Thermoresponsive Polymers with Tunable Cloud Point Temperatures Grafted From Chitosan via Nitroxide Mediated Polymerization

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Abstract

The heterogeneous grafting of chitosan with thermoresponsive (oligoethylene)glycol methacrylate (OEGMA)/diethyleneglycol methacrylate (MEO_2MA)/acrylonitrile (AN) was accomplished by nitroxide mediation polymerization (NMP) with SG1-based BlocBuilder unimolecular initiators. Homogeneous OEGMA/MEO_2MA/AN terpolymerizations in solution were done first at 120 ^oC to confirm the cloud point temperatures (CPTs) of the thermoresponsive chains that were to be grafted onto the chitosan (CPT tuning from 30-65 ^oC was done by varying OEGMA:MEO_2MA in the initial monomer composition). Grafting was accomplished by reacting chitosan with acryloyl choride and the subsequent acrylamide grafted chitosan was reacted by a 1,2 intermolecular radical addition with BlocBuilder followed by NMP with OEGMA/MEO_2MA/AN monomers. TGA revealed 65-80% of the composite material was due to the grafted polymer. CPTs of the chitosan-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) reflected those of the homogeneous case, but there was extensive hysteresis as the composite particles could not readily disentangle upon cooling below the CPT.

Keywords: nitroxide mediated polymerization; thermoresponsive polymers; heterogeneous grafting.

1. Introduction

Thermoresponsive polymers change morphology/configuration sharply after a small change in temperature in solution. Many exhibit a lower/ upper critical solution temperature (LCST, UCST), meaning that they are soluble and free flowing at certain temperatures, but agglomerate as temperature is changed, this transition temperature is often termed the cloud point temperature (CPT) [1]. Such polymers have potential applications in the biomedical field, such as drug delivery and other therapeutics [2]. For example, poly(N-isopropylacrylamide) (poly-(NIPAAm)) and poly(2-(dimethylamino)ethyl methacrylate) (poly(DMAEMA)) exhibit LCSTs of 32 and 46ºC, respectively, in aqueous media [3]. Many methods are possible to tune the LCST to the desired temperature by using hydrophobic or hydrophllic co-monomers to incorporate them into the final copolymer to modify the LCST. Recently, Lutz et al recently showed that tuning the LCST is possible by adjusting the ratios of two monomers: oligo (ethylene glycol) methyl ether methacrylate (OEGMA, with 8-9 EO segments per monomer) and 2-(2-methoxyethoxy) ethyl methacrylates (MEO_2MA) [4] resulting in easily tunable LCSTs from 20 to 90ºC. Lutz's group used atom transfer radical polymerization (ATRP), a controlled radical polymerization (CRP) method, to synthesize their copolymers. Previously, reversible addition-fragmentation chain transfer (RAFT) polymerization was also used to control the molecular weight and distribution and the composition of OEGMA-based copolymers [5-7]. While ATRP and RAFT do provide polymers with well-controlled microstructure, ability to form block copolymers and low dispersity, and have been readily applied to many bio-oriented systems [8-12], they often involve post-polymerization modifications to remove undesirable metallic species or thio-ester groups that may be detrimental to some applications [13] [14]. Nitroxide mediated

polymerization (NMP) is another controlled radical polymerization method that is simple to apply and requires little or no post-polymerization modification. Although historically developed earlier, NMP has lagged behind ATRP and RAFT in the development of polymers for biological applications [15] [16].

The first generation of NMP initiators based on 2,2,6,6 tetramethylpiperidinyl-1-oxy (TEMPO) was limited to styrenic polymerizations [17] [18] . However, with the advent of new alkoxymine initiators such as 2,2,5-trimethyl-4-phenyl-3-azahexane-3-oxyl (TIPNO) [19] and the N-*tert*-butyl-N-[1-diethylphosphono-(2,2-dimethylpropyl)] nitroxide (SG1) families [20], a wider range of monomers can be controlled such as acrylates and acrylamides. Even methacrylates can now be polymerized, often requiring a small amount ~ 5-10 mol% of co-monomer such as styrene and acrylonitrile. Methacrylates including methyl, ethyl, butyl, benzyl, and poly(ethylene glycol) methyl ether methacrylate [21-24] using commercially available BlocBuilder, (N-(2-methylpropyl)-N-(1-diethylphosphono-2,2-dimethylpropyl)-O-(2-carboxylprop-2-yl) hydroxylamine), with the presence of about 10% excess of free SG1 relative to the BlocBuilder initiator, resulted in controlled polymerizations as defined by a linear growth of number average molecular weight M_n versus conversion and low dispersities Đ ~ 1.2-1.3 [21] [25] [26]. It should be noted that methacrylate homopolymerization by NMP, without any controlling co-monomer, has been reported [27-30]. Using BlocBuilder, Lessard et al performed a controlled copolymerization of OEGMA and MEO_2MA using 9-(4-vinylbenzyl)-9H-carbazole (VBK) as a controlling co-monomer [31]. Acrylonitrile (AN) was also chosen as a controlling agent because of its relatively low K, implying it

might be an effective controlling co-monomer [13] [32]. Moreover, Nicolas et al were also able to synthesize a poly(OEGMA-*ran*-AN) using AN as a controlling agent that exhibited linear increase of M_n with conversion and low dispersity ~ 1.4 [14] The polymer was also shown to be non-cytotoxic [33]. Another attractive feature about the OEGMA-MEO_2MA system is its non-ionic nature and its solubility in aqueous media is independent of pH. The first part of our study copolyemrized OEGMA/MEO_2MA system using AN as a controlling agent and we report the effect of a small fraction of AN on the LCST tunability.

Chitin is the second most abundant natural polysaccharide [34]. They are derived from many natural sources such as crustacean shells, fungi cell walls, and squid beaks [35]. Through deacetylation, chitosan can be obtained. Recently, chitosan has been applied towards drug delivery, tissue engineering, wastewater treatment, and packaging [35] [36] [37] because of its properties like biocompatibility, biodegradability, hypoallergenicity, antibacterial properties, and wound-healing effects. For example, chitosan can encapsulate a drug in controlled delivery applications, and boost its antimicrobial properties because of the amine and the ammonium salts it produces in acidic media [38-40]. However, chitosan is not thermoresposive, and must be modified to impart this additional valuable functionality. Thus, combining polymers in composites with chitosan is an attractive method to impart anti-bacterial properties with thermoresponsive behaviour.

Grafting chitosan is a challenge because of chitosan's low solubility in many solvents and low reactivity [41]. Previous studies mainly focused on using free radical initiators, such

as ceric ammonium nitrate (CAN), ammonium persulfate (APS), and 2,2-azobisisobutyronitrile (AIBN) [42] to initiate free radical polymerization of grafted chains. However, conventional free radical polymerization is characterized by polymers with broad molecular weight distributions without active chain ends. Low Đ is especially important to obtain sharp responses to stimuli [43] [44]. ATRP and RAFT were used in chitosan grafting under homogeneous and heterogeneous conditions [45-48]. However, recently it was shown that BlocBuilder can be grafted onto a chitosan surface in a heterogeneous manner via NMP [49]. In this case, the chitosan surface functional groups (primary amine) were reacted with acryloyl chloride to form an acrylamide. Subsequently, an intramolecular 1,2 radical addition of BlocBuilder with the acrylamide was done to graft the initiator onto the surface. This step was followed by polymerizations of a methyl methacrylate/AN mixture and sodium 4-styrenesulfonate by grafting from the initiator-modified chitosan. A similar approach was adopted in the present paper (*Figure 1*). Before heterogeneneous grafting was done, OEGMA/MEO_2MA/AN homogeneous terpolymerizations were conducted to estimate the expected CPTs when grafting poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) chains onto the chitosan surface. This is the first attempt to produce thermoresponsive chains on the chitosan surface by NMP and hopefully provide a starting point towards further stimuli-responsive hybrid materials with chitosan via this route.

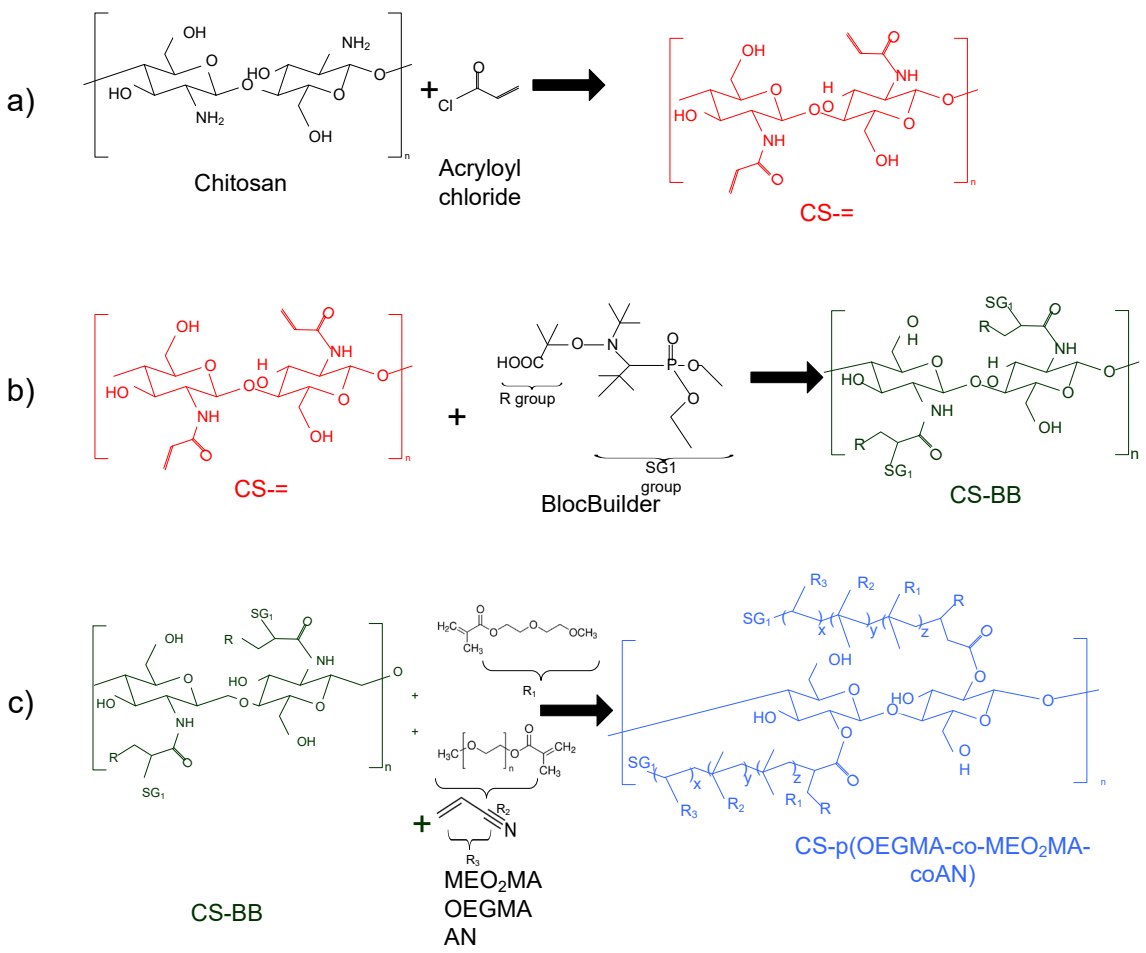


Figure 1: Chemical reaction schematic of chitosan conversion to the final product: a) First, the chitosan is reacted with acryloyl chloride to graft the vinyl groups onto the chitosan (CS-= is the acrylamide-modified chitosan); b) BlocBuilder is reacted with CS-= via an intramolecular 1,2 addition to place the initiator on the chitosan surface (CS-BB is the chitosan with BlocBuilder grafted; c) The polymer chains are grown from the surface heterogeneously to make CS-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) chitosan.

