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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 mediated polymerization (NMP) with SG1-based BlocBuilder unimolecular initiators. Homogeneous OEGMA/MEO_2MA/AN terpolymerizations in solution were done first at 120 °C to confirm the cloud point temperatures (CPTs) of the thermoresponsive chains that were to be grafted onto the chitosan (CPT tuning from 30 to 65 °C 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 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.

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 46 °C, respectively, in aqueous media [3]. Many methods are possible to tune the LCST to the desired temperature by incorporating hydrophobic or hydrophllic co-monomers 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 90 °C. 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 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

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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]. It should be noted that methacrylate homopolymerization by NMP, without any controlling co-monomer, has been reported with different specially designed initiators [26–29]. 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 [30]. 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, 31]. 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 Đ ~ 1.4 [14] The polymer was also shown to be non-cytotoxic [32]. 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 copolymerized the 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 [33]. They are derived from many natural sources such as crustacean shells, fungi cell walls, and squid beaks [34]. Through deacetylation, chitosan can be obtained. Recently, chitosan has been applied towards drug delivery, tissue engineering, wastewater treatment, and packaging [34–36] because of its properties like biocompatibility, biodegradability, hypoallergenicity, antibacterial activity, and wound-healing effects. For example, chitosan can encapsulate a drug in controlled delivery applications, and boost its antimicrobial properties because of the amine groups that can be converted to ammonium salts in acidic media [37–39]. 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 [40]. Previous studies mainly focused on using free radical initiators, such as ceric ammonium nitrate (CAN), ammonium persulfate (APS), and 2,2-azobisisobutyronitrile (AIBN) [41] 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 [42, 43]. ATRP and RAFT were used in chitosan grafting under homogeneous and heterogeneous conditions [44–47]. However, recently it was shown that BlocBuilder could be grafted onto a chitosan surface in a homogeneous and heterogeneous manner via NMP [48, 49]. In the case of heterogeneous modification, 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 (Fig. 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.

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 ^{1}H 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 aluminium 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. ^{1}H 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) with HPLC grade THF as the mobile phase running at a flow rate of 0.3 ml/min and heated to 40 °C. The GPC is equipped with a guard column and with 3 Waters Styragel HR columns with the molecular weight ranges are given: HR1: 10^2–5×10^3 g mol^{-1}, HR2: 5×10^2–2×10^4 g mol^{-1}, HR3: 5×10^3–6×10^5 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 −20 °C, and then lyophilized for 5 h to obtain the final terpolymer, which was analyzed via the UV–Vis spectrometer.