Synthesis of Well-Controlled Nanogels via Block Copolymer Self-Assembly: A Systematic Characterization of the Properties and Potential Applications

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Synthesis of Well-Controlled Nanogels via Block Copolymer Self-Assembly: A Systematic Characterization of the Properties and Potential Applications

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November 1, 2013
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Abstract

In the United States there is a growing need for materials that can probe and fight diseases of the heart, brain, and body as a whole. In this study nanogels, block copolymers, and copolymers were synthesized using solution polymerization and directed self-assembly. Results indicate that the nanogels are highly responsive to changes in pH and temperature and they are of a size (29 nanometers) that makes them viable drug delivery devices. Additionally, these nanogels macrogel at very low nanogel concentrations (10 – 15 weight %), which makes them reasonable candidates for dental repairs and enhancements. The present study also characterizes the process by which these nanogels and their precursors (block copolymers and copolymers) were synthesized. Our findings illustrate that solution polymerization followed by self-assembly is the most appropriate synthetic pathway for the synthesis of nanogels required for biomedical and dental interventions because the process allows the nanogels to retain the selected behavioral characteristics of the block copolymer and copolymer and, as this study specifically demonstrates, enhance certain copolymer and block copolymer traits. The copolymer, block copolymer, and nanogels that were synthesized in this study, therefore, could potentially be used to help researchers understand and treat some of the more deleterious illnesses such as Alzheimer’s disease, post-traumatic stress disorder, and Type II diabetes.
Introduction

The annual cost of healthcare in the US is expected to exceed $3 Trillion in 2013 (Centers for Medicare and Medicaid Services, 2011). A significant fraction of that cost is spent on prescription drug development and interventions for diseases of the cardiovascular and nervous systems. Alzheimer's disease (AD), for instance, cost the US $203 Billion in 2012 (Alzheimer’s Association). Similarly, coronary heart disease (CHD) costs the US $108.9 Billion per annum (Heidenreich et al., 2011). Other conditions like Type II diabetes, post-traumatic stress disorder (PTSD), and breast cancer all require significant pharmacological and/or behavioral interventions, which are highly invasive and do not necessarily benefit the person with the disorder.

Another cost that is not as easy to measure is that associated with people being unable to fulfill their duties because of a disability, the emotional toll for people who are acquainted with someone who has a condition, and the number of people who die as a result of the disease. Both the measurable and immeasurable costs compound upon each other such that people with disabilities cannot get the support and treatment they need, while their family members scramble to accommodate them. In the meantime, the financial cost of diagnosing and treating these and other ailments continues to rise even as people become less able to afford these expensive and, occasionally, unnecessary tests and therapies. A variety of new bioengineering and materials science approaches are being directed towards enhancing and extending biomedical applications. Recently nanogels have been advanced as a potential materials-based pathway toward addressing aspects of this health care conundrum.

Nanogels are hyperbranched, polymeric networks that are discrete globular particles less than 100 nanometers (nm) in diameter. Nanogels themselves are not new, but the current process
by which we are synthesizing them is. Since the 1930s and up until now, microgels and nanogels were made using a process called emulsion polymerization. Recently, solution polymerization and nanogel self-assembly (part of the solution polymerization process) have been added to the polymer scientists’ repertoire of synthetic techniques. These two polymerization processes reflect the synthetic needs of the US at the time the processes were developed and utilized. Emulsion polymerization, the form that is used now, was developed and patented by M. Luther and C. Heuck in 1932 (Erbil, 2000). Solution polymerization has been used widely as a processing mode in the preparation of linear polymers. However, solution polymerization coupled with block copolymer self-assembly was first used in the 1960s (Mai and Eisenberg, 2012).

Traditional emulsion polymerization is a process that involves a radical initiator, water as the medium, hydrophobic monomers, and a surfactant. Traditional emulsion polymerization, also called oil-in-water polymerization, is accomplished by adding the monomer to the continuous aqueous phase (deionized water), mixing the two phases with a surfactant (soap), emulsifying the mixture with stirring or a sonicator, and adding the initiator. The surfactant and energy input largely determine the particle dimensions. Emulsion polymerization was first used to synthesize latex and synthetic rubbers, making it a valuable synthetic technique before and during World War II, when it was the only process by which rubber and nylon could be produced to make tires, insulation for electrical wires, and stockings. After WWII, demand for plastics did not change, but there was a shift from the wartime plastics to plastics that were suited to civilian life. Tupperware, washing machines, and radios were some of the items that were made post-WWII using emulsion polymerization techniques.