2. Experimental Section

2.1 Materials

N,N-Dimethylformamide (DMF, >95%, certified ACS), tetrahydrofuran (THF, >99.5%, certified ACS), tetrahydrofuran (THF, >99.5%, HPLC grade), and dichloromethane

(certified ACS) were obtained from Fisher Scientific and used as received. Deuterated chloroform ($CDCl_3$, >99%) used for ^1H NMR spectroscopy was obtained from Sigma-Aldrich. Acrylonitrile (AN, ≥99%, contains 35-45 ppm monomethyl ether hydroquinone as inhibitor), oligo(ethylene glycol) methyl ether methacrylate (OEGMA, average Mn 500 g/mol, contains 100 ppm MEHQ as inhibitor, 200 ppm BHT as inhibitor, 8 to 9 ethylene glycols chains), and di(ethylene glycol)methyl ether methacrylate (MEO_2MA, 188 g/mol, 95%, 100 ppm hydroquinone monomethyl ether as inhibitor, two ethylene glycol chains) were obtained from Sigma-Aldrich. Low molecular weight chitosan (CS, number average molecular weight M_n 67 000 g mol^{-1}), acryloyl chloride (97.0%, contains <210 ppm MEHQ as stabilizer), and triethylamine (≥99%) were obtained from Sigma-Aldrich. Calcium hydride (>99.99%, trace metal basis) and aluminum oxide (activated, basic) were obtained from Sigma-Aldrich. 2-([*tert*-butyl[1-(diethoxyphosphoryl)-2,2 dimethylpropyl] amino]oxy)-2-methylpropanoic acid (BlocBuilder-MA, 99%) and [*tert*-butyl[1-(diethoxyphosphoryl)-2,2- dimethylpropyl]amino]oxidanyl (SG1, >85%) were obtained from Arkema and used without further purification.

2.2 Instrumentation

Fourier transform infrared (FTIR) spectroscopy was performed using Spectrum Two IR Spectrometer from Perkin Elmer using an attenuated total reflectance (ATR) diamond crystal.

Both solution and solid state nuclear magnetic resonance (NMR) was used. ^1H NMR was performed using an Agilent 300 MHz Varian VNMRS with $CDCl_3$ as a solvent for the terpolymer. Solid state ^{13}C NMR was performed using a 400 MHZ Varian VNMRS for

the chitosan and its subsequent modified composites with the terpolymer chains initiated from its surface.

The molecular weight distribution was measured using gel permeation chromatography (GPC, Water Breeze) using HPLC grade THF as the mobile phases running at a flow rate of 0.3 ml/min and heated to 40ºC. The GPC is equipped with a guard column and with 3 Waters Styragel HR columns with the molecular weight ranges are given: 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 differential refractive index detector (RI 2410). The molecular weights were determined by calibration against linear, nearly monodisperse poly(methyl methacrylate) (PMMA) standards supplied by Varian.

Dialysis was performed with a Pur-A-Lyzer Mega Dialysis Kit, MWCO 3.5 kDa (Sigma-Aldrich) to purify the polymer after the reaction is complete. The contents containing polymer, unreacted monomer, and solvent was put into the dialysis tube against distilled water for a week. This permitted monomers and solvent to flow out of the tube and displaced with water, while trapping the polymer inside. The distilled water was changed every two days. Once dialysis was complete, the contents were frozen at -20ºC, and then lyophilized for 5 hours to obtain the final terpolymer, which was analyzed via the UV-Vis spectrometer.

UV-Vis measurements were performed with a Cary 5000 UV-Vis-NIR spectrometer (Agilent Technologies) at 600 nm equipped with a Peltier thermostatted (6x6) multicell holder equipped with a temperature controller and magnetic stirring. The transmittance was recorded every 0.5ºC.

Thermal gravimetric analysis (TGA) was done using the TGA Q500 from TA Instruments equipped with both air and nitrogen gas. The sample is heated from room temperature to 550ºC under a nitrogen atmosphere, and from 550ºC to 700ºC under air at a heating rate of 10ºC min^{-1}.

Monomers (OEGMA, MEO_2MA, AN) and THF used in reactions were purified in a column. Typically, 250 mL of monomer was poured through a bed of CaH_2/Al_2O_3 (0.75 g CaH_2 plus 15 g Al_2O_3). After passing through the column, the purified monomer and solvents were stored under a head of nitrogen in a sealed round bottle flask until required.

2.3 Synthesis of terpolymer poly(OEGMA-ran-MEO2MA-ran-AN)

All copolymerization were performed in a 50 mL three-neck round-bottom glass reactor equipped with a condenser (cooled with 10% ethylene glycol to water mixture being circulated with a chiller). The reactor is heated using a heating mantle in combination with a thermocouple located in a thermal well and a temperature controller. The formulations are listed in *Table 1* with the addition of 50 wt% DMF as a solvent (usually around 10 mL). For example, for OM10:90, 0.261 g AN (0.0049 mol), 1.992 g OEGMA (0.00443 mol), 7.5 g MEO_2MA (0.0398 mol), 0.12 g of BB (0.315 mmol), and 0.00926 g of SG1 (0.0315 mmol) are mixed with 10 mL of DMF in the reactor. The contents are then stirred using a magnetic stir bar and purged under nitrogen gas for 30 minutes before heating the reactor to 120ºC at a heating rate of 10ºC min^{-1}. The reaction times were typically one hour (30 minutes for the chain extension), and samples were taken every 10 minutes to be analyzed via ^1H NMR and GPC. The start of the polymerization is arbitrarily set as the time when the reactor temperature reaches 100ºC. After the reaction is complete, the contents are cooled and the product is placed in a dialysis tube against

water for a week. Afterwards, the contents are frozen and lyophilized, recovering approximately 60% (6.0 g) of the total mass from the monomers added.

Table 1: Experimental formulations for the terpolymers poly(OEGMA-*ran*-MEO2MA-*ran*-AN) performed at 120°C in a 50 wt % DMF solution.

Exp. ID[a]	[BB][b] (mmol)	[SG1] (mmol)	[AN] (mmol)	[OEGMA] (mmol)	[MEO2MA] (mmol)	f_{AN}/f_{MEO2MA} [c]	Target M_n[d] (kg mol^{-1})
OM5:95	3.15	0.0315	4.94	2.22	0.0422	0.10/0.86	29.2
OM10:90	3.15	0.0315	4.92	4.43	0.0398	0.10/0.81	31.0
OM20:80	3.15	0.0315	4.43	8.00	0.0319	0.10/0.72	31.2
OM40:60	3.15	0.0315	3.70	13.33	0.0199	0.10/0.54	31.6
		Macro-initiator mmol					
OM5:95-OM40:60[e]	N/A	0.023	1.85	6.67	0.00996	0.10/0.54	237.8

[a]The nomenclature for the samples is OMx:y, which means that the initial monomer composition is x = mol% of oligoethyleneglycol methacrylate (OEGMA) denoted by O and y = mol% of diethyleneglycol methacrylate (MEO_2MA) demonted by M in the initial monomer mixture.
[b]BB = BlocBuilder unimolecular initiator
[c]f_{AN} = initial composition of acrylonitrile (AN) in the monomer mixture; f_{MEO2MA} = initial composition of diethyleneglycol methacrylate (MEO_2MA) in the monomer mixture.
[d]Target M_n is the number average molecular weight expected at complete conversion.
[e]Note the OM5:95-OM40:60 is a chain extension using OM5:95 as the macro-initiator.

2.4 Synthesis of acrylamide-modified chitosan (CS-=)

The synthesis follows similarly to Lefay et al's experimental procedure. [49] 2.0 g of chitosan are dispersed in 20 mL of anhydrous THF and 14 mL (0.1 mol) of triethylamine in a jacketed reactor with a coolant running at -2°C. The mixture is cooled, stirred via magnetic stir bar, and purged for 1 hour. 7.4 mL (0.09 mol) of acryloyl chloride and 10mL of anhydrous THF were mixed in an additional funnel at the top of the jacketed reactor and purged for 1 hour. The addition of the acryloyl chloride solution with the chitosan mixture was done drop-wise while making sure the temperature did not rise above 5°C. After adding all of the acryloyl chloride, the mixture is then heated at 40°C for 19 hours followed by 90 minutes at 55°C. The mixture is then washed successively with THF, acetone, and dichloromethane each in a 30 minute bath followed by a vacuum

236 filtration via Whatman Type 4 filter paper. The mixture is then stirred overnight in an
237 acetone bath. This mixture is then filtered and placed in a methanol bath overnight. The
238 mixture is then filtered, and then finally washed with methanol for a last time for 30
239 minutes. Then, this mixture is filtered and dried in a vacuum oven at 30^{o}C for 3 hours
240 before analysis via FTIR and solid state ^{13}C NMR. The resonances corresponding
241 between 50 and 120 ppm correspond to C-O groups in the unmodified chitosan. Peaks
242 between 200 and 160 ppm represent C=O functional groups. Peaks between 160 and 120
243 ppm represent C=C functional groups. Peaks observed at less than 35 ppm represent
244 alkane peaks. To verify that there are no impurities left, the sample of the last methanol
245 wash was analyzed via ^{1}H NMR to ensure no impurities were present.

246 ## *2.5 Synthesis of BlocBuilder-modified chitosan (CS-BB)*

247
248 The synthesis follows similarly to Lefay et al's experimental procedure [49]. 1.5 g of the
249 acrylated chitosan (CS-=) is mixed in a solution of 5.32g of BlocBuilder and 15 mL of
250 DMF. The contents are placed in a three-neck round-bottom reactor, and uses a similar
251 setup for the terpolymer poly(OEGMA-*ran*-MEO$_2$MA-*ran*-AN) synthesis mentioned
252 earlier. After 30 minutes of purging and stirring, the solution is heated to 90^{o}C for two
253 hours. The resulting powder, CS-BB, is then washed three times with THF and once in
254 pentane in 30 minute baths each to eliminate all residual free BlocBuilder and SG1
255 nitroxides. The CS-BB is then dried in the vacuum oven at 30^{o}C for 3 hours before
256 analysis via FTIR and solid state ^{13}C NMR.
257

2.6 Synthesis of Chitosan-graft-poly(OEGMA-ran-MEO2MA-ran-AN)

The formulations used for the *grafting from* experiments are shown in *Table 2*. The contents were mixed with 10 mL of DMF and placed in a three-neck round-bottom reactor, and uses a similar setup used in the terpolymer poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) synthesis mentioned earlier. For example, using CS-OM5:95 as an example, 0.5 g of CS-BB was mixed with 0.106 g of AN, 0.411 g of OEGMA, 3.267 g of MEO_2MA, and 5.0 g of DMF. The mixture is stirred and purged in nitrogen gas for 30 minutes before heating up to 120ºC. Samples were taken periodically for TGA analysis. After three hours of reaction, the mixture is washed in THF three separate times in two hour baths, and placed in the vacuum oven at 30ºC for 3 hours. The resulting solid was light brown before analysis via FTIR, solid state ^{13}C NMR, TGA, and UV-Vis spectroscopy.

Table 2: Experimental formulations for the chitosan grafted with terpolymer poly(OEGMA-r*an*-MEO2MA-*ran*-AN) performed at 120ºC in a 50 wt % DMF solution.

