

Facile RAFT Precipitation Polymerization for the Microwave-Assisted Synthesis of Well-Defined, Double Hydrophilic Block Copolymers and Nanostructured Hydrogels

Zesheng An,[†] Qihui Shi,[†] Wei Tang,[†] Chia-Kuang Tsung,[†] Craig J. Hawker,^{*,†,‡} and Galen D. Stucky^{*,†,‡}

Contribution from the Department of Chemistry and Biochemistry, Department of Materials, Materials Research Laboratory, University of California, Santa Barbara, California 93106-9510

Received August 5, 2007; E-mail: hawker@mrl.ucsb.edu; stucky@chem.ucsb.edu

Abstract: Water-soluble macromolecular chain transfer agents (Macro-CTAs) were developed for the microwave-assisted precipitation polymerization of *N*-isopropylacrylamide. Two types of Macro-CTAs, amphiphilic (Macro-CTA1) and hydrophilic (Macro-CTA2), were investigated regarding their activity for the facile formation of nanoparticles and double hydrophilic block copolymers by RAFT processes. While both Macro-CTAs functioned as steric stabilization agents, the variation in their surface activity afforded different levels of control over the resulting nanoparticles in the presence of cross-linkers. The cross-linked nanoparticles produced using the amphiphilic Macro-CTA1 were less uniform than those produced using the fully hydrophilic Macro-CTA2. The nanoparticles spontaneously formed core-shell structures with surface functionalities derived from those of the Macro-CTAs. In the absence of cross-linkers, both types of Macro-CTAs showed excellent control over the RAFT precipitation polymerization process with well-defined, double hydrophilic block copolymers being obtained. The power of combining microwave irradiation with RAFT procedures was evident in the high efficiency and high solids content of the polymerization systems. In addition, the "living" nature of the nanoparticles allowed for further copolymerization leading to multiresponsive nanostructured hydrogels containing surface functional groups, which were used for surface bioconjugation.

Introduction

Reversible addition-fragmentation chain transfer (RAFT) polymerization,¹ along with other controlled/living free radical polymerization (LFRP) techniques including atom transfer radical polymerization (ATRP)² and nitroxide-mediated polymerization (NMP),³ has been extensively studied and exploited for the production of well-defined polymers with predicted molecular weight, composition, and architecture.⁴ Compared with the tremendous success of RAFT polymerization in homogeneous systems (solution/bulk),^{5,6} successful implementation of RAFT in heterogeneous polymerization systems has been limited.^{7,8} Polymerization in heterogeneous systems, especially in aqueous media, has industrially important advantages such as high solids content, low viscosity, and the use of environ-

mentally benign water as the primary dispersant. In addition the isolation of the product is facile, and waste is limited.⁹ Successful adaptation of the RAFT process to aqueous dispersed media and to functional monomers therefore holds enormous potential for the efficient production of novel polymer materials with improved quality and expanded applications.

Increasing attention has recently been directed toward responsive polymers, macrohydrogels, and nano/microgels based on *N*-isopropylacrylamide (NIPAm) due to their potential use in optical/electronic devices,¹⁰⁻¹² biomedical therapies,¹³⁻¹⁷ and responsive surfaces.^{18,19} The successful preparation of PNIPAm homopolymers²⁰⁻²⁵ and block/random copolymers²³⁻²⁹ with

[†] Department of Chemistry and Biochemistry.

[‡] Department of Materials and Materials Research Laboratory.

- (1) Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffery, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559-5562.
- (2) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921-2990.
- (3) Hawker, C. J.; Bosman, A. W.; Harth, E. *Chem. Rev.* **2001**, *101*, 3661-3688.
- (4) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200-1205.
- (5) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379-410.
- (6) Favier, A.; Charreyre, M.-T. *Macromol. Rapid Commun.* **2006**, *27*, 653-692.
- (7) McLeary, J. B.; Klumperman, B. *Soft Matter* **2006**, *2*, 45-53.
- (8) Save, M.; Guillaneuf, Y.; Gilbert, R. G. *Aust. J. Chem.* **2006**, *59*, 693-711.

- (9) Herk, A. V., Ed. *Chemistry and Technology of Emulsion Polymerization*; Oxford: New York, 2005.
- (10) Weissman, J. M.; Sunkara, H. B.; Tse, A. S.; Asher, S. A. *Science* **1996**, *274*, 959-963.
- (11) Sidorenko, A.; Krupenkin, T.; Taylor, A.; Fratzl, P.; Aizenberg, J. *Science* **2007**, *315*, 487-490.
- (12) Dong, L.; Agarwal, A. K.; Beebe, D. J.; Jiang, H. *Nature* **2006**, *442*, 551-554.
- (13) Bromber, L. E.; Ron, E. S. *Adv. Drug Delivery Rev.* **1998**, *31*, 197-221.
- (14) Das, M.; Mardiyani, S.; Chan, W. C. W.; Kumacheva, E. *Adv. Mater.* **2006**, *18*, 80-83.
- (15) Nayak, S.; Lee, H.; Chmielewski, J.; Lyon, L. A. *J. Am. Chem. Soc.* **2004**, *126*, 10258-10259.
- (16) Murthy, N.; Xu, M. C.; Schuck, S.; Kunisawa, J.; Shastri, N.; Fréchet, J. M. J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 4995-5000.
- (17) Kiser, P. F.; Wilson, G.; Needham, D. *Nature* **1998**, *394*, 459-462.
- (18) Luzinov, I.; Minko, S.; Tsukruk, V. V. *Prog. Polym. Sci.* **2004**, *29*, 635-698.
- (19) Ionov, L.; Stamm, M.; Diez, S. *Nano Lett.* **2006**, *6*, 1982-1987.