Emulsion polymerization was suited to make such plastics because the process can be carried out by machines, the reactants are easy to procure, and the total amount of time necessary
to obtain the final plastic product can be on the order of hours to days (compared to weeks with other polymerization techniques). Also, the structural characteristics of emulsion polymers make them highly attractive in the industrial and business sectors that rely heavily on moldable thermoplastic materials. The polymers, once purified, are ready to use for whatever function they have been appropriated; little time is required to modify the polymers (if any modifications are necessary); the particles are nearly identical in size; and, in the case of high-density polyethylene, there are a number of practical uses for the modified and crude polymer products.

Emulsion polymerization has several disadvantages, however. The major disadvantages of using emulsion polymerization to synthesize polymers are the resultant polymeric density, the types of modifications that can be made to the polymers (micro), and the limitations on the particle size that can be achieved. The particles, because they are initially dispersed in an aqueous environment, form a shell around their hydrophobic interior that blocks the passage of solvent from the environment into the interior of the polymer structure. This characteristic is maintained throughout all steps in the polymerization process and in the case of internally crosslinked polymers, precludes the possibility of swelling the particle with organic or aqueous solvents post-polymerization and it also prevents the synthetic chemist from accessing the internal space of the particles. The inaccessibility of the interior of the particle also limits the degree to which the polymer can be modified during and after polymerization has occurred.

Although the surface of the emulsion polymer can be functionalized with metallic particles and chemical groups that crosslink particles together (Ouadahi et al., 2012), the interior cannot be manipulated. Finally, the lower end of the size spectrum of nanogels that can be made via emulsion polymerization is roughly 40 nm (Kabanov and Vinogradov, 2009), while the
The typical nanogel diameter is 100 nm or more. These limitations make it difficult to use emulsion-based nanogels for biomedical interventions, especially in contrast to solution-based nanogels.

Solution polymerization applied to nanogels was developed to overcome the shortcomings of emulsion polymerization and to allow polymer scientists to engineer nanogels to interact with their target substrates in a more exclusive manner (and discourage nonspecific interactions). Self-assembly of nanogels in solution polymerization gives researchers even more control over the sorts of conditions to which the nanogel and nanogel-precursor polymers respond. The development of this polymer synthesis and modification technique was spurred in part by the prospect of improved health of the denizens of the United States. Solution polymerization creates products currently used for a wide variety of biomedical interventions such as dental restorations, tissue engineering, and drug delivery.

Nanogels prepared using solution polymerization and self-assembled post-polymerization can be targeted to biological systems because they are small, their core and surface can be modified during and after polymerization to make them responsive to specific changes in pH, temperature, and pressure, and their structure is supple (unlike emulsion polymers). Solution polymerization-derived nanogels have one major problem with which they are associated: a fundamental lack of characterization. Solution polymerized nanogels as a whole have been extensively studied, but one class has not been successfully characterized: the nanogels that are less than ten nanometers in diameter. Preliminary studies of these nanogels are inconclusive with regards to viscosity effects, glass transition temperature, particle size, and modulus.

This study was motivated by the above gap in our knowledge of these polymers, particularly since, once they are characterized, these gels can be designed further to prevent diseases and conditions like gingivitis (dental sealants) and osteoarthritis; and, mitigate or repair...
the damage done by disorders like Parkinson’s and chronic inflammation (pharmacologically mediated). The specific aims of this current study are to synthesize nanogels with an average diameter of 10 nm (or less); characterize the nanogels and their precursors to determine what properties are conserved across the stages of polymerization; and, characterize the behavior of the nanogels in a range of organic and inorganic solvents to ascertain their potential applications. The results of this study, if we successfully synthesize and characterize these nanogels, could provide a foundation for future investigation and exploitation of these tiny particles, which could have a significant impact on the healthcare of people with conditions that are not well treated or understood.
Experimental

**Materials.** Poly(ethylene glycol) methacrylate (Mₙ = 360, PEGMA), 4-vinylbenzyl chloride (90%), sodium azide (99.5%, NaN₃), 2-(cyano-2-propyl) benzodithioate (97%, CPBD), 2-(dimethylamino) ethyl methacrylate (98%, DMAEMA), styrene (99%, St), propargyl ether (98%), azobisisobutyronitrile (98%, AIBN), tin (II) ethyl hexanoate (95%), and hexamethylene diisocyanate (99%, HDI) were purchased from Sigma Aldrich. PEGMA, DMAEMA, and St were purified with activated alumina (basic, Brockman I); AIBN was recrystallized twice in methanol to remove any impurities. 4-Azidomethyl styrene (AzMSt), the only monomer not commercially available, was synthesized according to the protocols of Roth et al. (2009). All other reagents were used as received. All solvents were purchased from Fisher Scientific and used as received.

