Ultrasound Assisted Synthesis of Cross-linked Poly(ethylene glycol) Nanostructures with Hydrophobic Core and Hydrophilic Shell

Haiyan Zhu, Francesca Cavalieri* and Muthupandian Ashokkumar*

Ms Haiyan Zhu, Prof. Muthupandian Ashokkumar
School of Chemistry, University of Melbourne, Victoria 3010, Australia
E-mail: masho@unimelb.edu.au
Dr. Francesca Cavalieri
Department of Chemical Engineering, University of Melbourne, Victoria 3010, Australia
E-mail: francesca.cavalieri@unimelb.edu.au

One-pot synthesis of polymeric nanostructures by using ultrasound without adding initiators and surfactants is a straightforward approach that has attracted significant attention in polymer science. In this study, the ultrasonic polymerization technique is employed to synthesize poly(ethylene glycol) based nanoparticles with a hydrophobic core and a hydrophilic shell. We performed interfacial polymerization using an oil-in-water emulsion containing oligo (ethylene glycol) methyl ether methacrylate (OEG-MA) as a water-soluble monomer, and ethylene glycol dimethacrylate (EGDMA) acting as an oily-cross-linker phase. Both the radicals and physical effects generated by acoustic cavitation are crucial to conduct this surfactant and initiator-free process and obtain uniform nanoparticles endowed with a hydrophobic core and hydrophilic shell. We show that the nanoparticles core can be loaded with hydrophobic compounds. This technique can be applied to different PEG monomers containing various functional moieties such as amines and carboxyl groups to obtain multifunctional nanoparticles.

FIGURE FOR ToC_ABSTRACT

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/macp.201800353.

This article is protected by copyright. All rights reserved.
Core-shell poly(ethylene glycol) nanoparticles and nanocapsules have been obtained by one-pot ultrasonic synthesis approach. Acoustic cavitation generates nanodroplets and radicals to promote surfactant- and initiator-free interfacial polymerization and crosslinking of oligo(ethylene glycol) methyl ether methacrylate. Uniform nanostructures with hydrophobic core, hydrophilic shell and carboxyl groups were obtained.

1. Introduction

Poly(ethylene glycol) (PEG) is a widely used polymer in biological fields, especially for drug delivery systems.[1-3] Since PEG possesses hydrophilicity and stealth properties, drug carriers and conjugates are commonly coated with PEG to minimize their binding to proteins.[2] The stealth properties of PEG chains can reduce kidney excretion and prolong blood circulation time of the nanoparticles and drug-polymer conjugates.[2] As a result of long circulation half-life, these polymeric carriers and conjugates can bind to specific tissue receptors and deliver drug to targeted sites, reducing side effects and enhancing the availability of drug.[4] In addition, PEG is a surface active polymer and can be used as a stabilizer during nanoparticles synthetic process[5-7] without adding other surfactants. Therefore, synthesis of polymers based
on PEG has attracted significant attention in polymer chemistry, pharmaceutics, and biomaterial science.\[^{3, 4, 8, 9}\]

Jung et al. utilized atom transfer radical polymerization (ATRP) method to synthesize nanometer-sized colloidal nanoparticles based on OEGMA in inverse miniemulsion using cyclohexane as the organic solvent.\[^{10}\] Cui et al. reported the engineering of PEG hydrogel nanoparticles using mesoporous silica templating method and investigated their in vivo properties.\[^{9}\] The previous studies on the synthesis of PEG nanoparticles have shown that initiators, organic solvents and surfactants or silica templates are required to obtain uniform nanoparticles.\[^{4, 9-13}\] The free-radical polymerization promoted by ultrasound (without the use of initiators and surfactants) has emerged as a one-pot technique to synthesize various polymeric structures and recently have been also used in combination with RAFT agents.\[^{14-17}\]

