Macromolecules

ARTICLE

pubs.acs.org/Macromolecules

Synthesis of Biodegradable Hydrogel Nanoparticles for Bioapplications Using Inverse Miniemulsion RAFT Polymerization

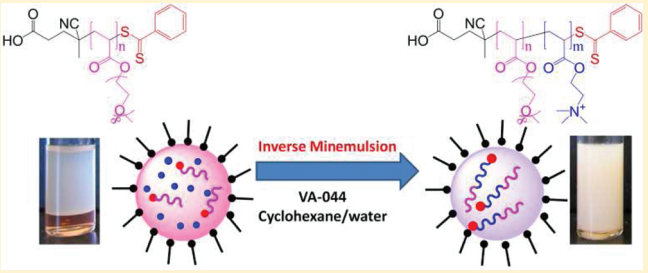
Marco Antonio M. Oliveira,[†] Cyrille Boyer,[‡] Marcio Nele,[†] José Carlos Pinto,[†] Per B. Zetterlund,[*,‡] and Thomas P. Davis[*,†]

[†]Programa de Engenharia Química/COPPE, Universidade Federal do Rio de Janeiro, Cidade Universitária, CP:68501, Rio de Janeiro, 21941-972 RJ, Brazil

[‡]Centre for Advanced Macromolecular Design (CAMD), School of Chemical Engineering, The University of New South Wales, Sydney, New South Wales 2052, Australia

Supporting Information

ABSTRACT: We report the synthesis of biodegradable hydrogel nanoparticles using a RAFT inverse miniemulsion cross-linking polymerization process with 2-(dimethylamino)ethyl methacrylate (DMAEMA) as monomer. The experimental conditions were optimized to yield a colloidally stable miniemulsion polymerization, which required protonation of DMAEMA using aqueous hydrogen chloride to ensure minimal partitioning to the continuous phase (cyclohexane). The nanoparticles were cross-linked using a disulfide cross-linker, thereby enabling subsequent degradation of the polymer network to its constituent primary chains by exposure to a reductive environment. The molecular weight distributions of the constituent primary chains were consistent with satisfactory control/livingness during the polymerization. These biodegradable hydrogel nanoparticles may have potential application as nanocarriers for encapsulation and controlled release of siRNA.



INTRODUCTION

Hydrogel nanoparticles (hydrophilic cross-linked polymeric nanoparticles) possess a number of advantageous properties in drug delivery applications.[1−4] The particle size can be tuned with relatively good precision, there is a large total surface area that can be employed for bioconjugation, and the particle interior can be used to embed drugs. By controlling the structure of the cross-linked network density of the hydrogel nanoparticle, it is possible to create a matrix with a defined porosity, thus allowing precise control of the loading and release behavior of the polymer network.[3] A number of criteria need to be fulfilled for optimization of the performance of hydrogel nanoparticles in drug delivery applications, including long-term stability for circulation *in vivo*, the presence of chemical functionality on the nanoparticle surface (introduced by postmodification to improve the recognition of the nanoparticle by receptors on specifics cells), a particle size less than 200 nm for accumulation by the enhanced permeation effect (EPR), and the ability of the nanoparticle to undergo biodegradation on exposure to external stimuli (e.g., pH, presence of enzymes). Biodegradation is important to control the delivery of the drug and also to facilitate removal of the "empty" nanoparticles or their residues.

Controlled/living radical polymerization (CLRP)[5,6] enables the precise synthesis of polymers with a multitude of architectures and has now been adapted to be applicable to a wide range of dispersed systems (e.g., emulsion, miniemulsion, microemulsion), thus providing a route to polymeric nanoparticles comprising well-defined polymers.[7,8] CLRP can also be applied to cross-linking polymerizations, whereby more well-defined and more homogeneous polymer networks can be prepared.[9−12] CLRP in dispersed systems thus offers an attractive route to hydrogel nanoparticles for drug delivery and gene therapy applications, consisting of polymer networks built from tailored and well-defined structures.[1−3] Reversible addition−fragmentation chain transfer (RAFT) polymerization is arguably the most versatile CLRP method[6,13] and appears advantageous for the preparation of drug delivery systems because of the low toxicity of some RAFT agents.[14−16]

Synthesis of hydrogel nanoparticles by cross-linking heterogeneous CLRP requires the use of an inverse system, i.e., a system where the continuous phase is the oil phase and the dispersed phase is hydrophilic (a water-in-oil system), stabilized by an oil-soluble surfactant.[7] The heterogeneous polymerization technique most well-suited for implementation of CLRP, as well as for synthesis of organic/inorganic hybrid nanoparticles,[17−19] is miniemulsion polymerization.[20,21] Miniemulsion polymerization is based on the direct transformation of monomer droplets into polymer particles, thus circumventing diffusion of monomer

Received: July 5, 2011
Revised: August 3, 2011
Published: August 18, 2011

(and other reactants) across the aqueous phase as in an emulsion polymerization. Miniemulsions are thermodynamically unstable and energy is required for their formation (normally provided via high-energy homogenization), although low-energy approaches have been reported.[22−24] In an inverse miniemulsion system,[25−27] a hydrophilic monomer is the main component of the dispersed phase (e.g., acrylamide, acrylic acid, and *N*-isopropylacrylamide), while an organic solvent is the main constituent of the continuous phase. To date, there exist only few reports describing inverse miniemulsion CLRP employing atom transfer radical polymerization (ATRP)[1,28−30] or RAFT.[6,31] For instance, Schork and co-workers described inverse RAFT miniemulsion polymerization of acrylamide,[32−34] acrylic acid,[34] and *N*-isopropylacrylamide,[35] while inverse RAFT microemulsion polymerization of *N*,*N*-dimethylacrylamide has also been reported by McCormick and co-workers.[36]

In this paper, we study the inverse miniemulsion polymerization of another important hydrophilic monomer, i.e., 2-(dimethylamino)ethyl methacrylate (DMAEMA).[37] This particular monomer can be used to prepare thermo- and pH-responsive polymers that can be employed in the preparation of new drug or gene delivery systems.[38−40] DMAEMA-based polymers have attracted special interest due to their ability to interact with DNA or siRNA, forming a polymer−plasmid complex which can be taken up by cells.[41−44] Indeed, the amino side groups can be easily quaternized to yield a positively charged polymer, which then can be employed to interact with siRNA by electrostatic interactions.[45−48] It was reported that (co)polymers obtained from DMAEMA can act as efficient transfection agents. Recently, CAMD researchers developed several platforms based on poly(2-(dimethylamino)ethyl methacrylate (PDMAEMA), such as biodegradable hyperbranched polymers[37,49] or hybrid inorganic/organic nanoparticles[50] for the delivery of DNA or siRNA.

In the present work, we have developed a methodology for RAFT inverse miniemulsion polymerization of DMAEMA to produce biodegradable cross-linked PDMAEMA hydrogel nanoparticles. To our knowledge, this is the first successful description of the use of DMAEMA in an inverse miniemulsion RAFT polymerization (or inverse miniemulsion CLRP in general).

