

Note

THERMO-RESPONSIVE BLOCK COPOLYMERS BASED ON LINEAR-TYPE POLY(ETHYLENE GLYCOL): TUNABLE LCST WITHIN THE PHYSIOLOGICAL RANGE

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Abstract Thermo-responsive block copolymers poly(ethylene glycol)-*block*-poly(*N*-acryloyl-2,2-dimethyl-1,3-oxazolidine), PEG-*b*-PADMO, based on linear PEG were prepared *via* a versatile reversible addition-fragmentation chain transfer (RAFT) polymerization. PEG₂₂ ($M_w = 1000$) was used as the hydrophilic component, whose dehydration was the main driving force for the phase transition of these copolymers, as demonstrated by the ¹H-NMR spectra. Their lower critical solution temperatures (LCSTs) could be tuned in the range of 20°C to 35°C, by adjusting the degree of polymerization (DP) of PADMO between 14–27. Furthermore, a sharp phase transition at *ca.* 33°C, close to the physiological temperature with minimal hysteresis, was observed for the PEG₂₂-*b*-PADMO₁₄ copolymer. Moreover, excellent reversibility and reproducibility were displayed for the same copolymer over 10 cycles of repeated temperature change between 25°C (below the LCST) and 40°C (above the LCST).

Keywords: Thermo-responsive; Block copolymers; Linear PEG; Phase transitions.

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INTRODUCTION

Thermo-responsive water-soluble polymers with lower critical solution temperatures (LCST) have attracted significant attention due to their wide applications across many fields. Examples include smart surfaces, sensors, drug and gene carriers and coatings^[1–5]. Above the LCST, these polymers can be separated from both the aqueous solution and the cross-linked analogues, which would display a dramatic change in volume. Some classical examples of thermo-responsive polymers include poly(*N*-isopropylacrylamide) (PNIPAM)^[6, 7], poly(dimethylaminoethyl methacrylate) (PDMAEMA)^[8], poly(*N*-vinylcaprolactam) (PVCL)^[9, 10] and poly(2-isopropyl-2-oxazoline) (PiPrOx)^[11]. PNIPAM is the typical benchmark for many temperature sensitive polymer investigations due to its sharp transition at approximately 32°C^[12, 13]. Despite the great developments and applications for PNIPAM-based polymers, their use in biomedical fields is largely limited due to their non-biocompatibility and non-degradability^[14, 15].

Poly(ethylene glycol) (PEG) is a water-soluble, biocompatible, non-toxic, FDA-approved polymer, which has been popularly utilized in biomedicine and pharmacy. In principle, linear PEG chains can dehydrate in an aqueous medium upon heating. Nevertheless, the LCST of PEG is typically higher than 100°C and increases with decreasing molecular weight^[16, 17]. Over the past decades, a great deal of effort has been made to develop

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thermo-responsive PEG-analogues, as they are promising materials for biological application. Oligo(ethylene glycol) (OEG)-based non-linear PEG analogues have been explored for the preparation of smart materials due to their biocompatibility and versatility^[18–23]. Thermo-responsive polymers, constructed from OEG macromonomers, exhibit a defined LCST in aqueous media, depending on the length of the OEG^[20]. Lutz *et al.*^[21] reported the copolymerization of 2-(2-methoxyethoxy) ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA) to form a thermo-responsive polymer. The LCST values could be tuned between 26°C and 90°C by adjusting the copolymer composition.

So far, thermo-responsive di-block copolymers, based on linear PEG as a hydrophilic motif, have also been widely reported. However, the thermo-sensitive functionality of PEG is often ignored due to its high phase transition temperature. In our group, Cui *et al.*^[24] presented novel thermo-sensitive copolymer micelles, comprising a hydrophilic PEG ($M_w = 2000$) corona and a hydrophobic PADMO core. A good thermo-sensitivity was observed for copolymers within a wide temperature range, which resulted from the attractive interactions between the densely packed PEG chains. However, the transition temperature can only be tailored to a minimum of 40°C, which is not ideal for the specialist biomedical application. In addition, a large hysteresis was evident for the polymer micellar solution with an LCST of 40°C. To meet this challenge, PEG with a low molecular weight was introduced to lower the LCST to the appropriate range.

