Video Article

Facile Synthesis of Worm-like Micelles by Visible Light Mediated Dispersion Polymerization Using Photoredox Catalyst

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Abstract

Presented herein is a protocol for the facile synthesis of worm-like micelles by visible light mediated dispersion polymerization. This approach begins with the synthesis of a hydrophilic poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGMA) homopolymer using reversible addition-fragmentation chain-transfer (RAFT) polymerization. Under mild visible light irradiation (λ = 460 nm, 0.7 mW/cm^2), this macro-chain transfer agent (macro-CTA) in the presence of a ruthenium based photoredox catalyst, $Ru(bpy)_3Cl_2$ can be chain extended with a second monomer to form a well-defined block copolymer in a process known as Photoinduced Electron Transfer RAFT (PET-RAFT). When PET-RAFT is used to chain extend POEGMA with benzyl methacrylate (BzMA) in ethanol (EtOH), polymeric nanoparticles with different morphologies are formed *in situ* according to a polymerization-induced self-assembly (PISA) mechanism. Self-assembly into nanoparticles presenting POEGMA chains at the corona and poly(benzyl methacrylate) (PBzMA) chains in the core occurs *in situ* due to the growing insolubility of the PBzMA block in ethanol. Interestingly, the formation of highly pure worm-like micelles can be readily monitored by observing the onset of a highly viscous gel *in situ* due to nanoparticle entanglements occurring during the polymerization. This process thereby allows for a more reproducible synthesis of worm-like micelles simply by monitoring the solution viscosity during the course of the polymerization. In addition, the light stimulus can be intermittently applied in an ON/OFF manner demonstrating temporal control over the nanoparticle morphology.

Video Link

The video component of this article can be found at https://www.jove.com/video/54269/

Introduction

The synthesis of nonspherical (and other) nanoparticle morphologies has traditionally been accomplished using a multistep self-assembly procedure starting with the synthesis and purification of well-defined amphiphilic diblock (or multiblock) copolymers. One of the most common self-assembly techniques was popularized by Eisenberg in the 1990s and involves the dissolution of the amphiphilic block copolymer in a common solvent for both polymer blocks followed by the slow addition of a solvent selective for one of the blocks[1-3]. As the selective solvent (typically water) is added, the block copolymer undergoes self-assembly to form polymeric nanoparticles. The final morphology (or mixtures of morphologies) of the nanoparticles are determined by a large number of factors such as the relative lengths of each polymer block, rate of water addition and the nature of the common solvent. However, this approach generally only allows for the production of nanoparticles at relatively low solids content (less than 1 wt%) and so limits its practical scalability[4]. In addition, the reproducible formation of "intermediate" phases such as worm-like micelles can be difficult owing to the narrow range of parameters required to stabilize this nonspherical morphology[5].

The polymerization-induced self-assembly (PISA) approach partially addresses the drawbacks of the Eisenberg approach by utilizing the polymerization process itself to drive self-assembly *in situ* allowing for nanoparticle synthesis at much higher solids content (typically 10-30 wt %)[6-8]. In a typical PISA approach, a living polymerization process is used to chain extend a solvent soluble macroinitiator (or macro-CTA) with a monomer that is initially soluble in the reaction medium but forms an insoluble polymer. The PISA approach has been used to synthesize worm-like micelles by systematically testing a number of experimental parameters and using detailed phase diagrams as a synthetic "roadmap"[5,9].

Despite their challenging synthesis, there is great interest in worm-like nanoparticles due to their interesting properties relative to their spherical counterparts. For example, we have demonstrated that drug loaded short and long worm-like micelles synthesized using a PISA approach have significantly higher *in vitro* cytotoxicity compared to spherical micelles or vesicles[10]. Others have shown a correlation between nanoparticle aspect ratio and blood circulation time in *in vivo* models[11]. Others have shown that the synthesis of worm-like nanoparticles using an appropriate PISA methodology yields a macroscopic gel due to the nanoscale entanglement of the nanoparticle filaments. These gels have demonstrated potential as sterilizable gels owing to their thermoreversible sol-gel behavior[12].

