ACS Macro Letters

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Reversible Addition–Fragmentation Chain Transfer Synthesis of a Micelle-Forming, Structure Reversible Thermosensitive Diblock Copolymer Based on the *N*-(2-Hydroxy propyl) Methacrylamide Backbone

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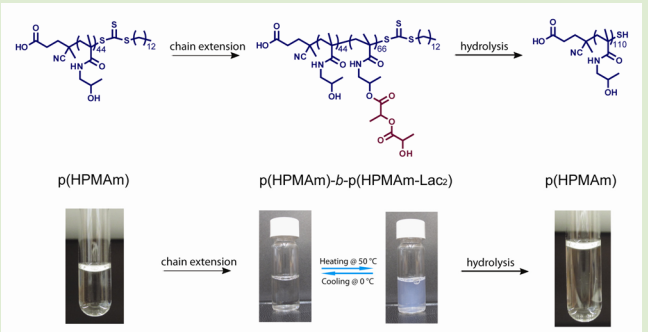
Supporting Information

ABSTRACT: A diblock copolymer composed of *N*-(2-hydroxy propyl) methacrylamide (HPMAm) as hydrophilic block and *N*-(2-hydroxy propyl) methacrylamide dilactate (HPMAm-Lac$_2$) as thermosensitive block was synthesized by reversible addition–fragmentation chain transfer (RAFT) polymerization. To this end, HPMAm was first polymerized with 4-cyano-4-[(dodecylsulfanylthiocarbonyl)-sulfanyl]-pentanoic acid as the chain transfer agent and azobisisobutyronitrile (AIBN) as the initiator. The polymerization showed a linear increase in M_n as a function of monomer conversion. The living p(HPMAm) chain (7 kDa) was subsequently extended with HPMAm-Lac$_2$ yielding a diblock copolymer (total M_n of 22 kDa). The copolymer showed reversible thermosensitivity in aqueous solution and self-assembled into micelles with a size of 58 nm (PDI 0.13) above its critical micelle temperature (CMT, 2.1 °C) and concentration (CMC, 0.044 mg/mL) and was soluble below the CMT. Paclitaxel, a hydrophobic chemotherapeutic drug, was encapsulated in the micelles with a loading capacity of 16.1 ± 1.2%. Hydrolysis of the dilactate side groups of the p(HPMAm-Lac$_2$) block converted the copolymer to the fully hydrophilic p(HPMAm) homopolymer, resulting in dissociation of the micelles. In conclusion, the livingness and versatility of RAFT polymerization provide possibilities to synthesize block copolymers with HPMAm and derivatives thereof.



Thermosensitive polymers have drawn great attention for biomedical applications such as drug and gene delivery[1−5] and tissue engineering.[6−8] Much work has been done regarding the syntheses and use of thermosensitive block copolymers for constructing drug delivery systems.[9,10] Block copolymers can turn from fully hydrophilic to amphiphilic when a thermosensitive block is combined in the same polymer chain with a permanently hydrophilic block and heated above its lower critical solution temperature (LCST) in aqueous solutions resulting in the formation of micelles or vesicles.[11−15] Polymeric micelles have shown great potential as vectors for targeted delivery of hydrophobic drugs.[16−21] Attractive features of polymeric micelles for pharmaceutical applications include (i) size below 150 nm; (ii) a good accommodation for poorly water-soluble drugs in the micellar core; and (iii) a hydrophilic corona endowing polymeric micelles with a stealth surface.[16,22]

Poly(*N*-(2-hydroxy propyl) methacrylamide) (p(HPMAm)) is a water-soluble polymer used for the development of polymeric prodrugs that have been clinically tested[23−27] as well as for the design of other functional biopolymers.[28,29] Chemical modifications of the hydroxyl group of *N*-(2-hydroxy propyl) methacrylamide (HPMAm) have resulted in various multifunctional monomers. As an example, the mono- and dilactate esters of HPMAm (HPMAm-Lac and HPMAm-Lac2) were synthesized, and the corresponding homopolymers and their random copolymers showed reversible thermoresponsiveness in aqueous solutions.[30] Hydrolysis of the lactate groups of HPMAm-Lac/HPMAm-Lac$_2$ results in an increased polarity and aqueous solubility of the corresponding polymers. When the resulting hydrophilic polymer has a molecular weight lower than 70 kDa, it will undergo renal excretion.[31]