**Instruments.** Sample conversion measurements were obtained using a Fourier-Transform Infrared (FT-IR) spectrometer (Nicolet 6700, ThermoFisher Scientific). The hydrodynamic (solvent swollen) dimensions of the nanogel were analyzed by a light-scattering detector (DLS, Viscotek 270 dual detectors). Scanning electron microscopy (SEM; JSM-6400, JEOL) images were taken to determine the dry nanogel dimensions. Proton nuclear magnetic resonance (¹H NMR) studies of the copolymer, block copolymer, and nanogel were conducted using a Bruker Avance-III HD (400 MHz) spectrometer. Chloroform-D (99.8%, CDCl₃) was used as the solvent for the analysis of the copolymer, block copolymer, and AzMSt; dimethyl sulfoxide (99%, DMSO) was used as the solvent for NMR analysis of the nanogel. Depending on the polymer being analyzed, CDCl₃ or DMSO was used as the internal calibration standard in the NMR spectra.
**Procedures**

**Synthesis of 4-Azidomethyl styrene (AzMSt, Figure 1).** 4-Vinylbenzyl chloride (4.9998 g, 32.76 mmol, 1 equivalent) was combined with sodium azide (6.3892 g, 98.28 mmol, 3 equivalents) in dimethylformamide (40 mL, DMF) and stirred at room temperature for 18.5 hours. After the reaction, the product was mixed with 100 mL of deionized water, and extracted three times with diethyl ether (100 mL) to remove any unreacted sodium (Na\(^+\)) or azide (N\(_3^–\)) salts. The collected organic layer was then washed three times with water containing dissolved lithium bromide to deactivate any residual azide groups (Bansal, 1998). The product dissolved in diethyl ether was dried with anhydrous sodium sulfate for thirty minutes. AzMSt was then concentrated via vacuum evaporation, as a yellow liquid at room temperature (5.13 g, 97.5%).

![Figure 1. The reaction scheme for the synthesis of 4-Azidomethyl styrene (AzMSt).](image)

**Synthesis of Copolymer, P(PEGMA-co-DMAEMA) (Figure 2).** PEGMA (6.017 g, 16.7 mmol, 20 mole %) and DMAEMA (10.5115 g, 66.9 mmol, 80 mole %) were dissolved in methyl ethyl ketone (48 mL, 80 weight %, MEK). To this solution the thermoinitiator AIBN (0.0696 g, 0.42 mmol, 0.5 mole %) and RAFT agent (Moad et al., 2010) CPBD (0.2774 g, 1.25 mmol, 1.5 mole %) were added. The mixture was purged with nitrogen (N\(_2\)) thirty minutes before the reaction started and until the reactants reached a sufficiently high conversion (87%).
Initially the reaction was heated to 65°C and allowed to react for 4 hours; however, after 4 hours essentially no reaction had taken place (confirmed by IR) and the temperature was increased to 75°C and allowed to react overnight. The product was isolated via Rotovap® evaporation as a pink, sticky solid (14.63 g, 87%). After the mass of the product was determined, the copolymer was dissolved into acetone to guarantee that it would continue to be soluble in traditional solvents.

\[ \text{Synthesis of Block Copolymer, } \text{P(PEGMA-co-DMAEMA)-b-P(St –co-AzMSt)} \]

(Figure 3). The copolymer (7.3 g, 33.5 mmol), was dissolved into MEK (37 mL) and reacted with St (5.88 g, 56.5 mmol, 3 equiv.) and AzMSt (3.0004 g, 18.9 mmol, 1 equiv.), with AIBN as the initiator (0.031 g, 0.188 mmol, 1 mole %) for 22 hours at 80°C (N₂ purging as before). Once reacted, the solution containing the block copolymer dissolved in MEK was dialyzed (Spectrum Labs, MWCO = 1000) in MEK followed by acetone. The product was then concentrated by Rotovap® and massed (8.75 g, 55%).