Exp. ID	CS-BB (g)	[AN] (mmol)	[OEGMA] (mmol)	[MEO2MA] (mmol)	$f_{AN,0}/f_{MEO2MA,0}$
CS-OM5:95	0.5	2.0	0.91	17.36	0.10/0.86
CS-OM10:90	0.5	2.0	1.83	16.44	0.10/0.81
CS-OM20:80	0.5	2.0	3.65	14.62	0.10/0.72
CS-OM40:60	0.5	2.0	7.31	10.96	0.10/0.54

Results and Discussion

To better understand the terpolymerizations required, as the cloud point is sensitive to the particular ratio of monomers incorporated, reactions were performed first under conditions without any chitosan. Lessard et al have performed similar studies using VBK as a controlling agent [31]. In this case, AN was used as the controlling co-monomer, and temperature was raised to 120ºC. A higher temperature was used here than is typical for methacrylate-rich copolymerizations with a controlling co-monomer (80-90 ºC usually)

as the reaction was sluggish with our initial attempts and the molecular weight distributions were somewhat broad. With the change in temperature, the robustness of the controlling co-monomer approach for NMP of methacrylates was tested.

During the reaction, samples were taken periodically for kinetic analysis with ^{1}H NMR and GPC. A typical ^{1}H NMR spectra is shown in *Figure 2*. The overall conversion was determined by monitoring the disappearance of the vinylic peak at approximately $\delta = 5.4$ ppm. Because the AN, OEGMA, and MEO_2MA C=C bond peaks are so close to each other, they were integrated atogether. The conversion was calculated via Equation 1.

$$X = 1 - \frac{\text{number of moles of monomer}}{\text{number of moles of monomer} + \text{number of moles of polymer}}. \quad \textbf{Equation 1}$$

Kinetic data at 120 ^{o}C was determined for the terpolymerizations and compared to the vast data set at lower temperature NMP using the co-monomer approach. The apparent slopes from the scaled conversion $\ln[(1\text{-}X)^{-1}]$ (X = monomer conversion) versus time can be related to the product of two parameters that influence the polymerization control: the average equilibrium constant K and the propagation rate constant k_p. K is defined in terms of the concentration of propagating macro-radicals [P•], free nitroxide [SG1•] and the dormant alkoxyamine terminated species [P−SG1] as follows.

$$K = \frac{[P\bullet][SG1\bullet]}{[P-SG1]} \quad \textbf{Equation 2}$$

The controlling monomer is necessary for BlocBuilder-mediated NMP as methacrylates have an elevated equilibrium constant K, resulting in high concentrations of propagating radicals leading to an irreversible termination at low conversion. By co-polymerizing the methacrylate with a monomer with a lower K such as styrene or acrylonitrile, the average K decreases, and results in a more controlled polymerization with the vast majority of

chains being terminated by the styrenic or acrylonitrile controlling co-monomer, characterized by a linear growth of the number average molecular weight versus conversion and relatively narrow molecular weight distribution [21, 50, 51] . *Equation 2* can be modified to extract kinetic data (the product k_pK, where k_p = propagation rate constant) from the kinetic plot. At the onset of the polymerization, the nitroxide concentration (SG1) is assumed to remain relatively constant and that [P-SG1] is approximately equal to the initial concentration of the initiator, $[BlocBuilder]_0$. After re-writing *Equation 2*, k_pK can be calculated from the slopes from **Figure 3**.

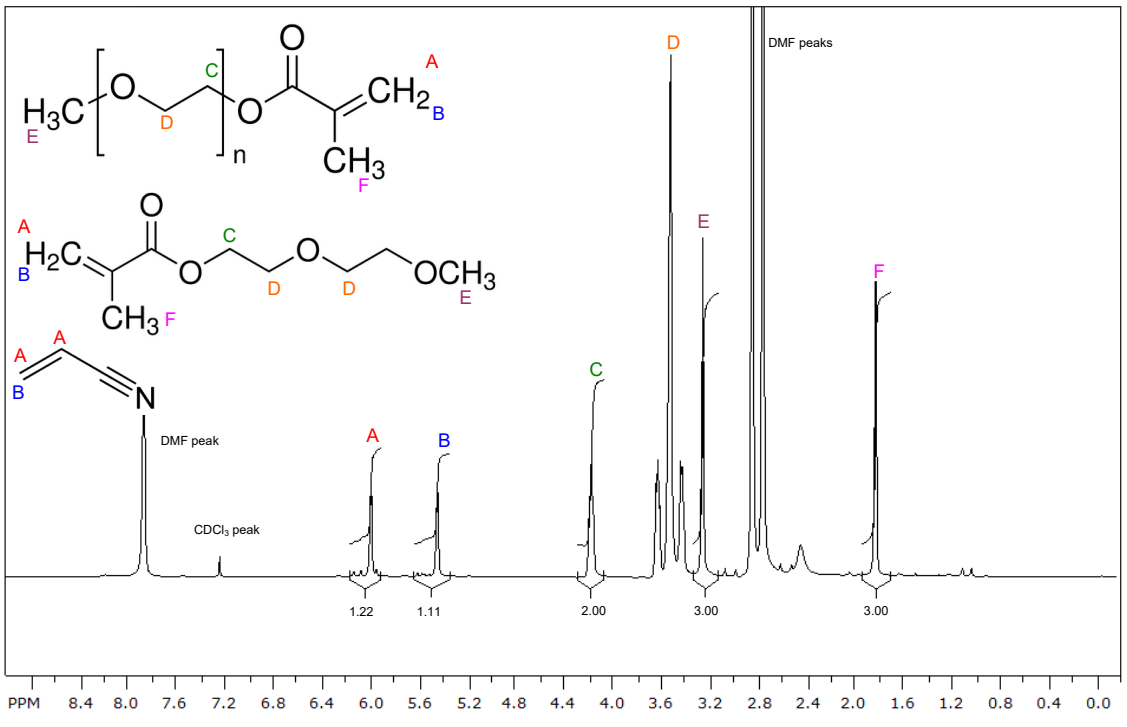
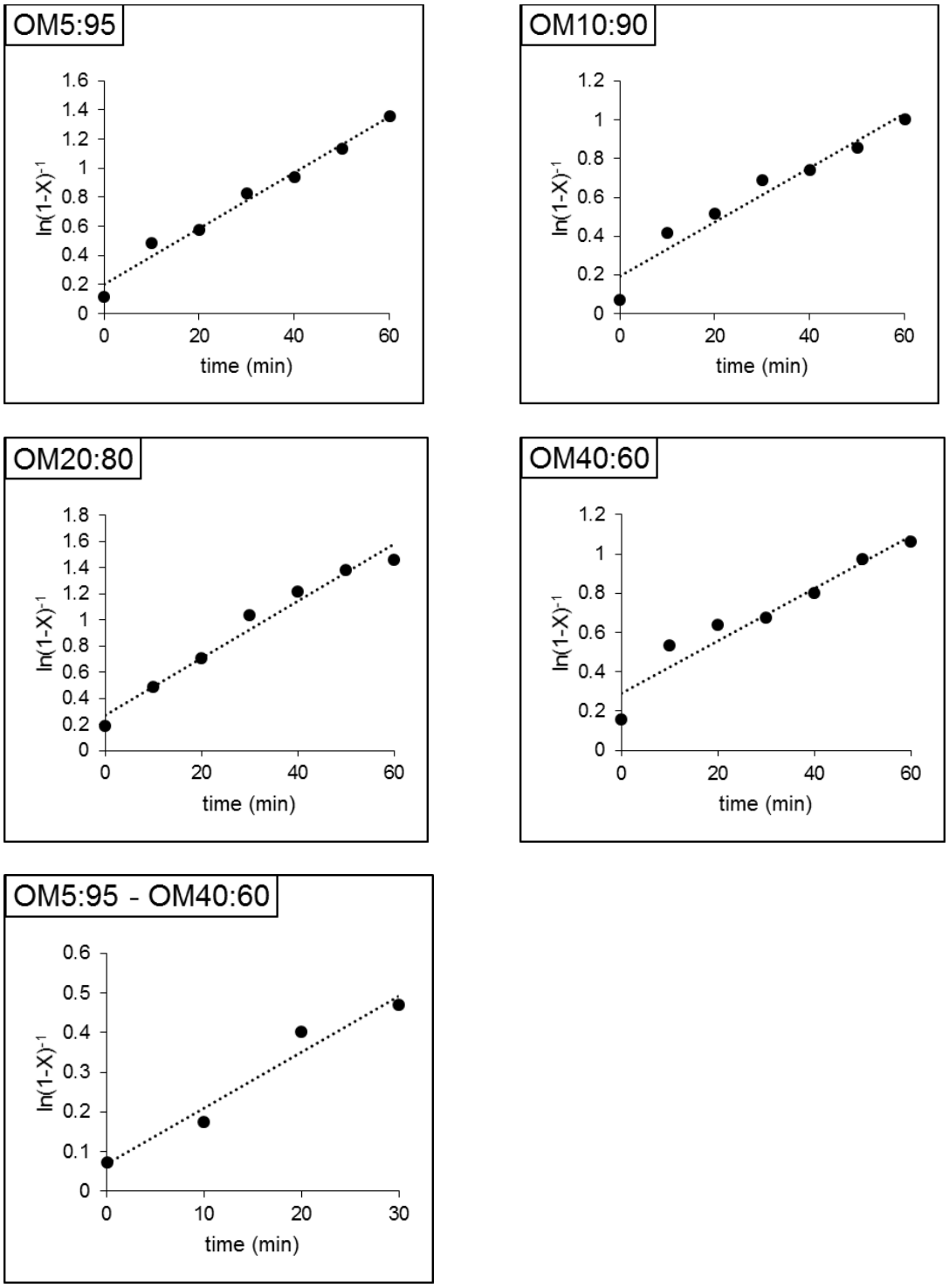


Figure 2: Sample ^{1}H NMR analysis done at 0 min in $CDCl_3$; Note that protons C on the OEGMA only refers to the first $–CH_2$ next to the ester group. Their reference is taken as group E where three protons are present for both OEGMA and MEO_2MA.



317
318 **Figure 3:** Characteristic semi-logarithmic plot of scaled conversion ($\ln((1-X)^{-1})$ (X =
319 conversion) versus time of different OEGMA:MEO_2MA ratio for poly(OEGMA-*ran*-
320 MEO_2MA-*ran*-AN) performed at 120ºC in 50 wt % DMF solutions. Note that the feed
321 composition of AN was constant in each case at 10 mol%.
322

$$k_p K \cong k_p \frac{[P\bullet][SG_1]_0}{[BlocBuilder]_0} = \underbrace{k_p [P\bullet] r}_{slope\ of\ \ln\left(\frac{1}{1-X}\right)\ vs.\ t\ plot} \qquad \textbf{Equation 3}$$

Note that in *Equation 3*, *r* is given as the ratio of free nitroxide relative to unimolecular initially. By examining *Figure 3*, the plots are linear and the slopes and thus k_pK have similar values, regardless of the feed composition (1.9 – 3.2 x 10^{-4} s^{-1} (*Table 3*)). Not surpisingly due to the temperature used, these values are much higher compared to those controlled by VBK at 80 ºC in more dilute DMF solutions (1.6 – 3.1 x 10^{-6} s^{-1}) [31]. Finally, using the GPC data to obtain the M_n and Đ at each conversion, a plot to compare how close the NMP resembled a truly living system was constructed (*Figure 4*). The various terpolymerizations resulted in linear plots and Đ = 1.3 – 1.5 for all cases in the conversion range studied. *Figure 5* shows the GPC chromatograms. In all cases, the peaks were monomodal and steadily shifted towards lower elution volume/time indicating steady chain growth. In many cases a slight low molecular weight tail was observed, indicating some irrerversible termination reactions during polymerization.

