Macromolecules

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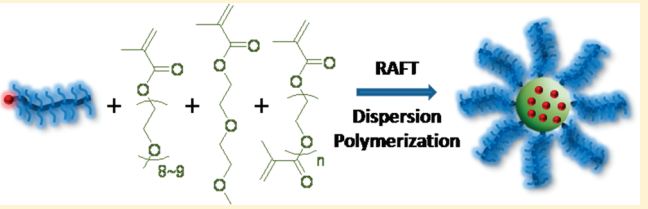
Biocompatible, Antifouling, and Thermosensitive Core−Shell Nanogels Synthesized by RAFT Aqueous Dispersion Polymerization

Wenqing Shen, Yanli Chang, Guangyao Liu, Haifang Wang, Aoneng Cao,* and Zesheng An*

Institute of Nanochemistry and Nanobiology, College of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, P. R. China

Supporting Information

ABSTRACT: Reversible addition−fragmentation chain transfer (RAFT) aqueous dispersion polymerization was used to synthesize a novel type of core−shell nanogel containing linear poly(ethylene glycol) (PEG) and/or nonlinear polymers with oligo(ethylene glycol) side chains. These nanogels with low polydispersities were synthesized efficiently with tunable sizes and thermosensitivities. The nanogels containing nonlinear polymers with oligo(ethylene glycol) side chains as the shell had enhanced stability during freeze−thawing process and in biologically relevant solutions including 1.5 M NaCl, 1% bovine serum albumin (BSA) and 100% fetal bovine serum (FBS) solutions. Aminolysis and hydrolysis of the chain transfer agents (CTAs) in the nanogels were studied and the nanogels exhibited enhanced stability in comparison with molecularly dissolved polymers. The chemical stability of the CTAs in the nanogels was well-correlated with the in vitro cell viability studies of the nanogels using lung cancer cells.



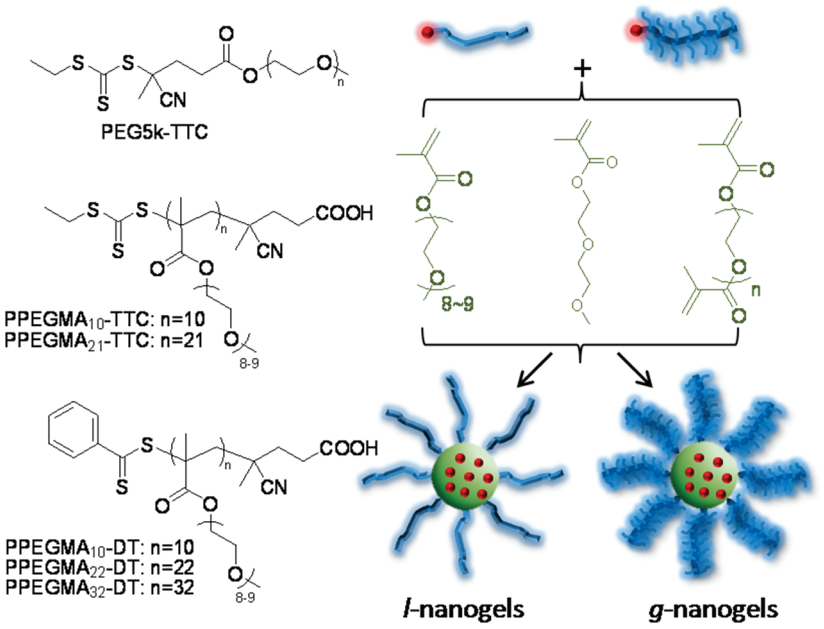
INTRODUCTION

Hydrogels, networks of cross-linked hydrophilic polymers, are an important class of soft materials with many of their properties similar to those of biological tissues. While these macroscopic materials have been intensively studied and exploited in various technological areas, their nanosized counterparts, the so-called nanogels, are currently attracting significant attention for their potential use in advanced technologies such as targeted drug delivery and imaging.[1−8] Nanogels suitable for drug delivery or imaging should be biocompatible and nontoxic, antifouling, biodegradable (if the size is above the renal threshold), and highly specific for target cells, to name but a few. However, meeting all these critical requirements still represents a grand challenge for clinical application of nanogels in particular and polymer nanoparticles in general.[9,10] Therefore, optimizing the design and synthesis of novel nanogels suitable for drug delivery is an important ongoing theme across several interdisciplinary areas.[11]

