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Control of Cationic Nanogel PEGylation in Heterogeneous ARGET ATRP Emulsion Polymerization with PEG Macromonomers

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Abstract

Crosslinked cationic nanoscale networks with hydrophobic cores are an environmentally robust alternative to self-assembled polymeric drug delivery carriers with respect to therapeutic encapsulation and stability to dilution. However, the ability to tune the degree of PEG incorporated into nanogels during synthesis is more challenging. In this work, biodegradable cationic nanogels were synthesized by ARGET ATRP emulsion polymerization in a single step. The density of PEG in the final nanogels ranged from zero to 40 wt % and was dependent on the feed concentration of PEG monomer, surfactant concentration, surfactant hydrophilic–lipophilic balance, and the ratio of cationic to nonionic surfactant. A comprehensive analysis of nanogel material properties as a function of PEG graft density is presented including analysis of composition, monomer conversion, thermal properties, size, surface charge, and degradation. This study provides a robust analysis for the synthesis of degradable cationic nanogels via a controlled radical polymerization with predictable degrees of PEGylation.

Keywords

ARGET ATRP; biodegradable; drug delivery; nanogel; poly(ethylene glycol)

INTRODUCTION

Cationic nanomaterials are excellent candidates as carriers for drug and gene delivery because positively charged polymers readily interact with negatively charged cell membranes.¹ Cationic carriers are of interest for delivery of siRNA, miRNA, sgRNA, and even proteins into cells thus forming the next generation of therapeutic delivery.^{2–4} Many natural and synthetic cationic materials have been explored for use as nanoscale carriers in drug and gene delivery including nanoparticles of cyclodextrin, poly(β -amino esters), tertiary amine (meth)acrylates, chitosan, conjugated inorganic materials such as mesoporous silica and gold, and lipid nanoparticles.^{5–12}

Ubiquitously, these carriers rely on polyethylene glycol (PEG) or similar hydrophilic materials to enhance circulation half-life, shield surface charge, and enhance stability in serum. Previous work in the drug delivery field has suggested that the use of PEG chains of at least 2 kDa is required to achieve adequate stealth properties.¹³ However, optimal PEG surface density for nanoparticles spans a wide range and is a balance of attaining a sufficient stealth coating to enhance circulation half-life, yet not too dense to hinder drug release at the site of action.¹³ Reports in the literature suggest PEG densities in the range of 5–20 mol % lead to the optimum PEG balance.¹³

The most common cationic formulations utilized for intracellular drug or protein delivery have been lipid nanoparticles and polymeric micelles.¹⁴ For lipid nanoparticles, PEG content can be modulated by the ratio of PEG-containing lipid incorporated during nanoparticle formation. Similarly, for polymeric micelles, PEG functionalized initiators are commonly utilized in controlled radical polymerizations to prepare diblock or triblock copolymers with a desired amount of PEG, prior to self-assembly.

Crosslinked nanopolymers are often preferred as they do not dissolve.¹⁵ Furthermore, crosslinked polymers containing ionizable groups can be utilized to synthesize nanopolymers with pH dependent swelling responses.¹⁶ However, the direct synthesis of nanoscale crosslinked polymers requires molecular separation of the crosslinking agent from other copolymerizing monomers so that a macroscale network is not formed. Nanoscale networks based upon 2-(diethylamino)ethyl methacrylate undergo a hydrophobic to hydrophilic transition near physiological pH (~7.4). Due to the chemical crosslinks, this transition imparts pH-dependent swelling properties. However, the increased hydrophobicity of the network at physiological pH values must be overcome to achieve colloidal stability in the collapsed state for therapeutic administration.

The synthesis of PEG-containing networks made from hydrophobic monomers typically requires postsynthesis crosslinking or postsynthesis PEGylation.^{17–24} Previously, we have demonstrated in our laboratory the use of an oil-in-water emulsion polymerization for the one-pot synthesis of cationic nanogels with hydrophobic cores and PEG tethers via both UVinitiated and ARGET ATRP schemes.^{25–30} Here, we report the ability to tune the degree of PEG decoration with respect to monomer feed and emulsion characteristics and a thorough characterization of the effect of the degree of PEGylation on the material properties of the biodegradable nanogels.