This protocol describes a method allowing for the *in situ* monitoring of the formation of worm-like micelles by simply observing the solution viscosity during the polymerization. Previous studies of similar worm-like micellar gels have demonstrated that above a critical temperature, these nanoparticles undergo a reversible worm-sphere transition and so form free-flowing dispersions at elevated temperatures. To date, these systems have utilized a thermally sensitive azo compound to initiate the controlled polymerization[13,14] and so gelation may not be readily observed in these systems during the thermal polymerization. From these studies, it was hypothesized that synthesizing PISA derived nanoparticles at lower temperatures may allow for observation of this gelation behavior *in situ*.

Recently we reported the use of a facile room temperature photopolymerization technique to mediate the PISA process to yield nanoparticles of different morphologies[15]. Here, a visualized protocol is presented for the reproducible synthesis of worm-like micelles by observing the solution viscosity behavior during the polymerization. The dispersion polymerization proceeds readily using commercially available light-emitting diodes (LEDs) (λ = 460 nm, 0.7 mW/cm^2).

Protocol

1. Synthesis and Characterization of POEGMA Macro-CTA

1. Add oligo(ethylene glycol) methyl ether methacrylate (OEGMA) (12 g, 4 × 10^{-2} mol), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB) (0.224 g, 8 × 10^{-4} mol), 2,2′-azobis(2-methylpropionitrile) (AIBN) (16.4 mg, 0.1 mmol) and 50 ml acetonitrile (MeCN) to a 100 ml round bottom flask.
2. Seal the flask with an appropriately sized rubber septum and steel wire and cool the flask from room temperature to < 4 °C in an ice-water bath.
3. Deoxygenate the flask for 30 min by bubbling nitrogen directly into the reaction mixture through a 21 G needle (0.8 mm x 120 mm) with a second 21 G needle (0.8 mm x 38 mm) acting as a vent.
4. Place the flask in an oil bath at 70 °C for 5.5 hr before quenching the polymerization by immersion in an ice-water bath and exposing the contents to air.
5. Remove the MeCN by agitation under a continuous stream of compressed air and re-dissolve the crude mixture in ~40 ml tetrahydrofuran (THF).
6. Add the contents of the flask dropwise to 400 ml of a rapidly stirred mixture of petroleum spirits (b.p. 40-60 °C) and diethyl ether (70:30, v/v) and continue to stir until the supernatant is no longer cloudy.
   Note: Cooling in an ice-bath may be used to accelerate the precipitation process.
7. Decant the supernatant and re-dissolve the polymer residue in ~40 ml THF.
8. Repeat the precipitation process (steps 1.5-1.7) at least two more times to ensure complete removal of the residual OEGMA monomer. Remove the excess solvent from the purified POEGMA macro-CTA firstly by agitation under a continuous stream of compressed air and drying in a vacuum oven (20 °C, 10 mbar) for 4 hr.
9. Determine the number average molecular weight of the POEGMA macro-CTA by Nuclear Magnetic Resonance (NMR) ($M_{n,\ NMR}$) using a previously reported method[15]. Using gel permeation chromatography[15] (GPC) (dimethylacetamide as mobile phase and appropriate standards for calibration) calculate the polymer dispersity (Đ).
   Note: Using the above synthesis (steps 1.1-1.8) should yield a POEGMA macro-CTA with $M_{n,\ NMR}$ = 9,000, and Đ < 1.15. If the molecular weight (and dispersity) of the synthesized POEGMA macro-CTA differs from the synthesis presented here (between 7,000 - 1,000 g/mol), the formation of worm-like micelles (as indicated by *in situ* gelation) can still occur using the subsequent PISA methodology presented in (section 2) albeit at a slightly altered reaction time.

