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pH and temperature-sensitive statistical copolymers poly[2-(dimethylamino)ethyl methacrylate-*stat*-2-vinylpyridine] with functional succinimidyl-ester chain ends synthesized by nitroxide mediated polymerization

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ABSTRACT

Statistical copolymerizations of 2-(dimethylamino)ethyl methacrylate (DMAEMA) with 2vinylpyridine (2VP) with 80-99 mol% DMAEMA in the feed utilizing a succinimidyl esterterminated alkoxyamine unimolecular initiator (NHS-BlocBuilder) at 80 °C in bulk were performed. The effectiveness of 2VP as a controlling comonomer is demonstrated by linear increases in number-average molecular weight versus conversion, relatively low PDI (1.5 - 1.6 with up to 98% DMAEMA) and successful chain extensions with 2VP. Additional free nitroxide does not significantly improve control for the DMAEMA/2VP copolymerizations. The succinimidyl ester on the initiator permits coupling to amineterminated poly(propylene glycol) (PPG), yielding an effective macroinitiator for synthesizing a doubly thermoresponsive block copolymer of PPG-*block*-P(DMAEMA/2VP). A detailed study of the thermo- and pH-sensitivities of the statistical and block copolymers is also presented. The cloud point temperature of the statistical copolymers is fine tuned from 14 to 75 °C by varying polymer composition and pH.

KEYWORDS (stimuli-responsive polymers, water-soluble polymers, nitroxide mediated polymerization, controlled radical polymerization, 2-(dimethylamino)ethyl methacrylate (DMAEMA))

INTRODUCTION

Polymers that have fast and reversible responses to environmental stimuli such as temperature, pH, light and ionic strength are of great interest for "smart" materials.^{1,2} Poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) is a water soluble polymer that is responsive to both temperature and pH.³⁻⁵ It exhibits lower critical solution temperature (LCST)-type phase separation upon heating in aqueous solutions at neutral or basic conditions while it protonates and becomes a polyelectrolyte in acidic media. Such polymers are very attractive for many potential applications, particularly in the biomedical field, such as drug/gene/protein delivery, biosensors, bioseparations, and tissue engineering.^{2,6-8}

The properties of a stimuli-responsive polymer such as PDMAEMA are closely related to the polymer microstructure. Traditionally, truly living polymerization techniques such as anionic polymerization⁹ were the only approaches to achieve precise control of molecular weight and molecular weight distribution. More recently, controlled radical polymerization (CRP) techniques such as reversible addition-fragmentation transfer (RAFT), atom transfer radical polymerization (ATRP) and nitroxide-mediated polymerization (NMP) were developed to superficially resemble living polymerization with relatively robust reaction conditions.¹⁰ For ATRP, metal catalysts are required and RAFT requires sulfur-based bimolecular chain transfer agents. Although these compounds can be removed by post-polymerization modification steps,¹¹ NMP requires simple purification procedures, often a single-step precipitation, since it only involves a single

initiator for controlling the polymerization. NMP has been touted as a convenient route towards synthesizing non-cytotoxic polymers for biomedical applications.¹²⁻¹⁴

However, NMP has not been as successful in controlling homopolymerization of methacrylates such as DMAEMA compared to the other CRP techniques.^{14,15} The very high equilibrium constant K of methacrylates that is caused by the slow recombination of nitroxides and the sterically-hindered poly(methacrylate) radicals has been claimed to be the main reason for poorly controlled methacrylate homopolymerizations via NMP.^{14,16} Additionally, other side reactions including irreversible terminations between propagating radicals and/or β -hydrogen transfers between propagating radicals and nitroxides,¹⁷ chain transfer to solvent/monomers,¹⁸ and decomposition of alkoxyamines¹⁴ can also deactivate the nitroxide group, limit achievable molecular weight and broaden the molecular weight distribution. Continuous efforts in modifying NMP initiator structures have given rise to alkoxyamines specifically synthesized for methacrylates.^{19,20} However, these alkoxyamines require extensive synthesis steps and lack versatility (eg. they are in some cases difficult to cross-over from one monomer to the other when performing block copolymerizations). Another approach to achieve controlled methacrylate polymerization via NMP is to use a small amount of a controlling comonomer.²¹ With a commercially available SG1-based unimolecular alkoxyamine called BlocBuilderTM (N-(2-methylpropyl-N-(1-diethylphosphono-2,2-dimethylpropyl)-O-(2-carboxylprop-2-yl) hydroxylamine, structures of SG1 and BlocBuilder[™] shown in Scheme 1 (a) and (b)), a range of methacrylates,^{13,21-24} including DMAEMA,²⁴ has been successfully controlled with a small amount (1 - 10 mol%) of comonomer in the feed and about 10% excess of free SG1

relative to the BlocBuilderTM. While styrene is the most popular choice as a comonomer,^{13,21,23} acrylonitrile (AN),²² sodium 4-styrene sulfonate (SS),²⁵ 9-(4-vinylbenzyl)-9H-carbazole (VBK),^{24,26} and pentafluorostyrene (PFS)²⁷ have also been reported as controlling comonomers for various methacrylates. The key properties of an effective controlling comonomer are much lower equilibrium constant K and low cross-propagation rate constant (k_p/reactivity ratio) compared to methacrylates.^{16,28,29} For the copolymerization systems of methyl methacrylate (MMA) with less than 10 mol% styrene (S), the favored MMA-S-SG1 chain end formation has been demonstrated critical for controlled SG1-mediated polymerization of MMA.^{30,31} This MMA-S-SG1 formation results in high alkoxyamine dissociation rate at low temperature of about 90 °C due to the penultimate unit effect from MMA and low active radical concentration allowed by the styrene-terminated macroalkoxyamines, ensuring fast initiation and controlled propagation. The same conclusion were also applied for MMA/SS²⁵ and MMA/AN²² copolymerization systems.

When copolymerizing a hydrophilic stimuli-responsive methacrylate with a hydrophobic comonomer via NMP, the benefits can be expanded from achieving a controlled polymerization to property tuning by simply varying copolymer composition.^{24,27,32,33} However, a hydrophillic poly(methacrylate) can be easily rendered water insoluble when a relatively small fraction of a hydrophobic comonomer was incorporated. Using specific examples of DMAEMA-rich copolymers, when more than 25 mol% of styrene,³³ 10 mol% PFS,²⁷ or as little as 5 mol% VBK²⁴ was copolymerized with DMAEMA, the resulting random copolymer became water-insoluble. This limits the applications of such random

copolymers and thus a less hydrophobic controlling comonomer would be desirable. On the other hand, sodium 4-styrene sulfonate, which was demonstrated to be effective as a comonomer to control methacrylic acid-rich copolymerizations, is totally water-soluble.²⁵ However, it has been reported that when LCST-type polymers were copolymerized with hydrophilic comonomers, the copolymer exhibited incomplete transitions and eventually no transitions as the hydrophilic comonomer content increased.^{34,35} This phenomena was explained by the dramatic reduction in hydrophobic interactions between the thermoresponsive units above their LCST.

In this work, 2-vinylpyridine (2VP) was chosen as a comonomer for DMAEMA-rich copolymerizations via NMP as it is structurally similar to styrene but less hydrophobic. Controlled homopolymerization of 2VP has been reported in the presence of TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl)^{36,37} and the reported kinetics of 2VP polymerization was not significantly different than that of styrene.³⁶ We thus expect 2VP to be a suitable controlling comonomer for methacrylates by NMP. P2VP is also pH-sensitive and becomes a polyelectrolyte in acidic media. Therefore, it can tune the properties of the random copolymer in its neutral form like other hydrophobic comonomers; it can also become totally water-soluble if it is protonated. Its pH sensitivity and polyampholytic properties have been utilized for pH-dependent micellization^{3,38,39} and forming polymer complexes.⁴⁰ By using 2VP as a comonomer, the resulting copolymer can potentially gain additional functionality besides enabling the controlled polymerization of a functional methacrylate.