narrow polydispersity has been well documented in various homogeneous systems via the RAFT process. However, polymerization of NIPAm by the RAFT process, and more generally LFRP techniques, in dispersed media (precipitation polymerization) has received little attention, and most studies of RAFT polymerization in heterogeneous systems have focused on hydrophobic monomers in emulsion polymerization and related techniques.^{7,8} For RAFT-mediated precipitation polymerization, there are only few relevant examples for precipitation polymerization in aqueous systems with no studies for NIPAm being reported. Recently, acrylamide was polymerized in a mixture of water and 2-propanol (4/1, v/v), however the dispersity of the resulting nanogels was poor, and in some cases gelation occurred.³⁰ The precipitation polymerization of NIPAm by ATRP using a PEG macro-initiator was studied; however, the polydispersity of the resulting polymers was greater than 1.6, and little control over functional group incorporation was demonstrated.³¹

In designing responsive PNIPAm materials based on LFRP techniques, the well-controlled nature of these systems offers a number of advantages when compared to the nature of traditional systems, such as more uniform response behavior and controlled size and structure.^{32–34} For example, macrohydrogels produced by the RAFT process exhibited faster shrinking kinetics and higher shrinking ratios than traditional hydrogels.^{35,36} To further extend the range of responsive materials, we report the microwave-assisted, precipitation polymerization of NIPAm and its subsequent copolymerization with *N*-isopropylmethacrylamide (NIPMAm) through seeded precipitation polymerization processes. The combination of microwave irradiation and RAFT procedures allows for the development of highly efficient, heterogeneous, and living polymerization strategies. In addition, the versatility of the RAFT process permits functional materials such as well-defined, double hydrophilic block copolymers and multiresponsive nanostructured hydrogel materials to be prepared with novel, core–shell nanogels having surface functionalities available for coupling to biologically relevant systems.

Results and Discussion

In order to evaluate the scope of the RAFT-mediated precipitation polymerization and, with the aim of developing novel, nanostructured hydrogel materials, water-soluble poly-(dimethylacrylamide)s (PDMA)s with different structural characteristics were examined as chain transfer agents (Macro-CTAs) for regulation of the radical polymerization of NIPAm (Scheme 1). These Macro-CTAs with diverse end groups and molecular weights bear several important features. First, they are water soluble and act as steric stabilizers in the formation of nanogel particles. Two types of Macro-CTAs were synthesized to investigate the role of the Macro-CTAs in the formation of the nanogel particles, Macro-CTA1a–c with hydrophobic dodecyl tails and fully hydrophilic Macro-CTA2a–c that are not surface active. Second, the Macro-CTAs were prepared via the RAFT process under microwave conditions (Supporting Information) with CTA *S*-1-dodecyl-*S'*-2-(2,2'-dimethylacetic acid)trithiocarbonate³⁷ for Macro-CTA1a–c and *S*-3-(propionic acid)-*S'*-2-(2,2'-dimethylacetic acid)trithiocarbonate³⁸ for Macro-CTA2a–c, respectively. The RAFT process not only endows the polymers with well-defined end groups, such as carboxylic acids, but the precipitation polymerization process translates these end groups into surface functionalities when nanogels are prepared using these macro-CTAs.

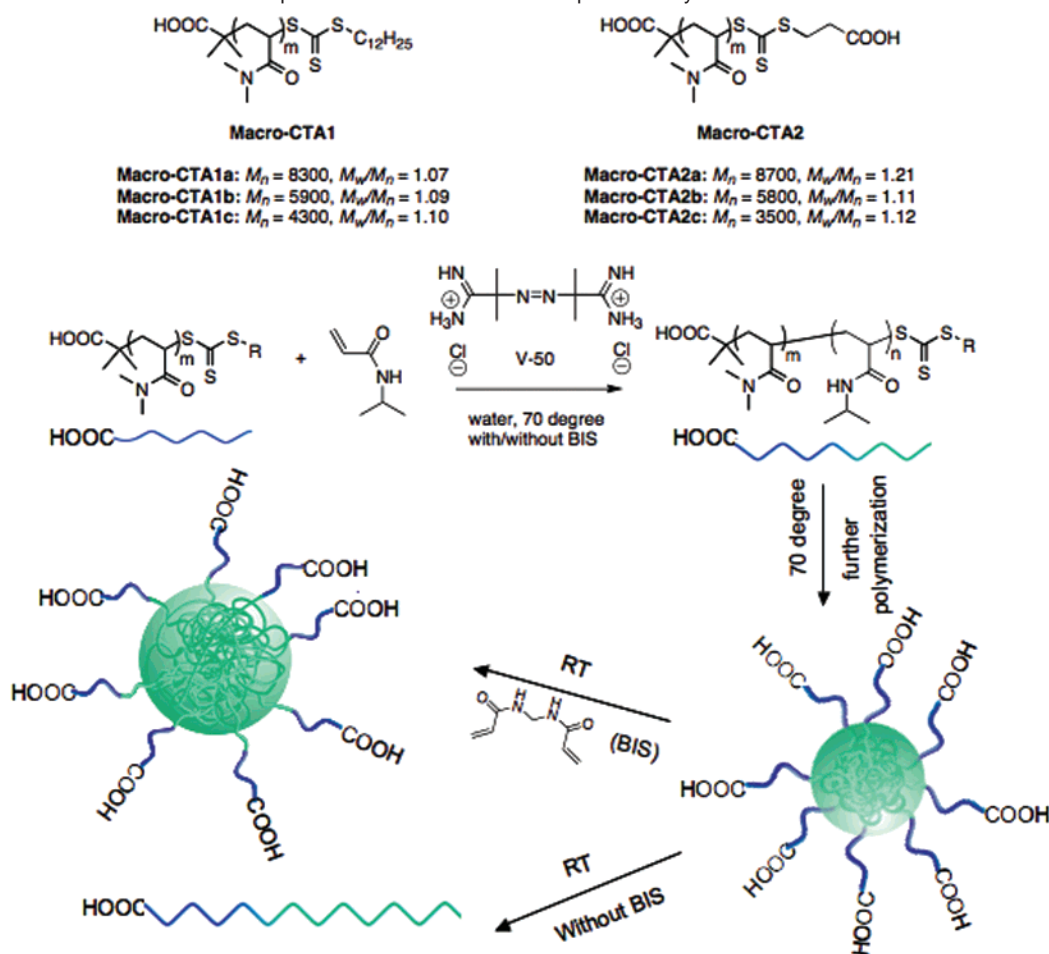
As illustrated in Scheme 1, when aqueous solutions of the monomers, Macro-CTAs, and initiator 2,2'-azobis(2-methylpropionamide) dihydrochloride (V-50) are irradiated with microwave to a polymerization temperature of 70 °C, chain extension of the existing Macro-CTAs with NIPAm monomers occurs to give chain-end functionalized PNIPAm. While PNIPAm prepared by traditional free radical polymerization typically shows a volume phase transition around a lower critical solution temperature (LCST) of 32 °C, the LCST was recently reported to be highly dependent on the molecular weights and end groups for narrow-dispersity PNIPAm prepared by LFRP techniques.^{32–34} Thus, in the precipitation polymerization of NIPAm mediated by the Macro-CTAs, the initially formed polymers show different solubility in water due to the dissimilar chain ends of the Macro-CTAs before the PNIPAm blocks become sufficiently long such that the PNIPAm chains collapse to form nanoparticle seeds stabilized by the water-soluble PDMA blocks. When the cross-linker *N,N'*-methylenebisacrylamide (BIS) is used, the growing polymers undergo cross-linking, and after cooling to room temperature, the resulting fully hydrophilic nanoparticles are in the swollen state with the integrity of the nanoparticles maintained. When no cross-linker is used, after cooling to room temperature, the nanoparticles dissociate into double hydrophilic block copolymers due to the transition of the PNIPAm block to being soluble below its LCST. As a result, the same precipitation polymerization process can lead to a novel range of materials, from responsive nanoparticles to double hydrophilic block copolymers with control over structure and functionality.