Ultrasound energy transmitted to a liquid medium produces both physical and chemical effects.\[^{18}\] When ultrasound passes through a liquid medium, the interaction between the bubbles in liquid and sound waves results in acoustic cavitation\[^{18}\], defined as the growth and violent collapse of critical-sized bubbles. The implosion of the bubbles can generate extremely high temperature (\(~5000\) K) and pressure (>100 atm). The high temperature generated within cavitation bubbles can lead to homolysis of water molecules (if water is the medium) to form primary hydrogen and hydroxyl radicals\[^{19}\]. These highly reactive primary radicals, generated by sonolysis of water, can attack monomer molecules to commence the process of converting a monomer or a mixture of monomers into polymers.\[^{20}\] Besides, the growth and collapse of bubbles are accompanied by shock waves, micro-streamers, and micro-jets create shear forces, which can induce the emulsification of two immiscible liquids by improving the mass transfer.\[^{20}\]
Compared with conventional methods of vinyl-monomer polymerization, employing ultrasound to initiate the reaction by radicals is more straightforward. The emulsion polymerization of vinyl C=C monomers assisted by ultrasound has been extensively studied, examples include the emulsion polymerization of methyl methacrylate (MMA), n-butyl methacrylate (BMA), and 2-ethylhexyl methacrylate (2EHMA). However, previous work lacked the investigation of ultrasonic polymerization of vinyl monomers combined with PEG functional group, which can be applied to the biomedical field. Since OEG-MA is a monomer which combines reactive vinyl double bonds and surface-active PEG chains, we sought to investigate the use of low frequency ultrasound to perform the interfacial polymerization of OEG-MA at the water-oil interface provided by an OEG cross-linker (EGDMA). In this paper, we report the one-pot ultrasonic synthesis method to obtain PEG nanoparticles and discuss the mechanism of formation of such core-shell nanostructures. We also show the versatility of this innovative approach to i) encapsulate hydrophobic molecules in the nanostructures and ii) introduce carboxyl groups for tagging ligands.

2. Experimental Section

2.1. Materials

Oligo (ethylene glycol) methyl ether methacrylate, (OEG-MA, Mn 950), ethylene glycol dimethacrylate (EGDMA >98%), methacrylic acid and Nile red were purchased from Sigma-Aldrich. Both the monomer (OEG-MA) and cross-linker (EGDMA) were filtered twice through the basic aluminium oxide to remove the inhibitor, hydroquinone, and mequinol. The purified monomer and cross-linker were sealed and stored below 4°C until further use. Water used in all experiments was obtained from a Milli-Q Academic purification system with a typical resistivity of 18.2 MΩ·cm at 25 °C. High-purity nitrogen gas was used for purging the solutions before sonication to eliminate oxygen.
2.2. Ultrasonication

For this heterogeneous system, the unit employed was a Sonic System, 20 kHz ultrasound (Branson Digital Sonifier) generator with a standard titanium horn tip of 10 mm diameter. Custom-made reaction vessels containing the reaction mixture were equipped with a cooling jacket through which thermostated water was circulated to maintain the reaction temperature at 40 °C ±3 °C.

2.3. Synthesis

The synthesis of the polymeric nanoparticles was performed by a polymerization reaction, which were carried out at 20 kHz, and 40 W applied power with a total sample volume of 20 mL, a monomer (OEG-MA) concentration of 0.115 M (10% w/v), and a cross-linker concentration of 0.053 M (0.2 mL). The reaction mixture was sparged with high-purity nitrogen for 40 mins. A constant flow of nitrogen was kept above the liquid surface during the sonification process to prevent the formation of reactive oxygen species. The reaction temperature was maintained at 40 °C. After 150-min polymerization, nanoparticles were precipitated from solution by centrifuge (7000 rpm, 10 min) and washed by a mixture of ethanol and Milli-q water (v/v=1:1) for three times to remove the unreacted oil core and soluble polymers.

2.4. Analytical Techniques

2.4.1 Dynamic Light Scattering

The size and zeta potential of particles were analysed by Dynamic Light Scattering (DLS) with a Zetasizer Nano ZS from Malvern Instruments Ltd. The stability of resulting particle size was investigated by measuring the sample mixtures as the function of stock time. The samples were diluted 60 times and filtered through Millipore nylon filters (pore size 0.45 µm)
to prevent particles aggregation and eliminate dust and large contaminants. The size measurements were carried out in quartz cuvettes.