Thermosensitive block copolymers based on p(HPMAm-Lac$_2$) have been used to construct micellar drug delivery systems.[9,11] Their synthesis was done using a polyethylene glycol (PEG) modified azo-compound which initiates conventional radical polymerization of HPMAm-Lac$_2$. However, this strategy has some drawbacks including limited control over

Received: December 26, 2012
Accepted: April 22, 2013
Published: April 25, 2013

Table 1. HPMAm and HPMAm-Lac$_2$ Homopolymers Synthesized in DMAc at 70 °C with AIBN as an Initiator and CDTPA as a CTA

polymer	time (h)	conversion (%)	M_n (theory)[a]	M_n (GPC)	PDI (M_w/M_n)
p(HPMAm)	1	22.1	6700	6900	1.25
	2	38.9	11500	11400	1.24
	5	56.8	16600	15200	1.39
p(HPMAm-Lac$_2$)	2	3.5	2400	2400	1.16
	4	7.2	4500	4600	1.16
	6	8.9	5500	6000	1.26
p(HPMAm)-b-p(HPMAm-Lac$_2$)	1	11.5	13600	14700	1.32
	2	20.5	18800	19100	1.30
	4	25.0	21400	21700	1.37

[a] M_n (theory) = [monomer]/[CTA] × conversion × $M_{wmonomer}$ + M_{wCTA}/(macro-CTA), where [monomer], [CTA], $M_{wmonomer}$, and M_{wCTA} are initial monomer and CTA concentrations, molecular weights of monomer and CTA/(macro-CTA), respectively.

molecular weight and its dispersity as well as limited possibilities to build various polymer architectures. Reversible addition−fragmentation chain transfer (RAFT) polymerization has been utilized to synthesize (micelle-forming) block polymers.[32] RAFT synthesis of block copolymers from PEG-coupled chain transfer agents for self-assembly into micelles has been described by several groups.[33,34] However, pegylated drug carriers are reported to have the accelerated blood clearance (ABC) effect after repeated i.v. injection.[35−37] Consequently, non-PEG hydrophilic polymers have been studied for the design of stealth nanoparticles.[38−42]

RAFT polymerization provides possibilities to copolymerize various monomers in a controlled fashion and build up multiple blocks.[43−45] Therefore, we have investigated the possibility to synthesize PEG-free thermosensitive block copolymers from HPMAm and HPMAm-Lac$_2$, by sequential RAFT polymerizations. HPMAm was first polymerized by RAFT, and then the p(HPMAm) chain (macro-CTA) was extended with HPMAm-Lac$_2$. The block copolymer showed thermosensitivity in the aqueous environment and formed micelles by heating an aqueous polymer solution above its LCST. Hydrolysis of the dilactate ester groups of the thermosensitive block converted the block copolymer into a fully hydrophilic p(HPMAm) homopolymer, and therefore the micelles gradually dissociated at physiological temperature and pH as also observed for PEG-*b*-p(HPMA-Lac$_2$) micelles.[11]

RAFT polymerizations of HPMAm and HPMAm-Lac$_2$ were done under three conditions adapted from the literature,[46−51] namely, using AIBN as an initiator and CDTPA as a CTA in DMAc (condition A) or ABCPA as an initiator and CDTPA (condition B) or CPAD (condition C) as a CTA in methanol/buffer pH 5 (1/1 (v/v)). For HPMAm, the results (Table 1 and Table S1, Supporting Information) show that the polymers synthesized under condition C have the lowest PDI (1.14−1.16), whereas the PDIs of the polymers synthesized under conditions A and B were slightly higher (1.21−1.39 and 1.19−1.32, respectively). However, the monomer conversion under condition C was very low (6% in 6 h) and substantially higher under conditions A and B (57 and 43%, respectively). For HPMAm-Lac$_2$, the RAFT polymerization under the three selected conditions proceeds slower than that of HPMA with conversions of 4−17%. The polymers synthesized under conditions A and C had the lowest polydispersity (1.16−1.26 and 1.18−1.21, respectively), whereas that of the polymer synthesized under condition B had a relatively high PDI (1.45−1.51). It should be noted that this reaction mixture became turbid, likely because methanol is a bad solvent for p(HPMAm-Lac$_2$). Taken together, RAFT polymerization using AIBN as an initiator and CDTPA as a CTA (condition A) in DMAc (a good solvent for both polymers) gives an acceptable polymerization rate and relatively narrow molecular weight distribution of both p(HPMAm) and p(HPMAm-Lac$_2$). So, this condition was used for the synthesis of p(HPMAm)-*b*-(HPMAm-Lac$_2$).