Moreover, a modified SG1-based alkoxyamine bearing a N-succinimidyl ester (NHS-BlocBuilder, Scheme 1 (c)) was used as the initiator for this work. This alkoxyamine can be obtained by a single step coupling of BlocBuilder with N-hydroxysuccinimide⁴¹ or potentially by a copper metal-mediated reaction of succinimidyl halide with SG1 nitroxide.⁴² NHS-BlocBuilder has been shown effective in controlling homopolymerization of styrene and n-butyl acrylate,⁴¹ as well as random copolymerization of glycidyl methacrylate with styrene⁴³ and DMAEMA with styrene³³ without the aid of additional SG1 nitroxide. The NHS-BlocBuilder is particularly attractive as it can be used for post-polymerization bioconjugation.⁴⁴

In this paper, the polymerization kinetics, level of control, polymer livingness and the effects of additional SG1 nitroxide on DMAEMA/2VP random copolymerizations initiated by NHS-BlocBuilder will be discussed. The effect of copolymer composition on the pH- and thermo-sensitivities of the copolymers will also be presented. The succinimidyl ester moiety from the NHS-BlocBuilder initiator, since it readily reacts with nucleophiles or amine-containing materials⁴⁵ will permit additional functionalization of the copolymer. This will also be demonstrated by the coupling of amine-terminated poly(propylene glycol) to NHS-BlocBuilder to form a macroinitiator for subsequent polymerizations in this work.

EXPERIMENTAL

Materials

2-Vinylpyridine (2VP, 97%), 2-(dimethylamino)ethyl methacrylate (DMAEMA, 98%), N,N'-dicyclohexylcarbodiimide (99.9%), N-hydroxysuccinimide (98%), poly(propylene glycol)-bis(2-aminopropyl ether) ($M_n \simeq 2000$ g mol⁻¹), basic alumina (Brockmann, Type I,

150 mesh), and calcium hydride (90-95% reagent grade) were purchased from Sigma-Aldrich. Tetrahydrofuran (THF, 99.9%), pentane and hexane (certified grade) were obtained from Fisher; deuterated chloroform (CDCl₃) was obtained from Cambridge Isotope Laboratories Inc. 2-({*tert*-butyl[1-(diethoxyphosphoryl)-2,2-(dimethylpropyl]amino}oxy)-2-methylpropionic acid, also known as BlocBuilderTM (99%, Scheme 1 (b)), was obtained from Arkema. 2VP and DMAEMA monomers were purified by passing through a column of basic alumina mixed with 5 wt% calcium hydride; they were stored in a sealed flask under a head of nitrogen in a refrigerator until needed. All other compounds were used as received.

Homopolymerization of 2-vinylpyridine (2VP) using NHS-BlocBuilder as initiator

The initiator NHS-BlocBuilder (Scheme 1 (c)) was synthesized via coupling of BlocBuilder and *N*-hydroxysuccinimide following procedures described previously.^{33,41} The homopolymerization of 2VP was performed in a 50 mL three-neck round-bottom glass flask fitted with a reflux condenser, a magnetic stir bar, and a thermal well. The NHS-BlocBuilder (0.048 g, 0.10 mmol) was dissolved in 2VP monomer (3.0 g, $M_{n,target} = 30$ kg mol⁻¹). The solution was then deoxygenated by nitrogen bubbling for 30 min at room temperature prior to heating to 120 °C using a heating mantle with a heat controller at a rate of about 8 °C min⁻¹ while maintaining a nitrogen purge. The time when the solution reached 120 °C was taken as the start of the reaction (t = 0). Samples were taken periodically to monitor conversion and molecular weight. The reaction was precipitated in hexane, decanted and dried in vacuum at 40 °C overnight. The purified polymer (50%

conversion) has number-average molecular weight (M_n) = 15.8 kg mol⁻¹ and polydispersity (PDI) = 1.29. Conversion was determined by ¹H NMR (400 MHz Varian Gemini 2000 spectrometer, CDCl₃) using the peak of the aromatic proton closet to the nitrogen of the monomer (δ = 8.5 - 8.6 ppm) and of the polymer (δ = 8.3 – 8.5 ppm). Molecular weight and PDI of the samples were measured by GPC (Waters Breeze) relative to poly(styrene) standards (see *Gel Permeation Chromatography* section for full details).

Statistical copolymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA) and 2vinylpyridine (2VP)

The same setup and experimental procedures as the homopolymerizations were used for the statistical copolymerizations of DMAEMA and 2VP except for the feed composition with respect to DMAEMA ($f_{DMAEMA,0}$) was varied from 80 – 99 mol% and the synthesis was conducted at 80 °C (detail experimental conditions can be found in Table 1). Samples were taken periodically to determine conversion and molecular weight. Monomer conversion and copolymer composition were determined by ¹H NMR (400 MHz Varian Gemini 2000 spectrometer, CDCl₃). Conversion of DMAEMA was calculated using the two protons adjacent to the ester of the monomer ($\delta = 3.7 - 3.8$ ppm) and those corresponding to the polymer ($\delta = 3.9 - 4.1$ ppm). 2VP conversion was determined by using the proton adjacent to the nitrogen of the monomer ($\delta = 8.5 - 8.6$ ppm) and of the polymer ($\delta = 8.3 - 8.5$ ppm). Overall conversion was then calculated using the feed composition with respect to DMAEMA ($f_{DMAEMA,0}$, mol%, Conv._{ave} = $f_{DMAEMA,0} \times \text{Conv.}_{DMAEMA} + (1 - f_{DMAEMA,0}) \times \text{Conv.}_{2VP}$). Polymerization was stopped when the reaction mixture became too viscous to withdraw samples. The copolymers were precipitated in hexane, decanted and dried in vacuum at 40 °C overnight. Molecular weight and polydispersity of the samples were measured by GPC (Waters Breeze) (see *Gel Permeation Chromatography* section for full details). The molecular weight stated for the copolymers was relative to poly(methyl methacrylate) standards. A homopolymerization of DMAEMA was also attempted with the same setup and procedures as the statistical copolymerizations. However, the polymerization was highly exothermic and the viscosity of the reaction mixture increased sharply as soon as the temperature reached 80 °C, which forbade any samples to be drawn. The mixture was diluted with THF and the final polymer was precipitated in hexane, decanted and dried in vacuum at 40 °C overnight. Molecular weight and polydispersity of the PDMAEMA homopolymer was measured by GPC (Waters Breeze) relative to poly(methyl methacrylate) standards (see *Gel Permeation Chromatography* section for full details).

Synthesis of poly(propylene oxide)-coupled BlocBuilder (PPO-BB) macroalkoxyamine

The following procedures were adapted from Vinas et al.¹ with slight modifications to produce a BlocBuilder terminated PPO. NHS-BlocBuilder (0.50 g, 1.05 mmol) was dissolved in THF (16 mL) and bubbled with ultra-pure nitrogen for 15 min at 0 °C. Poly(propylene glycol)-bis(2-aminopropyl ether) (PPO-di-NH₂) M_n ~ 2000 g mol⁻¹ (4.20 g, 2.10 mmol) was then added to the degassed NHS-BlocBuilder solution. Then, 4 times excess amine functional group from PPO-di-NH₂ was added to ensure only one NHS-BlocBuilder was coupled to each PPO-di-NH₂. The mixture was stirred at 0 °C for 2 hours, then filtered to remove the precipitated *N*-hydroxysuccinimide and dried under reduced pressure to remove THF. The final product is a mixture of PPO-coupled BlocBuilder (PPO-BB, Scheme 2) macroalkoxyamine and unreacted PPO-di-NH₂. ¹H NMR (400 MHz Varian Gemini 2000

spectrometer, CDCl₃, δ , ppm): 1.0 – 1.4 (3nH; CH₃ (PPO with degree polymerization of n) + 18H; 2 *tert*-butyl groups + 6H; 2 x (POCH₂**CH₃**) (BlocBuilder)), 1.6 – 1.9 (6H; (CH₃)₂CO (BlocBuilder)), 3.1 – 3.9 (3nH; OCH₂CH (PPO with degree polymerization of n)), 3.9 – 4.4 (4H; 2 x (PO**CH₂**CH₃) (BlocBuilder)).