EXPERIMENTAL

Materials

Poly(ethylene glycol) methyl ether methacrylate solution, henceforth designated as PEGMA2k, with M_n 2000, and in solutions 50 wt % in water, 2-(diethylamino)ethyl methacrylate (DEAEMA, 99%), tert-butyl methacrylate (tBMA, 98%), bis(2methacryloyl)oxyethyl disulfide (DSDMA), myristyl trimethylammonium bromide (MyTAB), ethyl α -bromoisobutyrate (EBiB, 98%), L-ascorbic acid (AA), and triethylamine were purchased from Sigma-Aldrich. Tris(2-pyridylmethyl)amine was purchased from TCI America. Brij 30, Brij 35, copper (II)bromide (991 %), acetone-d₆ (99.8% D), and deuterium oxide (99.8% D) were purchased from Acros Organics.

Tris(hydroxypropyl)phosphine (THP) and tris(2-carboxylethyl) phosphine (TCEP) were purchased from EMD Millipore. Tetrahydrofuran, acetone, hydrochloric acid, hexanes, and methylene chloride were purchased from Fisher Scientific. Poly(ethylene glycol) monomethyl ether monomethacrylate, M_n 1000 (PEGMA1k) was purchased from Polysciences. All chemical were used as received. For reactions in D₂O, PEGMA2k was prepared as described in the literature.³¹

Synthesis and Purification of Cationic Nanogels

Nanogels were synthesized via activators regenerated by electron transfer (ARGET) ATRP polymerization in an oil/ water emulsion polymerization as previously described by Forbes *et al.*²⁸ with modifications. Reactants were charged to 4 mL glass vials, sonicated into an emulsion using a 24-tip microtip sonicator probe, and initiated via the addition of excess ascorbic acid under nitrogen. Reactions were stopped via exposure to air and addition of 4 N HCl to a final concentration of 0.5 N. Nanogels were purified via addition of acid and precipitation with a low dielectric solvent (acetone or THF) as described previously.²⁵ Nanogels were collected via centrifugation at 4,000 rcf for 5 min. After the last cycle, nanogels were suspended in ultrapure deionized water and dialyzed against ultrapure deionized water for 3 days and lyophilized.

Characterization

Nanogel Composition—Lyophilized crosslinked polymer nanoparticles were characterized with Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) Spectroscopy (Thermo Scientific Nicolet iS10) with a germanium crystal. Background spectra were collected immediately before each sample and used for background subtraction. In all cases, spectra were averaged over 64 scans.

Nanogel composition was estimated using ¹H nuclear magnetic resonance (NMR) Spectroscopy. Spectra were obtained using a 400 MHz NMR (Varian Direct Drive 400 or Agilent MR 400) at 25 °C. To determine composition, dried nanogels were diluted in D₂O at 10–15 mg/mL and degraded via addition of tris(2-carboxylethyl)phosphine to a final concentration of 10 mM for 24 h prior to acquisition. Spectra were analyzed on MestReNova 10.0 software via integration of DEAEMA (δ 1.22, 6H), tBMA (δ 1.31, 9H), and PEGMA2k (δ 3.55 ppm, 176H).

Thermal Analysis

Thermal degradation of dried crosslinked nanogels (2–5 mg) was measured with thermal gravimetric analysis (TGA Q500, TA Instruments) from 30 to 550 °C with a 10 °C/min heat rate under nitrogen.

Thermal transitions were measured using dynamic scanning calorimetry (TA Q2000, TA Instruments). A 5 mg sample of dried crosslinked nanogels was tightly packed into an aluminum pan. Samples were heated to 130 °C at 10 °C/min to erase thermal history and cooled to 275 °C at 5 °C/min prior to heating to 155 °C at 10 °C/min.