Figure 1 (top) shows the kinetics of the RAFT polymerization (condition A) of both HPMAm and HPMAm-. It is

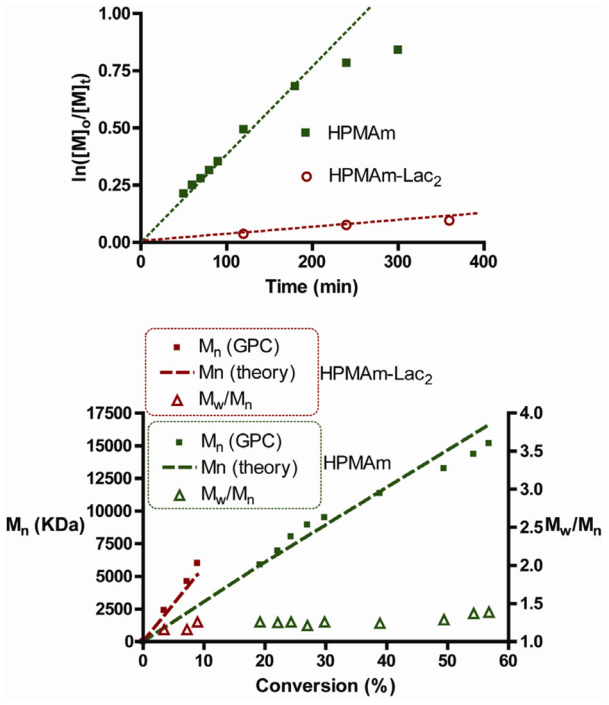


Figure 1. Plot of $\ln([M]_0/[M]_t)$ versus time for the RAFT polymerization of HPMA and HPMAm-Lac$_2$ (top) and plot of M_n (obtained from gel permeation chromatography (GPC)) versus conversion of HPMAm and HPMAm-Lac$_2$ with theoretical M_n calculated from the conversion and M_w/M_n (bottom).

shown that both polymerizations followed pseudofirst-order kinetics with deviation from linearity at high conversion of HPMAm (>50%), which can be ascribed to loss of active propagating radical species and/or a changed propagation rate constant, k_p.[51] A linear increase in M_n with the conversion of HPMAm and HPMAm-Lac$_2$, which is typical for a controlled radical polymerization,[51] was displayed in Figure 1 (bottom).

dx.doi.org/10.1021/mz300662b | ACS Macro Lett. 2013, 2, 403−408

For the synthesis of the block copolymer, we chose first to prepare a hydrophilic p(HPMAm) macro-CTA with a block length of 7 kDa, which is in the molecular weight range of p(HPMAm) used as stealth polymer[52−54] and which is close to the molecular weight of PEG that is frequently used as a "stealth" polymer.[55] Further, we aimed for a block copolymer with a total molecular weight far below 70 kDa, which allows its renal filtration in vivo.[31] The obtained p(HPMAm) macro-CTA was isolated by precipitation in diethyl ether (three times) to remove unreacted HPMAm, and the GPC trace of it is symmetric (Figure 1, top). Subsequently, the macro-CTA was extended with HPMAm-Lac$_2$ under the same conditions as for the synthesis of p(HPMAm) (Figure 2, middle). Different