In designing nanogels with desirable characteristics for drug delivery, linear poly(ethylene glycol)s (*l*-PEGs) and their nonlinear analogues such as polymers derived from oligo(ethylene glycol) (meth)acrylates (*g*-PEGs), are particularly attractive compositional polymers. Indeed, *l*-PEGs are frequently used to coat nanoparticles to prolong the blood circulation time by blocking adhesion of opsonins to the nanoparticles.[12,13] *g*-PEGs, synthesized by polymerization of oligo(ethylene glycol) (meth)acrylates, have been increasingly studied during the past several years.[14−16] *g*-PEGs share many important features of *l*-PEGs such as being highly hydrophilic and antifouling. For example, they have been used to decorate flat or nanoparticle surfaces to confer antifouling properties.[17−19] Also *g*-PEGs were proposed as alternatives for PEGylation of proteins and RNAs.[20,21] A recent study demonstrated that significantly improved pharmacokinetics (41-fold increase) was achieved for myoglobin conjugated with *g*-PEGs compared with the bare protein.[22] Importantly, *g*-PEGs have intriguing unique assets that *l*-PEGs do not possess.[14] *g*-PEGs are synthesized from (meth)acrylates via versatile radical polymerization processes, thus their molecular weights, architectures and functionalities can be easily tailored, especially via the controlled/living radical polymerization processes.[16,23−31] Furthermore, by varying the length of the side oligo(ethylene glycol) chains or by copolymerizing two monomers of different side chain lengths, *g*-PEGs can be made thermosensitive. Lutz showed that thermosensitive *g*-PEGs have properties rivalling those of poly(*N*-isopropylacrylamide),[32] one of the mostly studied thermosensitive polymers.[33,34] These thermosensitive polymers were used to thermally switch wetting properties when coated onto surfaces.[17,35] Recently, hyperbranched thermosensitive copolymers based on *g*-PEGs were reported by Davis and co-worker using reversible addition−fragmentation chain transfer (RAFT) polymerization,[36] and by Tai and Wang using *in situ* deactivation enhanced atom transfer radical polymerization (ATRP).[37] In addition, Alexander and co-workers reported that such *g*-PEGs can have dual thermo- and ion-responsiveness.[38] Microgels based on *g*-PEGs have also been reported. Hu and co-workers

Received: January 13, 2011
Revised: March 15, 2011
Published: March 25, 2011

Scheme 1. Macro-CTAs and the Formation of Core−Shell Nanogels via RAFT Dispersion Polymerization



prepared thermosensitive microgels of *g*-PEGs using traditional free radical precipitation polymerization with the aid of surfactants,[39−41] and Zhou and co-workers studied the potential of such microgels for drug delivery applications.[42] Matyjaszewski and co-workers reported thermosensitive microgels of *g*-PEGs using ATRP in miniemulsion using anisole as the oil phase.[43,44]

While there have been a few reports on microgels based on *g*-PEGs as mentioned above, they were either prepared via traditional free radical polymerization stabilized by labile surfactants or prepared using organic media. Architecturally well-defined nanogels with controlled formation of core and shell polymers prepared in water have yet to be developed. Among the several methods used for the preparation of nanogels,[2,3] surfactant-free, RAFT-mediated dispersion/precipitation polymerization using hydrophilic macromolecular chain transfer agents (Macro-CTAs) can efficiently generate nanogels with controlled core−shell architecture and spatially defined functional groups, in water and at high solids content. One of us used this strategy to prepare thermosensitive nanogels of poly(*N*-isopropylacrylamide).[45,46] Later, Charleux and co-workers reported thermosensitive PEGylated nanogels composed of poly(*N*,*N*-diethylacrylamide) using the same strategy,[47] while Li and Armes reported the formation of sterically stabilized nanolatexes and vesicles by RAFT dispersion polymerization of 2-hydroxypropyl methacrylate.[48]

Herein we report on a novel type of core−shell nanogel consisting of *l*-PEG and/or *g*-PEGs that is biocompatible, thermosensitive, and antifouling. These nanogels were synthesized via RAFT-mediated aqueous dispersion polymerization of di(ethylene glycol) methyl ether methacrylate (MEO_2MA) or copolymerization of MEO_2MA with poly(ethylene glycol) methyl ether methacrylate (M_n = 475) (PEGMA) (Scheme 1).

EXPERIMENTAL SECTION

Materials. α-Bromoisobutyric acid (98%), 4,4′-azobis(4-cyanovaleric acid) (98+%), poly(ethylene glycol) methyl ether (PEG, M_n = 5000), di(ethylene glycol) methyl ether methacrylate (MEO_2MA, 95%), poly(ethylene glycol) methyl ether methacrylate (PEGMA, M_n = 475), poly(ethylene glycol) dimethacrylate (PEGDMA, M_n = 750), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 99%), and 2,2′-azobis(2-methylpropionamidine)dihydrochloride (V-50, 97%) were purchased from Sigma-Aldrich and were used as received unless otherwise noted. 4-(Dimethylamino)pyridine (DMAP, 99%) from Alfa Aesar and ethanethiol (97+%) from Fluka were used as received. 2,2′-Azobis(isobutyronitrile) (AIBN, chemical grade, Sinopharm Chemical Reagent Co. Ltd.) were recrystallized twice from methanol prior to use. All monomers were passed through a column of Al_2O_3 to remove the inhibitor prior to polymerization.

