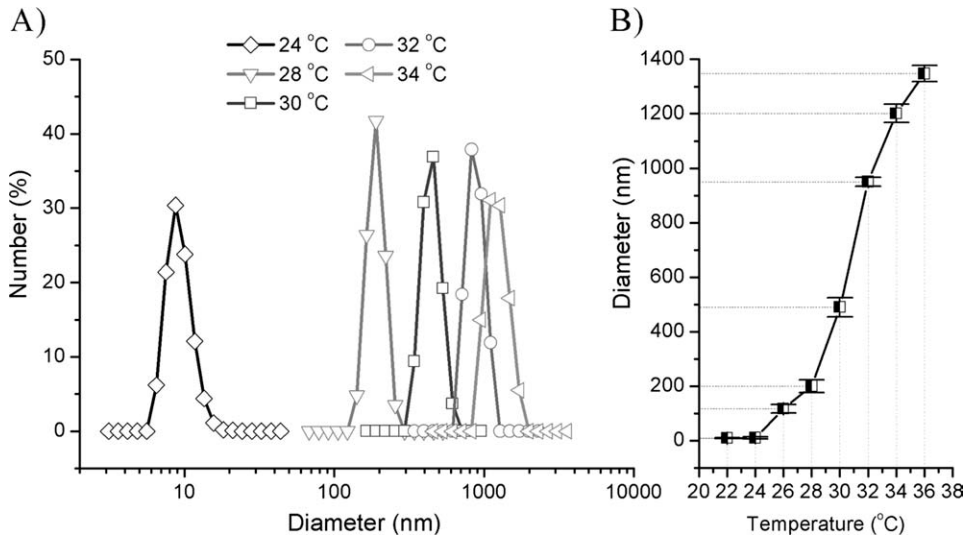


Figure 6. A: DLS plot of HPEI-star-PPO-2 aqueous solution at 24, 28, 30, 32, and 34°C, respectively. B: Particle size of HPEI-star-PPO-1 in aqueous solution measured by DLS over a temperature range of 22–36°C.

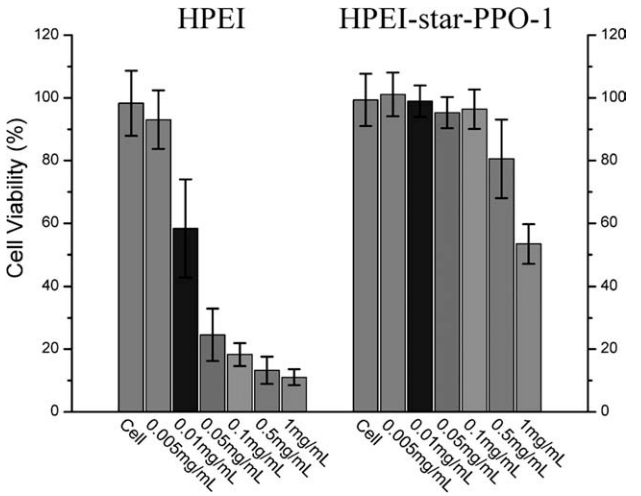


Figure 7. Cell viability of COS-7 cells against HPEI and HPEI-star-PPO-1 after 24 h of incubation with different concentrations.

PPOs aqueous solution in Figure 5(A), the linear relationship between LCST and PPO content could be obtained at different pH and was illustrated in Figure 5(B). The linear relationships were ($y = 328.6 - 3.74x$), ($y = 356.6 - 4.25x$), ($y = 345.1 - 4.20x$), and ($y = 342.6 - 4.23x$) at pH 6.5, 7.0, 7.4, and 8.0, respectively. So, the LCST of HEI-star-PPO may be predicted by controlling PPO content at different pH. For example, LCST of HPEI-star-PPO containing 73.4 mol % PPO would likely be 37°C at pH 7.4.