2. Preparation of POEGMA-*b*-PBzMA Nanoparticles Using PISA

1. Prepare a 1 mg/ml stock solution of $Ru(bpy)_3Cl_2 \cdot 6H_2O$ in ethanol (EtOH). Store the stock solution in the refrigerator to minimize solvent evaporation.
2. Plug a Pasteur pipette with a small wad of cotton wool using a second pipette to help pack it tightly. Pour basic aluminum oxide into the pipette with the cotton wool plug to give a column of approximately 5 cm. Remove the monomethyl ether hydroquinone inhibitor in commercial BzMA by passing ~3 ml of BzMA through the column and collecting the deinhibited BzMA eluent.
3. Add POEGMA macroCTA (~9,000 g/mol; 76.9 mg, 8.5 × 10^{-6} mol), deinhibited BzMA (0.301 g, 1.71 × 10^{-3} mol), $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (128 µg, 1.71×10^{-7} mol, 128 µl of a 1 mg/ml ethanolic stock solution), 0.383 ml MeCN and 1.402 ml EtOH (1.913 ml total solvent, 80 wt%, 20 v/v% MeCN) to a 4 ml glass vial.
4. Perform the deoxygenation procedure as outlined in steps 1.2-1.3.
5. Place the vial in a 2,000 ml glass beaker (**Figure 2**) lined with blue LED strips (λ_{max} = 460 nm, 0.7 mW/cm^2) and irradiate at room temperature with magnetic stirring. Monitor the reaction vial routinely after 20 hr and remove it from the reactor when the high viscosity solution forms a free standing gel when the vial is inverted (**Figure 3**).
   Note: The total time to yield a free-standing gel should be about 24 hr of blue light irradiation using the conditions presented here. Small differences in the light irradiation reactors (physical dimensions, intensity, *etc.*) may require slightly altered conditions (specifically reaction time) to achieve the *in situ* formation of worm-like micelles.
6. After removing from the reactor, quench the polymerization by exposing the nanoparticle gel to air for a few minutes and storing the closed vial upright in the dark.

3. Transmission Electron Microscopy (TEM) Imaging of Nanoparticle Morphology

1. Place approximately 40 mg of the crude nanoparticle gel (from section 2) in a 4 ml glass vial.
2. Continuously agitate the nanoparticle gel using a vortex mixer and add 4 ml of EtOH dropwise over a period of at least 5 min. The gel should become a free-flowing solution during the solvent addition.

Note: If the gel is diluted with EtOH too rapidly or not agitated adequately, some precipitation of the nanoparticles may occur. See step 3.3.

3. Remove any macroscopic aggregates from the diluted nanoparticles by filtering through glass wool.
4. Perform TEM imaging (with uranyl acetate staining) of the diluted sample according to a previously reported procedure.[15]

Representative Results

In this study, two-step polymerization protocol is used for the synthesis of worm-like micelles using a PISA approach (**Figure 1**). In the first step, the polymerization of OEGMA is performed yielding a POEGMA macro-CTA which can be used as a stabilizer in the subsequent polymerization step. The PET-RAFT polymerization proceeds under dispersion conditions owing to the insolubility of PBzMA in ethanol which ultimately leads to nanoparticle formation. During the polymerization, the initially transparent reaction mixture can be observed to become cloudy in accordance with a dispersion polymerization and eventually transitions to a highly viscous gel-like state indicating the formation of worm-like micelles (**Figure 3**). Indications of a "living" polymerization are apparent (**Figure 1A**) with low polymer dispersities (Đ < 1.3) and a good correlation between the molecular weight and monomer conversion. In addition, GPC traces (**Figure 1B**) indicate a predominantly unimodal distribution with varying conversion although some high molecular termination and low molecular weight tailing is observed in this system. Importantly, these "dead" polymer chains are not at a sufficient quantity to inhibit the formation of pure worm-like micelles. The shift of the molecular weight distribution with increasing conversion suggests the predominant formation of POEGMA-*b*-PBzMA diblock copolymers with a narrow distribution of chain lengths.

Figure 2A illustrates the light reactor setup used in this experiment in which a 1 meter commercial LED strip (λ = 460 nm, 4.8 W/m) is wound inside 2 L beaker. In our experiments, it was also determined that a household lamp with a similar blue light intensity (**Figure 1B**) could also be used in the PET-RAFT PISA process.

Figure 4 demonstrates that the formation of the worm-like micelle morphology is also achievable under different reaction conditions such as variable vial types and reagent compositions but also if the light source is applied in an intermittent fashion. This implies that despite the strong effect of light penetration on polymerization rates in most photopolymerization systems, the gelation behavior in the PET-RAFT PISA protocol can still be used as a reliable indicator for worm-like micelle formation. This is an important result since typically *ex situ* TEM imaging is required to provide evidence of worm-like micelle formation. Apart from the observed gelation behavior, formation of purely worm-like micelles should be confirmed by observing the morphology of a significant amount of nanoparticles (> 100) by TEM (with uranyl acetate staining). If partial vesicle morphologies are observed, the irradiation time should be decreased; conversely if spherical micelles are observed then irradiation time should be increased slightly.