Herein, we have devised a facile strategy to generate LCST tunable copolymers bearing a sharp phase transition near physiological temperature. This was achieved by both adopting short PEG chains ($M_w = 1000$) as the hydrophilic component and further regulating the hydrophilic/hydrophobic balance. The thermo-sensitive behaviors of these copolymers deriving from the PEG segment were determined by ¹H-NMR and turbidimetry. The development of these thermo-sensitive polymers with the appropriate LCST and reverse phase transition properties will be of benefit for potential biomedical and pharmaceutical applications.

EXPERIMENTAL

Reagents

N-acryloyl-2,2-dimethyl-1,3-oxazolidine (ADMO) and poly(ethylene glycol)-based chain transfer agent (PEG-CTA) were synthesized with a previously reported procedure^[24]. 2,2'-Azobis(isobutyronitrile) (AIBN) was refined by recrystallization from ethanol before use. 1,4-Dioxane was distilled over CaH₂. Other reagents were obtained from Beijing Chemical Reagent Company (China) and used as received. All aqueous solutions were prepared using deionized water.

Synthesis of PEG-*b*-PADMO

Herein, a series of block copolymers based on linear PEG₂₂ were synthesized *via* RAFT polymerization using PEG₂₂-CTA as macro chain transfer agent according to a previously reported procedure^[25]. To obtain diblock copolymers with different PADMO block lengths, the molar ratio of monomer to PEG₂₂-CTA was varied at 15, 20 and 30 respectively, while the molar ratio of PEG₂₂-CTA to initiator was kept at 10. As an example, a dry tube was charged with ADMO (0.35 g, 2.25 mmol), PEG-CTA (0.19 g, 0.15 mmol), AIBN (2.5 mg, 0.015 mmol) and 1,4-dioxane (5 mL) for synthesis of copolymer with PADMO length of 15 units. The solution was deoxygenated thoroughly by purging with nitrogen for 30 min, and the polymerization was carried out in water bath at 70°C for 24 h. The polymer was isolated by three times re-precipitations from THF to hexane and dried under vacuum at room temperature for 24 h.

Instruments and Methods

¹H-NMR spectra were taken with a Bruker DPX spectrometer operating at 400 Hz in deuterated solvents. Temperature-dependent ¹H-NMR spectra of the copolymers in D₂O were recorded with a Bruker DMX-300 NMR spectrometer.

The relative molecular weights and molecular weight distributions (PDI, M_w/M_n) were determined by gel permeation chromatography (GPC), using a Waters 1525 binary HPLC pump equipped with a Waters 2414 refractive index detector and three Waters Styragel columns. THF was used as the eluent at a flow rate

1 mL·min⁻¹, and calibration curves were obtained by linear polystyrene.

The light transmission of the polymers was determined on a Hitachi U-3900 UV-Vis spectrophotometer together with a Brookfield temperature controller. The measurements were performed with a wavelength of 600 nm, and the sample solutions were equilibrated for 10 min before each measurement. The cloud points of polymer solutions were defined at the temperature showing a 50% drop from the original transmittance.

The morphologies of aggregates were analyzed by TEM (JEOL JEM-2100) at an accelerating voltage of 200 kV. The sample was prepared by placing a drop of the copolymer solution onto a copper grid coated with carbon film, it was then stained with 10 g/L uranyl acetate solution and dried at low temperature before measurements.

RESULTS AND DISCUSSION

Characterization of Block Copolymers

Structures of copolymers were confirmed by ¹H-NMR spectroscopy (see supporting information), and their molecular weights were determined through ¹H-NMR spectroscopy and GPC methods. The GPC traces for the block copolymers are shown in Fig. 1. The numbers of ADMO units within the different di-block copolymers were estimated to be 14, 19 and 27, as calculated from ¹H-NMR and close to the feed compositions. The corresponding copolymers are denoted as E₂₂A₁₄, E₂₂A₁₉ and E₂₂A₂₇, respectively, where E and A represent the PEG and PADMO blocks, respectively. The characterization data for the various PEG-*b*-PADMO di-block copolymers are summarized in Table 1.