Novel Nanostructured Hydrogels by Precipitation Polymerization. The precipitation polymerization of NIPAm was carried out under microwave conditions in the presence of Macro-CTAs and 2 mol % BIS at a pH of 2.5. As monitored

- (20) Convertine, A. J.; Lokitz, B. S.; Lowe, A. B.; Scales, C. W.; Myrick, L. J.; McCormick, C. L. *Macromol. Rapid Commun.* **2005**, *26*, 791–795.
- (21) Convertine, A. J.; Ayres, N.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Biomacromolecules* **2004**, *5*, 1177–1180.
- (22) Ganachaud, F.; Monteiro, M. J.; Gilbert, R. G.; Dourges, M. A.; Thang, S. H.; Rizzardo, E. *Macromolecules* **2000**, *33*, 6738–6745.
- (23) Zhang, P.; Liu, Q.; Qing, A.; Shi, J.; Lu, M. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 3312–3320.
- (24) Liu, B.; Perrier, S. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 3643–3654.
- (25) Yang, C.; Cheng, Y.-L. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1191–1201.
- (26) Convertine, A. J.; Lokitz, B. S.; Vasileva, Y.; Myrick, L. J.; Scales, C. W.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2006**, *39*, 1724–1730.
- (27) Li, Y.; Lokitz, B. S.; McCormick, C. L. *Macromolecules* **2006**, *39*, 81–89.
- (28) Schilli, C. M.; Zhang, M.; Rizzardo, E.; Thang, S. H.; Chong, Y. K.; Edwards, K.; Karlsson, G.; Müller, A. H. E. *Macromolecules* **2004**, *37*, 7861–7866.
- (29) Savariar, E. N.; Thayumanavan, S. S. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 6340–6345.
- (30) Taton, D.; Baussard, J.-F.; Dupayage, L.; Poly, J.; Gnanou, Y.; Ponsinet, V.; Destarac, M.; Mignaud, C.; Pitois, C. *Chem. Commun.* **2006**, 1953–1955.
- (31) Kim, K. H.; Kim, J.; Jo, W. H. *Polymer* **2005**, *46*, 2836–2840.
- (32) Plunkett, K. N.; Zhu, X.; Moore, J. S.; Leckband, D. E. *Langmuir* **2006**, *22*, 4259–4266.
- (33) Xia, Y.; Yin, X.; Burke, N. A. D.; Stöver, H. D. H. *Macromolecules* **2005**, *38*, 5937–5943.
- (34) Furry, S.; Zhang, Y.; Ortiz-Acosta, D.; Cremer, P. S.; Bergbreiter, D. E. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 1492–1501.
- (35) Liu, Q.; Zhang, P.; Lu, M. *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2615–2624.
- (36) Liu, Q.; Zhang, P.; Qing, A.; Lan, Y.; Lu, M. *Polymer* **2006**, *47*, 2330–2336.

(37) Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754–6756.

(38) Wang, R.; McCormick, C. L.; Lowe, A. B. *Macromolecules* **2005**, *38*, 9518–9525.