Table 3: Molecular Characterization for the terpolymers poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) performed at 120ºC in a 50 wt % DMF solution.

Exp. ID	M_n (kg mol^{-1})[a]	Đ[a]	$k_p \cdot K$ (sec^{-1})[b]	CPT (ºC)[c]
OM5:95	21.7	1.48	3.2 * 10^{-4}	32
OM10:90	19.6	1.32	2.3 * 10^{-4}	35
OM20:80	24.0	1.46	1.6 * 10^{-4}	52
OM40:60	20.7	1.39	2.2 * 10^{-4}	65
OM5:95 – 40:60	85.3	1.53	1.9 * 10^{-4}	40 and 63

[a]The number average molecular weight M_n and the dispersity Đ reported were obtained by gel permeation chromatography (GPC) relative to poly(methyl methacrylate) standards in THF at 40 ºC.
[b]Estimates of k_pK (k_p = propagation rate constant, K = equilibrium constant) were estimated from the linear regions of the semi-logarithmic plots of $\ln[(1\text{-}X)^{-1}]$ (X = monomer conversion) versus polymerization time.
[c]CPT is the cloud point temperature of 0.5 wt % solutions of the copolymer in water from UV-Vis measurements.

To conclusively prove the activity of the chain ends, a chain extension was performed by using OM5:95 as a macro-initiator for an OM40:60 mixture as a second batch of

monomer(s). This was done intentionally as two distinct LCSTs, tuned by the respective compositions for each block, would be observed, confirming block copolymer formation (*Figure 3-E*). The chain-extended polymer continues to grow linearly with time and remained nearly monomodal and Đ did not dramatically increase from 1.48 to 1.53. However, there is a slightly larger tail in the chromatogram data compared with the original terpolymers, which is likely due to some irreversible termination reactions.

Given that we can control the homopolymerization well, grafting form chitosan wan now attempted following the steps outlined in *Figure 1*. As described in the experimental section, the samples were washed copiously before proceeding to the grafting reaction. Cleaning and drying is important to isolate only the modified chitosan. Since chitosan and its subsequent modifications do not dissolve in any organic media, the modified chitosan can easily be captured on filter paper, while dissolving any soluble impurities that are present can be removed. For example, BlocBuilder is soluble in pentane. After the CS-BB reaction, it is imperative to remove all unreacted BlocBuilder from the system. The pentane wash guaranteed that all BlocBuilder was removed while only modified chitosan remains. After the first pentane wash, a second wash was performed, and the pentane was analyzed via ^{1}H NMR to ensure that only pentane remains. If the second wash showed no evidence of BlocBuilder, it is safe to assume that all that remained is modified chitosan. After drying in the vacuum oven, only the modified chitosan remains and any evidence of BlocBuilder shown in the analysis must be chemically grafted onto the chitosan [52]. The amounts used for the polymerization of CS-BB to CS-*graft*-p(OEGMA-*ran*-MEO_2MA-*ran*-AN) are shown on *Table 2.*

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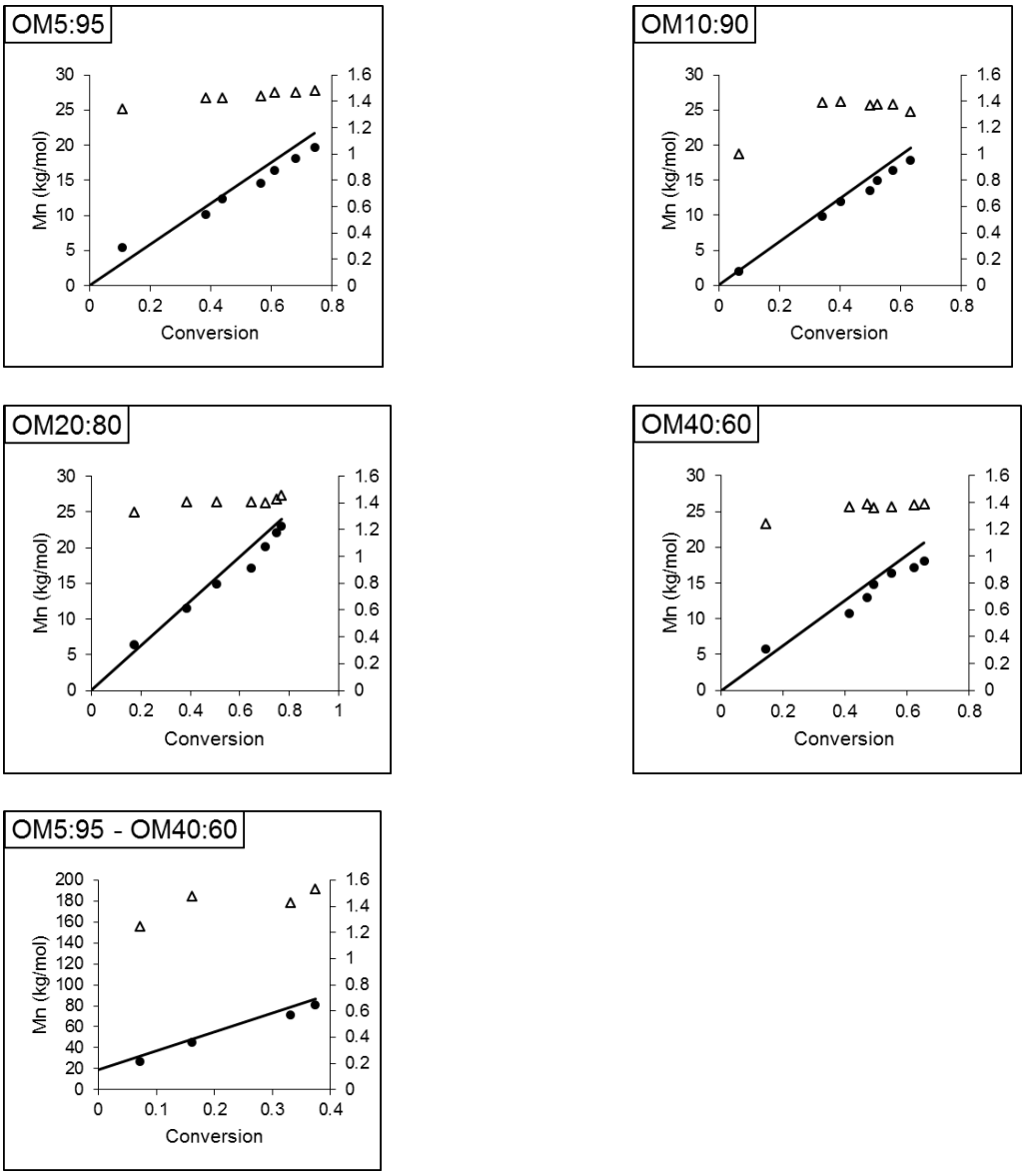


372 **Figure 4:** Number-average molecular weight M_n and dispersity (right vertical axis)
373 versus conversion of different OEGMA:MEO_2MA ratio for poly(OEGMA-*ran*-
374 MEO_2MA-*ran*-AN) performed at 120ºC in a 50 wt % of DMF. Note that the feed
375 composition of AN was constant in each case.

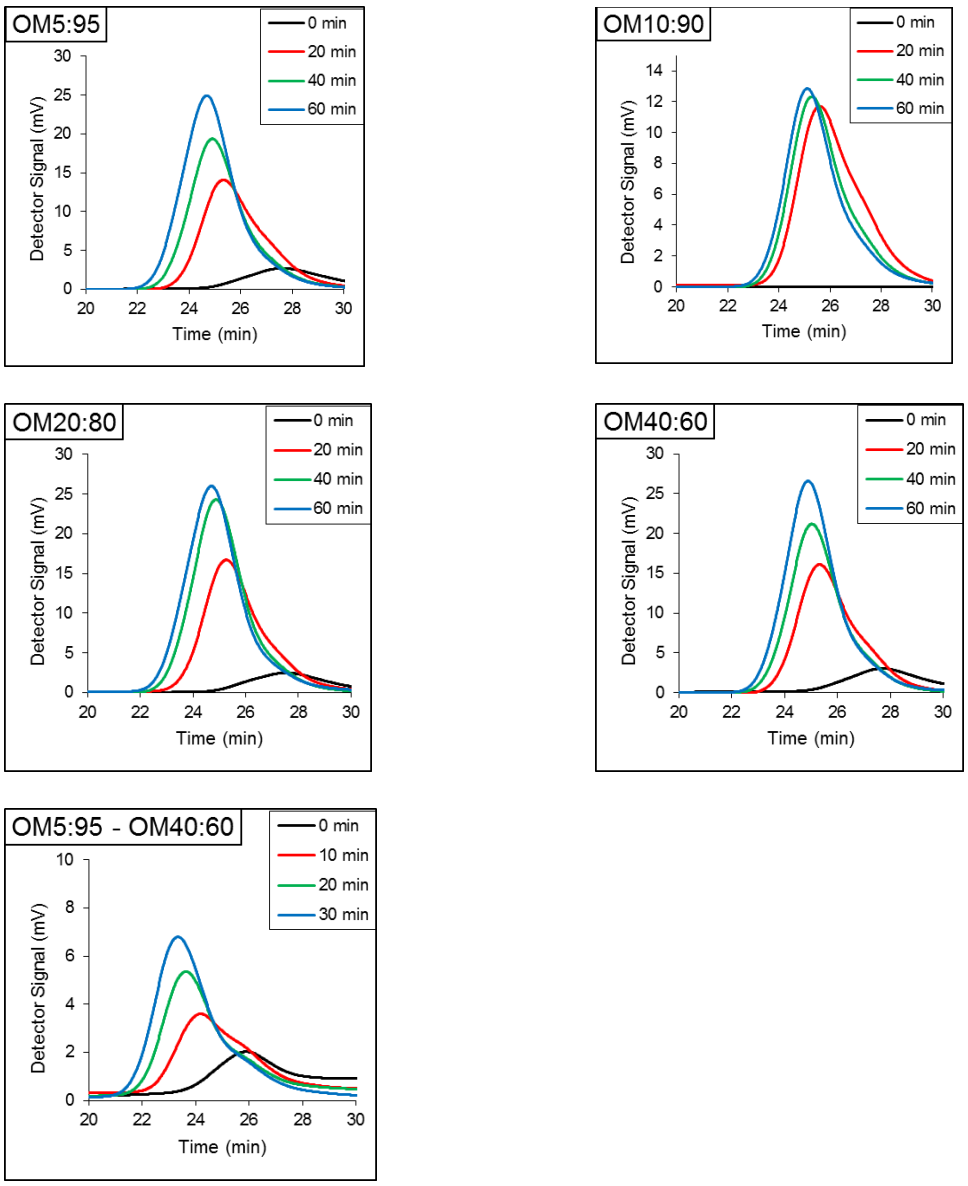
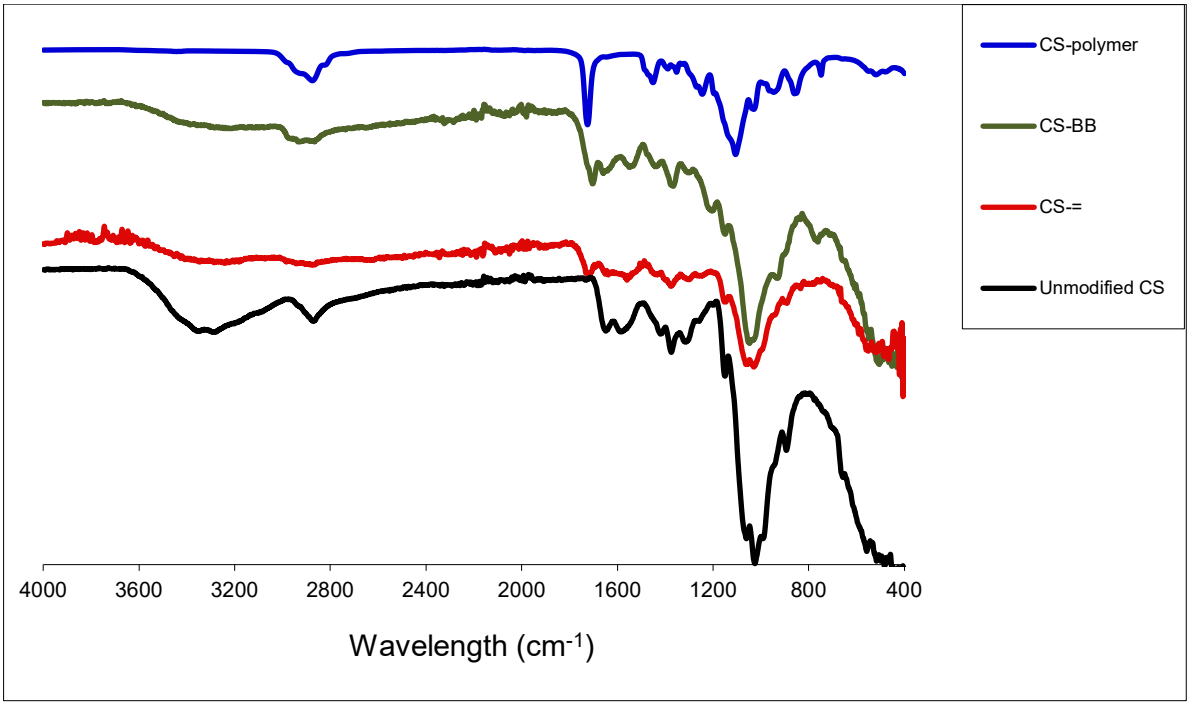