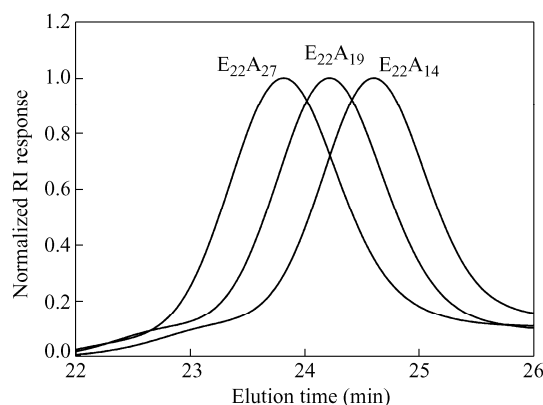


Fig. 1 GPC curves of PEG-*b*-PADMO block copolymers prepared by RAFT polymerization

Table 1. Polymerization conditions and properties for the di-block copolymers

Sample	[M] ₀ /[CTA] ₀ ^a	<i>M</i> _n (NMR) × 10 ⁻³ ^b	<i>M</i> _n (GPC) × 10 ⁻³ ^c	<i>M</i> _w (GPC) × 10 ⁻³ ^c	PDI ^c (<i>M</i> _w / <i>M</i> _n)	LCST ^d (°C)
E ₂₂ A ₁₄	15	3.4	2.9	3.5	1.21	33.4
E ₂₂ A ₁₉	20	4.2	3.3	4.1	1.23	25.3
E ₂₂ A ₂₇	30	5.4	4.3	4.9	1.14	19.2

^a [M]₀/[CTA]₀ is the initial monomer to PEG-CTA ratio; ^b Determined from ¹H-NMR spectra;

^c Determined by GPC in THF, relative to linear polystyrene; ^d Accessed by turbidity measurements

Self-assembly of Block Copolymers

The water-solubility of the amphiphilic block copolymers will decrease with increasing levels of the hydrophobic portion in the copolymer. Fortunately, all products synthesized in this study are soluble in water at temperatures below LCSTs. Amphiphilic PEG-*b*-PADMO copolymers form micelle-like aggregates in aqueous media, with a hydrophobic PADMO core and a hydrophilic PEG shell^[25]. In this work, the morphology of aggregates was investigated by TEM technique. As a typical example, the TEM micrograph of block copolymer (E₂₂A₂₇) is presented in Fig. 2. The self-assembled micelles formed and were nearly spherical in shape with size around 25 nm. Furthermore, to better understand the core-shell structure of the micelles, ¹H-NMR spectra in

different solvents were studied (Fig. 3). It can be seen from the ^1H -NMR in CDCl_3 that the characteristic resonance signals of PADMO and PEG are visible. However, the characteristic peak for the PADMO ($\delta = 1.10\text{--}3.00$) remains unclear, due to its restricted motion in water. Thus, this result indicates that the micelles with a hydrophilic PEG shell and hydrophobic PADMO core have successfully formed in water.

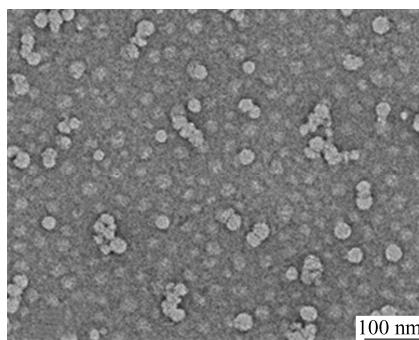


Fig. 2 Transmission electron microscopic image of $\text{E}_{22}\text{A}_{27}$ micelles