Scheme 1. Macro-CTAs and Schematic Representation of the RAFT Precipitation Polymerization Process

with dynamic light scattering (DLS), the nanogels quickly reached their final size within 10–30 min, and the conversion of the monomers was essentially quantitative after a similar reaction time. The pH of the polymerization media was controlled at 2.5 in order to minimize hydrolysis of the Macro-CTAs, since hydrolysis of CTAs at elevated pH in homogeneous aqueous polymerization systems has been shown to detrimentally affect the molecular weights and polydispersity of the RAFT polymers.^{20,39,40} As shown in Figure 1, the size of the nanogels decreases with increasing concentration of Macro-CTAs, **1b** and **2b**, and is relatively independent of the end groups and surface activity of the Macro-CTAs, suggesting that both Macro-CTAs function as stabilization agents in the formation of nanoparticles. At low concentrations of Macro-CTAs, the polydispersity of the nanoparticles is low. However, above 1.0 mM, the polydispersity of the amphiphilic system, **1b**, increases significantly when compared to that of the fully hydrophilic system, **2b**. From the amphiphilic structure of Macro-CTA**1b**, it can be postulated that Macro-CTA**1b** should behave as a surfactant to stabilize the nanoparticles. Indeed, the critical micellar concentration (CMC) of Macro-CTA**1b** was determined to be 0.23 mM using a spectroscopic method with rhodamine 6G as the optical probe (Supporting Information). For the polymerization systems with Macro-CTA**1b** in the concentration range of 0.43–1.71 mM, Macro-CTA**1b** is

expected to exist in an equilibrium of free molecules and micelles. Because the monomer and initiator are water soluble, initiation should occur in the homogeneous solution. After initiation, there are two different fates for the formed oligomeric radical and/or dormant species; they can either form particle seeds via a homogeneous nucleation mechanism by reacting with the free Macro-CTAs, or they can enter the Macro-CTA micelles (radical entry process) via a micelle nucleation mechanism.⁴¹ As a consequence, there are two competing particle nucleation mechanisms for Macro-CTA**1b**-mediated precipitation polymerization systems, resulting in the higher polydispersity in particle size (DLS in Figure 1 and AFM in Figure S2) than that found for the polymerization systems mediated by Macro-CTA**2b** in which a single homogeneous nucleation process is expected. These results further indicate that the Macro-CTAs function as steric stabilizers for the nanogels and that Macro-CTAs with a surfactant-like structure are not necessary for the stabilization of the formed particles.

A positively charged free radical initiator, V-50, was selected for these experiments as it offers an opportunity to differentiate two possible nanoparticle structures: traditional nanostructure **A** vs novel core–shell nanostructure **B** (Figure 2). If the precipitation polymerization process occurs via a traditional process, i.e. nanoparticles that are electrostatically stabilized by the radical initiator fragments, the surface of the nanoparticles should be positively charged. In contrast, if the precipitation

(39) Thomas, D. B.; Sumerlin, B. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2003**, *36*, 1436–1439.

(40) McCormick, C. L.; Lowe, A. B. *Acc. Chem. Res.* **2004**, *37*, 312–325.

(41) Gilbert, R. G., Ed. *Emulsion Polymerization: A Mechanistic Approach*; Academic Press: London, 1995.

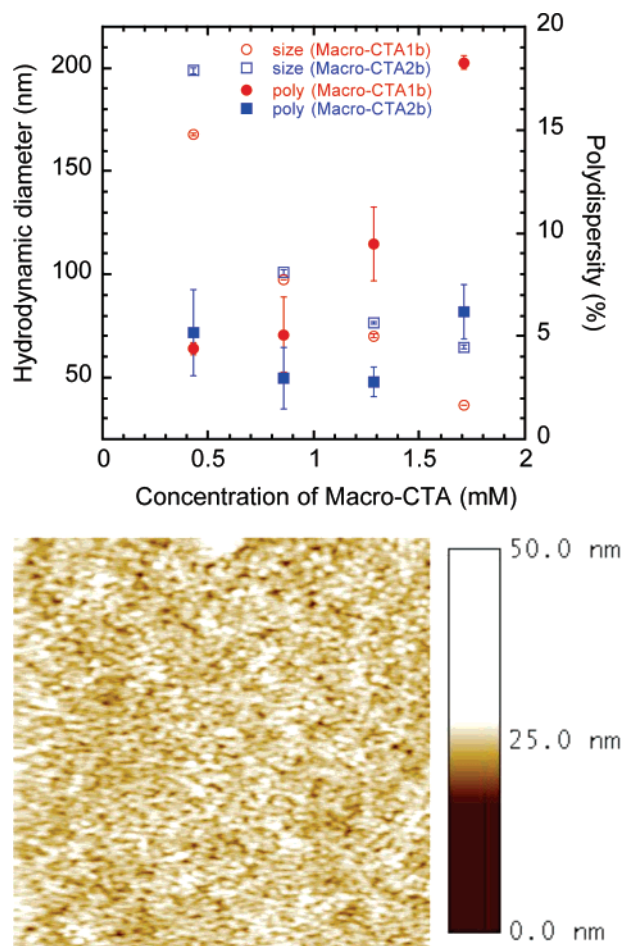


Figure 1. (Top) Evolution of nanoparticle size and polydispersity with the concentration of Macro-CTAs, [NIPAm] = 0.15 M, [BIS] = 3.0 mM, [V-50] = 0.9 mM, 70 °C, 1 h. (Bottom) AFM micrograph (5 μm \times 5 μm) of the dried nanogels prepared with 0.9 mM Macro-CTA1b.