Figure 5: Gel permeation chromatograms of different OEGMA:MEO_2MA ratios of different OEGMA:MEO_2MA ratio for poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) performed at 120°C in a 50 wt % DMF solution. Note that the feed composition of AN was constant in each case. Also note that OM5:95–OM40:60 chromatogram is not consistent with the OM:95 chromatograms. This is because the GPC analysis was done on a different day using a different calibration curve.

To prove each reaction was successful, FTIR was performed at each step. As can be seen in *Figure 6*, CS-= is different from CS at a wavelength of 1700 cm^{-1} indicating an ester C=O stretch. There is not much change between CS-= and CS-BB because the change in structure is not very evident via FTIR. However, confirmation of the C=O peak at 1700

387 cm^{-1} at least confirms that BlocBuilder could be attached based on the carbonyl group on
388 the BlocBuilder. The spectra of CS-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) shows
389 that the bare chitosan can no longer be detected. However, the strong absorption at 1700
390 cm^{-1} shows that C=O from the methacrylate grafting is present, and the peak at 2900 cm^{-1}
391 is due to the ether groups from the ethylene glycol repeat units of the OEGMAs in the
392 polymer.
393

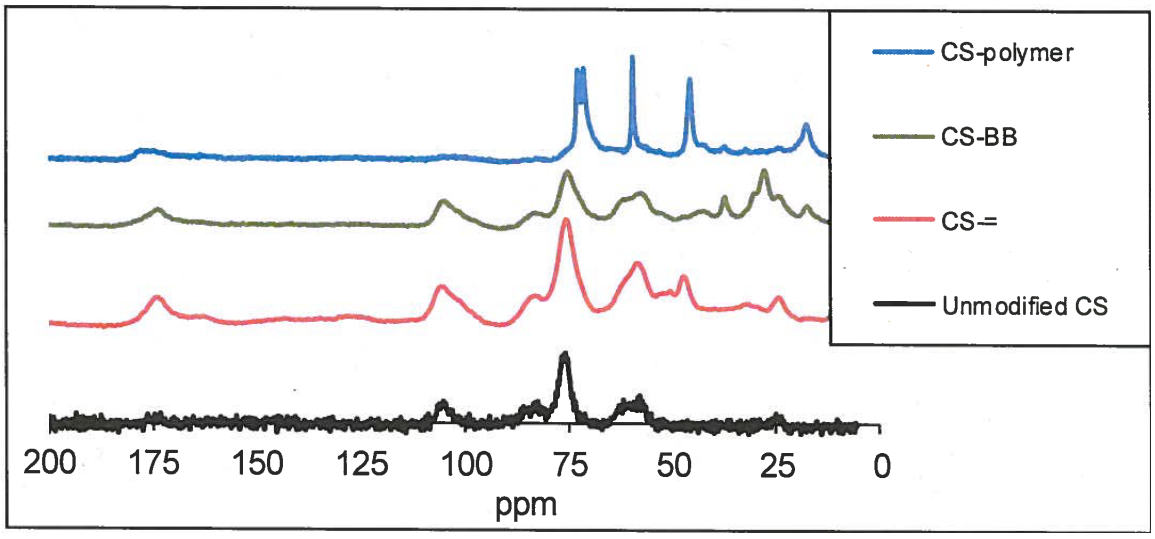


394

395 **Figure 6:** FTIR spectroscopy of chitosan and each of its subsequent reactions:
396 unmodified chitosan (black); acrylamide grafted chitosan (CS-=, red); BlocBuilder
397 grafted chitosan (CS-BB, green); poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) grafted onto
398 chitosan (CS-polymer).
399

400 The ^{13}C solid-state NMR was also performed to confirm the grafting reactions (*Figure 7*).
401 Chitosan has peaks from 50-110 ppm. Another subtle peak is seen at 130 ppm which
402 indicates the C=C carbons. There are also peaks below 50 ppm. It is not clear where these
403 peaks come from, but they most likely represent alkane groups from the triethylamine

404 that was used when the chitosan was reacted with the the acryloyl chloride. This is
405 because the peaks near 25 and 50 ppm are also the peaks for pure triethylamine. This
406 may mean potential side reactions on the C=C of the acrylamide from the acryloyl
407 chloride reaction, reducing the efficiency of the reaction of BlocBuilder to the vinyl
408 groups of the acrylamide. Examining the sample that represents the conversion of CS-=
409 to CS-BB, the C=O group remains.
410



411

412 **Figure 7:** ^{13}C NMR showing the various stages of modification of the chitosan (black),
413 followed by grafting of the methacrylic acid to place acrylamide groups on the surface
414 (CS-=, red), intermolecular radical addition (IRA) of the BlocBuilder unimolecular
415 initiator (CS-BB, green) followed by polymerization from the surface with
416 OEGMA/MEO_2MA/AN monomer mixture.
417

418 Furthermore, the disappearance of the C=C bond at 130 ppm is evidence for the
419 successful IRA. There is also a proliferation of peaks below 50 ppm. It is thought that
420 these peaks represent the alkane groups on the BlocBuilder. Unfortunately, NMR data of
421 CS-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) shows that chitosan can no longer be
422 detected. Indeed, all that can be seen now is the polymer grafted on the surface. The

peaks represent mainly the EG units from the side chain of the OEGMA/ (C-O-C) repeat units of the monomers between 40 and 75 ppm. There is an alkane peak at 15 ppm and a small peak at 180 ppm representing the C=O from the methacrylic units. Due to chitosan's insoluble nature, it was not possible in this study to measure the molecular weight distribution of CS-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) unless the chitosan was completely cleaved from the polymer. However, because the terpolymerization study confirms that the NMP is well controlled and able to initiate clearly a second batch of new monomer, it is highly probable that the polymerization initiated from the surface was effective in providing chains with the desired thermal response. Moreover, TGA analysis confirmed the presence of chitosan and its grafted polymer.

TGA was performed from 25ºC to 550ºC under nitrogen, and 550ºC to 700ºC in air. As seen in *Figure 8*, the terpolymer OM5:95 decomposes at 200ºC and 350ºC and burns completely after 400ºC, leaving only behind 1% ash content. Unmodified chitosan, however, decomposes at 280ºC and has an ash content of 35% at 550ºC before the switch to atmospheric conditions. Consequently, the ash content of the modified chitosan with the polymer attached should be somewhere between 1% and 35% at 550ºC. As seen in *Figure 9*, the modified chitosan has an ash content that continues to decrease as reaction time increases. The final ash content of the modified chitosan is 12%. Consequently, a crude estimate suggests chitosan accounts for 35% of the total mass of CS-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN).

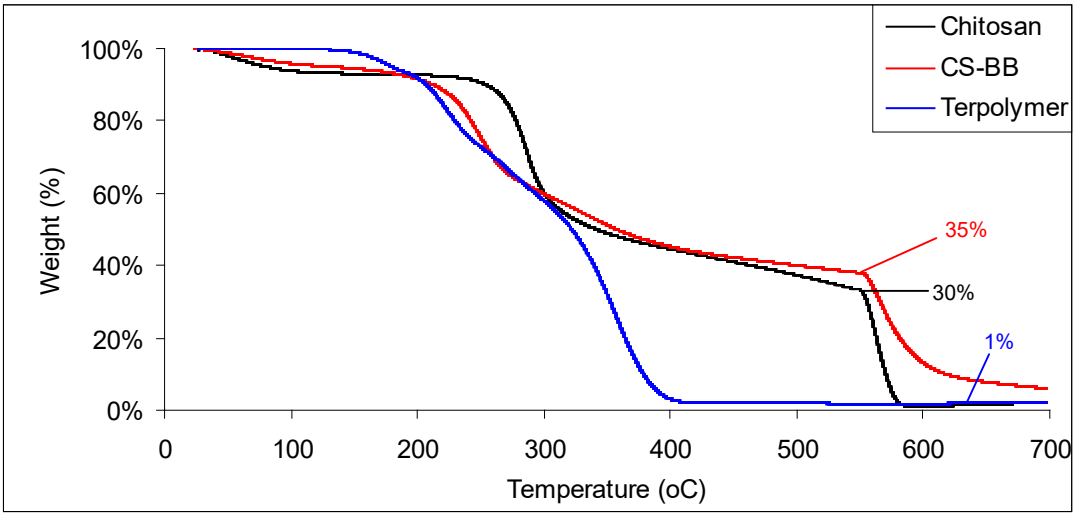


Figure 8: Comparison of TGA profiles between chitosan, CS-BB, and terpolymer poly(OEGMA-*ran*-MEO2MA-*ran*-AN) OM5:95.