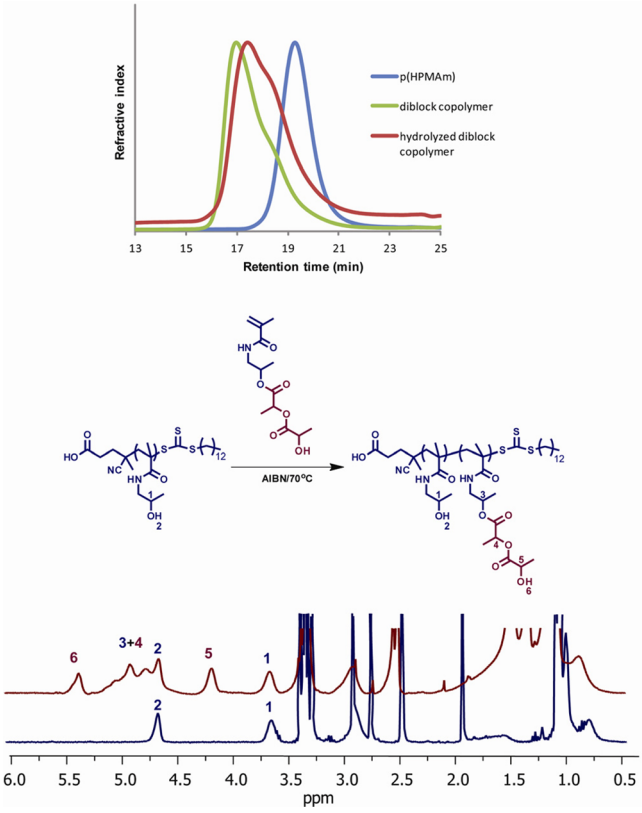


Figure 2. GPC traces of polymers before and after chain extension of p(HPMAm) macro-CTA with HPMAm-Lac$_2$ for 4 h (top). Reaction scheme of the copolymerization (middle). ^{1}H NMR spectra of p(HPMAm) (in blue) and p(HPMAm)-*b*-p(HPMAm-Lac$_2$) (in dark red).

block lengths of HPMAm-Lac$_2$ were aimed by increasing the polymerization time. Successful chain extension was proven by GPC and ^{1}H NMR spectroscopy (Figure 2). Copolymers obtained with chain extension showed a decreased GPC retention time (Figure 2, top) giving evidence that the molecular weight increased. The polymers have relatively low PDIs (<1.4) which points to a good control over the RAFT chain extension polymerization of HPMAm-Lac$_2$. The GPC trace of the polymer after chain extension for 4 h is asymmetric and shows slight tailing, which can be ascribed by dead chains forming during the chain extension. Such dead chains are commonly seen when diblock copolymers via RAFT are synthesized.[57] Table 1 also showed that with increasing reaction time the molecular weight of the block copolymer and thus that of the HPMAm-Lac$_2$ block increased. The M_n of the block copolymer after 4 h of chain extension was 22 kDa (PDI of 1.37) as measured by GPC.[56] The copolymer composition was also studied by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) shows repeating units of both HPMAm and HPMAm-Lac$_2$ (Figure 2, bottom). The theoretical M_n of the copolymer calculated from the HPMAm-Lac$_2$ conversion (25.0% at 4 h) was 21 kDa. By comparing the integration areas of resonances from the methine protons of HPMAm at 3.60 ppm and that of the methine protons of HPMAm-Lac$_2$ at 4.20 ppm, the M_n of the block copolymer can also be calculated (23 kDa). Overall, the experimental M_n by GPC measurement and ^{1}H NMR analysis is close to the theoretical M_n calculated by monomer conversion.

The p(HPMAm)-*b*-(HPMAm-Lac$_2$) diblock copolymer showed reversible thermoresponsive behavior in aqueous solution. The polymer can be dissolved in ammonium acetate buffer (pH 5.0, 120 mM) at a concentration of 10 mg/mL at 0 °C, while the solution turned opalescent after rapid heating at 50 °C for 1 min (Figure 3, top). The size of the polymeric

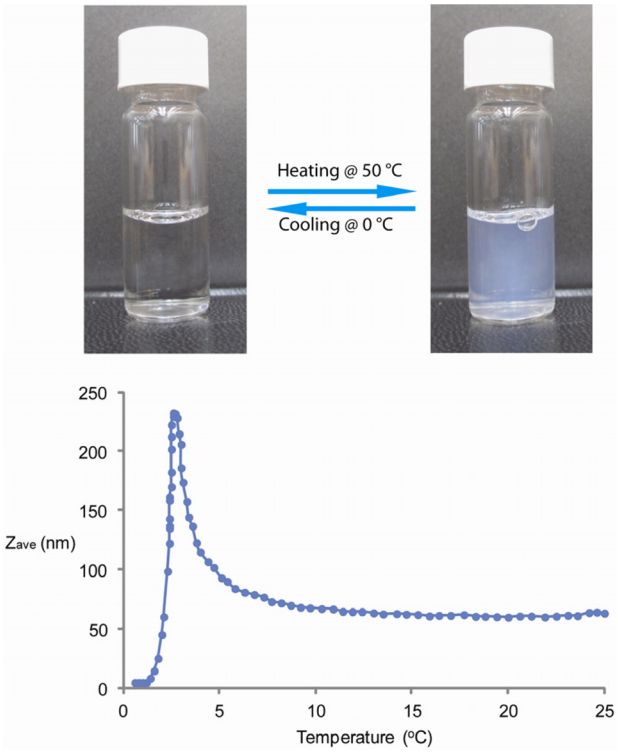


Figure 3. Photographs of an aqueous solution of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) (10 mg/mL) after heating (at 50 °C) or cooling (at 0 °C) (top). Change of Z-average diameter (by DLS) of p(HPMAm)-*b*-(HPMAm-Lac$_2$) micelles in an aqueous solution as measured during cooling (bottom).