Laser light scattering (DLS) was used to provide further understanding of the thermoresponsive behavior. The change in the size distribution of HPEI-star-PPO-2 was measured by DLS in its pH = 7.4 aqueous solution at various temperature, and the results are shown in Figure 6. When the temperature is below 24°C, the particle size of HPEI-star-PPO-1 in aqueous solution is about 10 nm, indicating the formation of nanomicelles. Because HPEI-star-PPOs were composed of hydrophobic PPO chains and hydrophilic PEI chain, they were expected to be amphiphilic in water and dispersed as nanomicelles with PPO as core and HPEI as shell. The HPEI can provide positive shell and prevent them from aggregation at low temperature and keep the hydrophilic–hydrophobic balance. An increasing of the solution temperature could destroy this balance, resulting in aggregation of nanomicelles to form larger particles. As shown in Figure 6(B), the hydrodynamic size of HPEI-star-PPO-1 became large at 26°C and finally increased to 1300 nm at 36°C. DLS experiments confirm the results of UV–vis measurements.

In Vitro Cytotoxicity

Because of their dual response, HPEI-star-PPOs can be used as promising material for biomedical applications. To evaluate the potential for biomedicine, *in vitro* evaluation of HPEI-star-PPOs was conducted. The cytotoxicity of HPEI-star-PPOs against COS-7 cells was studied and compared to HPEI by using MTT assay. The MTT assay is based on the ability of a mitochondrial dehydrogenation enzyme in viable cells to cleave the tetrazolium rings of the pale yellow MTT and form formazan crystals with a dark blue color.[54,55] The number of surviving cells is directly proportional to the level of formed formazan. COS-7 cells, a cell line derived from kidney cells of the African green monkey, were incubated with varying concentration of the polymers, and HPEI was used as a control. Considering the thermoresponsive behavior of HPEI-star-PPOs, only HPEI-star-PPO-1 was used to evaluate the cytotoxicity at 37°C. Figure 7 gives the cell viability after 24-h incubation with HPEI and HPEI-star-PPO-1 at various concentrations, and no significant cytotoxicity was observed at the concentration up to 0.1 mg/mL after 24-h incubation. On contrast, HPEI showed cytotoxicity when the concentration was above 10 μg/mL. At 1 mg/mL, less than 15% of the cells survived. It was reported that the strong positive charge of primary amino groups can greatly increase the cytotoxicity.[56] Therefore, the low cytotoxicity might be attributed to the absence of primary amino groups in HPEI-star-PPOs. Also, the PPO chains had similar chemical structure with poly(ethylene glycol), which has low cytotoxicity and wide bioapplication.[57–60] The MTT evaluation demonstrated that this new thermoresponsive HPEI-star-PPOs had low cytotoxicity and exhibited potential for biomedical applications.

CONCLUSIONS

A series of novel double stimuli-responsive HPEI-star-PPOs comprised hydrophilic HPEI and hydrophobic PPO were prepared successfully via Michael addition of HPEI, PPO-DM, and 2-mercaptoethanol in ethanol. The structures and properties of HPEI-star-PPOs were analyzed by NMR, FTIR, UV, and DLS. The obtained polymers exhibited response to temperature with well-tunable LCST. By changing the feed ratio, the LCST values decreased from 60.25 to 13.80°C in pH = 7.4 aqueous solution. Moreover, these polymers exhibited pH-dependent LCST values. *In vitro* evaluation was investigated by MTT assay, and it demonstrated that the polymers showed low cytotoxicity. These novel characterizations made these thermo and pH dual-responsive HPEI-star-PPOs as potential smart carriers for drug and gene-delivery systems.

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REFERENCES

1. Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004,** *29*, 1173.
2. Liu, F.; Urban, M. W. *Prog. Polym. Sci.* **2010,** *35*, 3.
3. Kasgoz, H.; Orbay, M. *Polymer* **2003,** *44*, 1785.
4. Zhao, C. S.; Nie, S. Q.; Tang, M.; Sun, S. D. *Prog. Polym. Sci.* **2011,** *36*, 1499.
5. Dayananda, K.; He, C.; Park, D. K.; Park, T. G.; Lee, D. S. *Polymer* **2008,** *49*, 4968.
6. Srinivas, G.; Harikrisna, D.; Aliasgar, S.; Mansoor, A. *J. Control. Release* **2008,** *126*, 187.