Characterization. UV−vis absorption spectra were collected on a Shimazu UV-1800 spectrometer. Gel permeation chromatography (GPC) was performed on a Waters 1525 system equipped with two PLgel mix-D columns (bead size 5 μm, exclusion limit 400 000), a Waters 2414 refractometer, using dimethylformamide with 0.5 M LiBr as the eluent at a flow rate of 1 mL/min at 80 °C. Molecular weight and polydispersity index of polymers were calculated using PEO standard (TOSOH). NMR spectra were collected on a Bruker AV 500 MHz spectrometer and chemical shifts were reported using the solvent residue as the reference. Dynamic light scattering (DLS) was performed on a Malvern Zetasizer 3000HSA at 25 °C. Variable temperature DLS was performed on a Malvern ZS90. Atomic force microscopy (AFM) was performed on a Shimadzu SPM-9600 in the tapping mode.

Synthesis of *g*-PEG Macro-CTAs. The *g*-PEG Macro-CTAs (PPEGMAs) were synthesized via RAFT mediated solution polymerization in ethanol or dioxane using the corresponding CTAs (Scheme 1). As an example for the synthesis of $PPEGMA_{32}$-DT, 4-cyano-4-(ethylsulfanylthiocarbonyl)sulfanylpentanoic acid (82 mg, 0.295 mmol), PEGMA (7.00 g, 14.737 mmol), AIBN (12 mg, 0.0731 mmol), 1,3,5-trioxane (0.200 g, internal standard), and ethanol (15 mL) were added into a 50 mL two-necked flask. After degassed with nitrogen for 40 min in an ice−water bath, the flask was dipped into an oil bath heated at 70 °C. The polymerization was terminated after 3.5 h at the monomer conversion of 64% by cooling the flask with an ice−water bath. The polymerization mixture was diluted with a minimum amount of

Table 1. Parameters of Nanogels Synthesized by RAFT Aqueous Dispersion Polymerization, [MEO_2MA] = 0.053 mol/L, Macro-CTA:PEGDMA:V-50 = 1:3:0.4, 70 °C

entry	Macro-CTA	DP,[a] MEO_2MA	DP,[a] PEGMA	diameter[b] D_h (nm)	PDI[b]	swelling ratio[c]
1	PEG5k-TTC	100	-	54	0.16	
2		150	-	56	0.06	
3		200	-	64	0.13	
4		150	7.5	62	0.11	1.2
5		150	15	82	0.15	1.1
6		150	22.5	81	0.14	1.2
7	$PPEGMA_{21}$-TTC	150	-	154	0.10	
8	$PPEGMA_{22}$-DT	150	-	73	0.09	
9		150	-	52	0.12	
10		200	-	61	0.06	1.3
11[d]		200	-	73	0.09	
12[e]		200	-	gel	-	
13	$PPEGMA_{32}$-DT	250	-	82	0.04	
14		190	10	68	0.08	1.4
15		180	20	61	0.08	
16		170	30	53	0.19	
17		160	40	*f*	*f*	

[a] Number-average degree of polymerization (DP). [b] Dynamic light scattering results measured at 25 °C. [c] Swelling ratio = hydrodynamic diameter in the swollen state/hydrodynamic diameter in the collapsed state. [d] [MEO_2MA] = 0.106 mol/L. [e] [MEO_2MA] = 0.159 mol/L. [f] No measurable results.

dichloromethane and was then precipitated into *n*-hexane to yield a viscous red material. The precipitation process was performed three times. After drying under vacuum, 2.7 g of a viscous red material was obtained in 60% yield. ^{1}H NMR (500 MHz, $CDCl_3$): 7.86−7.35 ppm (m, C_6H_5−), 4.06 ppm (s, $COOCH_2$−), 3.78−3.50 ppm (m, $-O(CH_2)_2O-$), 3.37 ppm (s, $-OCH_3$), 2.0−1.5 ppm (backbone $-CH_2-$), 1.0−0.8 ppm (s, $-CH_3$). M_n = 5300, M_w/M_n = 1.10, by GPC; M_n = 15500 by ^{1}H NMR.

General Procedure for the Synthesis of Nanogels Using MEO_2MA Monomer. The polymerizations of MEO_2MA were performed in water at 70 °C using V-50 as the initiator, PEGDMA as the cross-linker. The molar ratio of Macro-CTA, PEGDMA, and V-50 (1:3:0.4) was kept constant, and the molar ratio between Macro-CTA and the monomer was varied to prepare nanogels of different size and thermosensitivity. A general procedure for the synthesis of nanogels using MEO_2MA monomer is as follows. PEG5k-TTC (18.6 mg, 3.55 μmol), MEO_2MA (105.0 mg, 0.531 mmol), PEGDMA (8.0 mg, 10.63 μmol), and water (10 mL) were added in a 25 mL two-necked flask. The solution cooled with an ice−water bath was degassed with nitrogen for 40 min, which was then placed in a preheated oil bath (70 °C) under stirring. After the temperature was stabilized, degassed V-50 (50 μL, 28.4 mM) solution was injected. The polymerization was allowed to continue under protection of nitrogen for 4 h, which was finally quenched by exposing to air and immersing the flask into iced water. A bluish dispersion was obtained. D_h = 58.6 nm, PDI = 0.08, at 25 °C.