EXPERIMENTAL SECTION

Materials. All chemicals listed below were used as received: 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%, Aldrich), 2,2′-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044, 97%, Waco), 3,3′-dithiopropionic acid (99%, Sigma-Aldrich), 4-(dimethylamino)pyridine (DMAP, Aldrich, 99%), 4,4′-azobis(4-cyanopentanoic acid) (98%, Fluka), acetonitrile (99.7%, Ajax Finechem), benzyl chloride (99%, Sigma-Aldrich), cyclohexane (99%, Ajax Finechem), deuterated chloroform ($CDCl_3$, 99.8%, Cambridge Isotope Laboratories), deuterated dimethyl sulfoxide (DMSO-d_6, 99.9%, Cambridge Isotope Laboratories), deuterium oxide (D_2O, 99.9%, Cambridge Isotope Laboratories), dichloromethane (DCM, 99.5%, Ajax Finechem), diethyl ether (99%, Ajax Finechem), diphenyl ether (99%, Sigma-Aldrich), DL-dithiothreitol (99%, Sigma-Aldrich), elemental sulfur (APS, 99.3%), ethyl acetate (99.5%, Ajax Finechem), hydrochloric acid (HCl, 32%, Ajax Finechem), methanol anhydrous (99.8%, Sigma-Aldrich), *n*-hexane (95%, Ajax Finechem), triethylamine (99%, Sigma-Aldrich), *N*,*N*-dicyclohexylcarbodiimide (DCC, Fluka, 99%), *N*,*N*-dimethylacetamide (DMAc; 99.9%, Sigma-Aldrich), petroleum spirit (BR 40−60 °C, Ajax Finechem), poly(ethylene glycol) methacrylate (M_n = 526 g/mol, Aldrich), poly(ethylene glycol) methyl ether methacrylate (M_n = 475 g/mol, Sigma-Aldrich), potassium ferricyanide (III) (98%, Sigma-Aldrich), silica gel (Grace), sodium chloride (99%, Sigma-Aldrich), sodium hydroxide (97%, Ajax Finechem), sodium methoxide solution 25 wt % in methanol (Sigma-Aldrich), sodium sulfate (99%, Ajax Finechem), Span 80 (sorbitan oleate; Fluka), tetrahydrofuran (THF, Honeywell, HPLC grade), and toluene (99.5%, Ajax Finechem). 2,2′-Azobis(isobutyronitrile) (AIBN, 98%, Sigma-Aldrich) was recrystallized twice from acetone.

4-Cyano-4-(phenylcarbonothioylthio)pentanoic Acid. This RAFT agent was prepared based on a method described previously.[51]

Dithiobenzoic Acid (DTBA). Sodium methoxide (25% solution in methanol, 108.0 g, 0.5 mol), anhydrous methanol (125.0 g), and elemental sulfur (16.0 g, 0.5 mol) were added to a three-necked round-bottomed flask. Benzyl chloride (31.5 g, 0.25 mol) was then added dropwise via addition funnel over a period of 1 h at room temperature under stirring. The system was heated in an oil bath at 67 °C overnight. The reaction mixture was cooled to 0 °C using an ice bath, the precipitated salt was removed by filtration, and the solvent removed by rotary evaporation. Deionized water (250 mL) was added to the viscous residue, and the resulting solution was filtered and transferred to a separation funnel. The sodium dithiobenzoate solution was washed first with diethyl ether (300 mL) + HCl 1 N (250 mL) and then with deionized water (100 mL) + NaOH 1 N (250 mL). This washing process was repeated two more times to finally yield a solution of sodium dithiobenzoate.

Di(thiobenzoyl) Disulfide. Sodium dithiobenzoate solution (175 mL) was transferred to a three-necked round-bottomed flask, and a potassium ferricyanide solution (16.5 g, 0.05 mol in 250 mL of deionized water) was added dropwise via an addition funnel over a period of 1 h under vigorous stirring at room temperature. The resulting red/purple precipitate was filtered and washed with deionized water until the washings became colorless. The solid was dried in vacuo at room temperature overnight.

4-Cyanopentanoic Acid Dithiobenzoate. Ethyl acetate (80.0 mL), 4,4′-azobis(4-cyanopentanoic acid) (8.5 g, 0.03 mol), and di(thiobenzoyl) disulfide (5.7 g, 0.018 mol) were charged into a round-bottomed flask. The reaction solution was heated and allowed to reflux overnight. The solvent was removed by rotary evaporation, and the product was isolated by column chromatography (silicagel 60 Å, 70−230 mesh) using ethyl acetate:petroleum spirit (50/50 v/v) as eluent. Fractions that were red/pink in color were combined, and the solvent was removed by rotary evaporation. The final red oil was stored in a freezer. ^{1}H NMR (300 MHz, $CDCl_3$)/δ ppm: 1.94 (s, 3H, CH_3); 2.39−2.77 (m, 4H, CH_2CH_2); 7.42 (m, 2H, m-ArH); 7.59 (m, 1H, p-ArH) and 7.92 (m, 2H, o-ArH).

MacroRAFT Agent. Poly(ethylene glycol methyl ether) methacrylate (8.0 g, 16.8 mmol; M_n = 475 g/mol), AIBN (27.2 mg, 0.16 mmol), 4-cyanopentanoic acid dithiobenzoate (232.0 mg, 0.8 mmol), and acetonitrile (8.0 g, 2 mol) were charged into a round-bottomed flask which was capped and degassed with nitrogen for 1 h at 15 °C. The solution was stirred for 6 h at 70 °C, and subsequently the excess solvent was removed with air at room temperature. The viscous product was dialyzed at room temperature using a cellulose membrane (MW 3500 g/mol), first with deionized water and then with methanol. The excess solvent was removed with air at room temperature, and the final red viscous product was stored in a freezer. The viscous product was analyzed by ^{1}H NMR in $CDCl_3$ and D_2O. ^{1}H NMR (300 MHz, $CDCl_3$)/δ ppm: 1.50−2.00 (m, 3H, CH_2−CH); 3.36 (s, 3H, CH_3); 3.60 (m, nH, OCH_2CH_2O); 4.10 (s, 2H, $COOCH_2$); 7.36 (m, 2H, m-ArH); 7.50 (m, 1H, p-ArH) and 7.87 (m, 2H, o-ArH). Two different batches of macroRAFT agent were used. MacroRAFT$_1$ (miniemulsion polymerizations without cross-linker): M_n = 16 100 g/mol (^{1}H NMR); M_n = 13 650 g/mol, M_w/M_n = 1.11 (GPC). MacroRAFT$_2$ (miniemulsion

Table 1. Miniemulsion Recipes for Polymerizations at 60 °C

continuous phase	dispersed phase	amount (g)	notes
cyclohexane		20	
Span 80		1	
	$HCl_{(aq)}$[a] (1%)	2.5	
	NaCl	0.05	
	VA-044	0.0051	[M]:[I] = 400:1
	DMAEMA	1	[M]:[RAFT] = 100:1
	macroRAFT	0.87[b] or 0.90[c]	based on M_n of macroRAFT
	DMA-PEOSS	0.05 (if used)	

[a] pH of the dispersed phase was adjusted to 2−3 by addition of drops of $HCl_{(aq)}$ (20%). [b] $MacroRAFT_1$ (miniemulsion polymerizations without cross-linker): M_n = 13 650 g/mol, M_w/M_n = 1.11. [c] $MacroRAFT_2$ (miniemulsion polymerizations with cross-linker): M_n = 14 200 g/mol, M_w/M_n = 1.09.

polymerizations with cross-linker): M_n = 20 100 g/mol (^{1}H NMR); M_n = 14 200 g/mol, M_w/M_n = 1.09 (GPC).