By knowing the ratio between chitosan and polymer, the length of polymer chains can be estimated by using an approximation of the molecular weight of the chitosan and estimating the number of functional groups on the surface. For example, take CS-5:95 where the final ash content is 12.4%. Because CS-BB has an ash content of 35%, that means that CS-5:95 has a chitosan content of 35wt% and polymer content of 65wt%. To transform this into a molar ratio, the average molecular weight for both CS-BB and monomer are needed. For the case of CS-BB, chitosan's molecular weight is given as 67 000 g mol^{-1} from the supplier. It is calculated that there are approximately 194 functional groups on the surface of chitosan assuming that chitosan is a sphere. Assuming 100% efficiency and chitosan particle size to be approximately 10 microns, CS-BB would have an average molecular weight of 151 000 g mol^{-1} given that BlocBuilder has a molecular weight of 381 g mol^{-1} and the acrylamide group has a molecular weight of 52 g mol^{-1}. The compositionally averaged weight for the monomer is taken from the three monomers (OEGMA, MEO_2MA, AN), and calculated to be 186 g mol^{-1}. Knowing this, the

chitosan:polymer mass ratio can be converted into a chitosan:polymer molar ratio. In this case, it is calculated to be 1 480 mol monomer: 1 ml CS-BB. Because it was previously calculated that there are 194 functional groups per CS-BB, it can be approximated that the average chain length is 7.6. This value seems very low and thus the assumption of 100% efficient grafting should be questioned. This is further verified from the initial ^{13}C NMR studies performed on CS-=. Because there are some peaks found in the alkane region between 0 and 40 ppm as seen in *Figure 7,* it can be assumed that these side reactions prevented the vinyl group formation, and thus, not allowed the BlocBuilder to react with the chitosan. Moreover, as seen in *Table 4,* the ash content, and consequently the chitosan content, decreases as the ratio between OEGMA/MEO_2MA increases. This is because of the increase of the average molecular weight, as the OEGMA:MEO_2MA increases.

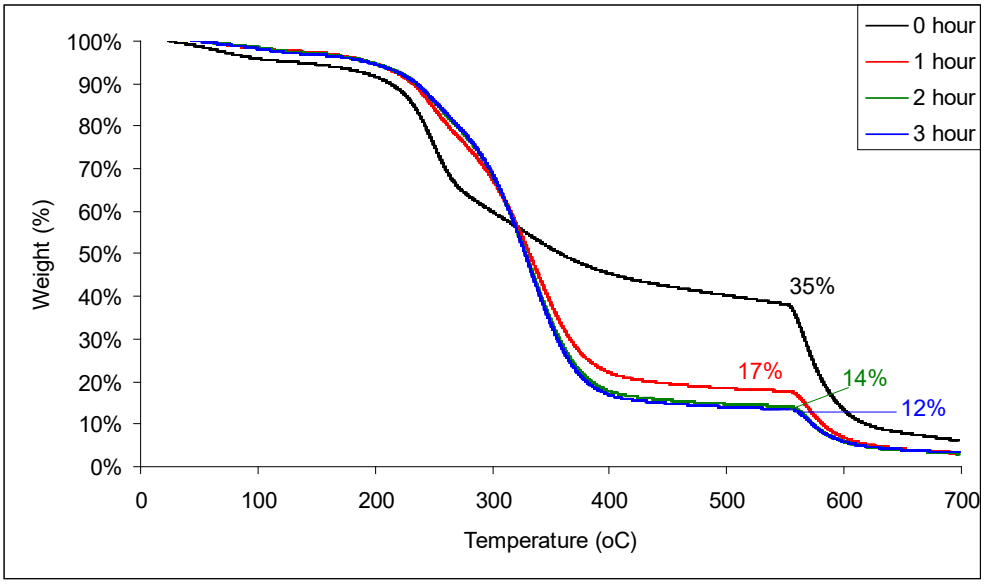


Figure 9: TGA analysis of sampled CS-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) at different reaction times.

Table 4: TGA summary CS-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) at different ratios

Sample name	Ash content (wt %)	Chitosan content (wt %)	Monomer (mol):Chitosan (mol)	Average chain length
CS-5:95	12.4	35.4	1480	7.6
CS-10:90	9.0	25.7	2200	11.4
CS-20:80	8.1	23.1	2260	11.6
CS-40:60	6.8	19.4	2330	12

Given that there was sufficient grafting of polymer onto chitosan, thermoresponsive properties were measured for the hybrid material. At first, only the terpolymers that were polymerized in homogeneous conditions were analyzed as shown in *Figure 10* in order to assess what CPT would be expected to be observed for a given composition. As can be seen, the more the OEGMA:MEO_2MA ratio increased, the higher the CPT. As explained previously, polymers in solutions above the LCST start aggregating, and precipitate eventually. With the heating and cooling of the solution, the data indicates a fully thermo-reversible transition, with a hysteresis of around 5ºC for the terpolymer, typical for this polymer [4] [31]. *Figure 10* also shows the results of the chain-extended species in water: OM5:95 – 40:60. As can be seen, there are two CPT's in the transmittance, one at 40ºC and the other at 65ºC (*Figure 11*). A hysteresis effect is visible for both transitions. The chain extension was determined to be largely successful because of the clean shift in the GPC traces and the dual CPTs shown in the UV-Vis experiments.

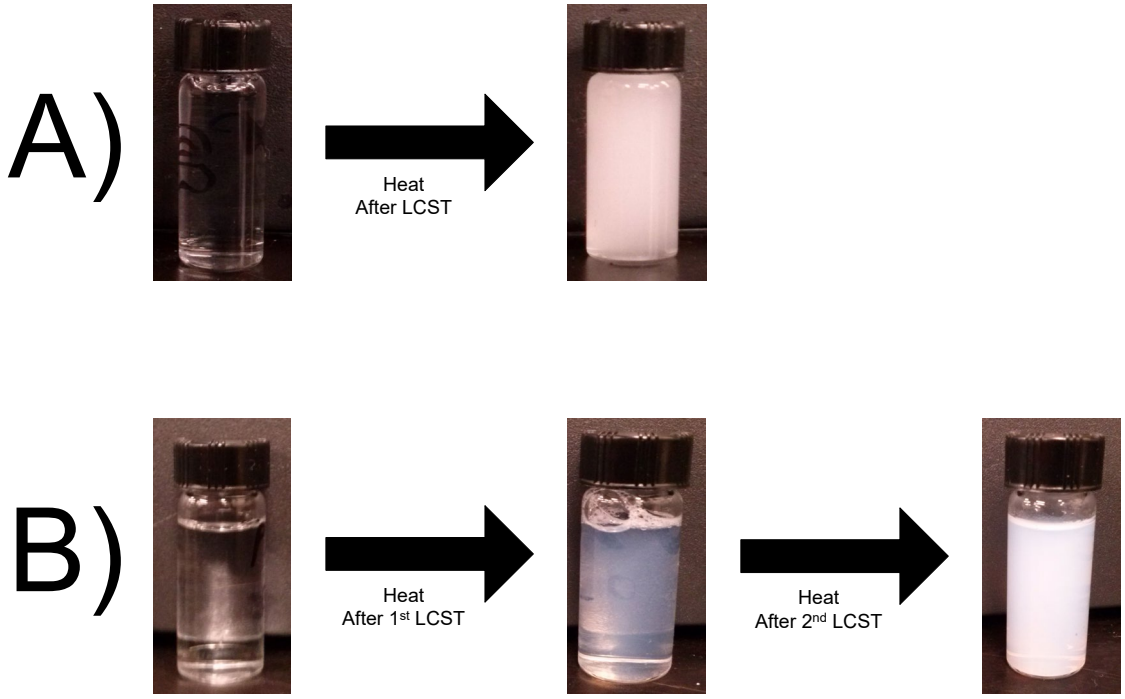


Figure 10: poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) terpolymer (see Table 4 for complete characterization and identification of samples) in water at 5 g L^{-1} solutions before and after the cloud point A) single terpolymer OM5:95; B) The chain extended block copolymer OM:5:95-OM40:60 after the first and second cloud point upon heating. Note that this is reversible, and cooling the solution rendered it clear again.

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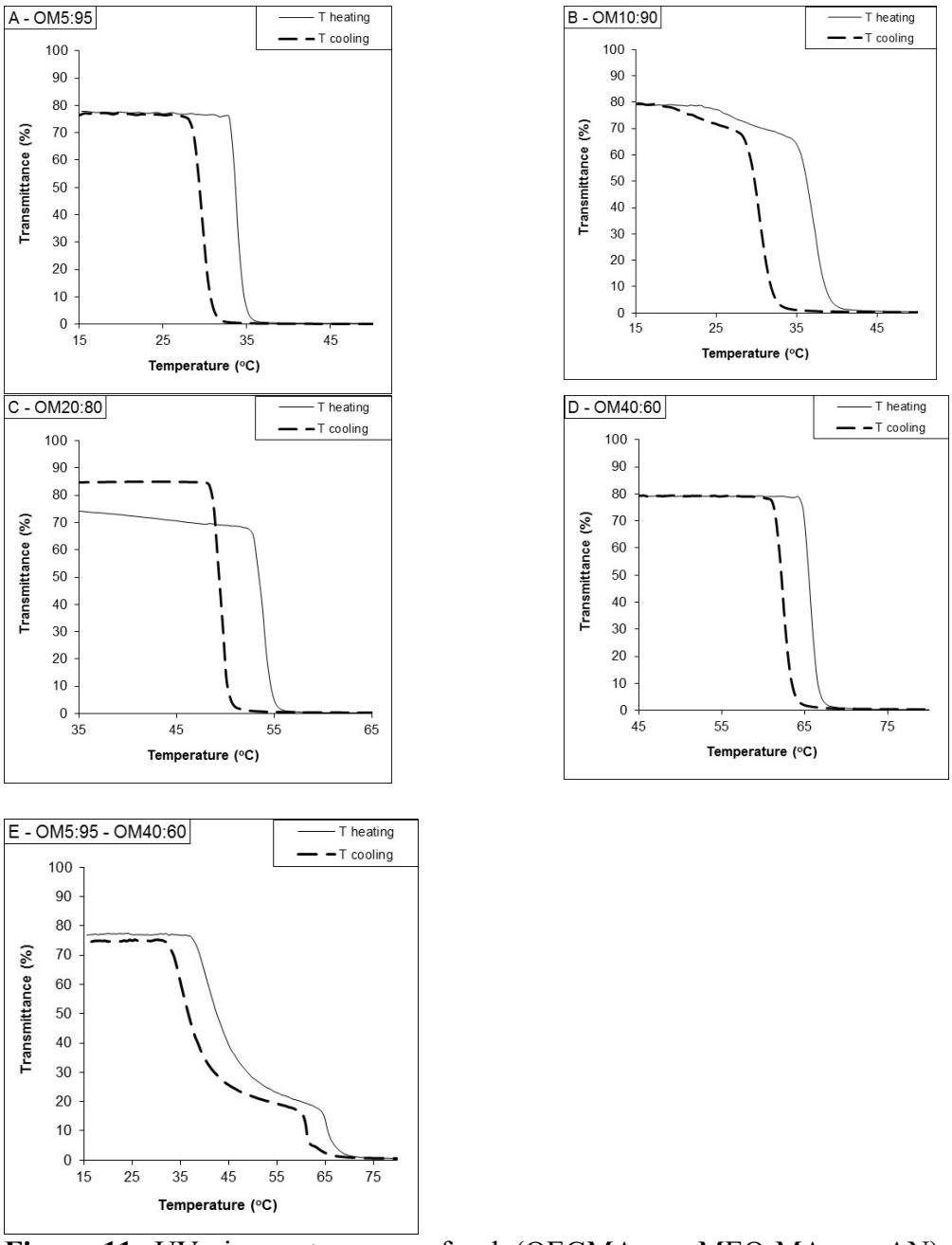

504 **Figure 11:** UV-vis spectroscopy of poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) terpolymers
505 (see for complete characterization and identification of samples) in water at 5 g L^{-1}
506 solutions for transmittance versus temperature. Note that the dashed line represents
507 cooling and shows the reversibility of the solution's transmittance.
508

509 Next, the modified chitosan was also analyzed via UV-Vis spectroscopy. While chitosan

510 is compatible with water and swells in solution, it does not dissolve in it and it eventually

settles [41]. In order to analyze chitosan suspended in solution, the solution must be continuously stirred. As the UV-Vis spectrometer is equipped with a stirrer, the chitosan suspended can be continuously stirred. However, when heated, aggregation is occurring. However, in contrast to the terpolymers made in homogeneous conditions, because of the chitosan core, they form gross aggregates. This observation is clearer as the aggregates drop out of solution (*Figure 12*). Once the suspension is heated, the aggregate grow and some get so large that they start floating on the water surface. This transition gives a good indication of where the CPT begins for the modified chitosan. The CS-BB was grafted using different OEGMA-MEO_2MA ratios. The absorbance was measured across the temperature change using the UV visible spectrometer.