**Figure 2.** The reaction scheme for the synthesis of the copolymer, P(PEGMA-co-DMAEMA).
Cross-linking of Block Copolymer (Figure 4). Methanol (13 mL) was added drop-wise to a 20 mL solution (2.28 g) of block copolymer in acetone. To this solution, acetone (17 mL) was added to ensure that the block copolymer was still dissolved. Propargyl ether (0.0538 g, 0.57 mmol, 1 equiv.), the cross-linking agent, was added to the solution while it was magnetically stirred followed by the addition of the copper (II)/PMDETA catalyst (excess). Ascorbic acid was then added to reduce the copper (II) to copper (I). The solution was stirred overnight, with N\textsubscript{2} purging. No byproduct formation occurred, so IR was necessary to confirm that the reaction had indeed occurred. The size of the nanogel (cross-linked block copolymer) was determined using DLS and SEM.
Secondary Polymerization of Nanogel (20%, Figure 5). The nanogel (0.2 g, 2 equiv. in PEGMA) dissolved in acetone (5 mL) was dissolved in DMF (0.8023 g, 80 weight %) and the acetone was removed via Rotovap®. HDI (0.0175 g, 0.104 mmol, 1 equiv.) was added as the cross-linking agent, and tin (II) ethyl hexanoate (trace amount) was the catalyst. The solution was mixed overnight without stirring or N₂ purging. To test if the urethane-forming intra- and inter-particle cross-linking reaction was successful, we inverted the 20 mL vial that contained the initially dissolved nanogel.

Secondary Polymerization of Nanogel (15%, Figure 5). The nanogel (0.15 g, 2 equiv. in PEGMA) dissolved in acetone (~4 mL) was dissolved in DMF (0.85 g, 85 weight %) and the acetone was removed via Rotovap®. HDI (0.0131 g, 0.078 mmol, 1 equiv.) was added as the cross-linking agent, and tin (II) ethyl hexanoate (trace amount) was the catalyst. The solution was mixed overnight without stirring or N₂ purging. To test if the urethane-forming intra- and inter-particle cross-linking reaction was successful, we inverted the 20 mL vial that contained the initially dissolved nanogel.

Secondary Polymerization of Nanogel (10%, Figure 5). The nanogel (0.1 g, 2 equiv. in PEGMA) dissolved in acetone (2.5 mL) was dissolved in DMF (0.90 g, 90 weight %) and the acetone was removed via Rotovap®. HDI (0.088 g, 0.052 mmol, 1 equiv.) was added as the cross-linking agent, and tin (II) ethyl hexanoate (trace amount) was the catalyst. The solution was mixed overnight without stirring or N₂ purging. To test if the urethane-forming intra- and inter-particle cross-linking reaction was successful, we inverted the 20 mL vial that contained the initially dissolved nanogel.
Figure 5. Secondary cross-linking of the nanogel.
Results

*Nuclear Magnetic Resonance (NMR) Imaging.* Analysis of the structure of AzMSt using $^1$H NMR spectroscopy confirmed that we had, in fact, synthesized the product of interest. The structure of AzMSt can be seen superimposed upon its NMR spectra (Figure 6). Each of the chemical shifts, denoted by letters a-e, is represented in the NMR. Mathematical analysis of the integrated spectra indicated that the compound was roughly 90% pure. The spectra for the copolymer, block copolymer, and nanogel are more informative than that of AzMSt: as the process progresses along the polymerization pathway we can use NMR to determine the presence, and absence, of groups we selected for at the beginning of the synthesis.

![Figure 6. NMR of AzMSt](image)

The NMR of the copolymer (Figure 7) has seven peaks that are noteworthy: the double bond (labeled with blue arrows), DMAEMA peaks (blue boxes), and PEGMA (alcohol) peak.
A peak that is not visible in this NMR is one that corresponds to the 7 – 8 ppm chemical shift of CPBD. The presence and characteristics of these peaks evinces that the concentration of the double bonds in the copolymer is low enough to guarantee that the copolymer is pure and the reactants reached a fairly high conversion because the double bond (Figure 2) is converted into a linear chain as DMAEMA and PEGMA react. Additionally, the presence of the DMAEMA peaks indicates that there is an appreciable concentration of DMAEMA in the copolymer; and, because the peaks are broader than those of monomeric DMAEMA (Sigma Aldrich), also confirms that the copolymer is a polymer (long chain length), rather than an oligomer (short chain length). This conclusion is echoed by the PEGMA spectrum, which has a widened alcohol peak, and by the absence of an absorption peak for CPBD. The lack of a CPBD peak indicates that the concentrations of PEGMA and DMAEMA relative to CPBD are high enough that the signal for CPBD, which adds to the ends of polymer chains, cannot be visualized.