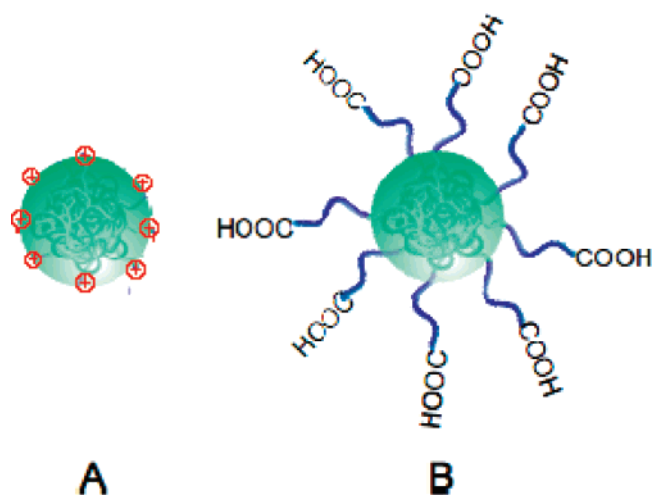


Figure 2. (A) Nanostructure by traditional free radical precipitation polymerization. (B) Novel, core-shell nanostructure by RAFT-mediated precipitation polymerization.

polymerization is mediated by the RAFT agents, the nanoparticles would have a core-shell structure with the carboxylic acid groups located at the surface and therefore at $\text{pH} > \text{pK}_a$ would have a negatively charged surface. For polymerizations mediated by both Macro-CTA1b and 2b at various concentrations, ζ -potential measurements showed that the nanoparticles

were all negatively charged (Supporting Information), suggesting that the precipitation polymerization is mediated by the RAFT process with the Macro-CTAs located on the surface of the nanoparticles giving rise to a defined core-shell nanostructure with surface functionalities. Control experiments without Macro-CTAs, however, only led to the formation of flocculate when V-50 concentration was in the range of 0.9–90 mM.

In order to further assess their stabilization ability, Macro-CTAs with different molecular weights were used in the precipitation polymerization process, and the results demonstrate a strong dependence of the nanoparticles size on the molecular weight of the Macro-CTAs. For these experiments, the concentration of NIPAm was controlled at 0.15 M and the ratio of NIPAm/Macro-CTA/V-50 was 333:1:2. As the molecular weight of Macro-CTA1 decreased from 8300 (Macro-CTA1a) to 5900 (Macro-CTA1b), the size of the nanoparticles increased from 129 to 168 nm. Further decreasing the molecular weight to 4300 (Macro-CTA1c) resulted in the formation of agglomerate. A similar trend was also observed for Macro-CTA2; the nanoparticle size was 185 and 199 nm for Macro-CTA2a ($M_n = 8700$) and Macro-CTA2b ($M_n = 5800$), respectively, and agglomeration was observed for Macro-CTA2c ($M_n = 3500$). These results further confirm the stabilization role of the Macro-CTAs and show a significant molecular weight effect.

Livingness of the RAFT-Mediated Precipitation Polymerization Process. Having established the character of NIPAm precipitation polymerizations mediated by the RAFT process, the properties of the double hydrophilic block copolymers produced in the absence of cross-linkers were examined. Under similar conditions but without BIS, the initially formed nanoparticles dissociated into double hydrophilic block copolymers, PDMA-*b*-PNIPAm, upon cooling to room temperature, allowing for the characterization of the RAFT process from the aspect of the generated block copolymers under precipitation polymerization conditions. As shown in Figure 3, while maintaining the concentrations of V-50 and the monomer, an increase in the Macro-CTA concentration leads to a decrease in the molecular weight of the PNIPAm block. Furthermore, at constant V-50 concentration, increasing the Macro-CTA concentration increases the ratio between Macro-CTA and V-50 from 0.5 to 2. Accordingly, the polydispersity index (M_w/M_n) (PDI) of the block copolymers decreases from 1.56 to 1.13 for the block copolymers mediated with Macro-CTA1b and from 1.82 to 1.18 for the block copolymers mediated with Macro-CTA2b. The increased control over molecular weight distribution with increasing CTA/initiator ratio resembles that for the RAFT-mediated polymerization in homogeneous polymerization systems.

It was previously reported that gel permeation chromatography (GPC) using tetrahydrofuran (THF) overestimates the molecular weights of PNIPAm homopolymers due to the formation of intermolecular hydrogen-bonding.²² However, overestimation of molecular weights was not observed for these PDMA-*b*-PNIPAm block copolymers (Supporting Information) with THF GPC. Examination of the GPC traces reveals small shoulders on the higher molecular weight side, which is especially clear for the block copolymers with narrower peaks. These higher molecular weight shoulders (<5%) are attributable to higher molecular weight polymers formed by radical-radical coupling reactions. While higher molecular weight shoulders