Dithiopropionyl Poly(ethylene glycol) Dimethacrylate (DMA-PEOSS). This biodegradable cross-linker was prepared as described elsewhere,[29] with some experimental modifications. First, poly(ethylene glycol) methacrylate (M_n = 526 g/mol) was purified as described elsewhere.[29] A solution of 3,3′-dithiopropionic acid (2.3 g, 10 mmol) in THF (30 mL) was added dropwise to a solution of the purified poly(ethylene glycol) methacrylate (10.0 g, 19 mmol) in DCM (60 mL) containing DCC (3.9 g, 19 mmol) and a catalytic amount of DMAP (0.5 g) kept in an ice bath over 20 min, and the reaction mixture was subsequently stirred at room temperature overnight. The formed solids were removed by vacuum filtration twice, and the solvents were removed by rotary evaporation at 30 °C. The resulting yellow oil was stored at in a refrigerator prior to use. ^{1}H NMR (300 MHz, DMSO-d_6)/δ ppm: 1.9 (s, 6H, CH_3-), 2.7 (t, 4H, $-C(O)-CH_2-CH_2-SS-$), 2.9 (t, 4H, $-O(O)C-CH_2-CH_2-SS-$), 3.3−3.7 (m, 72H, $-(CH_2CH_2O)_n-$), 4.0−4.3 (m, 8H, $-C(O)O-CH_2-$ and $-CH_2-O(O)C-$), 5.7 (s, 2H, CH=), 6.0 (s, 2H, CH=).

Miniemulsion Polymerizations. The basic inverse miniemulsion recipe was developed based on the work of Schork and coworkers[32,33] (Table 1). First, a solution of all components of the aqueous phase (monomer, salt, initiator, RAFT agent, and cross-linker (if present)) were dissolved in an aqueous solution of $HCl_{(aq)}$ (1%). The pH of the dispersed phase was adjusted to 2−3 by adding a few droplets of $HCl_{(aq)}$ (20%). In a separated flask, a solution of the continuous organic phase was prepared by dissolving the surfactant Span 80 in cyclohexane. The aqueous (dispersed) phase was mixed with the continuous (organic) phase for 30 min at 15 °C and ultrasonicated (Branson Digital Sonifier model 450) for 10 min at 70% of amplitude, while using an ice bath to cool the sample. The resulting miniemulsion was transferred to a rounded-bottom flask and degassed with N_2 for 30 min at 15 °C.

The polymerizations were carried out at 60 °C under magnetic stirring. Samples taken at predetermined times for characterization. Acetone was added to the miniemulsion, followed by centrifugation for 15 min at 7500 rpm. The acetone/cyclohexane was subsequently removed, and the precipitate was dried under vacuum at room temperature. 10 mg of the dried samples from the conventional (no RAFT agent) and RAFT polymerizations was treated with a small amount of triethylamine for 30 min (in order to neutralize the positive charge of the polymers) followed by dissolution in DMAc (GPC) or ^{1}H NMR solvent. Polymer samples from cross-linking polymerizations (RAFT with cross-linker) were treated with 100 μL of 0.5 M aqueous solution of DL-dithiothreitol for 1 h to break the disulfide cross-links (resulting in primary chains remaining, assuming complete cleavage of cross-links). The resulting aqueous solution was freeze-dried, and the recovered dry polymer was dissolved in DMAc/^{1}H NMR solvent.

Gel Permeation Chromatography (GPC). GPC analyses were performed in DMAc (0.03% w/v LiBr, 0.05% 2, 6-dibutyl-4-methylphenol (BHT)) at 50 °C and a flow rate of 1 mL/min using a Shimadzu modular system comprising an LC-20AT pump, a CTO-10A column oven, and a RID-10A refractive index detector. The system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2), followed by four linear PL (Styragel) columns (10^5, 10^4, 10^3, and 500 Å) calibrated with polystyrene standards ranging from 500 to 10^6 g/mol.

Theoretical number-average molecular weights ($M_{n,th}$) were calculated based on eq 1 (without cross-linker) and eq 2 (with cross-linker, after cleavage of disulfide bonds):

$$M_{n,th} = \frac{[M]_0 MW_{mon} \alpha}{[macroRAFT]_0} + MW_{macroRAFT} \quad (1)$$

$$M_{n,th} = \frac{[M]_0 MW_{mon} \alpha}{[macroRAFT]_0} + \frac{[cross\text{-}linker]_0 MW_{cross\text{-}linker} \alpha}{[macroRAFT]_0} + MW_{macroRAFT} \quad (2)$$

where α denotes the fractional conversion of DMAEMA. Note that two different batches of macroRAFT agent were used (see macroRAFT synthesis section above).

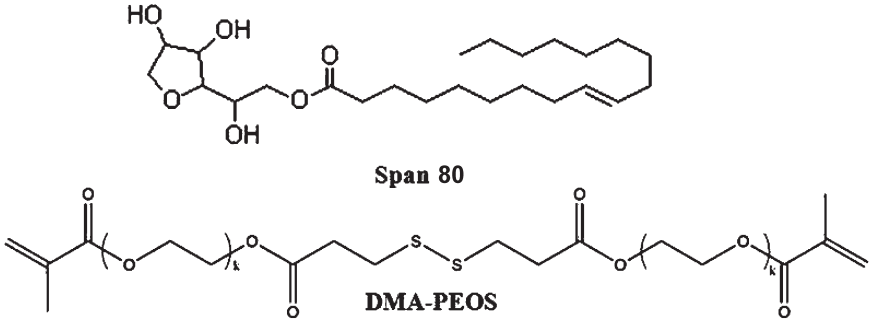
Dynamic Light Scattering (DLS). Dynamic light scattering measurements were conducted using a Malvern Zetasizer Nano ZS instrument equipped with a 4 mW He−Ne laser operating at λ = 633 nm and with an avalanche photodiode detector with high quantum efficiency. The emulsions were analyzed without dilution.