2.4.2 Scanning/Transmission Electron Microscopy

The size and morphologies of the particles were observed using a scanning electron microscope (FEI quanta) at an acceleration voltage of 10.0 keV and for preparation, the particles were air-dried and then sputter-coated with a thin gold film. TEM images were recorded on a FEI Tecnai transmission electron microscope at 120 keV. The samples were mounted on a carbon-film copper grid from ProSciTech and loaded with a single tilt holder.

2.4.3 Fluorescence Microscopy

In order to evaluate the encapsulation of a hydrophobic drug in the nanostructures, fluorescence properties of the nanoparticles (dye labelling) was observed by using an inverted Olympus IX 71 wide-field fluorescence microscope with 60X objective lens. A CCD camera (Cool SNAP FX, Photometrics, and Tucson, AZ) was mounted on the left-hand port of the microscope.

3. Results and discussion

OEG-MA (0.115 M) was first dissolved in an aqueous solution, EGDMA was layered at the top of the aqueous solution and low-frequency ultrasound (20 kHz, 40 W) was applied for a maximum duration of 150 min. In this heterogeneous system, the shear forces provided by ultrasound are required to mix the two phases and obtain a well dispersed emulsion. After the ultrasonic polymerization the formation of nanoparticles was observed. The resulting nanoparticles were purified by washing in ethanol: H₂O (1:1) for three times. The nanoparticles were not soluble in most of the solvents, including ethanol and acetone, indicating that the polymer chains have been cross-linked during sonication. [4]
To evaluate the monomer conversion, the reaction product was analysed as a function of sonication times Figure 1a. The conversion of monomers to nanoparticles was calculated by weighing the collected samples after purification at different reaction time. The conversion increased with increasing sonication time from 30 min to 120 min and reached a limiting value beyond 120 min, which indicates that the polymerization of OEG-MA and EGDMA was almost completed after 120-min sonication (Figure 1a).

The morphology and size of the PEG nanoparticles obtained after 150 min sonication were studied by TEM and SEM analysis.

Figure 1. The characterization of PEG nanoparticles: a) conversion of polymer into nanoparticles as the function of sonication time; b) SEM image of resulting particles after different sonication time (SEM inset shows the nanocapsule structure; c) TEM image of resulting nanoparticles after 150-min sonication (TEM inset shows the core shell structure of
nanoparticles); d) size distribution of resulting particles after 150-min sonication and e) the stability of the particle size as the function of storage time.

The TEM image (Figure 1 b) shows the morphology of PEG nanoparticles. More than 100 particles were measured to calculate the size distribution. The size distribution shown in Figure 1d indicates the nanoparticles’ size was approximately 159 nm ± 16 nm. Figure 1b inset shows that the inner core and the outer layer have a different contrast indicating a core-shell structure. We speculate that the outer layer is composed of PEG hydrophilic chains deriving from OEG-MA polymerization whereas the core is made of hydrophobic crosslinked EDGMA repeating units.

The SEM image, Figure 1c, shows that the smaller size particles retain their structure while large particles appear to collapse when drying and under vacuum. This suggests that the large particles may be hollow nanocapsules rather than solid nanoparticles. Overall the TEM and SEM images infer that such particles synthesized by ultrasound possess core-shell structures.

Further, the stability of the particles as the function of storage time was determined by DLS measurements (Figure 1e). The resulting nanoparticles were stored for five months for the stability study, the particles size appears to be similar as a function of time which suggests that the cross-linked polymeric particles are highly stable in suspension and do not aggregate. The particle size determined by DLS is in agreement with that measured using TEM images.

The overall ultrasonic polymerization process is shown in Figure 2. The physical effects generated by the ultrasound (Figure 2a) are needed to form the emulsion stabilized by the OEG-MA monomer (Figure 2b). Previous studies on ultrasonic polymerization have suggested that the monomer molecules adsorb at the interface of the cavitation bubbles\cite{28}. In the initiation step, the OH and H radicals generated by ultrasound can attack the C=C bond present in the OEG-MA moieties in aqueous solution. In principle, the chain propagation
steps can take place in the aqueous phase, in the oil phase and at the water-oil interface. As the radicals are generated in the aqueous phase, they will likely and primarily react with the water soluble monomer OEG-MA to form growing soluble poly (ethylene glycol) methacrylate chains. In fact, we noticed an increase in viscosity in the aqueous phase. Next, the growing chains residing at the water-oil interface (Figure 2c) can react with the cross-linker triggering the polymerization/crosslinking of the oily core. The latter process promotes the formation of core-shell nanoparticles (Figure 2d).