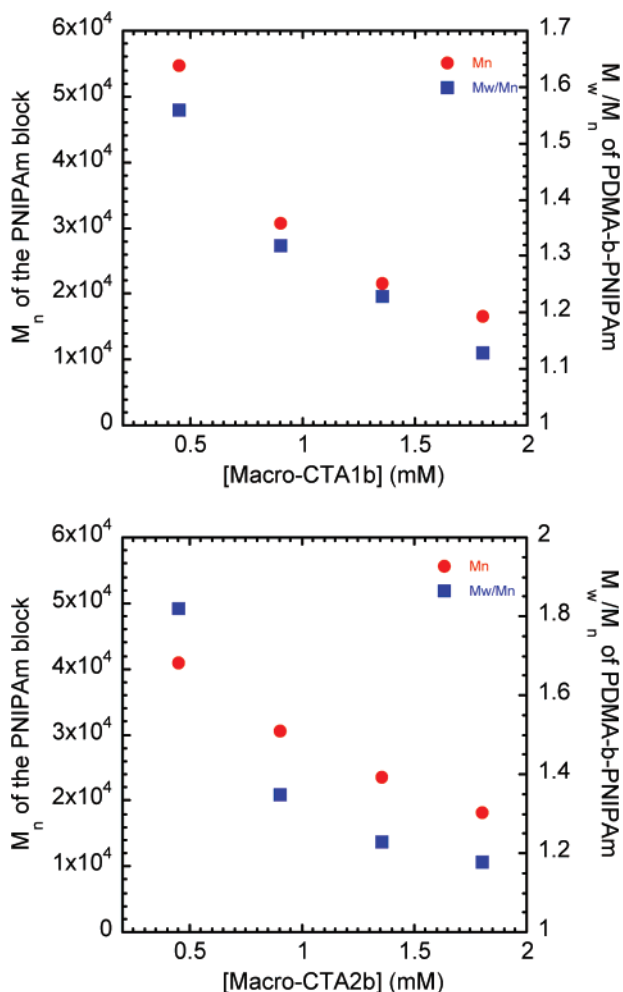


Figure 3. Dependence of molecular weights and polydispersity indices of double hydrophilic block copolymers, PDMA-*b*-PNIPAm, on the concentration of Macro-CTAs, [NIPAm] = 0.15 M, [V-50] = 0.9 mM, 70 °C, 1 h.

are sometimes observed in the RAFT-mediated homogeneous polymerization systems,^{27,42,43} it is possible that in heterogeneous systems the nanoparticles may contain two or more active radical species which would favor coupling. Nevertheless, when the ratio of Macro-CTA/V-50 is increased to 2, low PDIs (<1.2) can still be achieved, suggesting that the amount of both higher molecular weight polymers formed by radical–radical coupling reactions and Macro-CTA residue is small. Provided that the ratio of CTA/initiator is routinely maintained larger than 10 in order to gain good control over the RAFT process in homogeneous systems, the obtained block copolymers with a Macro-CTA/V-50 ratio of 2 are of high quality.

The living nature of the precipitation polymerization process was further evaluated by increasing the ratio of Macro-CTA/V-50 to 5 and the concentration of NIPAm to 1.18 M with a ratio of NIPAm/Macro-CTA/V-50 of 2600:5:1. As shown in Figure 4, the obtained molecular weights mediated by both Macro-CTA1a and Macro-CTA2b show good agreement with the expected values, suggesting excellent RAFT control of the precipitation polymerization process. The PDIs of the double hydrophilic block copolymers, PDMA-*b*-PNIPAm, mediated by

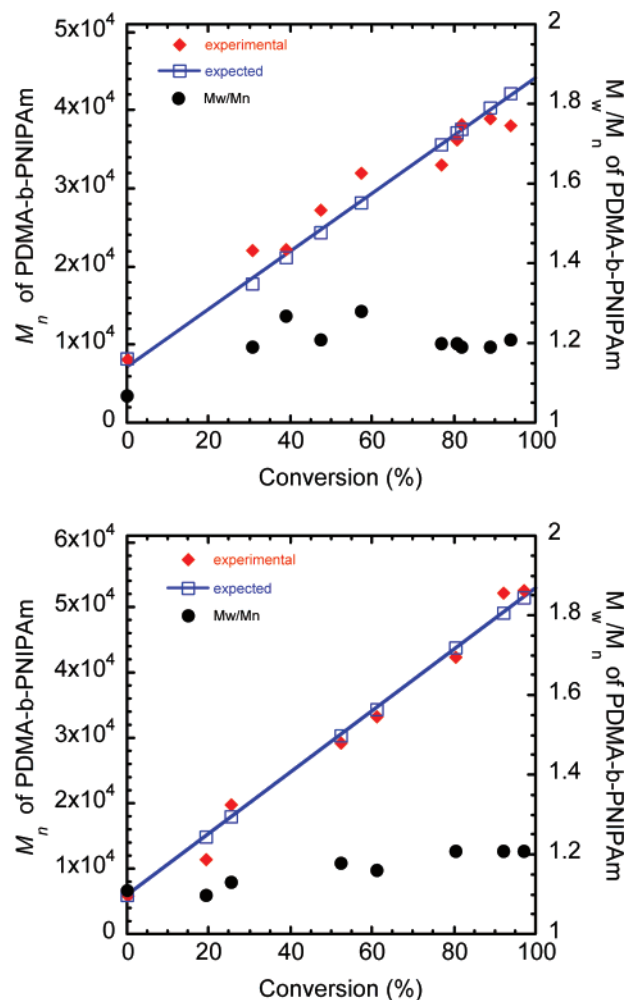


Figure 4. Evolution of molecular weights and polydispersity indices of block copolymers, PDMA-*b*-PNIPAm, with conversion of NIPAm monomer mediated by Macro-CTA1a (top) and Macro-CTA2b (bottom); 0% conversion represents data for the Macro-CTAs only, solid blue line represents linear fitting of conversions determined from ¹H NMR. [NIPAm] = 1.18 M, [V-50] = 0.45 mM, [Macro-CTA1a] = 3.46 mM, [Macro-CTA2b] = 3.50 mM, 70 °C.

Macro-CTA2b consistently fall in the narrow range of 1.1–1.2 throughout the entire conversion range, whereas the PDIs of the block copolymers mediated by Macro-CTA1a are larger than 1.2 at conversions <60% and drop to values around 1.2. Given that these two Macro-CTAs have similar structures with the PDMA as the leaving group (the R group) and the stabilization ability of the stabilizing group (the Z group) –C₁₂H₂₅ and –CH₂CH₂COOH being similar, these two types of Macro-CTAs are not expected to behave differently in their ability to control the RAFT polymerization process.