Monomer Conversion—Conversion was determined via ^1H NMR via dilution of nanogel polymerizations in acetone- d_6 at selected time points. Conversion of each monomer was followed via the disappearance of double bonds normalized to the initial time point utilizing TPMA as an internal standard. DEAEMA (δ 5.60, 1H, δ 6.03 ppm, 1H), tBMA (δ 5.50, 1H, δ 5.92, 1H) PEGMA2k (δ 5.62, 1H, δ 6.06, 1H), TPMA (δ 7.2–8.5, 12H).

Hydrodynamic Size and Electrophoretic Potential—Nanoparticle hydrodynamic diameters and electrophoretic potential were measured using a Malvern ZetaSizer NanoZS with a MPT-2 multi-purpose titrator. The hydrodynamic diameter was determined in 1× PBS from pH 4.0 to 8.5. Each data point was the average of three runs with a minimum of 12 subruns. Electrophoretic potential was measured in 5 mM phosphate buffer from pH 4.0 to 10.0. Each data point is the average of three measurements.

Transmission Electron Microscopy—Transmission electron microscopy images were collected on a FEI Tecnai Transmission Electron Microscope at a magnification of 105,000 ×. Lyophilized nanogels were diluted in ultrapure deionized water at 0.25 mg/mL, deposited on 400 mesh carbon-coated copper grids and stained with 2% uranyl acetate.

Nanoparticle Degradation—Nanoparticle degradation was investigated using dynamic light scattering count rates on a Malvern Zetasizer NanoZS. Nanogels were prepared degraded at 0.5 mg/mL in 1× PBS at pH 7.1 in 10 mM THP. Single 10 s measurements were acquired sequentially at a set attenuation value and measurement height. Data were normalized to the initial time point.

RESULTS AND DISCUSSION

Nanogel Composition

PEGMA2k is a hydrophilic yet bulky monomer. Homopolymerization of this monomer at low degrees of polymerization result in star-like morphologies.³¹ At higher degrees of polymerization, brush type morphologies are observed.³¹ In previous work, we have utilized PEGMA2k macromonomer in heterogeneous emulsion polymerizations to stabilize polycationic nanogels.^{25,28} However, instability of nanoparticles polymerized via ARGET ATRP with this emulsion polymerization method prompted investigation into the extent of PEG incorporation and emulsion characteristics affecting PEG macromonomer conversion.

Nanogel synthesis was conducted with increasing PEGMA2k feed concentration from 0 to 125 mM (Table 1). The PEG monomer was varied independently of the monomers in the organic phase. Concentrations of PEGMA2k higher than 125 mM were not investigated due to viscosity limitations. In this set of reactions, surfactant concentrations, organic phase volume, and organic phase composition were held constant.

Nanogels were synthesized, purified, and lyophilized prior to analysis of PEG content. Overall, increasing the nominal feed concentration of PEGMA2k led to a higher concentration of PEG in the final nanogel. Figure 1 clearly shows the increase in nanogel PEG content in the crosslinked state as determined by FTIR. Spectra were normalized to the characteristic methacrylate ester carbonyl stretching vibration at 1725 cm^{-1} . Methacrylate polymers also have an ester C-O-C stretching vibration at 1142 cm^{-1} . Here, the emergence of the ether asymmetric C-O stretch peak was observed with increasing concentration of PEG in the feed at 1106 cm^{-1} .³²

Analysis of degraded nanogels via ^1H NMR spectroscopy was used to estimate nanogel composition. (Table 1 and Supporting Information, Fig. S1) PEGMA2k mol fractions from 0% to 5.8% (40 wt %) were achieved in the final material by varying PEGMA2k feed concentration from 0 to 125 mM. The ratio of DEAEMA to tBMA remained constant across formulations.

In a similar manner, the feed concentration of PEGMA1k was varied from 0 to 200 mM. As expected, the PEGMA1k macromonomer behaved in a similar manner to PEGMA2k (Supporting Information, Table S1 and Supporting Information, Fig. S2). Higher mole fractions of PEGMA1k were incorporated onto nanogels compared to PEGMA2k with a range of PEG graft density from 0 to 16 mol % (52 wt %). In both cases, the mole fraction of PEG in the final nanoparticle trended linearly with feed concentration (Supporting Information, Fig. S3).