Figure 7. NMR spectra of the copolymer, P(PEGMA-co-DMAEMA).
Similarly, the spectra for the block copolymer (Figure 8) indicate that St and AzMSt were successfully added to the existing copolymer chain. The shift that is boxed and labeled with the letter “a” corresponds to the shift of aromatic compounds, which was not present in the NMR of the precursor copolymer (Figure 7). Because there are two aromatic peaks (though not easily distinguishable), we can safely assume that this shift is not solely due to St being added to the chain. Additionally, the signal of the aromatic peak (hydrophobic, integral of peaks in “a”) compared to those corresponding to PEGMA and DMAEMA (hydrophilic, integral of peaks in “b”) can provide the functional ratio of the two, which we calculated to be 5:3 (hydrophilic: hydrophobic), which is the ratio expected, given the ratios of reactants that were used.

Figure 8. NMR of the block copolymer, P(PEGMA-co-DMAEMA)-b-P-(St-co-AzMSt).
The NMR of the nanogel (Figure 9) confirmed that the cross-linking (also called copper azide-alkyne cycloaddition; CuAAC) reaction occurred. As the block copolymer cross-linking reaction (Figure 4) shows, the azide groups of the block copolymer become linked together to form what is called a triazole ring (Figure 9, inset molecule). The propargyl ether used in the reaction allows the azide groups of multiple block copolymer chains to become linked to each other in this tight ring structure, which shields them from the external (hydrophilic) environment. The remainder of the nanogel NMR is characteristic of a structure that has grown in size and, therefore, has many broad chemical shifts compared to its precursors (the copolymer and block copolymer).

![Figure 9. NMR of nanogel after cross-linking reaction.](image)

**Infrared Spectroscopy (IR).** The IR spectra of the block copolymer and nanogel (Figure 10) corroborate the NMR results for the nanogel after the cross-linking reaction (Figure 9). The
spectrum spanning the 2000 – 2200 cm\(^{-1}\) range represents the concentration of azide ("a"), whereas the 1600 – 1800 cm\(^{-1}\) peak (ketone, "b") was used as an internal calibration standard from which we calculated the change in azide concentration. Our calculations demonstrated that the concentration of azide groups was 5% (negligible for this reaction) after the reaction occurred, giving us a final conversion of 95% with respect to triazole ring formation.

Nanogel Characterization

The final portion of our analysis centered on characterizing the nanogel itself, either in isolation or compared to its polymer precursors. The four methods we used each approached this question from a different perspective.
Dynamic Light Scattering (DLS). Using dynamic light scattering techniques allowed us to expose the nanogel (while dissolved in water) to photons (633 nm wavelength) at an angle orthogonal (90°) to our sample. In response to this exposure, the nanogel produces a light scattering pattern from which we calculated the average swollen nanogel diameter. Results from the DLS experiment (Figure 11) indicate that the average swollen nanogel diameter is 29 nm (x-axis value at the vertex of the graph), which suggests that we had good experimental control of our final product.

Figure 11. DLS recording for the swollen nanogel

Scanning Electron Microscopy (SEM). Results from our scanning electron microscopy experiment of the nanogel (Figure 12) informed us of two properties: the dry diameter and hydrogel character of the nanogel. The image we obtained showed that the average dry nanogel
diameter was 20 nm, and that the nanogel could act as a hydrogel because the swollen diameter (DLS result) was greater than 20 nm.

Figure 12. SEM image of the dry nanogel.