Synthesis of statistical copolymer of DMAEMA and 2VP using PPO-BB macroalkoxyamine

The mixture of PPO-BB and residual PPO-di-NH₂ (0.85 g total, ~ 0.43 g or 2.12 mmol PPO-BB) was dissolved in a mixture of DMAEMA and 2VP monomers (total 6.0 g, $M_{n,target}$ = 30 kg mol⁻¹, $f_{DMAEMA,0}$ = 95 mol%). The same procedures as the random copolymerizations described earlier were followed. Samples were drawn periodically to monitor conversion and molecular weight. Monomer conversion and final polymer composition were determined by ¹H NMR (400 MHz Varian Gemini 2000 spectrometer, CDCl₃). All samples and the final polymer were precipitated in hexane where the monomers and the oligomeric PPO-di-NH₂ were soluble. The recovered block copolymer was then dried in an oven at 40 °C under vacuum. The final yield of the block copolymer was 0.8 g (32% conversion, low yield was mainly due to the loss from many samples drawn during the reaction) with M_n = 21.5 kg mol⁻¹ and PDI = 1.78 as determined by GPC (Waters Breeze) relative to poly(methyl methacrylate) standards (see *Gel Permeation Chromatography* section for full details). The composition of the DMAEMA/2VP block with respect to DMAEMA (*F_{DMAEMA}*, mol%) was 94% as determined by ¹H NMR.

Chain extension experiments

Selected DMAEMA/2VP statistical copolymers were chain-extended with 2VP at 120 °C in bulk. The setup and polymerization procedures were the same as the synthesis of homopolymer and statistical copolymers described earlier. The detailed experimental conditions can be found in Table 2. The experiments were stopped after 3 hours and the polymer was precipitated in hexane, decanted and dried in an oven at 40 °C under vacuum. The chain-extended block copolymers were characterized by GPC (Waters Breeze) calibrated with poly(methyl methacrylate) standards (see *Gel Permeation Chromatography* section for full details).

Gel permeation chromatography

Molecular weight and polydispersity index (PDI) of all polymers were characterized by gel permeation chromatography (GPC) (Waters Breeze) using THF as the mobile phase at 40 °C in this study. The GPC was equipped with three Waters Styragel HR columns (molecular weight measurement ranges: HR1: $10^2 - 5 \times 10^3$ g mol⁻¹, HR2: $5 \times 10^2 - 2 \times 10^4$ g mol⁻¹, HR3: $5 \times 10^3 - 6 \times 10^5$ g mol⁻¹) and a guard column. The columns were operated at 40 °C and a mobile phase flow rate of 0.3 mL min⁻¹ during analysis. The GPC was also equipped with both ultraviolet (UV 2487) and differential refractive index (RI 2410) detectors. The molecular weight measurements were calibrated relative to poly(styrene) narrow molecular weight distribution standards for 2VP homopolymer and poly(methyl methacrylate) narrow molecular weight distribution standards for all other polymers in this study.

Characterization of the aqueous solutions of the statistical copolymers and block copolymers

The transition temperature of the statistical copolymers in buffer solutions from pH 7 -

11 at 0.5 wt% concentration was characterized using a Cary 5000 UV-vis-NIR Spectrophotometer. Light transmittance of the solutions was monitored at 600 nm while the solution is heated/cooled at a rate of 0.5 °C min⁻¹. The cloud point temperature is defined as the temperature where the transmittance dropped to 50% during heating. Dynamic light scattering (DLS) was used to determine the hydrodynamic radius of the block copolymers in aqueous solutions at different pHs and temperatures using a Malvern ZetaSizer (Nano-ZS). The instrument was equipped with a He-Ne laser operating at 633 nm with a scattering angle of 173°, an avalanche photodiode detector, and a temperaturecontrolled cell. In all DLS measurements, the aqueous solutions were filtered using a 0.2 um pore size filter and the filtered solutions were monitored in the temperaturecontrolled cell while heated at a rate of about 0.5 °C min⁻¹. The pKas of the statistical copolymers and block copolymers were extrapolated from the titration curve obtained from an 888 Titrando automatic titrator with a pH probe calibrated with pH 4, 7, and 10 buffers. The copolymers were dissolved at pH 2 and titrated with a 0.05 mol L⁻¹ NaOH titrant.

RESULTS AND DISCUSSION

The polymerization results and polymer characterizations for homopolymerizations of 2VP and DMAEMA as well as statistical copolymerizations of DMAEMA/2VP are summarized in Table 3.

Homopolymerization of 2-vinylpyridine (2VP) using NHS-BlocBuilder

Homopolymerization of 2VP was performed using NHS-BlocBuilder as initiator to examine the level of control and to confirm if 2VP is indeed a good candidate as a controlling comonomer for this system. The homopolymerization was done in bulk at 120 °C with no additional free SG1 nitroxide.

The kinetic results shown in Figure 1 (a) indicate the reaction followed the first order kinetics as expected from a controlled polymerization. The number-average molecular weight (M_n) of the homopolymer increased linearly with conversion and had excellent agreement with the theoretical prediction as demonstrated in Figure 1 (b). The PDIs of the homopolymer remained below 1.30 throughout the reaction. These results illustrated that the 2VP homopolymerization using NHS-BlocBuilder was well controlled.

We have previously demonstrated that as little as 5 mol% styrene was effective in controlling 2-(dimethylamino)ethyl methacrylate (DMAEMA) polymerization using NHS-BlocBuilder and producing DMAEMA-rich copolymers capable of re-initiating a second batch of monomer.³³ In terms of both kinetics and level of control, the 2VP homopolymerization results were very similar to those of styrene homopolymerization

initiated by the same alkoxyamine at the same temperature.⁴¹ Therefore, we foresee 2VP as a capable controlling comonomer for DMAEMA-rich copolymerizations.

Statistical copolymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA) and 2vinylpyridine (2VP) using NHS-BlocBuilder

Effect of feed composition on polymerization kinetics and control

The homopolymerization of DMAEMA with NHS-BlocBuilder was attempted at 80 °C in bulk. The reaction was highly exothermic and the reaction mixture became very viscous within a minute after the temperature reached 80 °C. The final polymer was shown to have $M_n = 15.8$ kg mol⁻¹ and PDI = 2.01 when analyzed by GPC relative to poly(methyl methacrylate) standards. The extremely rapid kinetics and broad molecular weight distribution confirmed the uncontrolled nature of the DMAEMA homopolymerization by NMP and the necessity of using a controlling comonomer with this system (ie. polymerization initiated by NHS-BlocBuilder at 80 °C with a low concentration of a styrenic-type monomer)

For the DMAEMA/2VP statistical copolymerizations performed at 80 °C in bulk initiated by NHS-BlocBuilder, the initial amount of DMAEMA in the feed ($f_{DMAEMA,0}$) was varied from 80 – 99 mol%. All copolymerizations followed first-order kinetics as illustrated by linear kinetic plots presented in Figure 2 (a).