*Figure 2.* Schematic diagram of the interfacial polymerization via ultrasound and the obtainment of different nanostructures. a) physical effects generated by ultrasound; b) emulsification of OEG-MA droplets; c) growing soluble poly (ethylene glycol) methacrylate chains residing at the oil-water interface d) interfacial/crosslinking polymerization; e) cross-linker after sonication does not form nanoparticles; f) core-shell nanoparticles and g) hollow nanocapsules.

The sonication of the hydrophobic EGDMA cross-linker was performed without adding OEG-MA (Figure 2e); however we observed liquid-liquid phase separation and lack of
polymerization after three hours sonication. Therefore this indicates that the OEG-MA monomers plays an important role in acting as a surface-active and reactive monomer in the formation of nanoparticles and the dominant process is the interfacial polymerization. To explain the diversity in morphology observed in the SEM images between the small nanoparticles (Figure 2f) and large nanocapsules (Figure 2g), we hypothesize that in the larger nanodroplets the polymerization of the EGDMA cross-linker proceeds from the surface towards the inner core to the point where the steric hindrance of the highly crosslinked chains reduces the propagation rate and the termination reaction prevails. The unreacted cross-linker has been washed out during the purification steps by ethanol: H₂O (1:1), hence the soluble core is removed and the resulting nanoparticles exhibit a hollow structure.

3.2. Investigation of multi-functionalities for potential applications

To evaluate potential applications of these nanoparticles, we investigated the possibility of encapsulating a hydrophobic drug during the sonication. We used Nile red, a fluorescent dye that can mimic any hydrophobic drugs, to label the hydrophobic core of nanoparticles.

Figure 3. Loading hydrophobic molecules into the nanoparticles and nanocapsules a) optical and b) fluorescence microscopic images of Nile red loaded nanoparticles.
From the microscope images shown in Figures 3, it could be noted that the hydrophobic fluorescent dye can successfully label the hydrophobic component of nanoparticles. This suggest that hydrophobic drugs can be encapsulated in these nanostructures.

In addition, we also studied the possibility of introducing carboxylic groups into the nanoparticles, which can be used to tag bio-functional ligands. The copolymerization of methacrylic acid (MAA) and OEG-MA at the molar ratio (1:1) was performed using a similar approach. Zeta potential measurements showed that the nanoparticles' surface charge turned from neutral (0.165 ± 0.12 mV) to negative values -15.10 ± 0.21 mV when additional MAA monomer was involved in the interfacial polymerization. This result provides a clear evidence to confirm that the carboxyl groups can be introduced into the nanoparticles.

4. Conclusions

In summary, PEG based nanoparticles and nanocapsules have been synthesized using a one-pot ultrasonic method without adding initiator and surfactants in an aqueous medium. The particles are endowed with a hydrophobic core and hydrophilic PEG shell. PEG chains can be further functionalized with ligands and the hydrophobic core can accommodate hydrophobic drugs or contrast agents for potential biological applications.

Acknowledgements: This work was supported by the Australian Research Council (ARC) under a Future Fellowship (F. Cavalieri, FT140100873). We acknowledge the University of Melbourne and special thanks to Ms Sukhvir Kaur Bhangu and Dr. Meifang Zhou for SEM & TEM analyses.

Keywords: ultrasound, acoustic cavitation, interfacial polymerization, crosslinking, polymeric nanoparticles


Author/s:
Zhu, H; Cavalieri, F; Ashokkumar, M

Title:
Ultrasound-Assisted Synthesis of Cross-Linked Poly(ethylene glycol) Nanostructures with Hydrophobic Core and Hydrophilic Shell

Date:
2018-12-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/284705