Particle sizes were also measured for cross-linked particles redispersed in aqueous buffer solutions (10 mg in 4 mL): aqueous HCl 20% (pH = 1−2), purified water (pH = 7), aqueous Na_2CO_3 (pH = 12). To this end, acetone was added to the inverse miniemulsion to break the emulsion, followed by centrifugation at 7500 rpm for 15 min. The acetone/cyclohexane was then removed, and the remainder was dried in vacuo at room temperature. The zeta potential was measured for cross-linked particles redispersed in purified water.

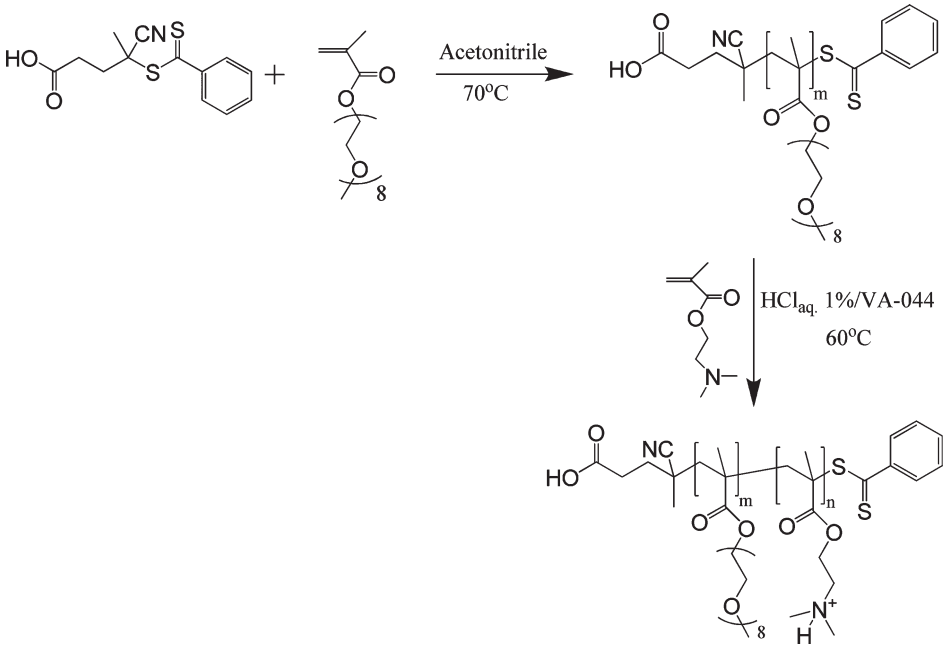
Gas Chromatography (GC). Monomer conversions were determined using a Shimadzu 17A gas chromatograph system equipped with an AT-Wax column (Heliflex capillary, Altech) using H_2 as carrier gas, with injector, column, and detector temperatures at 200, 120, and 250 °C, respectively. Samples of the inverse miniemulsion latex (0.1 g), taken during the polymerization, were diluted with 2 mL of a GC solution (cyclohexane:toluene = 1000:2; toluene used as internal standard), and then injected into the GC system.

Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). ^{1}H NMR spectra were recorded on a Bruker DPX 300, operating at 300 MHz, using deuterated chloroform ($CDCl_3$), deuterium oxide (D_2O), or deuterated dimethyl sulfoxide (DMSO) as solvents.

Scheme 1. Span 80 (Sorbitan Oleate) and Biodegradable Cross-Linker Dithiopropionyl Poly(ethylene glycol) Dimethacrylate (DMA-PEOSS)



Scheme 2. RAFT Polymerization of Poly(ethylene glycol) Methyl Ether Methacrylate (M_n = 475 g/mol) Employing 4-Cyanopentanoic Acid Dithiobenzoate To Generate Poly(ethylene glycol)-Based MacroRAFT Agent, Followed by RAFT Polymerization of 2-(Dimethylamino)ethyl Methacrylate (DMAEMA) Employing Poly(ethylene glycol)-Based MacroRAFT Agent in Water (in Inverse Miniemulsion)



RESULTS AND DISCUSSION

Miniemulsion Formation. It is a fundamental criterion of a miniemulsion polymerization that the monomer only exhibits limited solubility in the continuous phase. DMAEMA is miscible with a wide range of solvents that would normally be prime candidates as the continuous phase in an inverse miniemulsion system, e.g., cyclohexane, *n*-hexane, and heptane. To overcome this problem, the monomer was protonated using $HCl_{(aq)}$ to enhance its solubility in a neutral/acidic aqueous phase and reduce its miscibility with the organic phase.[52,53] This approach ensured that the monomer resided predominantly in the dispersed phase (no traces of monomer detected in the continuous organic phase by ^{1}H NMR analysis).

Inverse miniemulsions can be prepared using a surfactant with low HLB (hydrophilic lipophilic balance) value.[25,27] In order to prevent Ostwald ripening,[54] it is necessary to add an osmotic pressure agent, which is a low molecular weight compound that is soluble in the dispersed phase but exhibits extremely low solubility in the continuous phase, e.g., a salt such as NaCl, Na_2SO_4, or K_2SO_4 (equivalent to hexadecane in a normal (not inverse) miniemulsion).[25,27] In the present study, satisfactory emulsion stability was achieved employing the surfactant Span 80 (Scheme 1) and NaCl (Table 1).

The initial plan was to use the RAFT agent 4-cyanopentanoic acid dithiobenzoate, but this species proved to partition excessively to the continuous phase. This RAFT agent was therefore modified into a macroRAFT agent employing a PEG-based monomer (poly(ethylene glycol) methyl ether methacrylate (PEG-MA); M_n = 475 g/mol; Scheme 2), thereby increasing its solubility in the dispersed aqueous phase and reducing its solubility in the continuous phase (a similar strategy has been