micelles was 58 nm with a polydispersity of 0.13 as measured by dynamic light scattering (DLS). The formation of micelles composed of the p(HPMAm-Lac$_2$) core and the p(HPMAm) corona is due to the dehydration of the thermosensitive p(HPMAm-Lac$_2$) block upon heating above its LCST.[11,30] DLS analysis showed that when a micellar dispersion was slowly cooled from 25 to 0.1 °C, the size of the micelles did not change until the temperature reached around 14 °C and increased to a maximum value of around 240 nm at 3 °C, which indicates swelling of the micellar core caused by rehydration of the p(HPMAm-Lac$_2$) block below its LCST (6−13 °C[11,30]).

dx.doi.org/10.1021/mz300662b | ACS Macro Lett. 2013, 2, 403−408

Subsequently, the size decreased to lower than 10 nm at 1 °C (Figure 3), which means that the micelles dissociated and converted into unimers. The LCST (or critical micelle temperature, CMT) of the copolymer is 2.1 °C, similar to what has been observed for PEG-p(HPMAm-Lac$_2$), i.e., 6 °C.[11] Furthermore, the CMTs of the diblock copolymers slightly decreased from 2.4 to 2.1 °C with the molecular weights raised from 15 to 22 kDa (Table 1) which is in agreement of LCST data of p(OEGMA)-*b*-p(NIPAm) diblock copolymers of different molecular weights.[58]

Above the CMT of p(HPMAm)-*b*-p(HPMAm-Lac2), the copolymer can form micelles at concentrations above its critical micelle concentration (CMC) of 0.044 mg/mL which is close to that of the PEG-p(HPMAm-Lac$_2$).[11] The micelles were tested to incorporate a hydrophobic chemotherapeutic drug, i.e., paclitaxel (PTX), by the "fast heating" method which is accomplished in one minute and avoids the use of large amounts of organic solvents.[59] PTX was dissolved in ethanol at a concentration of 20 mg/mL and mixed with ice-cold polymer aqueous solution (10 mg/mL) at a volume ratio of 1/9 and then immediately incubated with vigorous shaking in a water bath at 50 °C for one minute. An opalescent micellar dispersion was obtained. After filtration of the micellar dispersion through a 0.45 μm membrane filter, the micelles were characterized by its size and drug loading capacity. The size of PTX loaded micelles was 88 nm with a polydispersity of 0.18. The PTX loading capacity (weight percentage of encapsulated drug to the sum of encapsulated drug and polymer) of the micelles and encapsulation efficiency (weight percentage of encapsulated drug to feed drug) were 16.1 ± 1.2% and 86.4 ± 7.7%, respectively.

HPMAm-Lac$_2$ is hydrolytically degradable ($t_{1/2}$ = 15.4 h at pH 7.5 and 37 °C).[60] Consequently, the thermoresponsive block copolymers will be converted into the fully hydrophilic homopolymer p(HPMAm) upon incubation in an aqueous environment. To investigate this, micelles of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) were incubated in pH 10.0 buffer (i.e., accelerated degradation conditions) at 37 °C; meanwhile, the size and light scattering intensity were monitored by DLS (Figure 4, top). The micelles had initially a size of around 60 nm and started to swell after around 50 min of incubation. The swelling was accompanied with an increase in light scattering intensity until 140 min. After that, the scattering intensity dropped during the next 20 min, indicating dissociation of the micelles. After 3.8 h, only free polymer chains remained in the solution (hydrodynamic size of 10 nm). This behavior can be explained as follows. The hydrolysis of the lactate side groups of the HPMAm-Lac$_2$ units results in an increase of the polarity of p(HPMAm-Lac$_2$) block, and therefore the LCST of the polymer gradually raises until above the incubation temperature of 37 °C. Consequently, the core of micelles becomes more hydrated and swollen, and eventually the micelles dissociate when the LCST of the p(HPMAm-Lac$_2$) block passes 37 °C. As shown by ^{1}H NMR spectroscopy, the dilactate groups of HPMAm-Lac$_2$ were completely removed after hydrolysis, and only p(HPMAm) remained (Figure 4, bottom). The GPC refractive index (RI) trace showed a slightly longer retention time of the hydrolyzed copolymer (Figure 2, top), which suggests that the copolymer after hydrolysis of the side groups has a smaller hydrodynamic size than the copolymer before hydrolysis. Additionally, it is worth mentioning that the CTA attached at the copolymer chain end was also substantially hydrolyzed under the described conditions as the GPC UV