Thermal Analysis of Nanogels

TGA studies were utilized to assess thermal degradation properties of the nanogels (Fig. 2). Mass loss under $100\text{ }^\circ\text{C}$ was attributed to bound water and accounted for 3–7 wt % of the nanogels. The fraction of mass loss between 180 and $285\text{ }^\circ\text{C}$ decreased as nanogel PEG content increased.

This trend was consistent with previous reports of ester group loss or anhydride formation with water liberation as a lower weight fraction of ester groups were present in the samples with higher PEG content.^{29,33} The fraction remaining was 2.8–4.3% and was highest for samples with no PEG. The broadening of the thermal degradation range with increasing PEG content was consistent with previous reports for PEG methacrylate chains.³⁴

DSC was used to determine thermal transitions (Fig. 2). A distinct PEG melting endotherm was present and increased with increasing PEGMA2k feed concentration. In addition, the melting point shifted closer to the homopolymer value ($54\text{ }^\circ\text{C}$) with increasing PEGMA2k feed concentration indicating an increase in the presence of PEG-rich domains.^{29,35}

Monomer Conversion in ARGET ATRP Emulsion Polymerization

Cho *et al.* recently reported that ATRP polymerizations of PEG macromonomers in organic solvents were sensitive to temperature, pressure, and feed concentration.³¹ In their work, concentrations of 50 mM PEGMA2k were required to observe any conversion.³¹ However, previous reports with PEGMA1k in ATRP inverse miniemulsion polymerization showed high conversion levels, indicating that high conversion can be achieved in aqueous reactions.³⁶

To understand the reactivity of the PEG macromonomers in this emulsion polymerization, monomer conversion was followed for nanogels with 0, 25, 50, and 100 mM PEGMA2k nominal feed concentration via ¹H NMR (Fig. 3). The protons of the vinyl peaks separated such that conversion of each monomer was followed independently with ¹H NMR (Supporting Information, Fig. S4). The organic phase monomers reacted to completion over the course of one hour independent of PEG feed concentration. After 4 h, conversion of PEGMA2k was 73, 81, and 92 mol % for 25, 50, and 100 mM feed concentrations, respectively. However, after 24 h, no detectable double bonds remained for any samples by ¹H NMR spectroscopy.

The reaction of PEGMA2k to high conversion in conjunction with the composition of nanogels indicated that a large fraction of the PEGMA2k monomer reacted but was not incorporated into nanogels. Here, Cu^{II}Br₂ and TPMA were used as the transition metal and ligand with EBiB as the initiator. These reagents are identical to those utilized for aqueous ARGET ATRP except for the substitution EBiB for the hydroxyl functionalized initiator 2-hydroxyethyl 2-bromoisobutyrate.²² Thus, it is likely that initiation occurred in the water phase leading to PEGMA2k homopolymers that were removed during purification.

Effect of Ligand, Copper, and Reducing Agent Concentration on Monomer Conversion

A brief investigation of monomer conversion as a function of copper, ligand, and reducing agent ratios was used to determine the influence of ATRP reagents on the emulsion polymerization. A 75 mM PEG2k feed concentration was utilized for all reactions with 150 ppm concentration of Cu^{II}Br₂ (Table 2).

No difference in the conversion of any monomer was observed when the ratio of TPMA was reduced by a factor of eight (Fig. 4). However, when the reducing agent was increased eightfold, DEAEMA reached 86% conversion in 20 min compared to 43% in 20 min for the other two conditions. In addition, PEGMA2k conversion reached 66% in 1 h with the eightfold increase in reducing agent and did not react further. The polymerizations with lower concentration of reducing agent reached full conversion after 24 h.