It should be noted, however, that when the precipitation polymerization proceeds in heterogeneous media, other factors such as radical entry/exit and mass transportation can affect and complicate the polymerization process. Previous reports on heterogeneous RAFT polymerization have shown that the molecular weights can significantly depart from the expected linear relationship between molecular weight and conversion. For example, lower molecular weights were observed in the mini-emulsion polymerization of styrene mediated by RAFT agents^{44,45} and in seeded emulsion copolymerization of *n*-butyl acrylate mediated by a xanthate.⁴⁶ The lower molecular weights

(42) Donovan, M. S.; Lowe, A. B.; Sumerlin, B. S.; McCormick, C. L. *Macromolecules* **2002**, *35*, 4123–4132.

(43) Schilli, C.; Lanzendörfer, M. G.; Müller, A. H. E. *Macromolecules* **2002**, *35*, 6819–6827.

were ascribed to direct polymerization by free radicals, instead of polymerization mediated by the RAFT process.^{44–46} In the above case, the excellent agreement observed for the molecular weights of the block copolymers with NIPAm conversion suggests negligible direct polymerization by free radicals and, instead, a well-controlled RAFT process, independent of the two competing nucleation mechanisms in the case of Macro-CTA1. The excellent RAFT control of the precipitation polymerization process can be in part attributable to the fast initiation under microwave conditions (vide infra) and to the water-soluble Macro-CTAs that function as both stabilization and RAFT agents, in contrast to the water-insoluble RAFT agents used in other heterogeneous polymerization systems in which mass transportation of the RAFT agents in the aqueous media is problematic.^{7,44–46} Through microwave irradiation,⁴⁷ the precipitation polymerization process was observed to proceed with high efficiency, and 90–100% conversion of monomers could be obtained within 5 min for thoroughly degassed samples, in contrast to hours of reaction under thermal conditions. The promotion effect for RAFT processes by microwave irradiation was also previously observed for polymerizations in homogeneous systems.^{48–50} In addition, a dramatic increase in the viscosity of the polymer solutions was observed when the noncross-linked colloidal nanoparticles dissociated into soluble polymers, demonstrating another advantage of precipitation polymerization which proceeds with low viscosity even at solids content of ca. 15%.

“Living” Nanoparticles for Multiresponsive Hydrogel Materials. Having established the role of the Macro-CTAs in the stabilization of the nanoparticles and in the regulation of precipitation polymerizations via the RAFT process, the exploitation of the functional groups at the surface of the nanoparticles and their ability to form nanostructured materials was studied. After precipitation polymerization, the nanogels are “living” in the sense that the RAFT moieties are still present in the nanoparticle and may act as initiating sites for further polymerization and/or functionalization. To investigate the scope of this secondary polymerization, nanoparticles consisting of cross-linked PDMA–PNIPAm chains were used as the particle seeds. Further polymerization was observed when feeding the seed solution with the same type of monomer (NIPAm) or a different type of monomer (*N*-isopropyl methacrylamide, NIPMAm), as evidenced from the increase in the particle size. When nanoparticles prepared with Macro-CTA2b (66 nm) were used as the “seeds”, reaction with different amounts of NIPAm (31 wt % and 57 wt % relative to NIPAm in the seed nanoparticle) in the presence of 2 mol % BIS gave nanoparticles with increased diameter (77 and 84 nm, respectively). The controlled nature of this subsequent polymerization can be judged by the small polydispersity (<5%) for the nanoparticles and the lack of formation of smaller secondary nanoparticles. In addition,

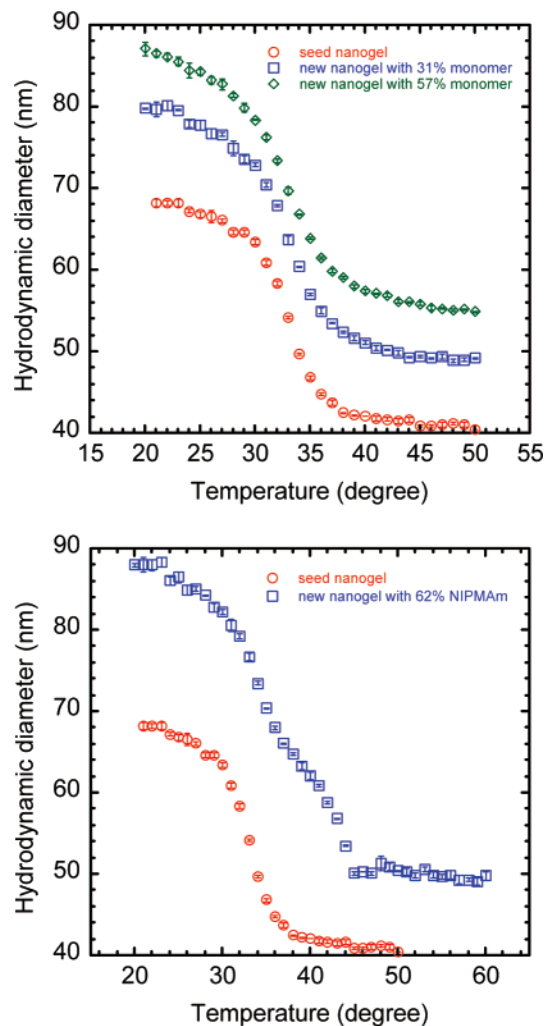


Figure 5. Thermosensitivity of nanogels by RAFT-mediated precipitation (co)polymerization. In both figures, red circle represents PDMA–PNIPAm seed nanogels that are used for subsequent seeded copolymerization with either 31% or 57% more NIPAm (top) and 62% NIPMAm (bottom) in the presence of 2% BIS.

the new nanoparticles were still negatively charged, indicating that the original “core–shell” structure was maintained, and with the phase transition temperature of 32–33 °C they displayed thermosensitivity similar to that of the original seed nanoparticles (Figure 5).