General Procedure for the Synthesis of Nanogels Using MEO_2MA and PEGMA Comonomers. The copolymerizations of MEO_2MA and PEGMA were performed in water at 70 °C with V-50 as the initiator and PEGDMA as the cross-linker. The molar ratio of Macro-CTA, MEO_2MA, PEGDMA, and V-50 was kept constant (1:150:3:0.4), and the molar ratio between MEO_2MA and PEGMA was varied. A general procedure for the synthesis of copolymer nanogels is as follows. PEG5k-TTC (18.6 mg, 3.55 μmol), MEO_2MA (105.0 mg, 0.531 mmol), PEGMA (25.2 mg, 53.13 μmol), PEGDMA (8.0 mg, 10.63 μmol), and water (10 mL) were added in a 25 mL two-necked flask. The solution cooled with an ice−water bath was degassed with nitrogen for 40 min. Then the flask was placed in a preheated oil bath (70 °C) under stirring. After the temperature was stabilized, degassed V-50 (50 μL, 28.4 mM) solution was injected. The polymerization was allowed to continue under protection of nitrogen for 4 h, which was finally quenched by exposing to air and immersing the flask into iced water. A bluish dispersion was obtained. D_h = 82.5 nm, PDI = 0.14, at 25 °C.

Synthesis of *t*-Nanogels Using Traditional Free Radical Polymerization. MEO_2MA (0.642 g, 3.4 mmol), PEGDMA (18.6 mg, 92.0 μmol), sodium dodecyl sulfate (8.0 mg, 30.0 μmol), and water (49.9 g) were added into a 100 mL two-necked flask. After the solution was degassed with nitrogen for 40 min in an ice−water bath, the flask was placed in a preheated oil bath (70 °C) under stirring. After the temperature was stabilized, degassed potassium persulfate solution (0.1 mL, 0.69 M) was injected. The polymerization was allowed to continue under protection of nitrogen for 6 h, which was finally quenched by exposing to air and immersing the flask into iced water. The as-prepared nanogel dispersion was purified via dialysis (MWCO = 14 000) by changing water twice everyday for a week. A white dispersion with a bluish hue was obtained. D_h = 76 nm, PDI = 0.07, at 25 °C.

In Vitro Cytotoxicity Assay. A549 cells were cultured in F-12K culture medium supplemented with 10% (v/v) fetal bovine serum (Lanzhou National Hyclone Bioengineering Co. Ltd., China) at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Cell viability was evaluated by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma). A549 cells were plated in 96-well plates (3×10^3 cells per well) and incubated for 24 h. Polymer samples were introduced separately to cells with different dose concentrations (0.5, 1, and 2 mg/mL) in the culture medium. Cells cultured in the medium without adding polymers were taken as the control. After 48 h incubation, the media was removed. Then, 100 μL of 0.5 mg/mL MTT solution (MTT stock solution diluted with culture medium) was added to each well and incubated for 4.5 h at 37 °C. Then, 100 μL lysis solution (10%(w/v)SDS, 5% (w/v) isobutanol, 0.012 mol/L HCl) to each well was added and left overnight at 37 °C. The optical density (OD) of each well at 570 nm was recorded on a Microplate Reader (Thermo, Varioskan Flash). The cell viability (relative to the control) is expressed as the percentage of $(OD_{test} - OD_{blank})/(OD_{control} - OD_{blank})$, where OD_{test} is the optical density of the cells exposed to polymer samples,

$OD_{control}$ is the optical density of the control sample and OD_{blank} is the optical density of the wells without A549 cells, respectively.