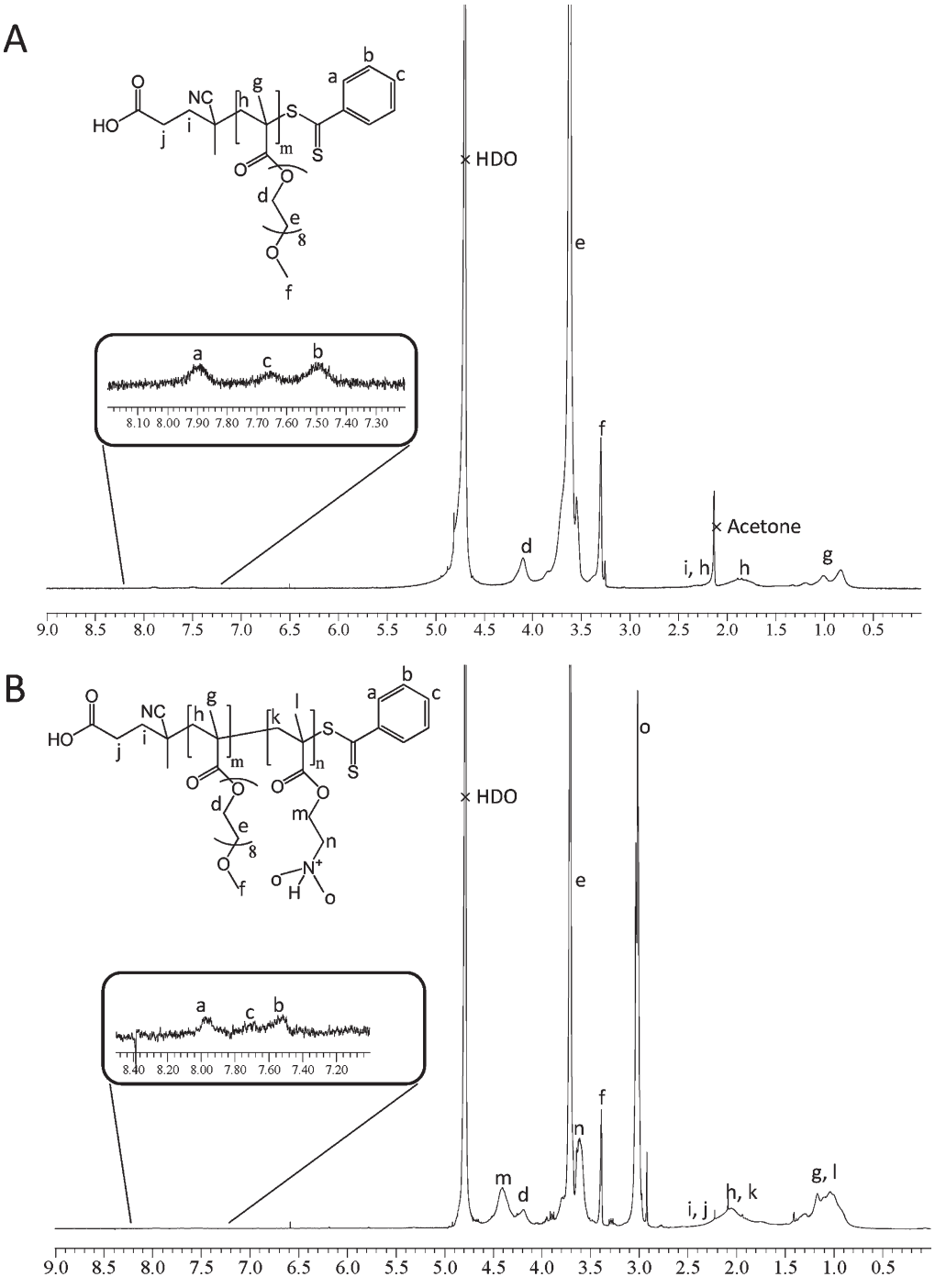


Figure 1. ^{1}H NMR spectra of (A) the purified poly(PEG-MA) macroRAFT agent and (B) the purified PDMAEMA-*b*-P(PEG-MA) macroRAFT agent in D_2O.

employed previously[55]). The synthesis of P(PEG-MA) macroRAFT was confirmed by ^{1}H NMR analysis (Figure 1A), where peaks related to the "Z-group" of the original RAFT agent (aromatic protons at 7.2−7.8 ppm) and poly(ethylene glycol) methyl ether methacrylate (4.1 (−CH_2O−), 3.6 (CH_2O), and 3.3 ppm (CH_2O)) could be observed (see Experimental Section for details).

Miniemulsion Polymerization. Inverse miniemulsion RAFT polymerization of DMAEMA using the macroRAFT agent was conducted at 60 °C using the aqueous phase (dispersed phase) initiator VA-044 according to the recipe in Table 1. The polymerization generates a block copolymer of the structure shown in Scheme 2. Figure 2 shows photographs of miniemulsions before and after polymerization as well as before ultrasonication. Identical polymerizations were also carried out in the absence of the macroRAFT agent. Similar polymerization rates were observed in the presence and absence of RAFT agent. Both polymerizations were rapid (close to 70% conversion in 1 h) and proceeded beyond 80% conversion in 2.5 h (Figure 3). The RAFT polymerization proceeded to near-complete conversion

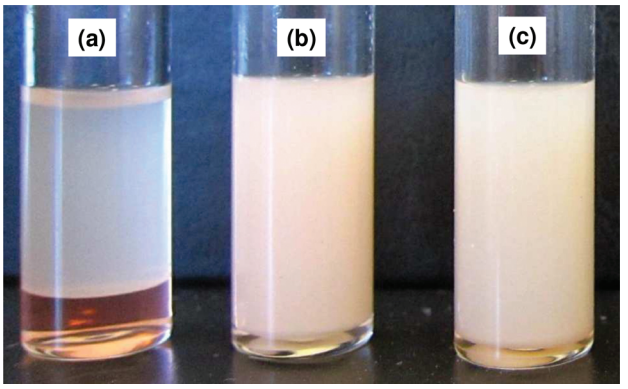


Figure 2. (a) Reaction mixture (as per recipe in Table 1) for preparation of emulsion with macroRAFT agent but without cross-linker before ultrasonication, (b) after ultrasonication, and (c) after polymerization for 30 min (36% conversion).

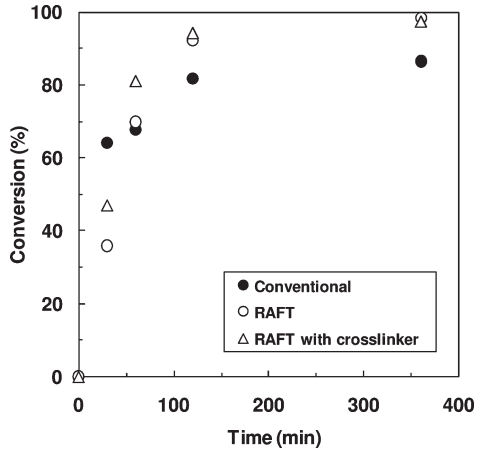


Figure 3. Conversion−time data for inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 at 60 °C without RAFT agent/cross-linker ("conventional"), with poly(ethylene glycol)-based macroRAFT agent, and with poly(ethylene glycol)-based macroRAFT agent and the cross-linker DMA-PEOSS based on recipes in Table 1.

(>98%) in 6 h, whereas the conventional radical polymerization appeared to reach a limiting conversion below 90%. Interestingly, the dithioester did not degrade during the polymerization despite the low pH. ^{1}H NMR analysis of the purified copolymer (Figure 1B) confirms the presence of the RAFT end group at 7.2−7.8 ppm, and the copolymer is pink in color.