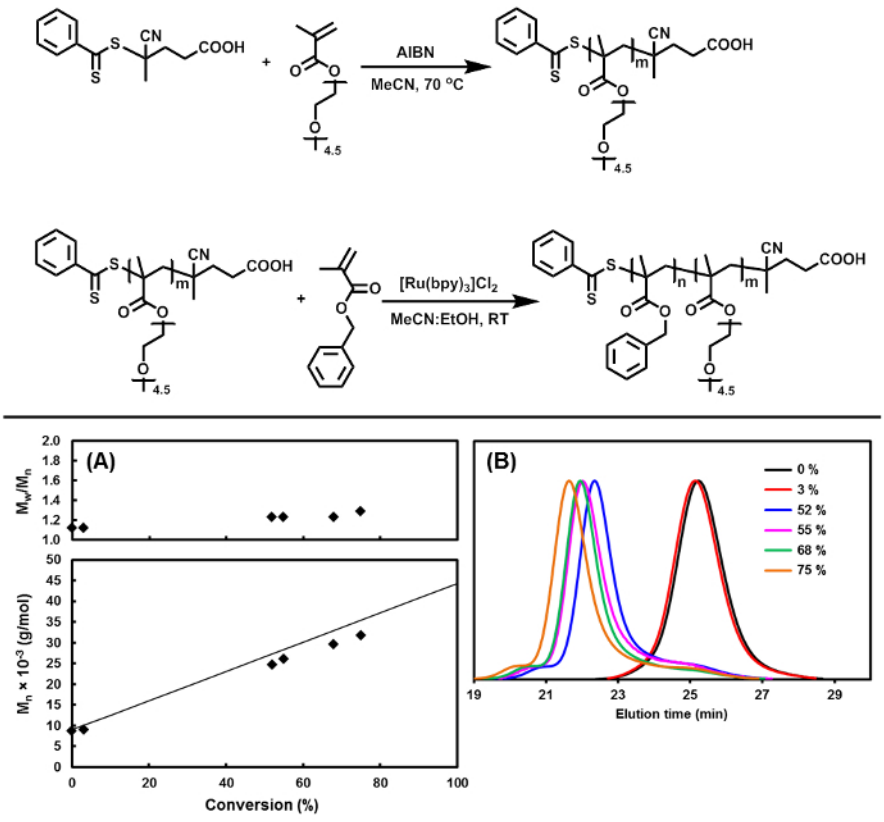

Figure 1. Reaction scheme for synthesizing worm-like micelles using PET-RAFT, a living photopolymerization technique. (Above) Two step approach for the synthesis of worm-like micelles using a PISA approach. (Below) Kinetic study demonstrating (**A**) the evolution of molecular weight and polymer dispersity during the PISA polymerization and (**B**) the evolution of the molecular weight distribution from gel permeation chromatography (GPC) with conversion. Adapted with permission from ref[15]. Copyright (2015) American Chemical Society. Please click here to view a larger version of this figure.

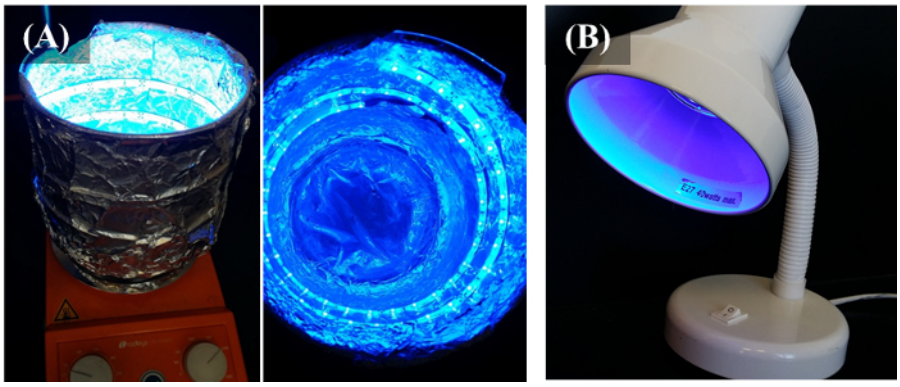

Figure 2. Digital photographs of different visible light reactors. (**A**) The circular reactor used in this study lined with blue LED strips (λ_{max} = 460 nm, 0.7 mW/cm^2). (**B**) A household lamps fitted with a 5 W bulb that can also be used in this protocol. Please click here to view a larger version of this figure.