RESULTS AND DISCUSSION

In order to synthesize biocompatible and antifouling nanogels, we chose to employ Macro-CTAs of *l*-PEG (PEG5k-TTC[49]) and *g*-PEGs (PPEGMA$_x$-TTC and PPEGMA$_x$-DT, where x represents number-average degree of polymerization, TTC stands for trithiocarbonate and DT stands for dithioester) as both stabilizing polymers for nanogels and RAFT-mediating agents, to elucidate the effects of *l*-PEGs and *g*-PEGs in the stabilization, antifouling property of the nanogels. PPEGMA-TTC and PPEGMA-DT with different molecular weights and low polydispersity indices were synthesized by polymerization of PEGMA using the corresponding CTAs (Table S1, Supporting Information). In dispersion polymerization, the Macro-CTAs, monomers (MEO_2MA, PEGMA and cross-linker PEGDMA) and initiator (V-50) form a homogeneous solution. Polymers of MEO_2MA and its copolymers with PEGMA are thermosensitive with the lower critical solution temperature (LCST) being 26 °C for $PMEO_2MA$ and in the range of 26−90 °C for their copolymers, depending on the composition.[14] It is therefore expected that as the polymerization proceeds, chain extension to the Macro-CTAs occurs. When the second block grows beyond a critical length, the polymers collapse from the solution to form core−shell nanoparticles as the polymerization temperature (70 °C) is higher than the LCST. The nanogels are composed of *l*-PEGs or *g*-PEGs as the shell and poly(MEO_2MA-*co*-PEGDMA) or poly(MEO_2MA-*co*-PEGMA-*co*-PEGDMA) as the core. It is noticeable that these nanogels have a high percentage of ethylene glycol units in the range of 61−68 wt % and thus excellent biocompatibility is expected. Nanogels prepared using *l*-PEG (PEG5k-TTC) and *g*-PEG (PPEGMA-TTC and PPEGMA-DT) Macro-CTAs are denoted as *l*-nanogels and *g*-nanogels, respectively. For comparison, we also used traditional free radical precipitation polymerization with the aid of surfactant to make nanogels without shell stabilizing polymers,[39] and these nanogels are denoted as *t*-nanogels (D_h = 76 nm, PDI = 0.07).

The dispersion polymerizations proceeded with high efficiency and no induction period was observed. 94% conversion was achieved in 3 h using PEG5k-TTC containing the trithiocarbonate group, whereas 5 h were required for PPEGMA-DT bearing the dithioester group to reach a similar conversion (93%) (Figure S1, Supporting Information). In most cases, nearly monodisperse nanogels (Table 1) of 50−80 nm were obtained, a size range well suited for drug delivery to cancerous tissues.[5,9] Recently, Lyon demonstrated that microgels can pass through pores of size 10 times smaller under pressures of renal filtration due to the excellent flexibility and compressibility of microgels.[50] It is therefore reasonable to infer that small-sized nanogels may likely go through renal filtration (~8 nm) without the incorporation of any degradation mechanism, which not only simplifies the design of nanogels but also preempts any undesirable byproducts from degradation processes. The molecular weights of *g*-PEGs can be easily controlled such that the number-average degree of polymerization (DP) on the stabilization effect of Macro-CTAs can be investigated. With increasing DP, the size of nanogels becomes smaller (entries 8, 9), providing a means for tuning the size of nanogels. However, no well-defined nanogels could be obtained using PPEGMA$_{10}$-TTC and PPEGMA$_{10}$-DT with a low DP (DP = 10). The size of nanogels can also be tuned by

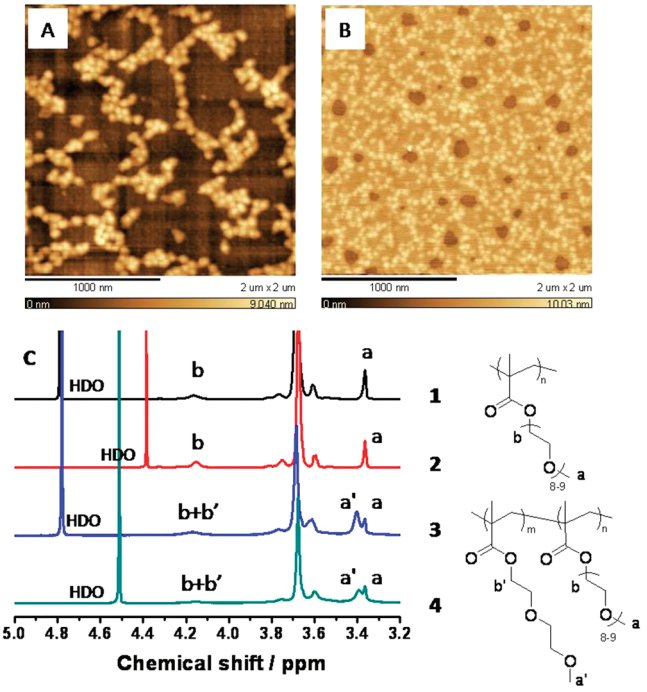


Figure 1. Atomic force microscopy (AFM) micrographs: (A) *l*-nanogel (entry 2, Table 1); (B) *g*-nanogel (entry 13). (C) Variable temperature ^{1}H NMR spectra in D_2O for (1) PPEGMA$_{32}$-DT at 22 °C, (2) PPEGMA$_{32}$-DT at 50 °C, (3) *g*-nanogel (entry 9, Table 1) at 23 °C and (4) *g*-nanogel (entry 9, Table 1) at 40 °C. HDO peaks at different temperatures were corrected.[51]

varying the molar ratio of Macro-CTA/monomer. For example, the nanogel size is tuned from 52 to 82 nm when the molar ratio of PPEGMA$_{32}$-DT/MEO_2MA is increased from 1:150 to 1:250 (entries 9, 10, and 13). Increasing the concentration of the polymerization system leads to an increase in the nanogel size (entries 10 and 11), but further increase in concentration results in the formation of macrogels (entry 12). Dispersion copolymerization of MEO_2MA and PEGMA of adjustable molar ratios leads to nanogels with tunable thermosensitivity. The molar ratio of the monomers also affects the size of nanogels. When the incorporation of PEGMA is high (entry 17), no nanogels are formed possibly due to the significantly increased hydrophilicity of the resulting polymers which could not collapse to form nanogels at the polymerization temperature.