Figure 4a shows molecular weight distributions at different monomer conversions, revealing how the distributions shift to higher molecular weight with increasing conversion, consistent with a controlled/living process. There is however a low molecular weight shoulder, indicative of some fraction of macroRAFT agents not having participated in the polymerization at a given conversion. This may be caused by dead chains (i.e., chains not containing a RAFT end group) of the initial macroRAFT agent, or it may be related to the heterogeneous polymerization itself. The M_n values increased linearly with conversion close to $M_{n,th}$, and M_w/M_n remained below 1.3 (Figure 5). The molecular weights were much higher, as expected, in the absence of RAFT agent (of the order of 10^5 g/mol; Table S1 in the Supporting Information). The molecular weight distributions were slightly bimodal (Figure S1), probably as a result of dependence of the instantaneous molecular

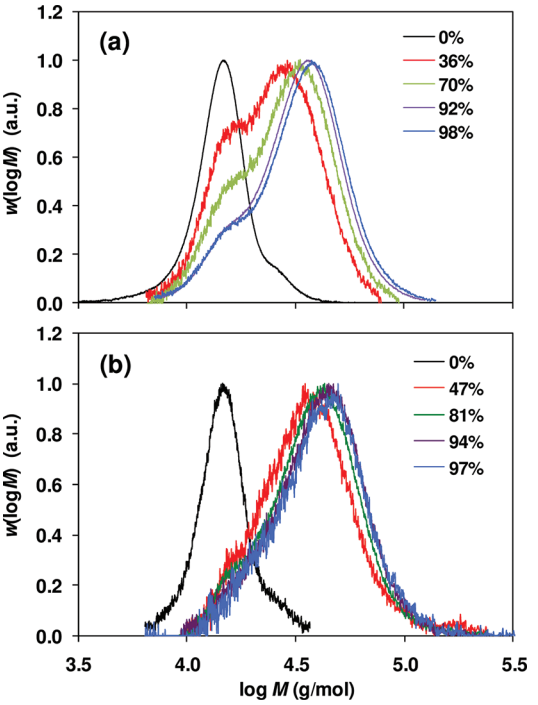


Figure 4. Molecular weight distributions obtained in inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 at 60 °C (a) with poly(ethylene glycol)-based macroRAFT agent and (b) with poly(ethylene glycol)-based macroRAFT agent and the cross-linker DMA-PEOSS after degradation by treatment with DL-dithiothreitol to break the disulfide cross-links.

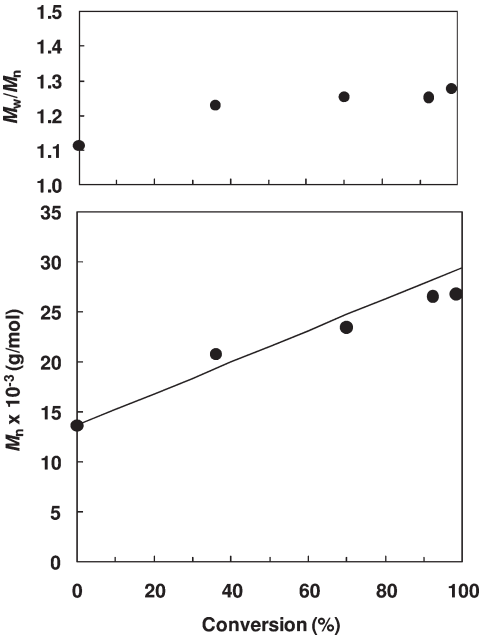


Figure 5. M_w/M_n and M_n vs conversion for inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 at 60 °C with poly(ethylene glycol)-based macroRAFT agent based on recipe in Table 1.

weight on conversion (reactant concentrations and the termination rate coefficient varying with conversion).

Figure 6 shows particle diameters vs conversion for miniemulsion polymerizations with and without RAFT agent. The number-average

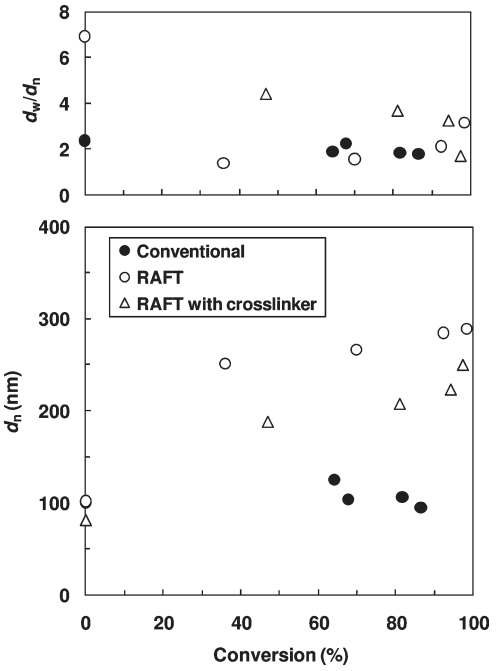


Figure 6. Particle size measurements for inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 at 60 °C without RAFT agent/cross-linker ("conventional"), with poly(ethylene glycol)-based macroRAFT agent and with poly(ethylene glycol)-based macroRAFT agent and the cross-linker DMA-PEOSS based on recipes in Table 1. The initial value of d_w/d_n for RAFT with cross-linker was 20 (not shown in plot).

particle diameters before polymerization were very similar to without RAFT agent ($d_n \approx 100$ nm). In the absence of RAFT agent, the particle size appeared to remain relatively constant with conversion (the d_n data point for conventional radical polymerization and the RAFT data point at zero conversion are superimposed). In the presence of RAFT agent, a significant increase in particle size was observed in the conversion range 0−30%, beyond which d_n remained in the range 250−290 nm. It is well established that it is more difficult to maintain colloidal stability in a CLRP system relative to its conventional radical polymerization counterpart as a result of superswelling,[7,56] i.e., oligomers (formed by CLRP) promoting swelling of nucleated monomer droplets by monomer from not yet nucleated monomer droplets. The particle size distributions by weight at high conversion (Figure 7) revealed that a small population of larger particles (micrometer-sized) were present in the RAFT system, but not in the conventional system. The values of d_w/d_n were similar for both systems throughout the polymerizations, although the system with RAFT agent had a considerably broader initial droplet size distribution (higher d_w/d_n) than the conventional system (Figure 6).

Synthesis of Cross-Linked Biodegradable Particles. Polymerizations were carried out as above using the macroRAFT agent in the presence of the biodegradable cross-linker DMA-PEOSS according to the recipe in Table 1. The polymerization rate was very similar to the case without the cross-linker (Figure 3). The number-average particle sizes were somewhat smaller in the presence of cross-linker, with $d_n \approx 200$ nm in the conversion range 30−100% conversion, but the particle size distributions were significantly broader (higher d_w/d_n with cross-linker; Figure 6). Also in the presence of cross-linker, a population of larger micrometer-sized particles were detected in the weight distribution (Figure 7).