The apparent rate constants, indicated by the slopes of the kinetic plots, $\langle k_p \rangle$ [P•] (where $\langle k_p \rangle$ is the average propagation rate constant and [P•] is the propagating radical concentration) were plotted against feed composition in Figure 2 (b). The copolymerization rate increased rapidly with increasing amount of DMAEMA in the feed,

especially when there was more than 90 mol% DMAEMA in the feed. The $\langle k_p \rangle$ [P•] values were similar to those obtained from the copolymerization of DMAEMA with styrene ($\langle k_p \rangle$ [P•] ranged from 2.5 x 10⁻⁵ s⁻¹ to 9.2 x 10⁻⁵ s⁻¹ when 2VP was used as comonomer, and ranged from 2.7 x 10⁻⁵ s⁻¹ to 6.6 x 10⁻⁵ s⁻¹ when styrene was used as comonomer³³ as feed composition varied from 80 to 95 mol% DMAEMA).

Nitroxide mediated copolymerization kinetics are often expressed as the product of average propagation rate constant $\langle k_p \rangle$ and average equilibrium constant $\langle K \rangle$, $\langle k_p \rangle \langle K \rangle$, which can be expressed as a function of k_p s, Ks and reactivity ratios of both monomers.²¹ The equilibrium constant K is defined by Equation 1 when SG1 is used as the free nitroxide:

Equation 1

$$K = \frac{[SG1\bullet][P\bullet]}{[P-SG1]}$$

where [SG1 •] is the free nitroxide concentration and [P-SG1] is the concentration of the dormant initiator.

Methacrylates generally have higher propagation rate constants k_p and much higher equilibrium constants K than styrene.^{16,29,46,47} The values of k_p for 2VP and styrene were similar (96.5 L mol⁻¹ sec⁻¹ for 2VP⁴⁸ and ranged from 77 to 95 L mol⁻¹ sec⁻¹ for styrene,⁴⁶ both at 25 °C). To the best of our knowledge, the value of K for 2VP is not available in the literature. We assumed it to be similar to that of styrene considering 2VP homopolymerization had similar kinetics compared to styrene under the same conditions as discussed earlier. Therefore, it was expected that the $\langle k_p \rangle \langle K \rangle$ values and thus the polymerization rates increased rapidly as the feed was enriched with methacrylate, just as in the other cases with methacrylate/styrene copolymerizations controlled by BlocBuildertype initiators.^{21,33}

As the amount of DMAEMA in the feed was increased from 95 mol% to 98 mol% in this study with 2VP as a controlling comonomer, the $\langle k_{\rho} \rangle$ [P•] values continued to increase sharply. However, a slight decline in $\langle k_{\rho} \rangle$ [P•] value was observed for the copolymerization with feed composition $f_{DMAEMA,0}$ = 99 mol%. This slight decrease in apparent polymerization rate was not unexpected. In nitroxide-mediated copolymerizations of methacrylates with a very small amount of controlling comonomer (< 5 mol% in the feed), polymerization rate showed minimal increase^{22,26} or declined slightly^{24,32} with increasing amount of methacrylate in the feed. Note that in Figure 3 (a), the conversion of the first sample for D2VP-99 was 24% at a reaction time of 2 minutes, yet only an increase of 2% in conversion was obtained at 4-minute reaction time for the second sample. With these highly methacrylate-rich feeds, a significant amount of irreversible termination reactions can occur at early stages of the polymerization when the concentration of regulating nitroxide was below its equilibrium value, which leads to non-first-order kinetics, similar to methacrylate homopolymerizations.¹⁷ Therefore, the apparent rate constant $\langle k_p \rangle$ [P•] obtained from these higher conversion samples was likely lower than its true value.

A linear increase of M_n with conversion is one of the signatures of a living polymerization and is often used in the analysis of controlled polymerizations. In all of the copolymerizations of DMAEMA with 2VP with up to 99 mol% DMAEMA in the feed, linear trends of M_n versus conversion were observed up to about 45% conversion, as shown in Figure 3 (a). At higher conversions, many of the M_n versus conversion plots of the

copolymers still followed the theoretical trend but tended to plateau. This deviation has been observed previously and attributed to the adsorption of PDMAEMA onto the GPC columns.^{49,50} The column adsorption led to an increase in retention time and broadening of peaks which resulted in lower detected molecular weight and higher PDIs. Another possible reason for the $M_n < M_{n,theo}$ behavior can be related to chain transfer side reactions. Greszta and Matyjaszewski¹⁸ showed in their simulations of styrene polymerization in the presence of TEMPO nitroxide that the combination of chain transfer to monomers and alkoxyamine decomposition were the main causes for lower M_n and increasing PDI at high conversions. Zetterlund et al.⁵¹ demonstrated experimentally the consequences of chain transfer to monomer with TEMPO and SG1 mediated styrene polymerizations. New chains were created when the radicals transferred to monomer, which could still polymerize in a controlled manner, yet a significant fraction of the new chains remained at low Mn because of the continuous chain transfer. As a result, the overall M_n became lower than the expected values and the distribution broadened. Nonetheless, the deviations of M_n from theoretical values were moderate and the linear increases of M_n versus conversion were evidenced for the controlled nature of the copolymerizations of DMAEMA and 2VP with feed composition up to 99 mol% DMAEMA.

The PDIs of the final copolymers were about 1.5 – 1.6 for copolymerizations with feeds containing up to 98 mol% DMAEMA (Table 3) which were comparable to those of the statistical copolymers of DMAEMA with styrene³³ and 9-(4-vinylbenzyl)-(9H-carbazole) (VBK)²⁴ as controlling comonomers synthesized with BlocBuilder-type unimolecular initiators. Molecular weight distributions broadened significantly (PDIs increased from

~1.6 to 1.9) when the feed contained more than 98 mol% DMAEMA (Table 3) indicating polymerization becoming less controlled. The ability of these statistical copolymers to reinitiate a second batch of monomer was further investigated by experiments discussed in the *Livingness of selected copolymers* section.

Statistical copolymerization of DMAEMA and 2VP using PPO-BB macroalkoxyamine

A statistical copolymerization of DMAEMA with 2VP with feed composition $f_{DMAEMA,0}$ = 95 mol% was performed using a poly(propylene oxide)-coupled BlocBuilder (PPO-BB) as a macroinitiator at 80 °C in bulk. A linear kinetic plot (Figure 4 (a)) was obtained with a slope of $\langle k_p \rangle$ [P•] = 1.0 x 10⁻⁴ s⁻¹, which was similar to the result of the copolymerization with same feed composition initiated by NHS-BlocBuilder ($\langle k_p \rangle$ [P•] = 9.2 x 10⁻⁵ s⁻¹).

 M_n of the block copolymers increased linearly with conversion as shown in Figure 4 (b) as expected from a controlled polymerization. However, the experimental M_n s were consistently higher than their theoretical values throughout the polymerization. This result is likely due to the combination of slow initiation and low initiating efficiency. Slow initiation in radical polymerization is know to result in higher than expected molecular weight and broad PDIs.^{52,53} Low initiating efficiency in NMP has been previously reported for copolymerizations with methacrylates (75% at ~60% conversion for MMA/styrene²¹ and 50 – 80% at 40% conversion for MMA/VBK²⁶). The apparent initiating efficiency defined by the value of theoretical M_n divided by the experimental M_n was about 55% at 32% conversion. These low initiating efficiencies were mainly attributed to incomplete initiation of the alkoxyamine due to irreversible termination of primary and/or oligoradicals that could not be quantified by GPC.²¹ The GPC chromatograms (Figure S1 in the

Supporting Information) of intermediate samples during the polymerization continued to shift towards lower retention time as reaction proceeded indicating continued increases of M_n with conversion. Small shoulders at about 29 min retention time were evident in all traces which represent a small amount of PPO-di-NH₂ residue that was not removed completely from precipitation, the existence of inactive macroalkoxyamine or/and dead short chains.