Variable temperature ^{1}H NMR spectra were recorded in D_2O to investigate the thermosensitivity of the nanogels (Figure 1C). As an example, PPEGMA$_{32}$-DT remains well solvated both at 22 and 50 °C, as the peaks remain unchanged at both temperatures. For the nanogels composed of PPEGMA (PPEGMA$_{32}$-DT) as the shell and $PMEO_2MA$ as the core, the characteristic peaks corresponding to both polymers are visible with the methoxy groups for PPEGMA at 3.36 ppm and those for $PMEO_2MA$ at 3.40 ppm at 23 °C. When the temperature is increased to 40 °C, while the peak at 3.36 ppm remains unchanged, the peak at 3.40 ppm undergoes a noticeable reduction in intensity due to the dehydration of the $PMEO_2MA$ core. Meanwhile, the ester group peak at 4.16 ppm also exhibits a significant decrease in intensity because $PMEO_2MA$ accounts for about 80% of the total signal. For the nanogels, the fact that PPEGMA remains well solvated while $PMEO_2MA$ experiences thermally induced dehydration at elevated temperature also confirms that the Macro-CTAs have a

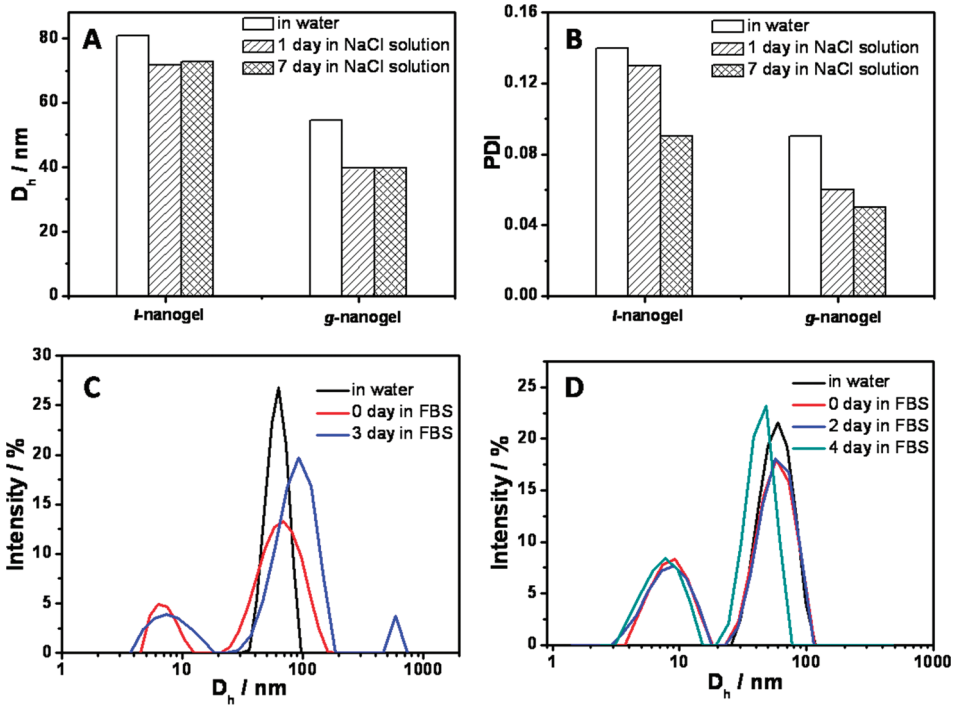


Figure 2. Dynamic light scattering (DLS) results of nanogels in NaCl solution (A and B) and FBS (C for *l*-nanogels and D for *g*-nanogels). In parts C and D, the peak below 10 nm was from FBS.

stabilization function for the nanogels which are characterized by a hydrophilic shell and a thermosensitive core.

For practical drug delivery or imaging applications, the ability of the nanogels to survive the formulation processes and their stability in biologically relevant milieux is of primary importance. In order to establish the rational design strategy of our core—shell nanogels mainly composed of ethylene glycol units, we performed a series of experiments to study the stability of the nanogels.