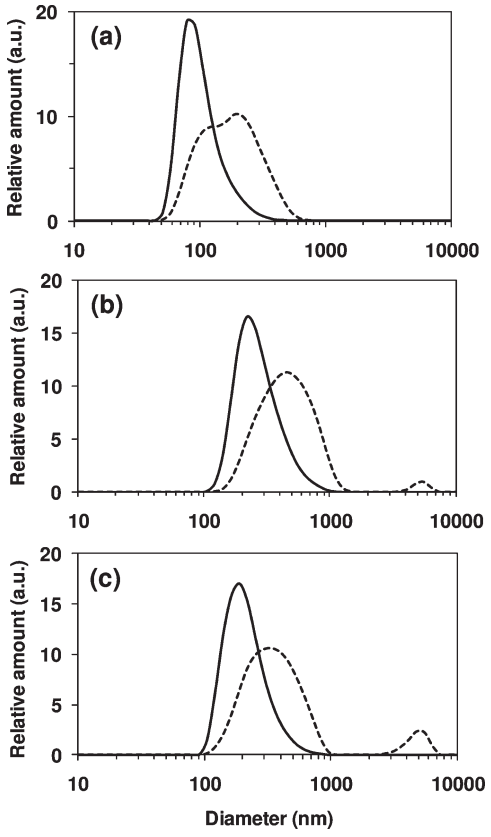


Figure 7. Particle size distributions by number (—) and volume (···) for inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 in at 60 °C (a) without RAFT agent/cross-linker ("conventional"; 82% conversion), (b) with poly(ethylene glycol)-based macroRAFT agent (92% conversion), and (c) with poly(ethylene glycol)-based macroRAFT agent and the cross-linker DMA-PEOSS (94% conversion) based on recipes in Table 1.

The obtained cross-linked networks of the hydrogel nanoparticles were subsequently subjected to induced degradation by treatment with DL-dithiothreitol to reduce the disulfide cross-links. This would result in formation of the primary chains of the network, which ideally would be of similar molecular weight distribution to the polymer formed in the corresponding polymerization in the absence of cross-linker.[10−12,57] Figure 4B shows molecular weight distributions of the polymer obtained after degradation, revealing relatively narrow monomodal distributions that shift to higher molecular weight with increasing monomer conversion. Interestingly, the fairly prominent low molecular weight shoulder present in the RAFT polymerizations without cross-linker (Figure 4A) is absent in this case. This suggests that the low molecular weight shoulder in the absence of cross-linker has its origin in the heterogeneous mechanism of the polymerization and is not caused by dead original macroRAFT agent. The values of M_n increased with increasing conversion but were somewhat higher than $M_{n,th}$, and M_w/M_n remained below 1.3 to high conversion (Figure 8).

After isolation, the purified hydrogel nanoparticles can be readily dispersed in water and exhibit pH-responsive behavior in aqueous dispersion.[38] The particle diameter decreased with increasing pH: d_n = 210 nm at pH = 4, d_n = 120 nm at pH = 7, and d_n = 94 nm at pH = 12. The decrease of the size is attributed to the decrease in water solubility of PDMAEMA at high pH. The zeta-potential of the purified particles redispersed in pure water

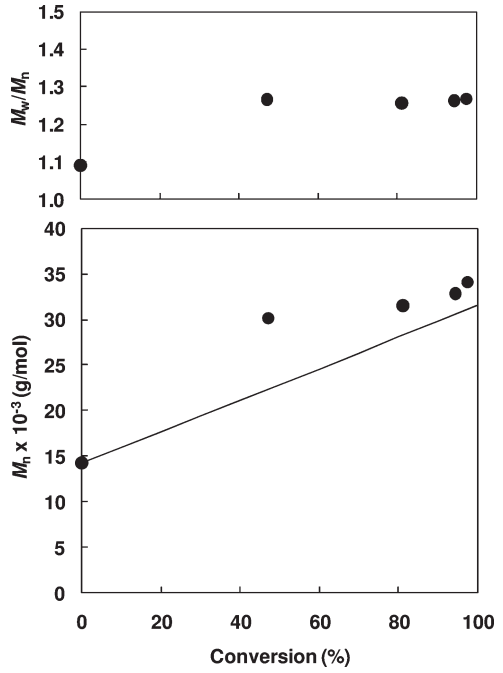


Figure 8. M_w/M_n and M_n vs conversion for inverse miniemulsion polymerizations of DMAEMA initiated by VA-044 at 60 °C with poly(ethylene glycol)-based macroRAFT agent and the cross-linker DMA-PEOSS after degradation by treatment with DL-dithiothreitol to break the disulfide cross-links.

(pH 6.5) was +42 mV. The presence of positive charges at the surface of the nanogels offers the possibility to conjugate siRNA or DNA and subsequently exploits these nanoparticles as gene nanocarriers.

CONCLUSIONS

Biodegradable hydrogel nanoparticles with diameters of ~200 nm have been synthesized via inverse miniemulsion RAFT polymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA). The experimental conditions were optimized so as to minimize partitioning of both monomer and RAFT agent to the continuous phase, thereby allowing the synthesis of nanoparticles comprising a well-defined network structure. Cross-linking (network formation) was achieved by use of a biodegradable disulfide cross-linker, thereby enabling us to subsequently severe the cross-links by exposure to a reductive environment. The molecular weight characteristics of the resulting primary chains were consistent with the cross-linking polymerization having proceeded via a controlled/living mechanism. These biodegradable hydrogel nanoparticles are currently being investigated for encapsulation and controlled release of siRNA.

ASSOCIATED CONTENT

Supporting Information. Molecular weight data in the absence of RAFT agent and cross-linker. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +61 2 9385 4331; Fax: +61 2 9385 6250; e-mail: p.zetterlund@unsw.edu.au (P.B.Z.), t.davis@unsw.edu.au (T.P.D.).

ACKNOWLEDGMENT

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq - Brazil) for the scholarship for M.A.M.O. In addition, we acknowledge significant research fellowship funding to T.P.D. (Federation Fellowship) and C.B. (Australian Postdoctoral Fellowship) from the Australian Research Council. P.B.Z. is grateful for strategic funding from UNSW.

