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Preparation of core cross-linked micelles using a photo-cross-linking agent

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ABSTRACT

A new method for preparing polymeric, core cross-linked (CCL) micelles has been developed using a bifunctional photo-cross-linking agent of di(4-hydroxyl benzophenone) dodecanedioate (BPD). An amphiphilic diblock copolymer of poly(ethylene glycol)-*b*-poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (PEG-*b*-P(HEMA-*co*-MMA)) was synthesized via atom-transfer radical polymerization (ATRP) using a PEG macroinitiator at 85 °C. The core domains of the PEG-*b*-P(HEMA-*co*-MMA) micelles containing BPD in aqueous solution were successfully photo-cross-linked by UV irradiation for only 30 min. The HEMA units incorporated in the hydrophobic block of PEG-*b*-P(HEMA-*co*-MMA) donated labile hydrogens to excited-state BP groups in BPD, and they were subsequently cross-linked by BPD through radical–radical combination. A sufficient degree of cross-linking was obtained at an equivalent ratio of the BP groups to the HEMA units.

1. Introduction

In order to enhance the structural integrity of block copolymer micelles, their stabilization via covalent cross-linking of the core or shell domain has been extensively explored [1–20]. The structures of the cross-linked polymeric micelles can remain intact even in dilute solutions or in organic solvents. They have potential as nano-sized carriers for poorly water-soluble drugs, proteins, and genes, imaging agents, and structural templates. Shell cross-linked (SCL) micelles can be prepared by direct reaction between the chain segments located within the polymer micelle shell, or via addition of multi-functional cross-linking reagents [1–8]. Their properties are intermediate between those of micelles, microgels, nano-particles, and dendrimers. Core cross-linked (CCL) micelles were originally reported by Liu et al. [9–13]. They synthesized various poly(2-cinnamoylethyl methacrylate) (PCEMA) block copolymers and used them to prepare stable CCL micelles by photo-cross-linking of the core domain. The shell of such micelles consists of a protective corona that stretches out to stabilize the micelle, and the micelle core can provide a carrier compartment for various agents. Core cross-linking is also beneficial because it leaves the micelle shell available to tailor according to a variety of uses.

In order to prepare SCL and CCL polymeric micelles, various cross-linking approaches, such as photo-cross-linking, free-radical polymerization within the core, condensation reaction, sol–gel chemistry, and click chemistry have been utilized [1–20]. Among these chemical reactions, the photo-cross-linking chemistry has significant advantages over other chemistries. It is nontoxic, cost-effective, and does not produce any byproducts that need removing after the reaction. It is known that the micellar characteristic features, such as micelle size and shape, are not affected by the photo-cross-linking process [18]. Recently, various photo-cross-linkable groups, such as thymine, coumarin, and benzophenone (BP) side groups, were used instead of conventional cinnamate groups, for the synthesis of CCL micelles. Saito et al. used thymine side groups in order to prepare bio-inspired, core–shell polymeric micelles [19]. Jiang et al. synthesized light-controllable polymer micelles using an amphiphilic block copolymer containing coumarin side groups [20]. These micelles can be cross-linked for stabilization, and subsequently de-cross-linked using light at two different wavelengths, with tunable cross-linking density. Chen et al. synthesized an amphiphilic block copolymer containing BP side groups [18]. UV irradiation generated excited-state BP triplets, followed by the abstraction of nearby benzylic or other hydrogens and subsequent core cross-linking.

In the present study, a new method for preparing polymeric CCL micelles has been developed using a bifunctional photo-cross-linking agent having two BP groups. An amphiphilic diblock copolymer of poly(ethylene glycol)-*b*-poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (PEG-*b*-P(HEMA-*co*-MMA)) was synthesized via atom-transfer radical polymerization (ATRP). In order to induce fast hydrogen abstraction reactions by excited-state BP groups and subsequent core cross-linking, small amounts of HEMA units were incorporated into the hydrophobic block of PEG-*b*-P(HEMA-*co*-MMA). The core domains of the PEG-*b*-P(HEMA-*co*-MMA) micelles containing the bifunctional photo-cross-linking

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agent in aqueous solution were photo-cross-linked by UV irradiation. The excited-state BP groups abstracted hydrogens from the hydroxyl groups in the HEMA units, resulting in subsequent chain cross-linking through radical–radical combination. To our knowledge, the preparation of polymeric CCL micelles using a bifunctional photo-cross-linking agent has never been reported.