7. Prabaharan, M.; Grailer, J. J.; Steeber, D. A.; Gong, S. Q. *Macromol. Biosci.* **2008,** *8*, 843.
8. Nayak, S.; Lee, H.; Chmielewski, J.; Lyon, L. A. *J. Am. Chem. Soc.* **2004,** *126*, 10258.
9. Mi, P.; Ju, X. J.; Xie, R.; Wu, H. G.; Ma, J.; Chu, L. Y. *Polymer* **2010,** *51*, 1648.
10. Meng, F. H.; Zhong, Z. Y.; Jan, F. *Biomacromolecules* **2009,** *10*, 197.
11. Yao, J.; Ruan, Y. L.; Zhai, T.; Guan, J.; Tang, G. P.; Li, H. R.; Dai, S. *Polymer* **2011,** *52*, 3396.
12. Guan, J.; Hong, Y.; Ma, Z.; Wagner, W. R. *Biomacromolecules* **2008,** *9*, 1283.
13. Xue, Y. N.; Huang, Z. Z.; Zhang, J. T.; Liu, M.; Zhang, M.; Huang, S. W.; Zhuo, R. X. *Polymer* **2009,** *50*, 3706.
14. Guo, C. P.; Chen, W. C.; Lin, S. D.; Li, H.; Cheng, D.; Wang, X. Y.; Shuai, X. T. *Polymer* **2012,** *53*, 342.
15. Xia, Y. M.; Wang, Y. M.; Wang, Y. P.; Tu, C. L.; Qiu, F.; Zhu, L. J.; Su, Y.; Yan, D. Y.; Zhu, B. S.; Zhu, X. Y. *Colloids Surf. B* **2011,** *88*, 674.
16. Stauffer, P.; Goldberg, N. *Int. J. Hyperthermia* **2004,** *20*, 671.
17. Godbey, W. T.; Wu, K. K.; Mikos, A. G. *J. Control. Release* **1999,** *60*, 149.
18. Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. *Angew. Chem. Int. Ed.* **2003,** *42*, 4640.
19. Shen, Y.; Kuang, M.; Shen, Z.; Nieberle, J.; Duan, H. W.; Frey, H. *Angew. Chem. Int. Ed.* **2008,** *47*, 2227.
20. Kojima, C.; Yoshimura, K.; Harada, A.; Sakanishi, Y.; Kono, K. *Bioconjugate Chem.* **2009,** *20*, 1054.
21. Pang, Y.; Zhu, Q.; Zhou, D. L.; Liu, J. Y.; Chen, Y.; Su, Y.; Yan, D. Y.; Zhu, X. Y.; Zhu, B. S. *J. Polym. Sci. Polym. Chem.* **2011,** *49*, 966.
22. Jia, Z. F.; Chen, H.; Zhu, X. Y.; Yan, D. Y. *J. Am. Chem. Soc.* **2006,** *128*, 8144.
23. Yuba, E.; Harada, A.; Sakanishi, Y.; Kono, K. *J. Control. Release* **2011,** *149*, 72.
24. Xia, Y. M.; Wang, Y. M.; Wang, Y. P.; Wang, D. L.; Deng, H. P.; Zhuang, Y. Y.; Yan, D. Y.; Zhu, B. S.; Zhu, X. Y. *Macromol. Chem. Phys.* **2011,** *212*, 1056.
25. Yu, B.; Jiang, X. S.; Yin, G. L.; Yin, J. *J. Polym. Sci. Polym. Chem.* **2010,** *48*, 4252.
26. Wang, R.; Jiang, X. S.; Yin, G. L.; Yin, J. *Polymer* **2011,** *52*, 368.
27. Schild, H. G. *Prog. Polym. Sci.* **1992,** *17*, 163.
28. Eliassaf, J. *J. Appl. Polym. Sci.* **1978,** *22*, 873.
29. Wei, H.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. *Prog. Polym. Sci.* **2009,** *34*, 893.
30. Çimen, E. K.; Rzaev, Z. M. O.; Pişkin, E. *J. Appl. Polym. Sci.* **2005,** *95*, 573.
31. Liu, Y.; Cui, Y. D. *J. Appl. Polym. Sci.* **2011,** *120*, 3613.
32. Tian, P.; Wu, Q. L.; Lian, K. *J. Appl. Polym. Sci.* **2008,** *108*, 2226.