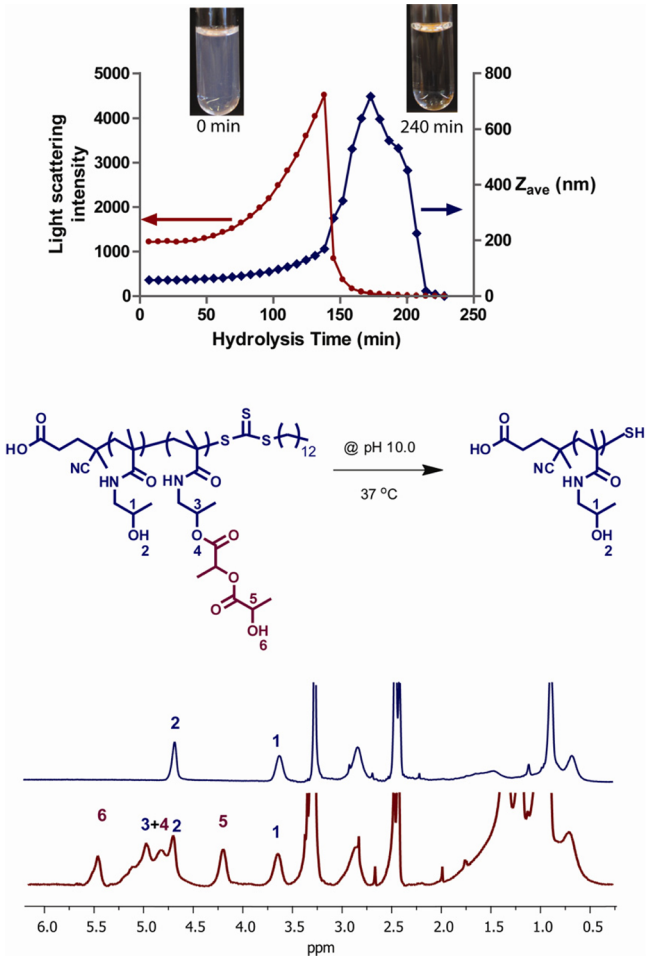


Figure 4. Effect of hydrolysis of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) micelles at pH 10.0 and 37 °C on *Z*-average diameter (Z_{ave}) and scattering intensity as studied by DLS (inset: photographs of the dispersion before and after hydrolysis) (top). Hydrolysis scheme of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) (middle). ^{1}H NMR spectra of p(HPMAm)-*b*-p(HPMAm-Lac$_2$) before (in dark red) and after hydrolysis (in blue) (bottom).

trace of the copolymer at 320 nm (which is specific for the CTA molecules) initially overlapped with the RI trace, but UV absorption of the CTA vanished upon hydrolysis. This is also a sign that the copolymer has an active CTA end group, and further chain extension with other monomers to synthesize triblock polymers is possible.

In conclusion, a thermosensitive block copolymer composed of p(HPMAm) as the hydrophilic block and p(HPMAm-Lac$_2$) as the thermosensitive hydrophobic block was successfully synthesized by RAFT. The block copolymer displayed reversible thermoresponsive behavior in aqueous environment; i.e., it was soluble under its CMT and formed micelles upon incubation at high temperature (50 °C). The hydrophobic drug paclitaxel can be encapsulated into the micellar hydrophobic core by the "fast heating" method. The micelles were hydrolytically degradable in aqueous solution, to yield p(HPMAm) which is hydrophilic and has a molecular weight lower than 22 kDa. These two aspects allow the degradation product to be cleared from the circulation in vivo by renal filtration. The degradability of the copolymer and expected controlled release behavior favor its biomedical applications, such as for drug delivery.

dx.doi.org/10.1021/mz300662b | *ACS Macro Lett.* 2013, 2, 403−408

■ ASSOCIATED CONTENT

Supporting Information

The detailed synthesis of the polymers, characterizations of the polymers by ^{1}H NMR spectroscopy and GPC, and preparation and characterizations of the micelles have been described. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research was partially supported by the China Scholarship Council.