When the methacrylamide derivative, NIPMAm, was used as the second monomer in the presence of 2 mol % BIS, a similar increase in the nanoparticle size was observed, from 66 to 86 nm (62 wt %), and significantly the nanoparticles now displayed a dual thermal sensitivity with two distinct volume phase transitions at 33 and 44 °C. This corresponds with the LCSTs for PNIPAm^{51–52} and PNIPMAm,⁵³ respectively. Noticeably, this dual thermosensitivity of the nanoparticles is similar to that of traditional core–shell microgels in which the second polymer is directly deposited onto the surface of the seed particles without any chemical connection between the polymers in the core and the shell.⁵³ These distinct volume phase transitions in the copolymer nanoparticles indicate that the two

(44) Tsavalas, J. G.; Schork, F. J.; de Brouwer, H.; Monteiro, M. J. *Macromolecules* **2001**, *34*, 3938–3946.

(45) Lansalot, M.; Davis, T. P.; Heuts, J. P. A. *Macromolecules* **2002**, *35*, 7582–7591.

(46) Smulders, W.; Monteiro, M. J. *Macromolecules* **2004**, *37*, 4474–4483.

(47) An, Z.; Tang, W.; Hawker, C. J.; Stucky, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 15054–15055.

(48) Zhu, J.; Zhu, X.; Zhang, Z.; Cheng, Z. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 6810–6813.

(49) Brown, S. L.; Rayner, C. M.; Graham, S.; Cooper, A.; Rannard, S.; Perrier, S. *Chem. Commun.* **2007**, *21*, 2145–2147.

(50) Brown, S. L.; Rayner, C. M.; Perrier, S. *Macromol. Rapid Commun.* **2007**, *28*, 478–483.

(51) Heskins, M.; Guillet, J. E. *J. Macromol. Sci. Chem.* **1968**, *A2*, 1441–1455.

(52) Gil, E. S.; Hudson, S. M. *Prog. Polym. Sci.* **2004**, *29*, 1173–1222.

(53) Berndt, I.; Richtering, W. *Macromolecules* **2003**, *36*, 8780–8785.

blocks of the polymers are in separate domains and are not phase mixed, similar to the observation of two glass transition temperatures for phase separated block copolymers. Experiments were also performed for seeded polymerization of NIPAm and NIPMAm without cross-linkers. The obtained nanogels from seeded polymerization processes without cross-linkers were also stable with low polydispersities, and their size was larger than that obtained in the corresponding seeded polymerization process with cross-linkers. These results suggest that the formed secondary polymers in the seeded polymerization process are chemically connected to the seed particles. If there were no chemical connection between the secondary polymers and the seed particles, the noncross-linked secondary polymers would dissolve in the solution, and the size increase would not be observed in the seeded polymerization process without cross-linkers. The ability of the RAFT-derived nanoparticles for further functionalization by polymerization from the embedded initiating sites demonstrates the “living” nature of the nanoparticles and the versatility of this methodology for the preparation of multiresponsive nanostructured hydrogel materials.

Surface Bioconjugation. One of the remarkable features of the RAFT nanogels is the distinct surface functionalities inherited from the end groups of the Macro-CTAs. These surface functionalities provide useful handles for preparing various biocomposites by coupling the nanoparticles with biologically interesting macromolecules. Conjugation with RAFT polymers for potential applications in biotechnology has recently received attention as the RAFT agents are highly amenable to various functionalizations.^{54–56} To demonstrate the utility of the surface functionalities and the feasibility for making biocomposites, the nanoparticles were coupled with fluorescein-labeled albumin by activation of the carboxylic acid groups on the nanogels with

1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide in aqueous solution. An excess amount of protein was used to suppress cross-coupling between nanoparticles. The nanoparticle–albumin composites showed an increase in size (120 ± 1 nm) as compared with the nanogels themselves (100 ± 1 nm) and an increase in polydispersity: $\sim 16\%$ for nanogel–Albumin vs 3.5% for the nanogels. The surface functionalization process was quantified by UV–vis spectroscopy, and it was found that over 250 wt % of the protein could be attached to the starting nanoparticles, indicating the functionalization process is very facile. As a result these nanostructured hydrogel materials are attractive in a variety of bionanotechnology applications, and research is ongoing to exploit these materials for targeted delivery in cancer cells.

Conclusions

We demonstrated a successful methodology for RAFT-mediated precipitation polymerization process, which showed excellent control over molecular weights with negligible side reactions from direct free radical polymerization. The polymerization process was highly efficient under microwave conditions at high solids content. The produced nanogels showed narrow dispersity and novel, core–shell nanostructures with distinct surface functionalities that were used for facile preparation of bioconjugates.

Acknowledgment. Financial support from Mitsubishi Chemical Center for Advanced Materials, MC-CAM, the National Institutes of Health through a Program of Excellence in Nanotechnology (HL080729) and the central facilities of the UCSB Materials Research Laboratory (NSF DMR05-20415) are gratefully acknowledged. Prof. Andrew B. Lowe at the University of Southern Mississippi is thanked for providing a CTA.

Supporting Information Available: Experimental details, characterization of nanogels and polymers. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA0756974

- (54) Bathfield, M.; D'Agosto, F.; Spitz, R.; Charreyre, M.-T.; Delair, T. *J. Am. Chem. Soc.* **2006**, *128*, 2546–2547.
(55) Scales, C. W.; Convertine, A. J.; McCormick, C. L. *Biomacromolecules* **2006**, *7*, 1389–1392.
(56) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *J. Am. Chem. Soc.* **2007**, *129*, 7145–7154.