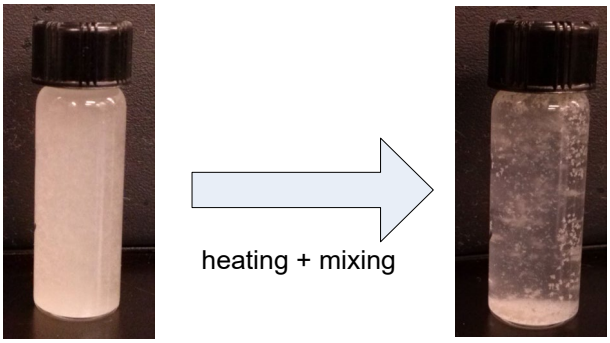

Figure 12: CS-*graft*-poly(OEGMA-ran-MEO2MA-ran-AN) in distilled water (solution concentration = 5 g L^{-1} before and after heating. The mixture is cloudy at room temperature. After heating and mixing, the CS-*graft*-poly(OEGMA-ran-MEO2MA-ran-AN) aggregates and clumps together, and starts either floating above the water or sinking to the bottom, rendering the mixture more clearer.

As can be seen in *Figure 13*, the transmittance increases as temperature increases. However, the CPT of the modified chitosan is not as well-defined as the pure terpolymer since the cooperative effective of neighbouring OEGMA/MEO_2MA chains is restricted by being grafted to the chitosan. This is manifested by the significant signal noise observed. It is also important to note that stirring time could be a factor.

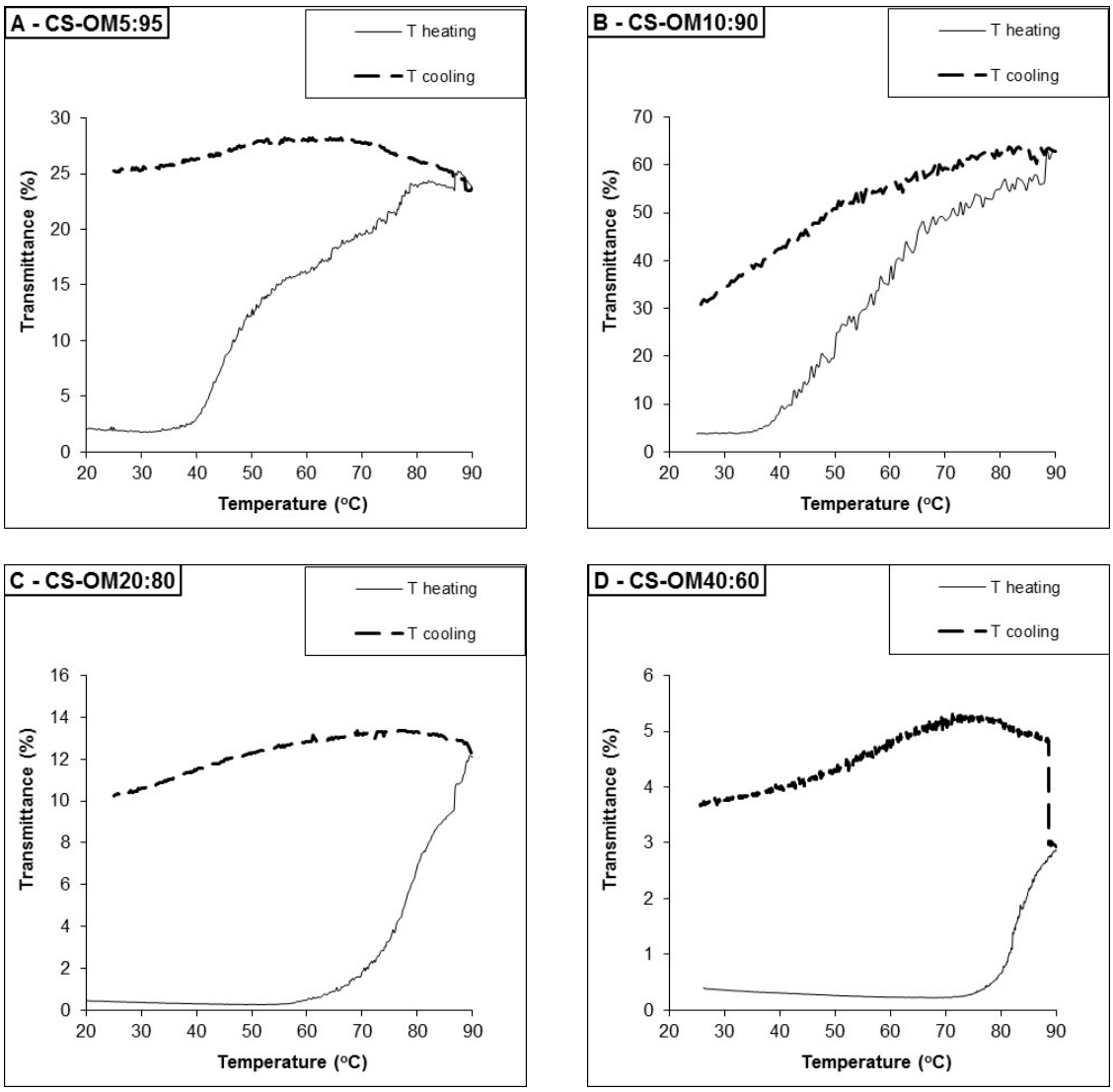

Figure 13: UV-vis spectroscopy of characteristic CS-*graft*-poly(OEGMA-ran-MEO2MA-ran-AN) terpoymer in neutral water at 5 g L^{-1} solutions for transmittance versus temperature.

All the trends in *Figure 13* show this phenomenon, but this is most evident in CS-OM40:60 where the temperature starts to decrease beginning at 90°C, but the transmittance continues to increase until 70°C. As such, when the temperature is decreasing, the transmittance decreases at a much slower rate. This is because the modified chitosan that floated above the water remained even when the solution is cooled.

543 The lack of reversibility of the aggregation process is obviously the key drawback here.
544 Many cases in the literature have made the chitosan organosoluble by methods such as
545 phthaloylation [46]. Perhaps if lower molecular weight water-soluble chitosan such as
546 that used by Lee et al (22 kg mol^{-1}) was used [47], the hysteresis would not be as severe.
547 Additionally, as the ratio of OEGMA:MEO_2MA increases, the CPT increases as well.
548 This is well correlated with the CPT of the terpolymer as seen in other studies [4] [31]
549 [53]. The modified chitosan has a higher CPT compared to the terpolymer poly(OEGMA-
550 *ran*-MEO_2MA-*ran*-AN). This may be due to chitosan's solubility, causing a slight
551 increase of the LCST. Also, note the transmittance increases even though temperature
552 starts to decrease. For example, looking at Figure -*D*, the transmittance increases from 2
553 to 4% despite being in the cooling cycle. This is because the continuous mixing is still
554 aggregating the clumps, rendering the solution clearer. While the solution is cooling, an
555 excessive hysteresis effect is observed, meaning that the CS-polymer does not readily
556 disperse back into the solution. However, this may be because the mixing is not vigorous
557 enough to re-mix the CS-polymer sufficiently, as it was observed that after the
558 experiment was complete in the UV-Vis spectrometer, the CS-polymer does in fact
559 disperse in water after manual vigorous shaking. In summary, we have shown chitosan
560 can be modified by grafting thermoresponsive polymers with tunable LCSTs via
561 heterogeneous NMP. Subsequent work will focus on using lower molecular weight
562 fractions of chitosan to improve the solubility and ensuing reversibility.
563
564
565

Conclusion

A series of poly(OEGMA/MEO_2MA/AN) terpolymers were synthesized in a controlled manner using BlocBuilder and additional SG1 free nitroxide at 120ºC. The terpolymerization indicated chains grew linearly over time up to conversions $X \approx 0.7$ with a relatively narrow molecular weight distribution with $Đ < 1.5$. The increase of the OEGMA:MEO_2MA ratio resulted in an increase in CPT between 30ºC to 90ºC. The terpolymer could be re-initiated as a macroinitiator for a second terpolymerization in the case of OM5:95-OM40:60. This resulted in a block copolymer that still had a narrow molecular distribution with two distinct CPT's and a moderate increase in dispersity.

The heterogeneous modification of chitosan was successfully performed using Lefay et al's procedure.[50] The addition of BlocBuilder onto the chitosan surface was shown to be successful by FTIR and ^{13}C NMR. Using the terpolymerization data of poly(OEGMA-*ran*-MEO_2MA-*ran*-AN), the monomers were successfully grafted from the chitosan, which was proven via TGA: the CS-*graft*-poly(OEGMA-*ran*-MEO_2MA-*ran*-AN) had between 65 and 80 wt% of polymer. The grafted chitosan was then shown not only to be thermally responsive, but also can have the LCST tuned just like the terpolymer poly(OEGMA-*ran*-MEO_2MA-*ran*-AN). Although the change is not as definitive as the pure terpolymer because of chitosan's insolubility and macro-aggregation that is dependent on mixing, the result showed that the polymers were grafted successfully by heterogeneous mean onto the chitosan, yielding a material with potential to combine thermoresponsive properties with antimicrobial properties due to the amine group and the formation of ammonium salts in acidic media with stimuli-responsive.

Acknowledgements

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References

[1] I. C. Sanchez and R. H. Lacombe, "Statistical thermodynamics of polymer solutions," *Macromolecules,* vol. 11, pp. 1145-1156, 1978.

[2] D. Schmaljohann, "Thermo-and pH-responsive polymers in drug delivery," *Advanced drug delivery reviews,* vol. 58, pp. 1655-1670, 2006.

[3] S. Wang, Z. Cheng, J. Zhu, Z. Zhang, and X. Zhu, "Synthesis of amphiphilic and thermosensitive graft copolymers with fluorescence P (St‐co‐(p‐CMS))‐g‐PNIPAAM by combination of NMP and RAFT methods," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 45, pp. 5318-5328, 2007.

[4] J.-F. Lutz, Ö. Akdemir, and A. Hoth, "Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: is the age of poly (NIPAM) over?," *Journal of the American Chemical Society,* vol. 128, pp. 13046-13047, 2006.

[5] C. R. Becer, S. Hahn, M. W. Fijten, H. M. Thijs, R. Hoogenboom, and U. S. Schubert, "Libraries of methacrylic acid and oligo (ethylene glycol) methacrylate copolymers with LCST behavior," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 46, pp. 7138-7147, 2008.

[6] C. Pietsch, M. W. Fijten, H. M. Lambermont‐Thijs, R. Hoogenboom, and U. S. Schubert, "Unexpected reactivity for the RAFT copolymerization of oligo (ethylene glycol) methacrylates," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 47, pp. 2811-2820, 2009.

[7] P. J. Roth, F. D. Jochum, F. R. Forst, R. Zentel, and P. Theato, "Influence of End Groups on the Stimulus-Responsive Behavior of Poly [oligo (ethylene glycol) methacrylate] in Water," *Macromolecules,* vol. 43, pp. 4638-4645, 2010.