REFERENCES

(1) Oh, J. K.; Siegwart, D. J.; Lee, H.; Sherwood, G.; Peteanu, L.; Hollinger, J. O.; Kataoka, K.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 5939–5945.
(2) Oh, J. K.; Drumright, R.; Siegwart, D. J.; Matyjaszewski, K. *Prog. Polym. Sci.* **2008**, *33*, 448–477.
(3) Hoare, T. R.; Kohane, D. S. *Polymer* **2008**, *49*, 1993–2007.
(4) Ryu, J. H.; Chacko, R. T.; Jiwpanich, S.; Bickerton, S.; Babu, R. R.; Thayumanavan, S. *J. Am. Chem. Soc.* **2010**, *132*, 17227–17235.
(5) Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* **2007**, *32*, 93–146.
(6) Boyer, C.; Stenzel, M. H.; Davis, T. P. *J. Polym. Sci., Part A: Polym. Chem.* **2011**, *49*, 551–595.
(7) Zetterlund, P. B.; Kagawa, Y.; Okubo, M. *Chem. Rev.* **2008**, *108*, 3747–3794.
(8) Cunningham, M. F. *Prog. Polym. Sci.* **2008**, *33*, 365–398.
(9) Ide, N.; Fukuda, T. *Macromolecules* **1999**, *32*, 95–99.
(10) Zetterlund, P. B.; Alam, M. N.; Minami, H.; Okubo, M. *Macromol. Rapid Commun.* **2005**, *26*, 955–960.
(11) Saka, Y.; Zetterlund, P. B.; Okubo, M. *Polymer* **2007**, *48*, 1229–1236.
(12) Gao, H.; Matyjaszewski, K. *Prog. Polym. Sci.* **2009**, *34*, 317–350.
(13) Barner-Kowollik, C. *Handbook of RAFT Polymerization*; Wiley-VCH: New York, 2008.
(14) Chang, C.-W.; Bays, E.; Tao, L.; Alconcel, S. N. S.; Maynard, H. D. *Chem. Commun.* **2009**, 3580–3582.
(15) Boyer, C.; Bulmus, V.; Davis, T. P.; Ladmiral, V.; Liu, J.; Perrier, S. *Chem. Rev.* **2009**, *109*, 5402–5436.
(16) Pissuwan, D.; Boyer, C.; Gunasekaran, K.; Davis, T. P.; Bulmus, V. *Biomacromolecules* **2010**, *11*, 412–420.
(17) Landfester, K. *Angew. Chem., Int. Ed.* **2009**, *48*, 4488–4507.
(18) Faucheu, J.; Gauthier, C.; Chazeau, L.; Cavaille, J. Y.; Mellon, V.; Lami, E. B. *Polymer* **2010**, *51*, 6–17.
(19) van Berkel, K. Y.; Hawker, C. J. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 1594–1606.
(20) Asua, J. M. *Prog. Polym. Sci.* **2002**, *27*, 1283.
(21) Landfester, K. *Macromol. Rapid Commun.* **2001**, *22*, 896.
(22) Spernath, L.; Magdassi, S. *Polym. Adv. Technol.* **2007**, *18*, 705–711.
(23) Guo, Y.; Liu, J. Q.; Zetterlund, P. B. *Macromolecules* **2010**, *43*, 5914–5916.
(24) Cheng, S. Q.; Guo, Y.; Zetterlund, P. B. *Macromolecules* **2010**, *43*, 7905–7907.
(25) Landfester, K.; Willert, M.; Antonietti, M. *Macromolecules* **2000**, *33*, 2370–2376.
(26) Hemingway, M. G.; Gupta, R. B.; Elton, D. J. *Ind. Eng. Chem. Res.* **2010**, *49*, 10094–10099.
(27) Capek, I. *Adv. Colloid Interface Sci.* **2010**, *156*, 35–61.
(28) Oh, J. K.; Perineau, F.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 8003–8010.
(29) Oh, J. K.; Tang, C.; Gao, H.; Tsarevsky, N. V.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2006**, *128*, 5578–5584.
(30) Oh, J. K.; Dong, H.; Zhang, R.; Matyjaszewski, K.; Schlaad, H. *J. Polym. Sci., Part A: Polym. Chem.* **2007**, *45*, 4764–4772.
(31) Moad, G.; Rizzardo, E.; Thang, S. H. *Aust. J. Chem.* **2005**, *58*, 379–410.

(32) Qi, G.; Jones, C. W.; Schork, F. J. *Macromol. Rapid Commun.* **2007**, *28*, 1010–1016.
(33) Qi, G.; Eleazer, B.; Jones, C. W.; Schork, F. J. *Macromolecules* **2009**, *42*, 3906–3916.
(34) Ouyang, L.; Wang, L.; Schork, F. J. *Macromol. Chem. Phys.* **2010**, *211*, 1977–1983.
(35) Lu, F.; Luo, Y.; Li, B.; Zhao, Q.; Schork, F. *Macromolecules* **2010**, *43*, 568–571.
(36) Sogabe, A.; McCormick, C. L. *Macromolecules* **2009**, *42*, 5043–5052.
(37) Tao, L.; Liu, J.; Tan, B. H.; Davis, T. P. *Macromolecules* **2009**, *42*, 4960–4962.
(38) Yuk, S. H.; Cho, S. H.; Lee, S. H. *Macromolecules* **1997**, *30*, 6856–6859.
(39) Liu, F.; Urban, M. W. *Macromolecules* **2008**, *41*, 6531–6539.
(40) Liu, X.; Ni, P.; He, J.; Zhang, M. *Macromolecules* **2010**, *43*, 4771–4781.
(41) Lim, D. W.; Yeom, Y. I.; Park, T. G. *Bioconjugate Chem.* **2000**, *11*, 688–695.
(42) van de Wetering, P.; Moret, E. E.; Schuurmans-Nieuwenbroek, N. M. E.; van Steenbergen, M. J.; Hennink, W. E. *Bioconjugate Chem.* **1999**, *10*, 589–597.
(43) Jiang, X.; Lok, M. C.; Hennink, W. E. *Bioconjugate Chem.* **2007**, *18*, 2077–2084.
(44) Lin, S.; Du, F.; Wang, Y.; Ji, S.; Liang, D.; Yu, L.; Li, Z. *Biomacromolecules* **2007**, *9*, 109–115.
(45) Wetering, P.; Zuidam, N. J.; Steenbergen, M. J.; Houwen, O.; Underberg, W. J. M.; Hennink, W. E. *Macromolecules* **1998**, *31*, 8063–8068.
(46) Cherng, J.; Wetering, P.; Talsma, H.; Crommelin, D. J. A.; Hennink, W. E. *Pharm. Res.* **1996**, *13*, 1038–1042.
(47) Wetering, P.; Cherng, J.; Talsma, H.; Crommelin, D.; Hennink, W. *J. Controlled Release* **1998**, *53*, 145–153.
(48) Wetering, P.; Cherng, J.; Talsma, H.; Hennink, W. E. *J. Controlled Release* **1997**, *49*, 59–69.
(49) Tao, L.; Chou, W. C.; Tan, B. H.; Davis, T. P. *Macromol. Biosci.* **2010**, *10*, 632–637.
(50) Boyer, C.; Priyanto, P.; Davis, T. P.; Pissuwan, D.; Bulmus, V.; Kavallaris, M.; Teoh, W. Y.; Amal, R.; Carroll, M.; Woodward, R.; St Pierre, T. *J. Mater. Chem.* **2010**, *20*, 255–265.
(51) Mitsukami, Y.; Donovan, M. S.; Lowe, A. B.; McCormick, C. L. *Macromolecules* **2001**, *34*, 2248–2256.
(52) Bütün, V.; Billingham, N. C.; Armes, S. P. *Chem. Commun.* **1997**, 671–672.
(53) Lee, A. S.; Gast, A. P.; Bütün, V.; Armes, S. P. *Macromolecules* **1999**, *32*, 4302–4310.
(54) Taylor, P. *Adv. Colloid Interface Sci.* **1998**, *75*, 107–163.
(55) Boyer, C.; Bulmus, V.; Liu, J.; Davis, T. P.; Stenzel, M. H.; Barner-Kowollik, C. *J. Am. Chem. Soc.* **2007**, *129*, 7145–7154.
(56) Luo, Y.; Tsavalas, J.; Schork, F. J. *Macromolecules* **2001**, *34*, 5501–5507.
(57) Li, Y.; Armes, S. P. *Macromolecules* **2005**, *38*, 8155–8162.