The ¹H NMR spectrum of the final diblock copolymer of PPO-D2VP-95 is presented in Figure 5. Degree of polymerization (DP) can be calculated using the PPO protons as a reference since it was known that the M_n of PPO was about 2000 g mol⁻¹ (equivalent to DP of about 34, confirmed by ¹H NMR). From the ratios of proton peaks in the NMR spectrum, the DP of DMAEMA was calculated to be about 117 and the DP of 2VP was about 9, giving a DMAEMA/2VP statistical copolymer block of about 19 kg mol⁻¹ with 93 mol% DMAEMA. Combined with the 2 kg mol⁻¹ PPO block, the M_n of the diblock copolymer was about 21 kg mol⁻¹. This result corresponded well with that obtained from GPC relative to PMMA standards (Table 3).

The PDIs of the block copolymers were ~ 1.7, which was broader than those initiated by NHS-BlocBuilder (PDI = 1.48 for D2VP-95, Table 3). The broadening of molecular weight distribution may be due to the slow initiation, the incomplete removal of the unreactive PPO-di-NH₂ residue and/or inactive macroalkoxyamine as well as dead oligoradicals. The diblock copolymer was chain extended to further investigate its livingness, which will be discussed in the *Livingness of selected copolymers* section.

Livingness of selected copolymers

The statistical copolymers with feed composition $f_{DMAEMA,0} = 95 - 99$ mol% and the diblock copolymer initiated by the PPO-BB macroalkoxyamine were chain extended by 2VP to examine their re-initiation ability. The results were summarized in Table 4.

Overall, the percentage of DMAEMA of the chain-extended block copolymers decreased from that of the macroinitiator as expected after the 2VP block was incorporated. M_n of the chain-extended 2VP block was about 15 – 20 kg mol⁻¹ after approximately 3 hours of reaction time. The PDIs of the block copolymers remained nearly the same compared to those of the macroinitiators. These results showed the ability of the macroinitiators to reinitiate a second batch of monomer and continue to polymerize, which is a feature of living polymers.

The chain extension results were also demonstrated in the GPC chromatograms shown in Figure 6. The chain-extended block copolymers (shown as solid lines) were all monomodal and shifted to lower retention times compared to their respective macroinitiators (shown as dashed lines). Low molecular weight tails were observed for all macroinitiators and some of the chain-extended block copolymers. Similar drifting/tailing has been reported previously for PDMAEMA homopolymer⁴⁹ and DMAEMA-rich copolymers^{24,33} and it was attributed to the adsorption of PDMAEMA to the GPC column.⁴⁹ The adsorption of the chain-extended products was less significant compared to the DMAEMA-rich macroinitiators as the 2VP concentration increased in the block copolymer composition. The reduced degree of adsorption to the column of the block copolymer compared to the

macroinitiator may explain the lower PDIs of some of the chain-extended polymer compared to their macroinitiators. For the PPO-D/2VP-95/5 diblock copolymer and its chain-extended triblock copolymer, the small shoulder in the macroinitiator chromatogram became negligible after chain extension, likely due to easier separation of the low molecular weight contaminants (inactive macroinitiator, short dead chains, and/or the unreactive PPO-di-NH₂ residue) from the higher M_n triblock copolymer.

Effect of additional SG1 free nitroxide

In previous NMP studies with BlocBuilder, a small amount of additional free nitroxide (about 5-10 % relative to the initiator) was beneficial in the reaction mixture with monomers that have relatively high k_p such as acrylates⁵⁴⁻⁵⁶ and methacrylates^{13,21,23,24} as well as acidic monomers that promote SG1 nitroxide degradation such as acrylic acid⁵⁷ to control the polymerization. The addition of free SG1 reduced the polymerization rate significantly and led to a linear increase in M_n with conversion following the theoretical trend as well as much lower PDIs.⁵⁴⁻⁵⁷ As demonstrated in this work and previous studies,^{33,41,43} by using NHS-BlocBuilder as the initiator, additional SG1 was no longer a requirement to achieve controlled polymerization of acrylates⁴¹ and more importantly, methacrylates.^{33,43} However, the control in these NHS-BlocBuilder systems was still thought to be able to be improved as the PDIs were still relatively high compared to many other controlled polymerization techniques.^{58,59} Therefore, 10% additional free SG1 relative to NHS-BlocBuilder was added in DMAEMA/2VP feeds containing 95 and 99 mol% DMAEMA to examine the effects of excess nitroxide for this system.

In a controlled polymerization, fast initiation and constant chain concentration are often assumed, meaning [P-SG1] \approx [Initiator]₀. With the large initial concentration of nitroxide, the nitroxide concentration [SG1] should remain relatively constant, close to its initial value [SG1]₀, throughout the polymerization. The product of propagation rate constant k_p and equilibrium constant K, k_pK , can then be estimated from the slopes obtained from the kinetic plots and the ratio of initial nitroxide concentration and initiator concentration, r (Equation 2).

Equation 2

$$k_p K = k_p \frac{[P \bullet][SG1 \bullet]}{[P - SG1]} \approx k_p [P \bullet] \frac{[SG1 \bullet]_0}{[P - SG1]_0} = k_p [P \bullet] r$$

The kinetic results of the copolymerizations with 10% free SG1 were compared to the ones with no additional SG1 in Table 5. The apparent rate constants $\langle k_p \rangle$ [P•] with additional SG1 were lower than those without. This was expected, as the dormant state should be favoured as the nitroxide concentration [SG1•] is increased. $\langle k_p \rangle \langle K \rangle$ values calculated according to Equation 2 were very similar to those reported for the DMAEMA/VBK system at the same temperature.²⁴

 M_n versus conversion was not strongly affected by the addition of free nitroxide, as shown in Figure 7. Plateauing at higher conversion still occurred with additional SG1. Further, provided that enough of the controlling comonomer was added (eg. when $f_{DMAEMA,0}$ = 95 mol%), the PDIs were not significantly affected by the addition of free SG1. Only in the case when the controlling comonomer concentration was low ($f_{DMAEMA,0}$ = 99 mol%), were slightly lower PDIs achieved (PDI \sim 1.8 with 10% free SG1 versus PDI \sim 1.9 when no SG1 was added). In summary, at the conditions used, the addition of free SG1 did not improve the control of DMAEMA/2VP copolymerizations significantly.

This result was not totally surprising as NHS-BlocBuilder has a very high dissociation rate constant ($k_d = 5 \text{ s}^{-1}$ for NHS-BlocBuilder compared to 0.32 s⁻¹ for BlocBuilder at 120 °C in *tert*-butyl benzene⁴¹) and relatively lower activation energy ($E_a = 105 \text{ kJ mol}^{-1}$ for NHS-BlocBuilder compared to 112 kJ mol⁻¹ for BlocBuilder⁴¹). The much higher dissociation rate constant for NHS-BlocBuilder provided sufficiently rapid release of the SG1 regulating nitroxide, which mimicked the addition of a small amount of free SG1 at the onset of polymerization when BlocBuilder was used.³³

In summary, various controlling comonomers have been previously tested to control methacrylate-rich polymerizations using BlocBuilder-based NMP approach.^{21,22,26} Obviously, minimizing the controlling comonomer concentration is often viewed as necessary so that the properties of the poly(methacrylates) are not compromised. The minimum amount of controlling comonomer content has been reported to be 4.4 mol% for MMA/styrene,²¹ 2.2 mol% for MMA/acrylonitrile,²² ~ 1 mol% for MMA/VBK²⁶ and ~2 mol% for DMAEMA/VBK.²⁴ To the best of our knowledge, this is the first time 2VP is reported as a controlling comonomer for methacrylate-rich polymerizations via NMP and is able to control DMAEMA polymerizations with as little as ~ 2 mol% 2VP in the feed. With only 1 mol% 2VP in the feed, although significant broadening of molecular weight distribution was observed, the copolymer was able to re-initiate to form a block copolymer, indicating its living nature.