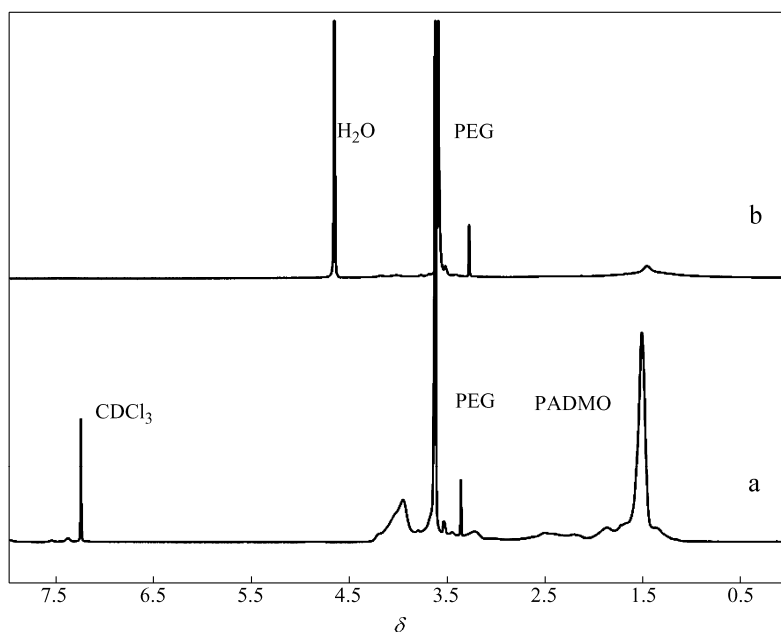


Fig. 3 ^1H -NMR spectra of polymer $\text{E}_{22}\text{A}_{27}$ dissolved in (a) CDCl_3 and (b) D_2O

Temperature Sensitivity of Block Copolymers

The temperature-dependent phase transition behaviors for the PEG-*b*-PADMO block copolymers in aqueous solutions were determined through turbidity measurements (Fig. 4). The results show that the cloud points are strongly influenced by the polymer composition, which is well known^[26–29]. The primary strategy was to introduce PEG₂₂ into the polymers to reduce the length of hydrophilic portion and subsequently decrease the LCST. In comparison to $\text{E}_{45}\text{A}_{28}$ (LCST *ca.* 60°C) in our previous report, the phase transition for $\text{E}_{22}\text{A}_{27}$, with a smaller hydrophilic/hydrophobic proportion, occurred at a much lower temperature (*ca.* 19.2°C). As expected, the LCST was reduced to a large extent *via* the introduction of low molecular weight PEG. Nevertheless, the hydrophilic/hydrophobic balance can also be controlled by altering the PADMO content, to bring the LCST closer to the physiological range. $\text{E}_{22}\text{A}_{19}$ and $\text{E}_{22}\text{A}_{14}$ copolymers, comprising short PADMO segments, were prepared and the results suggest that the LCST for PEG₂₂-*b*-PADMO shifts to higher temperature, from 19.2°C

to 33.4°C, which reflects a decrease in the proportion of PADMO. A rapid phase transition, within the physiological range, was finally achieved using an $E_{22}A_{14}$ polymer solution. As a consequence, the LCSTs for the amphiphilic copolymers could be improved to meet the requirement for physiological conditions. This renders the polymers suitable for biomedical and pharmaceutical applications.

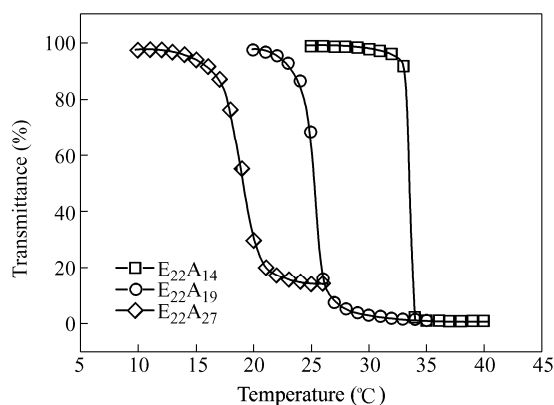


Fig. 4 Effect of temperature on the transmittance of the block copolymer solutions upon heating ($c_p = 5$ mg/mL)