[8] C. L. McCormick and A. B. Lowe, "Aqueous RAFT polymerization: recent developments in synthesis of functional water-soluble (Co) polymers with controlled structures," *Accounts of chemical research,* vol. 37, pp. 312-325, 2004.

[9] A. E. Smith, X. Xu, and C. L. McCormick, "Stimuli-responsive amphiphilic (co) polymers via RAFT polymerization," *Progress in Polymer Science,* vol. 35, pp. 45-93, 2010.

[10] P. D. Topham, N. Sandon, E. S. Read, J. Madsen, A. J. Ryan, and S. P. Armes, "Facile synthesis of well-defined hydrophilic methacrylic macromonomers using ATRP and click chemistry," *Macromolecules,* vol. 41, pp. 9542-9547, 2008.

[11] K. Matyjaszewski and J. Xia, "Atom transfer radical polymerization," *Chemical Reviews,* vol. 101, pp. 2921-2990, 2001.
[12] W. A. Braunecker and K. Matyjaszewski, "Controlled/living radical polymerization: features, developments, and perspectives," *Progress in Polymer Science,* vol. 32, pp. 93-146, 2007.
[13] M. Chenal, C. Boursier, Y. Guillaneuf, M. Taverna, P. Couvreur, and J. Nicolas, "First peptide/protein PEGylation with functional polymers designed by nitroxide-mediated polymerization," *Polymer Chemistry,* vol. 2, pp. 1523-1530, 2011.
[14] J. Nicolas, P. Couvreur, and B. Charleux, "Comb-like polymethacrylates with poly (ethylene glycol) side chains via nitroxide-mediated controlled free-radical polymerization," *Macromolecules,* vol. 41, pp. 3758-3761, 2008.
[15] J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes, and B. Charleux, "Nitroxide-mediated polymerization," *Progress in Polymer Science,* vol. 38, pp. 63-235, 2013.
[16] R. B. Grubbs, "Nitroxide-mediated radical polymerization: limitations and versatility," *Polymer Reviews,* vol. 51, pp. 104-137, 2011.
[17] C. Marestin, C. Noël, A. Guyot, and J. Claverie, "Nitroxide mediated living radical polymerization of styrene in emulsion," *Macromolecules,* vol. 31, pp. 4041-4044, 1998.
[18] L. I. Gabaston, R. A. Jackson, and S. P. Armes, "Living free-radical dispersion polymerization of styrene," *Macromolecules,* vol. 31, pp. 2883-2888, 1998.
[19] D. Benoit, V. Chaplinski, R. Braslau, and C. J. Hawker, "Development of a universal alkoxyamine for “living” free radical polymerizations," *Journal of the American Chemical Society,* vol. 121, pp. 3904-3920, 1999.
[20] S. Grimaldi, J.-P. Finet, F. Le Moigne, A. Zeghdaoui, P. Tordo, D. Benoit*, et al.*, "Acyclic β-phosphonylated nitroxides: a new series of counter-radicals for “living”/controlled free radical polymerization," *Macromolecules,* vol. 33, pp. 1141-1147, 2000.
[21] B. Charleux, J. Nicolas, and O. Guerret, "Theoretical expression of the average activation-deactivation equilibrium constant in controlled/living free-radical copolymerization operating via reversible termination. Application to a strongly improved control in nitroxide-mediated polymerization of methyl methacrylate," *Macromolecules,* vol. 38, pp. 5485-5492, 2005.
[22] J. Nicolas, C. Dire, L. Mueller, J. Belleney, B. Charleux, S. R. Marque*, et al.*, "Living character of polymer chains prepared via nitroxide-mediated controlled free-radical polymerization of methyl methacrylate in the presence of a small amount of styrene at low temperature," *Macromolecules,* vol. 39, pp. 8274-8282, 2006.
[23] B. Lessard and M. Marić, "Incorporating glycidyl methacrylate into block copolymers using poly (methacrylate ‐ ran ‐ styrene) macroinitiators synthesized by nitroxide ‐ mediated polymerization," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 47, pp. 2574-2588, 2009.
[24] C. Zhang, B. Lessard, and M. Maric, "Synthesis and characterization of benzyl methacrylate/styrene random copolymers prepared by NMP," *Macromolecular Reaction Engineering,* vol. 4, pp. 415-423, 2010.

[25] J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes, and B. Charleux, "Nitroxide-mediated polymerization," *Progress in Polymer Science,* 2013.
[26] J. Nicolas, S. Brusseau, and B. Charleux, "A minimal amount of acrylonitrile turns the nitroxide‐mediated polymerization of methyl methacrylate into an almost ideal controlled/living system," *Journal of Polymer Science Part A: Polymer Chemistry,* vol. 48, pp. 34-47, 2010.
[27] A. C. Greene and R. B. Grubbs, "Nitroxide-mediated polymerization of methyl methacrylate and styrene with new alkoxyamines from 4-nitrophenyl 2-methylpropionat-2-yl radicals," *Macromolecules,* vol. 43, pp. 10320-10325, 2010.
[28] Y. Guillaneuf, D. Gigmes, S. R. Marque, P. Astolfi, L. Greci, P. Tordo*, et al.*, "First effective nitroxide-mediated polymerization of methyl methacrylate," *Macromolecules,* vol. 40, pp. 3108-3114, 2007.
[29] C. Detrembleur, C. Jérôme, J. De Winter, P. Gerbaux, J.-L. Clément, Y. Guillaneuf*, et al.*, "Nitroxide mediated polymerization of methacrylates at moderate temperature," *Polymer Chemistry,* vol. 5, pp. 335-340, 2014.
[30] C. Detrembleur, A. Mouithys-Mickalad, P. Teyssié, and R. Jérôme, "Sodium nitrite and ascorbic acid: a metal-free combination that controls the free-radical polymerization of tert-butyl methacrylate in water," *e-Polymers,* vol. 2, pp. 53-68, 2002.
[31] B. H. Lessard, E. J. Y. Ling, and M. Marić, "Fluorescent, Thermoresponsive Oligo (ethylene glycol) Methacrylate/9-(4-Vinylbenzyl)-9H-carbazole Copolymers Designed with Multiple LCSTs via Nitroxide Mediated Controlled Radical Polymerization," 2012.
[32] M. Chenal, S. Mura, C. Marchal, D. Gigmes, B. Charleux, E. Fattal*, et al.*, "Facile synthesis of innocuous comb-shaped polymethacrylates with PEG side chains by nitroxide-mediated radical polymerization in hydroalcoholic solutions," *Macromolecules,* vol. 43, pp. 9291-9303, 2010.
[33] V. Delplace, S. Harrisson, A. Tardy, D. Gigmes, Y. Guillaneuf, and J. Nicolas, "Nitroxide‐Mediated Radical Ring‐Opening Copolymerization: Chain‐End Investigation and Block Copolymer Synthesis," *Macromolecular rapid communications,* vol. 35, pp. 484-491, 2014.
[34] M. F. Goosen, *Applications of Chitan and Chitosan*: CRC Press, 1996.
[35] M. N. Ravi Kumar, "A review of chitin and chitosan applications," *Reactive and functional polymers,* vol. 46, pp. 1-27, 2000.
[36] M. Rinaudo, "Chitin and chitosan: properties and applications," *Progress in polymer science,* vol. 31, pp. 603-632, 2006.
[37] M. Dash, F. Chiellini, R. Ottenbrite, and E. Chiellini, "Chitosan—A versatile semi-synthetic polymer in biomedical applications," *Progress in Polymer Science,* vol. 36, pp. 981-1014, 2011.
[38] S. A. Agnihotri, N. N. Mallikarjuna, and T. M. Aminabhavi, "Recent advances on chitosan-based micro-and nanoparticles in drug delivery," *Journal of Controlled Release,* vol. 100, pp. 5-28, 2004.
[39] O. Felt, P. Buri, and R. Gurny, "Chitosan: a unique polysaccharide for drug delivery," *Drug Development and Industrial Pharmacy,* vol. 24, pp. 979-993, 1998.

[40] N. Bhattarai, J. Gunn, and M. Zhang, "Chitosan-based hydrogels for controlled, localized drug delivery," *Advanced drug delivery reviews,* vol. 62, pp. 83-99, 2010.
[41] C. Pillai, W. Paul, and C. P. Sharma, "Chitin and chitosan polymers: Chemistry, solubility and fiber formation," *Progress in Polymer Science,* vol. 34, pp. 641-678, 2009.
[42] D. W. Jenkins and S. M. Hudson, "Review of vinyl graft copolymerization featuring recent advances toward controlled radical-based reactions and illustrated with chitin/chitosan trunk polymers," *Chemical Reviews,* vol. 101, pp. 3245-3274, 2001.
[43] B. Jeong and A. Gutowska, "Lessons from nature: stimuli-responsive polymers and their biomedical applications," *Trends in biotechnology,* vol. 20, pp. 305-311, 2002.
[44] C. Zhang and M. Maric, "Synthesis of stimuli-responsive, water-soluble poly [2-(dimethylamino) ethyl methacrylate/styrene] statistical copolymers by nitroxide mediated polymerization," *Polymers,* vol. 3, pp. 1398-1422, 2011.
[45] K. El Tahlawy and S. Hudson, "Synthesis of a well‐defined chitosan graft poly (methoxy polyethyleneglycol methacrylate) by atom transfer radical polymerization," *Journal of applied polymer science,* vol. 89, pp. 901-912, 2003.
[46] N. H. Munro, L. R. Hanton, S. C. Moratti, and B. H. Robinson, "Synthesis and characterisation of chitosan-*graft*-poly (OEGMA) copolymers prepared by ATRP," *Carbohydrate polymers,* vol. 77, pp. 496-505, 2009.
[47] D. Hua, J. Tang, J. Cheng, W. Deng, and X. Zhu, "A novel method of controlled grafting modification of chitosan via RAFT polymerization using chitosan-RAFT agent," *Carbohydrate polymers,* vol. 73, pp. 98-104, 2008.
[48] J. Tang, D. Hua, J. Cheng, J. Jiang, and X. Zhu, "Synthesis and properties of temperature-responsive chitosan by controlled free radical polymerization with chitosan-RAFT agent," *International journal of biological macromolecules,* vol. 43, pp. 383-389, 2008.
[49] C. Lefay, Y. Guillaneuf, G. Moreira, J. J. Thevarajah, P. Castignolles, F. Ziarelli, E. Bloch, M. Major, L. Charles, M. Gaborieau, D. Bertin, D. Gigmes. "Heterogeneous modification of chitosan via nitroxide-mediated polymerization," *Polymer Chemistry,* vol. 4, pp. 322-328, 2013.
[50] K. Bian and M. F. Cunningham,"Nitroxide-mediated living radical polymerization of 2-hydroxyethyl acrylate and the synthesis of amphiphilic block copolymers" Macromolecules, vol. 38, pp. 695-701, 2005.
[51] M. E. Thomson, A.-M. Manley, J. S. Ness, S. C. Schmidt, and M. F. Cunningham, "Nitroxide-mediated surfactant-free emulsion polymerization of n-butyl methacrylate with a small amount of styrene," *Macromolecules,* vol. 43, pp. 7958-7963, 2010.
[52] J. Vinas, N. Chagneux, D. Gigmes, T. Trimaille, A. Favier, Y. Guillaneuf*, et al.*, "Use of an SG1-based alkoxyamine bearing a n-succinimidyl ester to achieve advanced copolymer architectures" *Polymer Preprints,* vol. 49, p. 388, 2008.
[53] J. F. Lutz, "Thermo‐Switchable Materials Prepared Using the OEGMA‐Platform," *Advanced Materials,* vol. 23, pp. 2237-2243, 2011.