33. Chen, Y.; Yi, M. *Radiat. Phys. Chem.* **2001,** *61*, 65.
34. Cho, S. H.; Jhon, M. S.; Yuk, S. H.; Lee, H. B. *J. Polym. Sci. Polym. Phys.* **1997,** *35*, 595.
35. Fang, J.; Bian, F. L.; Shen, W. G. *J. Appl. Polym. Sci.* **2008,** *110*, 3373.
36. Liu, S. X.; Liu, M. Z. *J. Appl. Polym. Sci.* **2003,** *90*, 3563.
37. Bian, F. L.; Xiang, M.; Yu, W.; Liu, M. Z. *J. Appl. Polym. Sci.* **2008,** *110*, 900.
38. Mortensen, K.; Pedersen, J. S. *Macromolecules* **1993,** *26*, 805.
39. Malmsten, M.; Lindman, B. *Macromolecules* **1992,** *25*, 5446.
40. Jeong, B.; Bae, Y. H.; Kim, S. W. *Macromolecules* **1999,** *32*, 7064.
41. Maurdev, G.; Millington, K. R. *J. Appl. Polym. Sci.* **2009,** *113*, 2346.
42. Sosnik, A.; Cohn, D. *Biomaterials* **2004,** *25*, 2851.
43. Harpe, A. V.; Petersen, H.; Li, Y.; Kissel, T. *J. Control. Release* **2000,** *69*, 309.
44. Neu, M.; Fischer, D.; Kissel, T. *J. Gene Med.* **2005,** *7*, 992.
45. Godbey, W. T.; Barry, M. A.; Saggau, P.; Wu, K. K.; Mikos, A. G. *J. Biomed. Mater. Res.* **2000,** *51*, 321.
46. Fischer, D.; Bieber, T.; Li, Y. X.; Elsässer, H. P.; Kissel, T. *Pharm. Res.* **1999,** *16*, 1273.
47. Boussif, O.; Lezoualc'h, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. *Proc. Natl. Acad. Sci. USA* **1995,** *92*, 7297.
48. Abdallah, B.; Hassan, A.; Benoist, C.; Goula, D.; Behr, J. P.; Demeneix, B. A. *Hum. Gene Ther.* **1996,** *7*, 1947.
49. Bandyopadhyay, P.; Ma, X. M.; Linehan-Stieers, C.; Kren, B. T.; Steer, C. J. *J. Biol. Chem.* **1999,** *274*, 10163.
50. Türk, M.; Dinçer, S.; Yuluğ, I. G.; Pişkin, E. *J. Control. Release* **2004,** *96*, 325.
51. Liu, H. J.; Chen, Y.; Shen, Z. *J. Polym. Sci. Polym. Chem.* **2006,** *45*, 1177.
52. Kim, H.; Lee, S.; Noh, M.; Lee, S. H.; Mok, Y.; Jin, G. W.; Seo, J. H.; Lee, Y. *Polymer* **2011,** *52*, 1367.
53. Lukovkin, G. M.; Pshezhetsky, V. S.; Murtazaeva, G. A. *Eur. Polym. J.* **1973,** *9*, 559.
54. Mosmann, T. *J. Immunol. Methods* **1983;** *65*, 55.
55. Prabaharan, M.; Grailer, J. J.; Pilla, S.; Steeber, D. A.; Gong, S. *Macromol. Biosci.* **2009,** *9*, 515.
56. Stasko, N. A.; Johnson, C. B.; Schoenfisch, M. H.; Johnson, T. A.; Holmuhamedov, E. L. *Biomacromolecules* **2007,** *8*, 3853.
57. Kojima, C.; Toi, Y.; Harada, A.; Kono, K. *Bioconjugate Chem.* **2007,** *18*, 663.
58. Gajbhiye, V.; Kumar, P. V.; Sharma, A.; Jain, N. K. *Curr. Nanosci.* **2008,** *4*, 267.
59. Gajbhiye, V.; Kumar, P. V.; Tekade, R. K.; Jain, N. K. *Eur. J. Med. Chem.* **2009,** *44*, 1155.
60. Paleos, C. M.; Tsiourvas, D.; Sideratou, Z.; Tziveleka, L. *Biomacromolecules* **2004,** *5*, 524.