Cloud Point. The observation that the nanogel could act as a hydrogel was also supported by the results obtained from our cloud point tests (Tables 1 – 4). A cloud point test involves the dissolution of each of the two polymers (copolymer and block copolymer) and nanogel into three aqueous (water-based) solutions of varying pH. The three solutions had pH levels of 4 (acidic, protonating), 7 (neutral), and 10 (basic, deprotonating). The nine solutions were then heated until the solution became opaque or bubbles formed (100°C, boiling point of water). The quantitative results of the cloud point tests (Table 1) show that the copolymer, block copolymer, and nanogel all behave identically in an acidic solution; however, the nanogel in a neutral or basic solution behaves very differently from its polymer precursors.
These differences can be explained by the behavior of DMAEMA in these three different types of solutions. DMAEMA acts as a base (pKa = 8.4) in solutions of neutral and acidic pH, and deprotonates the hydronium ion (H$_3$O$^+$) or water molecule (H-OH) of the solvent. DMAEMA in its protonated form (Figure 13) is stable and causes the two polymers and nanogel to remain soluble at higher temperatures when in a solution of neutral or acidic pH. For the basic solution, DMAEMA groups on the same polymer structure form links to one another (due to the loss of their electron pairs) and the polymers and nanogel become insoluble at temperatures much lower than those observed for solutions of neutral and acidic pH.

This interpretation can be visually supported by the qualitative cloud point test results (Tables 2 – 4). Moving down the rows and across the columns, the loss of solubility can be seen as an increase in opacity and cloudiness as a function of temperature. These results demonstrate that the DMAEMA functionality, the ability of the polymers and nanogel to be responsive to changes in temperature and pH, has been preserved and enhanced across the stages of polymerization.

<table>
<thead>
<tr>
<th>pH/Polymer or Gel</th>
<th>Copolymer</th>
<th>Block copolymer</th>
<th>Nanogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Above 100</td>
<td>Above 100</td>
<td>Above 100</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>65</td>
<td>Above 100</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>42</td>
<td>58</td>
</tr>
</tbody>
</table>

Figure 13. Protonation of DMAEMA by water of pH 4 (1) and 7 (2).
Table 2. Cloud point test for the copolymer P(PEGMA-co-DMAEMA)-b-P(St-co-AzMSt).

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>40°C</th>
<th>70°C</th>
<th>100°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>![Image]</td>
<td>![Image]</td>
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</tr>
<tr>
<td>7</td>
<td>![Image]</td>
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<tr>
<td>10</td>
<td>![Image]</td>
<td>![Image]</td>
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</tbody>
</table>
Table 3. Cloud point test for the block copolymer, P(PEGMA-co-DMAEMA)-b-P(St-co-AzMS).

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>40°C</th>
<th>70°C</th>
<th>100°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td><img src="bcp_4_40C.png" alt="Image" /></td>
<td><img src="bcp_4_70C.png" alt="Image" /></td>
<td><img src="bcp_4_100C.png" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
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<tr>
<td>10</td>
<td><img src="bcp_10_40C.png" alt="Image" /></td>
<td><img src="bcp_10_70C.png" alt="Image" /></td>
<td><img src="bcp_10_100C.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Table 4. Cloud point test for the nanogel.

<table>
<thead>
<tr>
<th>Solution pH</th>
<th>40°C</th>
<th>70°C</th>
<th>100°C</th>
</tr>
</thead>
<tbody>
<tr>
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<td><img src="ng4.png" alt="Image" /></td>
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<tr>
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<tr>
<td>10</td>
<td><img src="ng10.png" alt="Image" /></td>
<td><img src="ng10.png" alt="Image" /></td>
<td><img src="ng10.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Gravity Test: Macrogelation. Finally, a simple gravity test can be used to demonstrate the potential the nanogel has for inter-particle cross-linking. The results of the gravity test indicate that the critical concentration for nanogel macrogelation is between 10 and 15 weight % (Figure 14). For the 10 weight % nanogel (Figure 14, left) the macrogel point was not reached as
demonstrated by its ability to “flow” even when not inverted. This indicates the 10 weight% nanogel concentration (in DMF) is below the percolation threshold, which leads to limited and covalently interconnected nanogel aggregates that are on the nano- to micrometer scale. The 15 and 20 weight% (right), alternately, do not flow even when fully inverted. These results illustrate the point at which the individual nanogel particles come close enough together that the small cross-linker HDI can cause the alcohol (PEGMA) groups from different nanogels to link together forming a nanogel-based mesh that, as depicted in the picture, is insoluble in any solution. Additionally, the fact that this point is reached for such a low weight % of nanogel also indicates that the distance between the nanogel particles was small before the cross-linking reaction began.