■ REFERENCES

(1) De las Heras Alarcón, C.; Pennadam, S.; Alexander, C. *Chem. Soc. Rev.* **2005**, *34*, 276.
(2) Bae, Y. H.; Okano, T.; Hsu, R.; Kim, S. W. *Macromol. Rapid Commun.* **1987**, *8*, 481.
(3) Soga, O.; van Nostrum, C. F.; Fens, M.; Rijcken, C. J. F.; Schiffelers, R. M.; Storm, G.; Hennink, W. E. J. *Controlled Release* **2005**, *103*, 341.
(4) Kurisawa, M.; Yokoyama, M.; Okano, T. J. *Controlled Release* **2000**, *69*, 127.
(5) Liu, R.; Fraylich, M.; Saunders, B. R. *Colloid Polym. Sci.* **2009**, *287*, 627.
(6) Censi, R.; Fieten, P. J.; di Martino, P.; Hennink, W. E.; Vermonden, T. *Macromolecules* **2010**, *43*, 5771.
(7) Vermonden, T.; Fedorovich, N. E.; van Geemen, D.; Alblas, J.; van Nostrum, C. F.; Dhert, W. J. A.; Hennink, W. E. *Biomacromolecules* **2008**, *9*, 919.
(8) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173.
(9) Talelli, M.; Rijcken, C.; Van Nostrum, C.; Storm, G.; Hennink, W. *Adv. Drug Delivery Rev.* **2010**, *62*, 231.
(10) He, C.; Kim, S. W.; Lee, D. S. J. *Controlled Release* **2008**, *127*, 189.
(11) Soga, O.; van Nostrum, C. F.; Ramzi, A.; Visser, T.; Soulimani, F.; Frederik, P. M.; Bomans, P. H. H.; Hennink, W. E. *Langmuir* **2004**, *20*, 9388.
(12) Wei, H.; Cheng, S. X.; Zhang, X. Z.; Zhuo, R. X. *Prog. Polym. Sci.* **2009**, *34*, 893.
(13) Topp, M.; Dijkstra, P.; Talsma, H.; Feijen, J. *Macromolecules* **1997**, *30*, 8518.
(14) Riess, G. *Prog. Polym. Sci.* **2003**, *28*, 1107.
(15) Arotçaréna, M.; Heise, B.; Ishaya, S.; Laschewsky, A. *J. Am. Chem. Soc.* **2002**, *124*, 3787.
(16) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2012**, *64*, 37.
(17) Oerlemans, C.; Bult, W.; Bos, M.; Storm, G.; Nijsen, J. F. W.; Hennink, W. E. *Pharm. Res.* **2010**, *27*, 2569.
(18) Gong, J.; Chen, M.; Zheng, Y.; Wang, S.; Wang, Y. J. *Controlled Release* **2012**, *159*, 312.
(19) Bromberg, L. J. *Controlled Release* **2008**, *128*, 99.
(20) Kedar, U.; Phutane, P.; Shidhaye, S.; Kadam, V. *Nanomed.: Nanotechnol., Biol. Med.* **2010**, *6*, 714.
(21) Deng, C.; Jiang, Y.; Cheng, R.; Meng, F.; Zhong, Z. *Nano Today.* **2012**, *7*, 467.
(22) Gaucher, G.; Marchessault, R. H.; Leroux, J. C. J. J. *Controlled Release* **2010**, *143*, 2.
(23) Ulbrich, K.; Šubr, V. *Adv. Drug Delivery Rev.* **2010**, *62*, 150.
(24) Hong, C. Y.; Pan, C. Y. *Macromolecules* **2006**, *39*, 3517.
(25) Charles, W.; Huang, F.; Li, N.; Vasilieva, Y. A.; Ray, J.; Convertine, A. J.; McCormick, C. L. *Macromolecules* **2006**, *39*, 6871.
(26) Duncan, R.; Vicent, M. J. *Adv. Drug Delivery Rev.* **2010**, *62*, 272.
(27) Kopeček, J.; Kopečková, P. *Adv. Drug Delivery Rev.* **2010**, *62*, 122.
(28) Treat, N. J.; Smith, D. D.; Teng, C.; Flores, J. D.; Abel, B. A.; York, A. W.; Huang, F.; McCormick, C. L. *ACS Macro Lett.* **2011**, *1*, 100.
(29) Yanjarappa, M. J.; Gujraty, K. V.; Joshi, A.; Saraph, A.; Kane, R. S. *Biomacromolecules* **2006**, *7*, 1665.
(30) Soga, O.; van Nostrum, C. F.; Hennink, W. E. *Biomacromolecules* **2004**, *5*, 818.
(31) Etrych, T.; Šubr, V.; Strohalm, J.; Šírová, M.; Říhová, B.; Ulbrich, K. J. *Controlled Release* **2012**, *164*, 346.