The increase in the rate of polymerization was consistent with a similar report for PEG graft monomers in miniemulsion with increasing concentrations of hydrazine.³⁷ Furthermore, the lower degree of conversion with an increase in reducing agent is consistent with previous reports of higher termination rate constants in water when using ascorbic acid.³⁸

Effect of Surfactant on Nanogel Composition

PEGMA2k is a very hydrophilic monomer. To participate in the reaction, the monomer must be able to interact at the emulsion boundary layer. As such, the concentration of surfactant relative to the concentration of PEGMA2k as well as the hydrophilic–lipophilic character of the surfactants used will be critical parameters in the final degree of PEGylation. It is important to note that the PEG monomer can also act as a stabilizer.³⁹

Surfactant concentration, the ratio of cationic surfactant to nonionic surfactant, and the ratio of two nonionic surfactant were varied in a set of 18 polymerizations. Surfactant concentration was adjusted between 5 and 40 mg/mL (0.5–4%) (Table 3).

Two primary end-point parameters were compared for each nanogel formulation, namely PEG molar density and collapsed hydrodynamic size. As shown in Figure 5, the degree of PEGylation increased with increasing surfactant concentration from 10 to 40 mg/mL. However, the lowest surfactant concentration did not follow this trend and had a higher degree of PEG of the surface.

At a surfactant concentration of 20 mg/mL, the influence of the ratio of cationic surfactant to nonionic surfactant as well as the ratio of two nonionic surfactants were analyzed. Specifically, 4 weight percentages of cationic surfactant (6.75, 13.5, 27, and 54 wt %) were studied with 3 combinations of nonionic surfactants (100% Brij 30, 100% Brij 35, or 50/50 Brij 30/Brij 35). The data are displayed on a scale of calculated hydrophilic–lipophilic balance (HLB) values using the following values (Brij 30 5 9.7, Brij 35 5 16.9, Mytab 5 22.35).^{40–43}

The best-fit regression for both collapsed hydrodynamic size and PEG incorporation was second order with respect to HLB and first order with respect to cationic surfactant fraction (Supporting Information, Table S2).

Decreasing the cationic surfactant ratio led to higher degree of PEG incorporation into nanogels, but also resulted in larger nanogels in the collapsed state. PEG incorporation into nanogels reached a minimum at an HLB of ~17.5, while collapsed hydrodynamic size reached a minimum at an HLB of ~14.5.

This experiment identified the tradeoff between HLB, surfactant concentration, and surfactant ratios. This approach can be utilized to target a desired degree of PEGylation, while minimizing surfactant concentration and targeting the desired size range for the application of interest.

Nanogel pH-Dependent Swelling and Zeta Potential

PEG is widely known to enhance particle colloidal stability and enhance circulation half-life. To investigate the responsive nature of nanogels with increasing PEG graft density, hydrodynamic size and electrophoretic potential were analyzed as a function of pH (Fig. 6). Nanogels were ~75 nm in the collapsed state and 120 nm in the swollen state.

Nanogels polymerized in the absence of PEG were unstable and aggregated above pH 6.5 (off scale). Nanoparticle with feed ratios of 25 mM resulted in slight aggregation at pH > 6.5

as observed by tailing in the size profile and higher polydispersity. However, formulations with above 50 mM PEGMA2k in the feed were stable over the range of pH tested with a characteristic sigmoidal swelling response and did not display a hysteresis effect upon titration from pH 4 to pH 8.5 and then pH 8.5 to pH 4 (Supporting Information, Fig. S5).

As described in the literature, increased concentrations of PEG will lead to increased charge shielding. At pH 7.4 the apparent zeta potential was ~20 mV compared to 5 mV for 100 mM formulations. Charge shielding has a major influence on cellular interactions, and thus the ability to tune this parameter is crucial. Furthermore, in modulating properties for this scheme, it is important to note that colloidal stability at neutral pH is primarily driven by PEG content and monomer hydrophobicity.

Representative TEM images (Fig. 7) demonstrate the spherical morphology of nanogels with diameters similar to the hydrodynamic diameter of the collapsed state.