Freeze-thawing or freeze-drying is a technique frequently used in the formulation of drug delivery systems. Even for PEGylated nanoparticles, cryoprotectants are needed to prevent aggregation of nanoparticles.[52−54] We performed dynamic light scattering (DLS) measurements for the three types of nanogels, i.e., *t*-nanogels, *l*-nanogels, and *g*-nanogels, without the addition of surfactants during freeze—thawing to compare their ability to survive the process (Table S2, Supporting Information). Both *t*- and *g*-nanogels survived freeze—thawing as evidenced by the fact that the nanogel size was essentially unchanged after freeze—thawing. However, for the *l*-nanogels tested, most of them underwent an increase in size and polydispersity. The *t*-nanogels (after removal of surfactant) are stabilized by the charged radical fragments derived from the initiator potassium persulfate. The combination of charge and hydrophilicity of the nanogels may together contribute to the stability of the *t*-nanogels during freeze—thawing process. *l*-PEGs are known to partially crystallize during freezing and as a consequence the nanoparticles coated with *l*-PEGs become resistant to redispersion.[54−57] In contrast, *g*-PEGs with multiple side chains of oligo(ethylene glycol) have a rich collection of conformations, which effectively prevents the crystallization process and thus a better stabilizing effect is achieved during freeze—thawing.

Next we investigated the stability of the nanogels in biologically relevant milieux by mixing a portion of aqueous dispersion of nanogels with 1.5 M NaCl solution, 1% bovine serum albumin (BSA) and 100% fetal bovine serum (FBS). While the *t*-nanogels flocculated shortly upon dispersion in all three types of solutions, the core—shell nanogels exhibited significantly enhanced stability. Both *l*- and *g*-nanogels had a long-term of stability (more than three months) in NaCl solution. Both *l*- and *g*-nanogels shrunk when transferred from water to NaCl solution due to dehydration and their PDI decreased due to their more compact nature (Figure 2, parts A and B). Both *l*- and *g*-nanogels remained stable in BSA solution for a week but flocculated afterward. In FBS, *g*-nanogels showed a superior stability as they were stable for 4 days while *l*-nanogels started to aggregate on the third day as indicated by the appearance of a larger size around 1 μm (Figure 2, parts C and D). The higher stability and thus the better antifouling capability of the *g*-nanogels suggest that the architecture of the PPEGMA shell can better protect against biomacromolecules.

Our nanogels are composed of *l*-PEGs and/or *g*-PEGs, both of which are nontoxic. However, these nanogels are prepared via RAFT polymerization and the CTAs for RAFT polymerization have been a concern of biosafety because they are susceptible to reactions such as hydrolysis.[58,59] Unlike molecularly dissolved polymers, the nanogels are 3D cross-linked, nanosized networks with the CTAs buried within. On the basis of this architectural difference, we envisioned that the nanogel architecture should present a large steric hindrance to the reactions of the CTAs. To test this hypothesis, we carried out aminolysis reaction with 20 folds of ethanolamine at 4 and 37 °C, where the nanogels are either swollen or collapsed, respectively (Figure S4, Supporting Information). At 4 °C, it took 5 days and 10 days, respectively, for $PPEGMA_{32}$-DT and *g*-nanogels to be completely aminolyzed. At 37 °C, a drastic reduction (~72%) in the absorption was seen within the first 7 h for $PPEGMA_{32}$-DT and it took 52 h for $PPEGMA_{32}$-DT to be completely aminolyzed. The reduction in

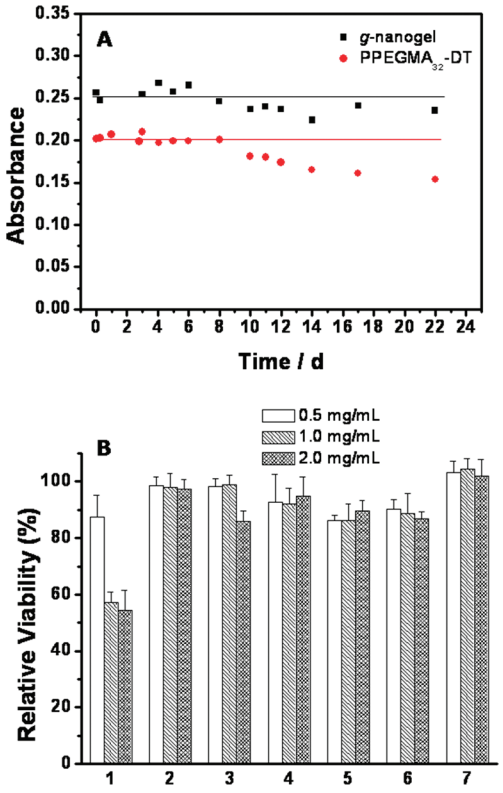


Figure 3. (A) Hydrolysis of the dithioester group in *g*-nanogel and $PPEGMA_{32}$-DT in 0.1 M PBS buffer (pH = 7.4) by monitoring the UV−vis absorbance at 304 nm for *g*-nanogel and 307 nm for $PPEGMA_{32}$-DT at 37 °C, [dithioester] = 0.354 mM. (The higher apparent absorbance for *g*-nanogel is due to scattering. Manually drawn straight lines for guidance only). (B) Cell viability of A549 cells after exposure to the polymers for 48 h at different dose of Macro-CTAs or nanogels: 1, PEG5k-TTC; 2, $PPEGMA_{21}$-TTC; 3, $PPEGMA_{32}$-DT; 4, *l*-nanogel (54 nm, entry 1, Table 1); 5, *l*-nanogel (56 nm, entry2, Table 1); 6, *g*-nanogel (150 nm, entry 7, Table 1); 7, *g*-nanogel (61 nm, entry 10, Table 1).