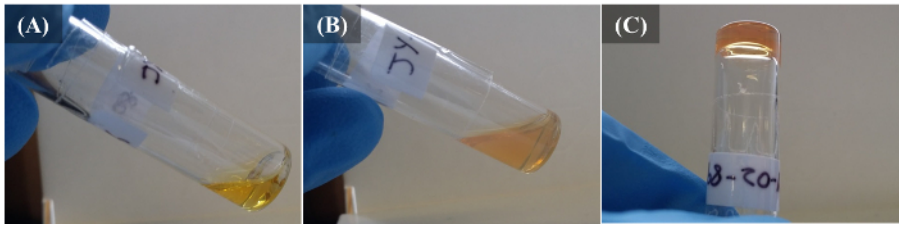

Figure 3. Representative digital photographs of a PET-RAFT mediated PISA polymerization. Images were taken (**A**) before polymerization, (**B**) after 15 hours and (**C**) after 24 hours of visible light irradiation. During the polymerization, the initially transparent reaction mixture becomes cloudy and eventually transitions to a free-standing gel state indicative of the *in situ* formation of worm-like micelles. Please click here to view a larger version of this figure.

Exp.	Vial type	POEGMA $M_{n,NMR}$ (g/mol), Đ	Total irradiation time (h)	Total solids content (wt%)	MeCN content (v/v%)	Conversion (%)	$M_{n,theo}$ (g/mol)	$M_{n,NMR}$ (g/mol)	$M_{n,GPC}$ (g/mol)	Đ
A	4 mL glass	9 000, 1.10	24	20	20	68	33 000	31 400	29 500	1.20
B	0.9 mL quartz	10 050, 1.13	39	20	20	78	36 500	33 800	31 000	1.15
C	4 mL glass	9 000, 1.10	24	10	10	61	30 500	29 300	25 000	1.23

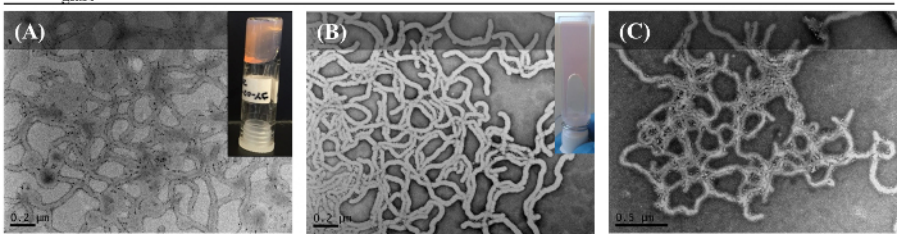

Figure 4. Characterization and TEM images of POEGMA-*b*-PBzMA diblock copolymers formed using a PET-RAFT PISA approach yielding worm-like micelles. TEM images (and digital photograph inserts) of worm-like micelles formed using different polymerization conditions. (**A**) and (**C**) were illuminated for 24 hours whereas (**B**) required a total ON/OFF irradiation time of 39 hours before gelation (when using a 10,000 g/mol POEGMA macro-CTA). In each case, a high viscosity gel is formed which is characteristic of the formation of worm-like micelles. Adapted with permission from ref[15]. Copyright (2015) American Chemical Society. Please click here to view a larger version of this figure.

Discussion

This visualized protocol demonstrates the ability to monitor the formation of worm-like micelles simply by observing the onset of gel-like behavior. The utility of this approach lies in the ability to monitor worm formation during the polymerization in comparison to other methods. This procedure can be performed using a two-step polymerization of two commercially available monomers (OEGMA and BzMA) to yield self-assembled POEGMA-*b*-PBzMA amphiphilic diblock copolymers.