Biodegradability of Nanogels

Another crucial parameter for the utility of crosslinked nanoparticle in drug delivery applications is the ability to degrade in response to environmental cues. Disulfide bonds are a popular choice for degradable nanoparticle systems due to the presence of intracellular glutathione.⁴⁴ Here degradable crosslinks were incorporated during polymerization with DSDMA. The degradable characteristics are linked to the degree of PEGylation. Here, that tradeoff is present in the rate of degradation at pH 7.1 in 1× PBS. As shown in Figure 8, as the degree of PEGylation increased the time required to degrade nanogels increased from 2 to 4 days. This trends with the degree of PEG charge shielding observed from zeta potential measurements. Finally, GPC refractive index traces indicate that polymers increase in molecular weight with increasing PEGMA2k in the feed (Supporting Information, Fig. S6).

CONCLUSIONS

Reaction conditions were developed for the tunable one-step incorporation of PEG grafts onto biodegradable pH responsive polymer nanoparticles in dispersed media. PEG incorporation into nanogels increased with increasing PEGMA2k feed and resulted in nanogels that contained up to 40 wt % PEG grafts. The modulation of surfactant chemistry utilized for polymerization further increased the ability to tune the degree of PEG grafts onto the surface of nanogels at a set PEGMA2k feed concentration. The pH-responsive properties of the nanogels demonstrated the tradeoff between the concentration of PEGMA2k needed to impart colloidal stability and the degree of charge shielding. The incorporation of a disulfide crosslinking agent rendered these polymers biodegradable, with biodegradation dependent on the degree of charge shielding. The work detailed here describes a method to tune the amount of PEG on the surface in a single step without the need for additional conjugation reactions or crosslinking steps. This approach will enable the efficient synthesis of cationic nanomaterials with desired material properties for intracellular drug delivery applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

ATR-FTIR spectra of cationic nanogels with varied PEGMA2k feed concentrations from 0 to 125 mM. All spectra are normalized to the ester carbonyl peak at 1725 cm^{-1} . Spectra shifted vertically for clarity.

FIGURE 2.

Thermogravimetric analysis (top) and dynamic scanning calorimetry (bottom) of nanogels synthesized with increasing PEGMA2k molar feed ratios. DSC curves are shifted vertically for clarity.

FIGURE 3.

Conversion of PEGMA2k (■), DEAEMA (▲), and tBMA (◆) as a function of time for nanogels polymerized 0, 25, 50, and 100 mM PEGMA2k in the feed measured via ^1H NMR. Conversion was determined via ^1H NMR via dilution of the reaction in acetone- d_6 .

FIGURE 4.

Conversion of PEGMA2k (■), DEAEEMA (▲), and tBMA (◆) as a function of time for nanogels polymerized 75 mM PEGMA2k in the feed with Cu(II): TPMA: ascorbic acid ratios of 1:8:2 (top), 1:1:2 (middle), and 1:8:16 (bottom) and polymerized under conditions from Table 2. Conversion was determined via ^1H NMR from dilution of the reaction in acetone- d_6 .

FIGURE 6.

Dynamic light scattering and electrophoretic potential of nanogels with PEGMA2k feed concentration 0–125 mM. Hydrodynamic size was measured with nanogels in 13 PBS at 0.5 mg/mL from pH 4 to pH 8.5. Nanogels synthesized with 0 mM PEGMA2k aggregated significantly and are off scale. All data points are the average of three measurements with a minimum of 12 subruns for each measurement. Electrophoretic potential was measured for nanogels at a concentration of 0.5 mg/mL in 5 mM phosphate buffer from pH 4 to 10. All data points are the average of 3 measurements.

FIGURE 7.

Transmission electron microscopy images nanogels with increasing PEG content (top left to right 0, 25, and 50 mM; bottom left to right 75, 100, and 125 mM) Nanogels were suspended at 0.25 mg/mL in ultrapure DI water and deposited on 400 mesh carbon-coated copper grids and stained with 2% uranyl acetate. Images were taken at 105,000 $\times$ magnification. Scale bar represents 100 nm.

FIGURE 8.