Figure 14. Gravity test for the 10, 15, and 20 weight% nanogels after reaction with HDI.
Discussion

The overall goal of this study was to characterize the behavior of a specific class of nanogels: those with a diameter of 10 nanometers or less. As the DLS (Figure 11) and SEM (Figure 12) results show, JianCheng Liu and I were not successful in making nanogels of this size, though the Stansbury group has been successful in making these nanogels using a direct synthesis pathway (Liu et al., 2012). However, we did successfully synthesize and characterize a class of amphiphilic block copolymer-based nanogels that possess certain properties that make them attractive in ways that can be exploited clinically. Additionally, based on the consolidation and enhancement of certain properties across the stages of polymerization, we have demonstrated that the polymerization process used to synthesize the nanogels and their precursors is more than viable for the synthesis of polymers that are required for a range of biomedical interventions, such as targeted drug delivery (Vinogradov et al., 2002) and dental fillings (Morães et al., 2011).

The clinically relevant properties that these nanogels possess are the selective ability to respond to changes in pH and temperature (cloud point), a non-rigid self-assembled structure that retains internal free volume to allow the nanogel to be functionalized pre- and post-polymerization in a number of locations, the appropriate polarity for gating water into their core, and solvent resistance (gelation) upon secondary polymerization. The relevance of the first property is understandable in the context of how the human body is structured in terms of temperature and pH. For drug related interventions there are a few sites in the body, each with their own pH and temperature range, which are potential target sites for nanogel-mediated drug interventions. The first is the mouth, as we have learned these nanogels act as hydrogels in solutions of physiologic pH (pH regulation) across the entire spectrum of physiological
temperatures (Akin, 2011). The mouth, which has a pH of 5.5, is well within the soluble pH range of the nanogel (4 – 7, Table 4). Given this result, the nanogels could be functionalized to meet the release requirements of the target substrate (Svenson and Prud’homme, 2012), swollen with water containing pharmacological agent of interest, and packaged into a pill or a biomedical device. Additional studies, however, would be necessary to determine the pharmacokinetics of the release mechanism and the pharmacodynamics of the nanogel as it is passed through the body (entry and exit), in addition to determining the appropriate drug concentration for the intervention to work, and its feasibility for the nanogel.

The same entry site, the mouth, can also be used for dental repair and enhancements. As illustrated by Bonomi et al. (2012), nanogels that are water sensitive cannot be used to replace damaged or decaying teeth that are not structurally sound; if the nanogel is still soluble in any solvent (namely saliva) crevices in the nanogel are eroded appreciably over a short time period. One study recently published by Dailing et al. (2013) highlights the importance of solution polymerization (our polymerization technique) in making nanogels that can resist such degrading forces once in situ. This current study reinforces that assertion, showing experimentally that secondary cross-linking of PEGMA with HDI (tin (II) ethyl hexanoate as a catalyst) yields a nanogel that is fully gelled and completely insoluble in any solvent.

Other parts of the body that can be used as nanogel drug delivery sites are blood vessels because, although we did not test the cloud point of the nanogel in the specific pH of blood (7.35 – 7.45), we determined that even at a pH of 10 the nanogel was soluble at 58°C (134.6°F), which exceeds even the highest reported fevers (107°F). In this case, the nanogel treatment would be the same as in an oral administration route, with the exception of the packaging step, because, as a water soluble entity, the nanogel can be injected systemically. However, if the target were the
brain, extra packaging would be necessary to allow the nanogel to pass through the blood-brain barrier, in a manner similar to the approaches mentioned by Douglas and Young (2006). The nanogel we synthesized, though not quite in the size class targeted, can be functionally applied in several areas in the fields of biomedical science and engineering; our study can also act as a foundation from which researchers can begin to develop nanogels in that smallest size range.