Reversibility of Phase Transition

Thermo-responsive intelligent materials, such as drug and gene carriers or sensors, highlight the importance of a rapid and reversible phase transition. A sharp phase transition at about 33°C was observed from the transmittance measurements for $E_{22}A_{14}$ in solution, which is comparable to that for PNIPAM. Furthermore, the optical transmittance of $E_{22}A_{14}$ only exhibited minor hysteresis (approx. 1°C, Fig. 5) throughout the cooling process, rendering it superior to PNIPAM^[23, 30]. The reversibility and stability of the sample during the temperature-induced transition process were also investigated through the application of repeated temperature cycles between 25°C (below the LCST) and 40°C (above the LCST). The transmittance changes, in response to the alternate temperature changes, are shown in Fig. 6. Remarkably, a highly reversible performance was exhibited by the $E_{22}A_{14}$ copolymer solution. The transparent solution became turbid at 40°C, but reverted to transparency state at 25°C. The highest and lowest transmittances for $E_{22}A_{14}$ polymer solution remained roughly constant over multiple heating-cooling cycles. In summary, the reversibility and reproducibility for the thermo-sensitive materials offer great potential in terms of biomedical and specialist material applications.

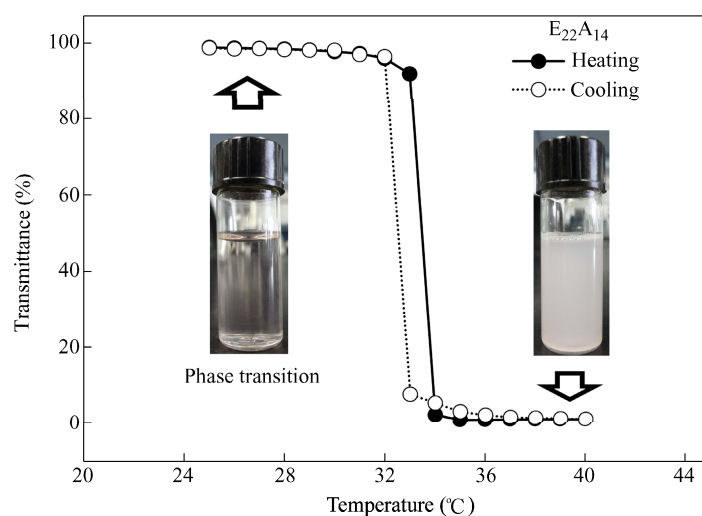


Fig. 5 Transmittance versus temperature plots for the aqueous $E_{22}A_{14}$ solutions over one heating-cooling cycle ($c_p = 5$ mg/mL)

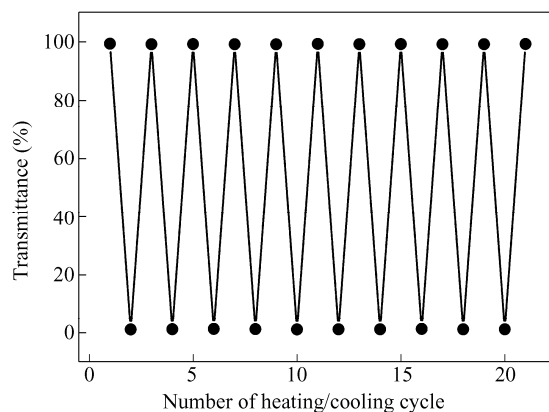


Fig. 6 Reversible changes in optical transmittance, in response to temperature fluctuations, for the micellar E₂₂A₁₄ solution ($c_p = 5$ mg/mL)