It should be noted here that reactors with different reactor geometries, light intensities, *etc.*, compared to those in **Figure 2** may require slightly altered conditions to yield worm-like micelle gels. Due to the absorption characteristics of the ruthenium-based catalyst, polymerization can only occur at a reasonable rate under blue visible light. In principle, other catalysts with different light absorption properties could also be used. Care should be taken to not leave the worm gels in the reactor too long otherwise macroscopic precipitates may begin to form. This occurs as the nanoparticles attempt to reorganize into vesicular structures but are inhibited by the highly viscous medium. In some cases, we have observed

the formation of partial vesicles (jellyfish or octopi structures) via TEM imaging when the polymerization was kept in the reactor beyond the first observation of a free-standing gel-like state.

To increase the accessibility of this technology, the PET-RAFT PISA polymerizations reported in this protocol were performed at room temperature without external temperature regulation (cooling fan, water bath *etc.*). Additionally, the low wattage LED strips do not generate observable increases in the vial temperatures during the course of the polymerization (less than 5 °C). Whilst it is well known that the polymerization rate has a strong dependence on temperature, inhibition of the ability of worm-like micelles to induce macroscopic gel-like behavior even when polymerizing at 50 °C has not been observed.

Obtaining shorter worm-like micelles (on average) is also possible by removing the light source before the reaction medium has reached a free-standing state but has a noticeable increase in viscosity. This approach may be favorable since dilution of these "softer" gels (without precipitation) for analysis is significantly easier compared to the free-standing gels. In a similar manner, spherical micelles may be obtained by reducing the irradiation time even further; typically after the first onset of cloudiness during the polymerization.

In principle, a range of different solvophillic monomers could be used instead of OEGMA (*e.g.*, poly(2-hydroxyethyl methacrylate), poly(methacrylic acid) however some optimization of the polymerization kinetics and self-assembly parameters would be required. A high livingness of the homopolymerization of the macro-CTA should be demonstrated in order to increase the efficiency of the subsequent PISA polymerization. However, as long as a sufficiently pure worm-like micelle phase exists during the course of the polymerization, gelation can still occur. The utility of the presented approach lies in the fact that different length macro-CTA stabilizers can be used without the need to significantly reoptimize the procedure for forming worm-like micelles. In this protocol, the POEGMA macro-CTA was synthesized using a thermally initiated RAFT protocol however, we have also demonstrated the ability to generate POEGMA with high chain-end fidelity using a homogenous PET-RAFT protocol[16]. Although structurally similar monomers to BzMA have also been reported to form worm-like micelle gels[17], it is likely that only a limited number of monomers are able to undergo controlled radical dispersion polymerization to yield worm-like micelles with significant gelation properties.

Although different reactor setups (including reaction vial geometry) can result in varying polymerization rates in most photopolymerization systems, the ability to visually monitor the *in situ* formation of worm-like micelles helps overcome this limitation when using a PET-RAFT PISA approach. As a result, the polymerization time can be altered depending on the precise reactor setup implemented. It is well known that the worm-like micelle phase can be difficult to produce in high purity and yield, however in the presented approach we are able to produce worm-like particles at a solids content > 10 wt%. Importantly, the formation of these particles can be monitored during the polymerization rather than previous reports whereby worm-like micelle synthesis can only be confirmed after quenching the polymerization and performing *ex situ* TEM imaging.

Importantly, the ability to reproducibly generate these high aspect ratio nanoparticles at high solids content has important implications for a number of applications particularly in the biological arena as drug delivery carriers. A number of studies have demonstrated the interesting behavior of non-spherical morphologies in biological environments such as an increased blood circulation time compared to their spherical counterparts[11] or varying cell-uptake behavior[10]. Whilst these particles are synthesized in ethanolic solution, we have previously demonstrated that under appropriate dialysis conditions the morphology of these PISA nanoparticles can be retained in aqueous solution[10]. The advantage of this approach lies in the ability to firstly encapsulate poorly water soluble therapeutics under ethanolic dispersion conditions before dialysis into water for biological study. In addition, it is likely that these elongated particles exhibit varied cell uptake behavior relative to spherical structures due to their virus-like morphologies.

Disclosures

The authors have nothing to disclose.

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