Lastly, as mentioned in the introduction, the polymerization process that we used in the preparation of the polymers and nanogel is not the *de facto* polymerization process of many polymer or material scientists. Emulsion, bulk, and suspension polymerization techniques are more often used because they are not labor intensive and they supply the experimenter with large quantities of product in a short period of time, especially compared to solution polymerization with self-assembly included. This study demonstrates that, while the yields of solution polymerization via self-assembly are not as high and the process is time-consuming and labor intensive, the degree to which we were able to select for a pair of behaviors (DMAEMA- and PEGMA-mediated) across all stages of polymerization is a singular advantage of solution polymerization. Solution polymerization with self-assembly, for these reasons, is the best way to engineer particles if they are to be used in a site-specific and directed fashion, as is necessary for dental repair, drug delivery, and tissue engineering.
Conclusions

In summary, our experimental approach and the nanogels that we synthesized did not quite accomplish our initial goals, but they are potentially useful for a number of therapies and future studies. These nanogels can be used to deliver drugs in a spatially and temporally specific manner, once they are functionalized for a transport to a target tissue or cell type, and they can be used to make dental materials that can withstand the deteriorating influences of chewing (mechanical stress) and saliva (chemical erosion). However, future studies are necessary to examine the properties of the nanogels that we attempted to synthesize in this experiment, so as to determine whether they will be well suited to these interventions. Secondly, this study has demonstrated that the solution polymerization process is a process versatile enough to produce biomaterials that will better inform us about diseases like AD, CHD, and PTSD, and perhaps eventually develop cures. Finally, this study provides scientists and corporations with a close examination of the potential utility of this polymerization process, and a thorough description of the ways it can be implemented.
Contributions

The methods and protocols necessary to complete this project covered a broad swath of techniques with which I have become familiar but by no means an expert. I was largely responsible for the synthesis of the materials that were characterized, but JianCheng Liu conducted much of the more technical testing on the various monomers, block copolymers and nanogels I synthesized here. In addition to technical testing, JianCheng helped me with the cloud point tests and gravity tests by taking pictures as changes were observed. JianCheng has also been instrumental in the interpretation of the NMR, DLS, and IR results, which I did not have the background to analyze on my own.

Acknowledgements

I could not have completed this project without the help and support of my thesis advisor, Dr. Jeffrey Stansbury, and my direct supervisor, JianCheng Liu. I would also like to thank Dr. Parag Shah for monitoring my progress through the entire project, and Sandra Medel for her willingness to respond to any and all questions I had about the materials I synthesized. I thank Dr. Jeffrey Stansbury and Dr. Veronica Bierbaum for the time and energy they spent critiquing the many drafts of this paper, their investment has made this paper into the manuscript it is today. I also extend thanks to my thesis committee members, Dr. Ryan Bachtell, Dr. Robert Spencer, Dr. Jeffrey Stansbury, and Dr. Veronica Bierbaum for their continued support and guidance. The overall funding support for the nanogel project was provided from an NIH/NIDCR R01DE022348 grant. On a slightly less conventional note, I would like to extend my thanks to Alison Balsom for recording music without which I would have been unable to complete this thesis.
Key Terms

AD: Alzheimer’s disease
AIBN: Azobisisobutyronitrile, thermoinitiator
AzMSt: 4-Azidomethyl styrene
CHD: Coronary heart disease
CPBD: 2-(Cyano-2-propyl) benzodithioate; RAFT agent
DMAEMA: 2-(Dimethylamino) ethyl methacrylate
DMSO: Dimethyl sulfoxide
DLS: Dynamic light scattering
FT-IR spectroscopy: Fourier-Transform Infrared spectroscopy
Gelation: The point at which a nanogel transitions from a structure composed of discrete unlinked particles to a structure that is composed of units that form intra-particle (micro) or inter-particle (macro) cross-links.

$^1$H NMR: Proton nuclear magnetic resonance
HDI: Hexamethylene diisocyanate, cross-linking agent
MEK: Methyl ethyl ketone
$M_n$: Number-average molecular mass
NaN$_3$: Sodium azide
$N_2$ purge: Purge a container with nitrogen to remove any oxygen, which would prevent product formation.

PEGMA: Poly(ethylene glycol) methacrylate
PTSD: Post-traumatic stress disorder
RAFT agent: Reversible addition/fragmentation chain transfer agent
SEM: Scanning electron microscopy
St: Styrene
References


Appendix

Emulsion polymerization

Solution polymerization


Self-assembly

Polymersomes: Self-Assembled Nanoparticles

Self-Assembly

Polymersomes

Cross-linking


Retrieved from http://www.mrsec.northwestern.edu/content/highlights/triblock.htm
Block copolymer

*diblock copolymers*

*triblock copolymers*

**ABA** type

**ABC** type

(ABCD, ...)

Percolation threshold

Below the Percolation Threshold

Above the Percolation Threshold

- Fill Particle

- Bulk Phase or Matrix