Mechanism of Phase Transition

The hydrophilic PEG chains collapse and expel water *via* the strong n-clustering attractive interactions upon heating. These lead to the aggregation of dehydrated micelles and result in an abrupt decrease in transmittance^[24]. The mechanism for the thermally induced phase transition can be demonstrated by temperature-dependent ¹H-NMR. Figure 7 shows the variable temperature ¹H-NMR spectra for E₂₂A₁₄ in D₂O. The proton signals for the PEG chains ($\delta = 3.7$) are sharp and clear at low temperatures. However, upon heating the polymer solution from 27°C to 40°C (higher than the setting temperature), a decrease in water solubility was observed from the reduced resonance intensities. This may be due to the aggregation of polymer chains, which confirms the occurrence of a phase transition. Meanwhile, all the peaks for PEG shift to a lower field upon heating, which may be due to the break-down of the ether-water hydrogen bonding at increased temperatures^[29]. All these results suggest that the thermally induced dehydration of the PEG chains is the main driving force for phase transition.

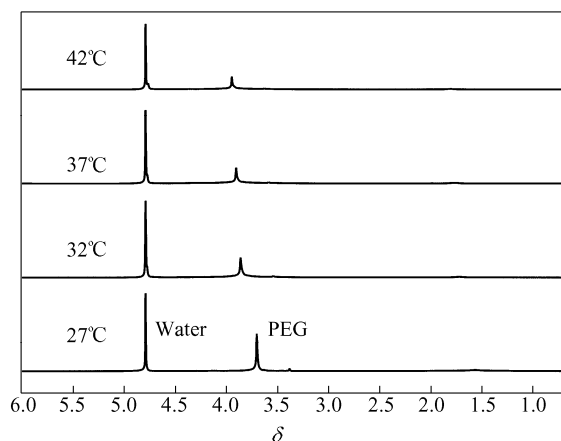


Fig. 7 Temperature-dependent ¹H-NMR spectra for E₂₂A₁₄ in D₂O (10 mg/mL)

CONCLUSIONS

Well-defined thermo-responsive block copolymers, based on linear PEG₁₀₀₀, have been prepared *via* a versatile RAFT polymerization. Their cloud points can be tailored within the range of 20–35°C by adjusting the length of hydrophobic blocks. Encouragingly, a sharp transition temperature response, with little hysteresis, was observed upon heating the aqueous E₂₂A₁₄ solution to *ca.* 33°C (within physiological conditions). Moreover, good reversibility and reproducibility were exhibited by the E₂₂A₁₄ copolymer from optical transmittance

measurements of the micellar solution over 10 repeated temperature cycles (between 25°C and 40°C). This new class of thermo-responsive polymers, with appropriate and tunable LCST, offers great potential for application within the biomedical and pharmaceutical fields.