Further, we demonstrated that NHS-BlocBuilder could be used without any additional free nitroxide for the controlled NMP of methacrylate-rich compositions. This could simplify greatly the processes for implementation of such polymerizations. Also, NHS-BlocBuilder was shown to be easily conjugated via a single-step coupling to amine-containing compounds, in this case, amine-terminated poly(propylene glycol), and remained an effective macroinitiator for initiating further polymerizations and yielding a block copolymer. It demonstrated an additional approach to couple different types of polymers to form block copolymers via NMP.

Temperature and pH sensitivities of the statistical copolymers

PDMAEMA is known to be sensitive to both temperature and pH;^{3,24,33,58,60,61} P2VP is water-insoluble at neutral and basic aqueous solutions while highly water-soluble in acidic media.^{3,38,62} It is therefore very interesting to investigate the phase behavior of the statistical copolymers of DMAEMA with 2VP at different temperatures and pHs, as the 2VP not only can serve as an NMP controlling comonomer for DMAEMA polymerizations, but also can impart different solution properties to the copolymers, such as tuning of the cloud point temperatures (CPT).

The statistical copolymers were dissolved in buffer solutions from pH 7 to 11 at 0.5 wt% concentration and their thermo-sensitivity was examined by UV-visible spectroscopy. The light transmittance of the solutions decreased sharply from 100% (transparent) to 0% (opaque) once the solution temperature is above a critical value (an example was shown in Figure S2 in the Supporting Information), which is typical of a lower critical solution

temperature (LCST)-type phase separation. The temperature where light transmittance dropped to 50% during the heating cycle was defined as the CPT. The CPT obtained from turbidity measurements and LCST defined as the onset of particle size increase observed by dynamic light scattering (DLS) by López-Pérez et al.⁶³ can be significantly different in some cases, especially when the polymer consists of a hydrophilic constituent.^{24,63} In cases of DMAEMA-rich copolymers with a small amount of a hydrophobic comonomer,^{24,33} the reported CPTs and LCSTs have been very similar.

The LCST of a thermo-responsive statistical copolymer can be affected by many parameters such as copolymer composition,^{24,33} molecular weight,^{64,65} solution concentration,^{33,65} and in the case of PDMAEMA, pH.^{33,61} We aimed to focus on examining the effects of pH and copolymer composition on the polymer phase behavior in aqueous media for this study, thus keeping solution concentration constant at 0.5 wt%. The M_n of the polymers ranged from about 10 to 16 kg mol⁻¹ as listed in Table 6. Although the LCST of PDMAEMA has been reported to be M_n-dependent,⁶⁴ it does not differ significantly within our M_n range. Therefore, we assumed the effect of M_n on the aqueous phase behavior of our polymers to be negligible in our studies. The results demonstrating the thermo- and pH-sensitivities of the copolymers are summarized in Table 6.

The pKa values of the polymers shown in Table 6 were obtained from titration. The pKa of PDMAEMA in water was reported to be 7.0^{64,66} and pKa of P2VP measured in a mixture of water and ethanol ranged from about 3 to 4 depending on the degree of ionization.⁶⁷ We obtained a pKa value of 7.0 for the PDMAEMA homopolymer, which was consistent with the literature.^{64,66} For the statistical copolymers with DMAEMA compositions from

76% to 93%, the measured pKas were very similar to the PDMAEMA homopolymer. P2VP is only water soluble when it is 75 – 80% ionized,⁶² or at pH < 2.5 according to its pKa of $3.0.^{67}$ Therefore, the 2VP units in the statistical copolymers likely stayed as a neutral hydrophobic constituent during the titration and did not alter the effective pKa values of the copolymers. It also explains why the measured pHs of all the copolymers dissolved in deionized water were not significantly different.

Effect of pH on the LCST of DMAEMA homopolymer

The CPT of the PDMAEMA homopolymer was examined at pHs from 7 to 11 and compared to the literature. As listed in Table 6, the CPT decreased sharply from 72 °C at pH 7 to 27 °C at pH 10. These sharp changes in thermo-sensitivity were closely related to the degree of ionization (DI) of the polymer as illustrated in Figure 8. As pH increased from 7 to 10, PDMAEMA was largely deprotonated, from about 50% to 0.1% ionized. Meanwhile, the molecular forces controlling chain conformations changed from predominantly repulsive forces between charged units to attractive intermolecular forces between the uncharged side groups,⁶² causing the polymer chains to become much more compact and hydrophobic. The CPT of 39 °C at pH 9 was consistent with the reported LCST of about 40 °C for PDMAEMA of similar M_n dissolved in water.^{64,65} At pH 10, the DI decreased to below 0.1%, a point at which the polymer can practically be considered neutral. A slight increase in CPT was observed at pH 11, likely due to a relaxation of attractive forces, analogous to pH-induced micelles that became less dense as they passed a critical pH value.³ These results well demonstrated the interesting combination of

temperature and pH sensitivities of PDMAEMA that make it so attractive for stimuliresponsive materials.

Effect of copolymer composition on the LCST of statistical copolymers

The LCST-type phase separation of the statistical copolymers was also investigated at pH 7 to 11. At these conditions, P2VP was totally deprotonated and therefore simply acted as a hydrophobic component of the copolymer. It is known that when DMAEMA is copolymerized with a hydrophobic comonomer, the CPTs of the resulting statistical copolymer decreases with increasing hydrophobic content.^{24,33} The same is applicable to the DMAEMA/2VP copolymer system, where the CPTs decreased as 2VP content increased in the statistical copolymer as shown in Table 6.

Moreover, the effect of copolymer composition on the CPT varied with pH. At pH 10 and 11, the effect of copolymer composition was very similar and the most profound, where the copolymer with 24% 2VP had a CPT 13 °C lower than the PDMAEMA homopolymer. The differences in CPTs with composition diminished as pH decreased. At pH 7, all copolymers with up to 24% 2VP had similar CPTs to the PDMAEMA homopolymer. These results made good sense if put together with the change in DI of PDMAEMA with pH as shown in Figure 8. At pH 10 and above, PDMAEMA is completely deprotonated and no longer sensitive to pH. Therefore, the copolymer composition became the sole variable that affected the CPT, which explains why the changes in CPT at pH 10 and 11 with composition were very similar. As pH decreased below 10, the DMAEMA units started to ionize and the repulsive forces between charged units soon

dominated the attractive intermolecular forces from the hydrophobic 2VP units. As a result, the effect of copolymer composition on the cloud point temperatures diminished with decreasing pH.

In previous studies of similar systems where DMAEMA was copolymerized with styrene³³ and VBK²⁴ via NMP, the CPTs of the resulting statistical copolymers were also strongly affected by the copolymer composition. The main difference between those systems and this work is that those copolymers became water-insoluble when DMAEMA content in the copolymer decreased to 81 mol% with styrene³³ as comonomer and 87 mol% with VBK²⁴ as comonomer because of the high hydrophobicity of these comonomers. At pH 10, the DMAEMA/VBK copolymers became water-insoluble even with as little as 2 mol% VBK and the DMAEMA/styrene copolymers were only water-soluble when the copolymer consisted of 89 mol% or more DMAEMA. In comparison, the DMAEMA/2VP copolymers in this study were all water-soluble and exhibited LCST-type phase separation from pH 7 to 11 with up to 24 mol% 2VP in the copolymer. These results demonstrated an obvious advantage of using 2VP as a comonomer with DMAEMA as compared to the aforementioned systems, as it not only led to controlled polymerization, but also retained the thermo-responsive properties of PDMAEMA and effectively finetuned the CPT of the copolymer in the full range of pH where PDMAEMA was pH sensitive.

Effect of composition and pH on reversibility of phase separation

Sharp transitions and reversibility are often cited as the two criteria for "smart" materials. For thermo-responsive polymers such as PDMAEMA, this means the phase

separation needs to not only happen quickly at the critical temperature, but also be redissolved at the same temperature. Thus, hysteresis was studied and it refers here to a difference in the transition temperature during heating and cooling.