absorption for the nanogels was however more gradual and was not complete within 100 h, at which time the nanogel sample was still colored due to the presence of the CTA. These results indeed pointed to the enhanced hydrolytic stability of the CTAs in the nanogels due to the steric hindrance. Our conclusion was further underpinned by the stability study of CTAs in PBS buffer (pH = 7.4) at 37 °C (Figure 3A). The UV−vis absorbance due to the dithioester group in the *g*-nanogels was only slightly attenuated, if at all, within 22 days. In contrast, the dithioester group in $PPEGMA_{32}$-DT started to degrade from the eighth day and a significant decrease (25%) in the absorbance was detected within 22 days. The enhanced long-term stability of the CTAs in the nanogels in PBS buffer, compared to that of the molecularly dissolved polymers, bodes well for biorelated applications of nanogels synthesized via RAFT dispersion polymerization.

In order to further evaluate the suitability of the nanogels for biorelated applications, we performed *in vitro* cell toxicity studies by MTT assay of the nanogels and the Macro-CTAs with lung cancer cell A549 (Figure 3B). Upon increasing the polymer dose from 0.5 mg/mL to 2.0 mg/mL, only the *l*-PEG-based PEG5k-TTC exhibits a significant decrease in cell viability, dropping from ~90% to ~60%, while both $PPEGMA_{21}$-TTC and $PPEGMA_{32}$-DT, regardless of the difference in their CTA structure, are essentially nontoxic, although there is some decrease for $PPEGMA_{32}$-DT at 2.0 mg/mL, because dithioesters are usually more susceptible toward hydrolysis/aminolysis than trithiocarbonates.[49] In principle, trithiocarbonate-type CTAs such as PEG5k-TTC and PPEGMA-TTC are more stable than dithioester-type CTAs such as PPEGMA-DT. However, recent results indicated that toxicity of RAFT polymers is closely related to the structure of the polymers. Different polymers bearing the same type of CTA can exhibit different levels of toxicity.[58] The toxicity of PEG5k-TTC, in comparison with that of $PPEGMA_{21}$-TTC and $PPEGMA_{32}$-DT, suggests that CTAs connected to linear polymers may be more prone to hydrolysis than those connected to nonlinear graft polymers, possibly due to the steric congestion conferred by nonlinear graft polymers. On the other hand, both the *l*-nanogels and the *g*-nanogels, ranging from 50 to 150 nm, exhibit high levels of cell viability. These results correlate well with our CTA aminolysis/hydrolysis studies and indicate that the enhanced stability of the CTAs in the nanogels, due to both the nanogel network and the steric congestion conferred by nonlinear graft polymers, contributes favorably to the biocompatibility of these materials.

CONCLUSION

In summary, RAFT aqueous dispersion polymerization has been used to prepare a novel type of core−shell, thermosensitive nanogel composed of mainly oligomeric ethylene glycol. This method can efficiently produce small nanogels with tunable size. Both *l*-nanogels and *g*-nanogels are stable in NaCl solution for more than three months and in BSA solution for 1 week. In FBS, *g*-nanogels are stable for 4 days while *l*-nanogels are stable only for 2 days. In addition, *g*-nanogels show superior stability against freeze−thawing in comparison with *l*-nanogels. The CTAs in the RAFT nanogels have enhanced chemical stability, which endows the RAFT nanogels with excellent biocompatibility.

ASSOCIATED CONTENT

Supporting Information. Polymerization kinetics, NMR spectra, thermal properties of nanogels, freeze−thawing data and aminolysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*E-mail: (Z.A.) an.zesheng@shu.edu.cn; (A.C.) ancao@shu.edu.cn.

ACKNOWLEDGMENT

This work is supported by National Basic Research Program of China (973 Program Nos. 2009CB930200 and 2011CB933402), National Natural Science Foundation of China (20904029), Shanghai Pujiang Program (09PJ140800), Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning, Foundation for Excellent Youth Scholar of Higher Education of Shanghai, and Shanghai Leading Academic Disciplines(S30109). We thank Prof. Ming Jiang and Dr. Guosong Chen (Fudan University) for their help on GPC, and Dr. Chao Lin and Dr. Yongyong Li (Tongji University) for their help on DLS.

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