Nanogel degradation in the presence of reducing agent. Dynamic light scattering count rate was measured sequentially for nanogels with 0, 50, or 100 mM PEGMA2k feed concentrations at 0.5 mg/mL in 1× PBS pH 7.1 with 10 mM THP. Data points represent single 10 s runs normalized to the initial time point.

Table 1

Molar Feed Ratio and Composition of Cationic Nanogels Synthesized with Increasing Feed Concentrations of PEGMA2k

[PEGMA2k] ₀ (mM) ^a	Nominal Molar Feed Ratio			Nanogel Molar Composition (¹ H NMR)		
	DEAEMA	tBMA	PEGMA2k	DEAEMA	tBMA	PEGMA2k
0	75.0	25.0	0.0	75.3	24.7	0.0
25	70.5	23.5	6.0	72.9	26.4	0.7
50	66.5	22.2	11.3	73.1	25.1	1.7
75	63.0	21.0	16.1	71.4	25.0	3.6
100	59.8	19.9	20.3	72.4	23.6	3.9
125	56.9	19.0	24.2	70.9	23.2	5.8

^aAll reactions were carried out at 25 °C for 16 h. For all reactions, the organic phase was maintained at 7.5 vol % and had a constant composition [I]₀/[DEAEMA]₀/[tBMA]₀/[DSDMA]₀ 54/75/25/1.25. The concentration of catalyst was maintained at [Cu^{II}Br₂]₀ = 150 ppm based on total volume with the following ratios [TPMA]₀/[Cu^{II}Br₂]₀/[AA]₀ 58/1/2. Surfactant concentration was held constant at 20 mg/mL with 5.4 mg/mL MyTAB, 7.3 mg/mL Brij 30, and 7.3 mg/mL Brij 35.

Table 2

Molar Ratios of ARGET ATRP Reagents Used for Synthesis of Cationic Nanogels

Molar Ratios for ARGET ATRP			
Entry ^a	Cu(II)	TPMA	AA
1	1	8	2
2	1	1	2
3	1	8	16

^aAll reactions were carried out at 25 °C for 24 h in deuterium oxide with an initial concentration of 75 mM PEGMA2k. The concentration of copper was maintained at [Cu^{II}Br₂] 0.5150 ppm based on total volume. The organic phase was maintained at 7.5 vol % and had a constant composition [I]/[DEAEMA]/[tBMA]/[DSDMA] 0.54/75/25/1.25. Surfactant concentration was held constant at 20 mg/mL with 5.4 mg/mL MyTAB, 7.3 mg/mL Brij 30, and 7.3 mg/mL Brij 35.

Table 3

Surfactant Concentrations Used for the Polymerization of Nanogels

Entry ^a	MyTAB	Brij 30	Brij 35	Total
1	1.35	1.83	1.83	5
2	2.7	3.65	3.65	10
3	5.4	7.3	7.3	20
4	5.4	7.3	7.3	20
5	5.4	7.3	7.3	20
6	8.1	10.95	10.95	30
7	10.8	14.6	14.6	40
8	1.35	18.65	0	20
9	1.35	9.33	9.33	20
10	1.35	0	18.65	20
11	2.7	17.3	0	20
12	2.7	8.65	8.65	20
13	2.7	0	17.3	20
14	5.4	14.6	0	20
15	5.4	0	14.6	20
16	10.80	9.20	0	20
17	10.80	4.60	4.60	20
18	10.80	0	9.20	20

^aAll reactions were carried out at 25 °C for 16 h. For all reactions, the oil phase was maintained at 7.5 vol % and had a constant composition $[I]_0/[DEAEMA]_0/[tBMA]_0/[DSDMA]_0 = 4/75/25/1.25$ with $[PEGMA2k]_0 = 100\text{mM}$. The concentration of transition metal was maintained at $[Cu^{II}Br_2]_0 = 150\text{ ppm}$ based on total volume with the following ratios $[TPMA]_0/[Cu^{II}Br_2]_0/[AA]_0 = 8/1/2$.