For polymers that exhibit LCST-type phase separation in water, a layer of clathrate-like structures is formed around a hydrophobic group of the polymer via hydrogen bonding between water molecules and a hydrogen-bond acceptor in the polymer (ie. an atom with unpaired electrons, usually nitrogen or oxygen), which increases the solubility of the polymer in water.^{68,69} As temperature increases above a critical point, the ordered water layer is interrupted and the polymer phase-separates from the aqueous solution, undergoing a "coil-to-globule" transition.⁷⁰ During cooling, the transition from globule to coil is more difficult because of the formation of intra-chain structures in the globule state through intra-chain hydrogen bonding, which retards the re-dissolution of the polymer globule.⁷⁰

For the DMAEMA/2VP copolymers, 1 to 5 °C of hysteresis was observed as plotted in Figure 9. Similar observations were reported for DMAEMA/VBK copolymers that contained only 3 mol% VBK.²⁴ Hysteresis increased to as high as 10 °C when VBK content was increased to 7 mol% in the copolymer.²⁴ The amplified hysteresis was attributed to the high hydrophobicity of VBK. In this study, we investigated the effects of both polymer composition and pH on the extent of hysteresis as they both played important roles on the copolymer thermo-responsiveness.

From Figure 9, the extent of hysteresis showed a strong correlation with pH. As discussed before, increasingly more DMAEMA units became charged as pH decreased.

These charged units remained in an extended form even at temperatures above the CPT because of the strong electrorepulsive forces. As a result, the polymer chains were less compact when they aggregated at lower pH and easier to be re-dissolved when cooled. Polymer composition did not have a significant impact on the extent of hysteresis according to Figure 9 in contrast to DMAEMA/VBK copolymers.²⁴ This is likely due to the much lower hydrophobicity of 2VP compared to VBK. In summary, the degree of hysteresis is relatively small for all the DMAEMA/2VP copolymers, indicating good reversibility in phase separation, which is a desirable property for "smart" materials.

Temperature and pH sensitivities of the block copolymers

pH-induced micellization at room temperature

Micellization can be induced by changing pH when two ionizable weakly basic (or acidic) polymers of different pKas^{3,71} or one ionizable block and a water-soluble neutral block³⁸ are covalently linked in the same molecule as a block copolymer. In this work, a DMAEMA/2VP statistical copolymer with 93 mol% DMAEMA was chain-extended with 2VP to form a block copolymer. The DMAEMA/2VP copolymer had a very similar pKa as PDMAEMA homopolymer (Table 6) and it is known that P2VP had a lower pKa than PDMAEMA.^{64,66,67} Such a block copolymer should form pH-dependent micelles.³

The titration curve of the sample D2VP-95-2VP diblock copolymer is presented in Figure 10. Because P2VP is only water-soluble when highly protonated at low pH, micelle formation was expected as pH is increased. When titration was started with a pH of about 2, the solution was clear. As pH increased to about 4, the solution developed cloudy

domains as the NaOH solution was added to the stirred solution of the block copolymer, indicating initial formation of micelles where local pH was higher. The domains disappeared when the solution became more homogenized, due to more stirring. When solution pH increased to greater than 5, the entire solution suddenly turned translucent, signifying the formation of stable micelles. From the titration curve, a plateau appeared at about pH 5 that coincided with this visual observation of micellar formation. Similar micellization of P2VP-b-poly(ethylene oxide) diblock copolymer was also reported to occur at pH 4.8 using titration.³⁸

To get better insight of the micellization process, dynamic light scattering (DLS) was used to examine the hydrodynamic radius (R_h) of D2VP-95-2VP diblock copolymer in different buffer solutions. The DLS results are shown in Figure 11. The R_h of the diblock copolymer was below 10 nm at pH 4, increased to about 40 nm at pH 5 and reached a plateau at about 60 nm at pH 6. This result demonstrates the transition of the diblock copolymer from well-dispersed unimers at pH 4 to initial micelles at pH 5 and finally stable micelles at pH 6, which agreed well with the observations from titration. Meanwhile, the dispersity of the particle size distribution (μ_2/Γ^2) was about 0.3 at pH 4, indicating loose unimers. The very low μ_2/Γ^2 value of below 0.01 at pH 5 revealed the critical pH where P2VP is completely deprotonated while PDMAEMA is still totally ionized, forming dense micelles.³ The value of μ_2/Γ^2 relaxed to a relatively constant value of about 0.15 at pH 6 and higher when PDMAEMA became partially deprotonated. These results showed good agreement with a previous study of micellization of PDMAEMA-P2VP diblock copolymer in aqueous solutions.³

Temperature sensitivity of D2VP-95-2VP diblock copolymer

The temperature sensitivity of the diblock copolymer D2VP-95-2VP was examined in different pH buffer solutions by DLS and the results are presented in Figure 12. Final pHs of the polymer solutions were confirmed using a calibrated pH probe. Surprisingly, some of the solution pHs were not the same as the buffer pHs. Specifically, polymer solutions in pH 3 and 4 buffers appeared to be pH 4 and 5, respectively, at room temperature when measured by a pH probe. It is likely that the basicity of the copolymer overcame the buffering capacity of the pH 3 and 4 buffers. Therefore, the pHs of those two solutions were expected to increase significantly with temperature.

From Figure 12, well-dispersed unimers with R_h under 10 nm were observed in a pH 4 solution up to about 37 °C and in a pH 5 solution up to about 10 °C. Again, these pHs only represent the solution pH at room temperature and the true pH varied with temperature. Formation of micelles were evidenced by the sharp increase in R_h to above 100 nm and then dropped to a stable size of about 60 nm in both solutions, indicating a critical pH was reached as temperature increased, consistent with the titration results. For a pH 6 solution, stable micelles were observed from 5 to 80 °C. This is expected because at pH 6 and lower, PDMAEMA is fully protonated and no longer thermo-sensitive. At pH 8, stable micelles were observed up to 47 °C and then R_h rapidly increased to above 1000 nm, representing collapse of micelles into aggregates. This result is consistent with the CPT of D2VP-95 statistical copolymer at 48 °C at pH 8 (Table 6).

Doubly thermo-responsive block copolymer of poly(propylene glycol)-b-poly(2dimethylamino ethyl methacrylate-stat-2-vinylpyridine) (PPO-D2VP-95)

A diblock copolymer consisting of a poly(propylene glycol) (PPO) block and a block of DMAEMA/2VP statistical copolymer with 95 mol% DMAEMA was synthesized using the macroalkoxyamine PPO-BB (Scheme 2). It has been previously shown that the solubility of PPO in water decreased with temperature due to diminishing hydrogen bonding between water and PPO.⁷² Therefore, the diblock copolymer PPO-D2VP-95 was expected to have additional thermo-sensitivity compared to DMAEMA/2VP statistical copolymers.

Both poly(propylene glycol) bis(2-aminopropyl ether) (PPO-di-NH₂) and the diblock copolymer PPO-D2VP-95 in pH 8 buffers were studied using DLS. As shown in Figure 13, PPO-di-NH₂ was found to exhibit LCST-type phase separation in water above a critical temperature of 19 °C. For the diblock copolymer, the R_h increased sharply from below 10 nm to about 100 nm, indicating micelle formation, at 19 °C. The coincidence of transition temperatures implied the PPO block was responsible for the formation of micelles as PPO became water-insoluble. At 41 °C, a second transition was observed, where micelles collapsed and formed large aggregates when the DMAEMA/2VP statistical copolymer segment became insoluble in water. The second transition temperature (41 °C) was lower than the CPT of DMAEMA/2VP statistical copolymer with the same composition (48 °C). This is likely due to the presence of the hydrophobic PPO block rendering the block copolymer more hydrophobic.