(32) Stenzel, M. H. *Chem. Commun.* **2008**, *30*, 3486.
(33) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81.
(34) Liu, S.; Weaver, J. V. M; Tang, Y.; Billingham, N. C.; Armes, S. P.; Tribe, K. *Macromolecules* **2002**, *35*, 6121.
(35) Szebeni, J. *Crit. Rev. Ther. Drug Carrier Syst.* **2001**, *18*, 567.
(36) Ishida, T.; Kiwada, H. *Int. J. Pharm.* **2008**, *354*, 56.
(37) Saadati, R.; Dadashzadeh, S.; Abbasian, Z.; Soleimanjahi, H. *Pharm. Res.* **2013**, *30*, 985.
(38) Koňák, Č.; Ganchev, B.; Teodorescu, M.; Matyjaszewski, K.; Kopečková, P.; Kopeček, J. *Polymer* **2002**, *43*, 3735.
(39) Hemmelmann, M.; Kurzbach, D.; Koynov, K.; Hinderberger, D.; Zentel, R. *Biomacromolecules* **2012**, *13*, 4065.
(40) Oupický, D.; Koňák, Č.; Ulbrich, K. *Mater. Sci. Eng., C* **1999**, *7*, 59.
(41) Bailly, N.; Thomas, M.; Klumperman, B. *Biomacromolecules* **2012**, *13*, 4109.
(42) Metselaar, J. M.; Bruin, P.; de Boer, L. W. T.; de Vringer, T.; Snel, C.; Oussoren, C.; Wauben, M. H. M.; Crommelin, D. J. A.; Storm, G.; Hennink, W. E. *Bioconjugate Chem.* **2003**, *14*, 1156.
(43) York, A. W.; Kirkland, S. E.; McCormick, C. L. *Adv. Drug Delivery Rev.* **2008**, *60*, 1018.
(44) Klumperman, B.; van den Dungen, E. T. A.; Heuts, J.; Monteiro, M. J. *Macromol. Rapid Commun.* **2010**, *31*, 1846.
(45) Boyer, C.; Bulmus, V.; Davis, T. P.; Ladmiral, V.; Liu, J.; Perrier, S. b. *Chem. Rev.* **2009**, *109*, 5402.
(46) Luo, K.; Yang, J.; Kopečková, P.; Kopeček, J. *Macromolecules* **2011**, *44*, 248.
(47) Scales, C. W.; Vasilieva, Y. A.; Convertine, A. J.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2005**, *6*, 1846.
(48) Yang, J.; Luo, K.; Pan, H.; Kopečková, P.; Kopeček, J. *React. Funct. Polym.* **2011**, *71*, 294.
(49) Jia, Z.; Wong, L.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2008**, *9*, 3106.
(50) Šubr, V.; Kostka, L.; Strohalm, J.; Etrych, T.; Ulbrich, K. *Macromolecules* **2013**, *46*, 2100.
(51) Charles, W.; Vasilieva, Y. A.; Convertine, A. J.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2005**, *6*, 1846.
(52) Koňáka, Č.; Ganchevb, B.; Teodorescuc, M.; Matyjaszewskic, K.; Kopečkovád, P.; Kopeček, J. *Polymer* **2002**, *43*, 3735.
(53) Barz, M.; Tarantola, M.; Fischer, K.; Schmidt, M.; Luxenhofer, R.; Janshoff, A.; Theato, P.; Zentel, R. *Biomacromolecules* **2008**, *9*, 3114.
(54) Lele, B. S.; Leroux, J. C. *Macromolecules* **2002**, *35*, 6714.
(55) Lukyanov, a. N.; Gao, Z. G.; Mazzola, L.; Torchilin, V. P. *Pharm. Res.* **2002**, *19*, 1424.
(56) Tao, L.; Liu, J.; Xu, J.; Davis, T. P. *Org. Biomol. Chem.* **2009**, *7*, 3481.
(57) Duréault, A.; Taton, D.; Destarac, M.; Leising, F.; Gnanou, Y. *Macromolecules* **2004**, *37*, 5513.
(58) Graaf, A. D. J.; Mastrobattista, E.; Vermonden, T.; van Nostrum, C. F.; Rijkers, D. T. S.; Liskamp, R. M. J.; Hennink, W. *Macromolecules* **2012**, *45*, 842.

(59) Neradovic, D.; Soga, O.; Van Nostrum, C.; Hennink, W. *Biomaterials* **2004**, *25*, 2409.
(60) Neradovic, D.; Van Steenbergen, M.; Vansteelant, L.; Meijer, Y.; Van Nostrum, C.; Hennink, W. *Macromolecules* **2003**, *36*, 7491.