REFERENCES

- 1 Stuart, M.A.C., Huck, W.T.S., Genzer, J., Müller, M., Ober, C., Stamm, M., Sukhorukov, G.B., Szleifer, I., Tsukruk, V.V., Urban, M., Winnik, F., Zauscher, S., Luzinov, I. and Minko, S., *Nat. Mater.*, 2010, 9: 101
- 2 Meng, F.H., Zhong, Z.Y. and Feijen, J., *Biomacromolecules*, 2009, 10: 197
- 3 Liu, R.X., Fraylich, M. and Saunders, B.R., *Colloid Polym. Sci.*, 2009, 287: 627
- 4 Motornov, M., Roiter, Y., Tokarev, I. and Minko, S., *Prog. Polym. Sci.*, 2010, 35: 174
- 5 Tan, S.W., Han, C., Wang, H.J., Liu, D.R., Tu, K.H., Jiang, H.L., Zhang, M.H. and Wang, L.Q., *Acta Polymerica Sinica* (in Chinese), 2011, (11): 1237
- 6 Li, Z.Q., Qian, J., Cao, X.L., Song, X.W. and Wu, F.P., *Acta Polymerica Sinica* (in Chinese), 2010, (3): 329
- 7 Cao, Z., Chen, T.Y., Guo, X.L., Zhou, X.J., Nie, J.J., Xu, J.T., Fan, Z.Q. and Du, B.Y., *Chinese J. Polym. Sci.*, 2011, 29(4): 439
- 8 Plamper, F.A., Ruppel, M., Schmalz, A., Borisov, O., Ballauff, M. and Müller, A.H.E. *Macromolecules*, 2007, 40: 8361
- 9 Su, X. and Liu, Y.Y., *Acta Polymerica Sinica* (in Chinese), 2009, (4): 390
- 10 Ramos, J., Imaz, A., Callejas-Fernández, J., Barbosa-Barros, L., Estelrich, J., Quesada-Pérez, M. and Forcada, J., *Soft Matter*, 2011, 7: 5067
- 11 Hoogenboom, R., *Angew. Chem., Int. Ed.*, 2009, 48: 7978
- 12 Wei, H., Cheng, S.X., Zhang, X.Z. and Zhuo, R.X., *Prog. Polym. Sci.*, 2009, 34: 893
- 13 Schild, H.G., *Prog. Polym. Sci.*, 1992, 17: 163
- 14 Jeong, B., Bae, Y.H., Lee, D.S. and Kim, S.W., *Nature*, 1997, 388: 860
- 15 Yang, Y.Y., Wu, J.Y., Liu, S.Q. and Heng, P.W.S., *J. Controlled Release*, 2005, 102: 361
- 16 Sacki, S., Kuwahara, N., Nakata, M. and Kaneko, M., *Polymer*, 1976, 17: 685
- 17 Ashbaugh, H.S. and Paulaitis, M.E., *Ind. Eng. Chem. Res.*, 2006, 45: 5531
- 18 Lutz, J.F., *J. Polym. Sci., Part A: Polym. Chem.*, 2008, 46: 3459
- 19 Medel, S., García, J.M., Garrido, L., Quijada-Garrido, I. and París, R.J., *Polym. Sci. Part A: Polym. Chem.*, 2011, 49: 690
- 20 Han, S., Hagiwara, M. and Ishizone, T., *Macromolecules*, 2003, 36: 8312
- 21 Lutz, J.F. and Hoth, A., *Macromolecules*, 2006, 39: 893
- 22 Feng, L., Hao, J.Y., Xiong, C.D. and Deng, X.M., *Chem. Commun.*, 2009, 4411
- 23 Lutz, J.F., Akdemir, Ö. and Hoth, A., *J. Am. Chem. Soc.*, 2006, 128: 13046
- 24 Cui, Q.L., Wu, F.P. and Wang, E.J., *J. Phys. Chem. B*, 2011, 115: 5913
- 25 Cui, Q.L., Wu, F.P. and Wang, E.J., *Polymer*, 2011, 52: 1755
- 26 Hoffman, A.S., Stayton, P.S., Bulmus, V., Chen, G.H., Chen, J.P., Cheung, C., Chilkoti, A., Ding, Z.L., Dong, L.C., Fong, R., Lackey, C.A., Long, C. J., Miura, M., Morris, J.E., Murthy, N., Nabeshima, Y., Park, T.G., Press, O.W., Shimoboji, T., Shoemaker, S., Yang, H.J., Monji, N., Mowinski, R.C., Cole, C.A., Priest, J.H., Harris, J.M., Nakamae, K., Nishino, T. and Miyata, T., *J. Biomed. Mater. Res.*, 2000, 52: 577
- 27 Cheng, C., Wei, H., Shi, B.X., Cheng, H., Li, C., Gu, Z.W., Cheng, S.X., Zhang, X.Z. and Zhuo, R.X., *Biomaterials*, 2008, 29: 497
- 28 Dong, A.J., Zhai, Y.L., Xiao, L., Qi, H.Y., Tian, Q., Deng, L.D. and Guo, R.W., *J. Polym. Sci. Part B: Polym. Phys.*, 2010, 48: 503
- 29 Fu, H.G., Gao, H., Wu, G.L., Wang, Y.N., Fan, Y.G. and Ma, J.B., *Soft Matter*, 2011, 7: 3546
- 30 Wang, X.H., Qiu, X.P. and Wu, C., *Macromolecules*, 1998, 31: 2972