These results confirmed the coupling of PPO-di-NH₂ with NHS-BlocBuilder was indeed successful. Vinas et al.⁴¹ demonstrated post-functionalization of a NHS-BlocBuiler-initiated

polymer with an amine functional compound was also possible. The use of NHS-BlocBuilder opened a new approach for obtaining block copolymers as opposed to the traditional chain extension method. With NHS-BlocBuilder, it is also easy to see how other amine functional materials can be coupled to the polymer chains, as has been demonstrated by bioconjugation of NHS-BlocBuilder to peptides.⁴⁴

CONCLUSIONS

DMAEMA was copolymerized with 2VP using NHS-BlocBuilder with feed compositions varied from 80 – 99 mol% DMAEMA, producing water-soluble, thermo- and pH-sensitive statistical copolymers. The copolymerization was controlled to relatively high conversions ~ 50% based on the linear increase in M_n versus conversion and monomodal molecular weight distributions with relatively low PDIs. As little as ~ 2 mol% 2VP in the initial composition was demonstrated sufficient in producing controlled, living DMAEMA-rich polymers. Feed containing 1 mol% 2VP yielded DMAEMA/2VP copolymer that was living but had significantly broader molecular weight distribution. With NHS-BlocBuilder, additional SG1 nitroxide was proven unnecessary and pre-functionalization of PPO-di-NH₂ with NHS-BlocBuilder was demonstrated successfully.

The CPTs of the statistical copolymers were tuned in the range of 14 – 75 °C by varying copolymer composition and solution pH. Only 1-5 °C of hysteresis was observed, which indicated good reversibility of the phase separation. The block copolymer obtained by chain extension of DMAEMA/2VP statistical copolymer with 2VP was found to form pH-dependent micelles as expected at a critical pH of 5 at room temperature and collapsed to

aggregates at higher temperature at pHs > 8 because of the thermo-responsive DMAEMA/2VP block. The PPO-D2VP-95 diblock copolymer synthesized from the PPO-BB macroalkoxyamine exhibited double thermo-responsiveness at transition temperatures of 19 °C (corresponding to the PPO block) and 41 °C (corresponding to the DMAEMA-rich block).

This study reported for the first time the use of 2VP as a controlling comonomer for a methacrylate-rich copolymerization without aid of free nitroxide by utilizing NHS-BlocBuilder. We showed that the advantages of 2VP as a comonomer include a very small amount (~2 mol%) necessary for controlling and low hydrophobicity for tuning LCST while maintaining water solubility within a wide range of pH. The versatility of NHS-BlocBuilder was also demonstrated by producing doubly thermo-responsive block copolymer by pre-functionalization of the NHS-BlocBuilder initiator with PPO-di-NH₂.

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SCHEME AND FIGURE CAPTIONS

Scheme 1: Structures of (a) SG1 nitroxide (b) BlocBuilder[™] (c) *N*-hydroxy succinimidyl estercoupled BlocBuilder (NHS-BlocBuilder).

Scheme 2: Synthesis route for a diblock copolymer containing a poly(propylene glycol) block and a DMAEMA/2VP statistical copolymer block by NMP.

Figure 1: Bulk homopolymerization of 2VP initiated by NHS-BlocBuilder at 120 °C. (a) Kinetic plot of $ln[(1-x)^{-1}]$ versus time (x = monomer conversion). (b) Number-average molecular weight (M_n) and polydispersity (PDI) versus conversion (M_n relative to poly(styrene) standards). Solid line represents theoretical M_n trend.

Figure 2: (a) Kinetic results $(\ln[(1-x)^{-1}]$ versus time) of statistical copolymerization of DMAEMA with 2VP initiated by NHS-BlocBuilder (x = monomer conversion). (b) Product of average propagation rate constant $\langle k_p \rangle$, and propagating radical concentration $[P^{\bullet}]$, $\langle k_p \rangle [P^{\bullet}]$ (slope of the kinetic plots in (a)), versus feed composition with respect to DMAEMA ($f_{DMAEMA,0}$); error bars represent standard deviation of the slopes from (a).

Figure 3: (a) Number-average molecular weight (M_n) and (b) polydispersity index (PDI) of the statistical copolymers of DMAEMA and 2VP measured by GPC relative to poly(methyl methacrylate) standards versus conversion; solid line in (a) represents the theoretical M_n trend expected.

Figure 4: (a) Kinetic plot of scaled conversion $ln[(1-x)^{-1}]$ (x = conversion) versus polymerization time, (b) number-average molecular weight (M_n) and polydispersity index (PDI) of DMAEMA/2VP copolymerization with 95 mol% DMAEMA in the feed initiated by PPO-BB macroalkoxyamine at 80 °C in bulk. M_n and PDI were obtained by GPC relative to poly(methyl methacrylate) standards; solid line in (b) represents the theoretical M_n trend.

Figure 5: ¹H NMR spectrum of poly(propylene glycol)-b-poly(dimethylaminoethyl methacrylatestat-2-vinylpyridine) diblock copolymer (PPO-*b*-P(DMAEMA-stat-2VP) in CDCl₃. Protons P, D, V correspond to the labeled protons on the chemical structures for PPO, DMAEMA, 2VP, respectively.

Figure 6: GPC chromatograms of macroinitiator (dashed line) and chain extended polymer (solid line) in the chain extension experiments.

Figure 7: Effect of additional SG1 free nitroxide on (a) number-average molecular weight (M_n , relative to poly(methyl methacrylate) standards) and (b) polydispersity index (PDI) of DMAEMA/2VP statistical copolymers with 95 mol% and 99 mol% DMAEMA in the feed; filled symbols represent results obtained with no free SG1 and open symbols represent results obtained with 10% free SG1, solid line in (a) represents theoretical M_n trend.

Figure 8: Cloud point temperature (CPT) of polymers of various compositions versus pH compared to the degree of ionization of PDMAEMA.

Figure 9: Hysteresis (difference in temperature at 50% light transmittance during heating and cooling) of polymers with various compositions versus pH of the solutions.

Figure 10: pH titration curve of D2VP-95-2VP diblock copolymer obtained from automatic titration (blue line) and the first derivative of the measured pH (red line).

Figure 11: (a) Hydrodynamic radius and (b) dispersity of the particle size distribution (μ_2/Γ^2) of D2VP-95-2VP diblock copolymers determined by dynamic light scattering at room temperature versus pH with solution concentration of 0.5 wt%.

Figure 12: Hydrodynamic radius (R_h) of D2VP-95-2VP diblock copolymer at various pH determined by dynamic light scattering versus temperature. Temperature was increased at a rate about 0.5 °C min⁻¹.

Figure 13: Comparison of hydrodynamic radius of poly(propylene glycol) bis(2-aminopropyl ether) (PPO-di-NH₂) and diblock copolymer PPO-D2VP-95 in pH 8 buffer solution measured by dynamic light scattering. Temperature was increased at a rate about 0.5 °C min⁻¹.

GRAPHICAL ABSTRACT

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pH and temperature-sensitive statistical copolymers poly[2-(dimethylamino)ethyl methacrylate-*stat*-2-vinylpyridine] with functional succinimidyl-ester chain ends synthesized by nitroxide mediated polymerization

This article describes a series of statistical copolymerizations of 2-(dimethylamino)ethyl methacrylate with 1 to 20 mol% 2-vinylpyridine in the feed utilizing a succinimidyl esterterminated BlocBuilder at 80 °C. All copolymerizations occured in a controlled manner with no additional SG1 free nitroxide. The initiator was conjugated to amine-terminated poly(propylene glycol), yielding an effective macroinitiator for synthesizing a block copolymer. Thermo- and pH-sensitivities of the statistical and block copolymers are comprehensively studied by UV-vis spectroscopy and dynamic light scattering.

GRAPHICAL ABSTRACT FIGURE