2. Experimental part

2.1. Materials

Methyl methacrylate (MMA, Duksan, 99.8%, Korea) and 2-hydroxyethyl methacrylate (HEMA, Acros, 96%) were passed through a column filled with neutral alumina. They were stirred over calcium hydride (CaH_2) and then distilled under reduced pressure. Acetonitrile (Acros, 99.5%) was stirred over CaH_2 and then distilled under reduced pressure. Tetrahydrofuran (THF, Duksan, 99.5%) was dried over Na and BP. Poly(ethylene glycol) methyl ether (MPEG, $M_n = 2000$ g/mol, Fluka), ethyl 2-bromoisobutyrate (Aldrich, 98%), copper(I) chloride (CuCl, Acros, 99.99%), 2,2′-bipyridine (bpy, Acros, 99+%), triethylamine (TEA, Acros, 99%), dodecanedioyl dichloride (Aldrich, 98%), 4-hydroxybenzophenone (Aldrich, 98%), sodium bicarbonate ($NaHCO_3$, Duksan, >99%), dichloromethane (Duksan, 99%), *N*,*N*-dimethylformamide (DMF, Duksan, 99.5%), and diethyl ether (Duksan, 99%) were all used as received.

2.2. Synthesis of di(4-hydroxyl benzophenone) dodecanedioate (BPD)

4-Hydroxybenzophenone (3.97 g, 20.0 mmol) was dissolved in THF (200 mL). TEA (3.49 mL, 25.0 mmol) and dodecanedioyl dichloride (3 mL, 12.0 mmol) were added dropwise to this solution, which was being kept in an ice bath. After stirring for 24 h, the amine salt formed was removed by filtration. After concentrating the solution, dichloromethane was added, and the solution was washed twice with 3% $NaHCO_3$ aqueous solution, and water. The resulting solution was dried over magnesium sulfate, and concentrated under reduced pressure. The product was purified by recrystallization from dichloromethane. The yield was 1.73 g (37%).

^{1}H NMR spectrum, δ (ppm): 1.3 (m, 2H), 1.56 (m, 2H, –$COOCH_2$–CH_2–), 2.23 (m, 2H –COO–CH_2–), 7.17 (m, 2H, –COO–ArH–), 7.83 (m, 2H, –CO–ArH–), 7.49–7.75 (m, 5H, –CO–ArH).

2.3. Synthesis of the poly(ethylene glycol) (PEG) macroinitiator

MPEG (6.0 g) was dissolved in THF (100 mL). TEA (0.60 g, 5.9 mmol) and 2-bromoisobutyryl bromide (0.44 g, 1.9 mmol) were added dropwise to this solution, which was being kept in an ice bath. After stirring for 48 h, the amine salt that had formed was removed by filtration. After concentrating the solution, the resulting polymer was precipitated in a large excess of diethyl ether. For further purification, the polymer solution in THF was reprecipitated in diethyl ether. The yield was 5.2 g (84%).

2.4. Synthesis of PEG-b-P(HEMA-co-MMA) diblock copolymer

A 100 mL reaction flask with a magnetic stirrer and a rubber septum was charged with the PEG macroinitiator (1.0 g, 0.5 mmol), CuCl (0.05 g, 0.5 mmol), bpy (0.23 g, 0.5 mmol), and acetonitrile (4.1 mL). The mixture was bubbled with N_2 for 10 min, and then HEMA (0.6 mL, 5.0 mmol) and MMA (2.14 mL, 20.0 mmol) were added. The mixture was bubbled with N_2 for 10 min, and then placed in a preheated oil bath at 85 °C. After 24 h, the mixture was diluted with acetone and passed though an aluminum oxide column to remove the copper catalyst. The solvent was removed by a rotary evaporator. The polymer was precipitated in a large excess of diethyl ether. The yield was 1.7 g (47%).

2.5. Photo-cross-linking of the BPD-containing PEG-b-P(HEMA-co-MMA) micelles

1.5 mL of a PEG-*b*-P(HEMA-*co*-MMA) solution in DMF (2.7 mg/mL), and a predetermined volume of a BPD solution in DMF (0.5 mg/mL) were added to a 50 mL vial. The final volume of the solution was adjusted to be 8 mL by adding pure DMF, after which 20 mL of deionized water was gradually added dropwise (1 mL/min) with vigorous stirring. The DMF was removed by dialysis against water for 24 h. The final concentration of polymer in each solution was 0.2 mg/mL. The BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles were cross-linked by UV irradiation at room temperature using a 500 W high pressure mercury lamp (Ushio UI-501-C). The UV lamp was kept at a distance of 15 cm from the sample surface.

2.6. Characterization

The molecular weight (MW) and polydispersity of the resulting polymers were determined by a gel permeation chromatography (GPC) system (Young Lin SP930D solvent delivery pump) coupled with a refractive index detector (RI 750F) and two columns (GPC KD-G and KF-806, Shodex). The eluent used was DMF at 40 °C with a flow rate of 1.0 mL/min. Poly(ethylene oxide) standards were used for calibration. ^{1}H nuclear magnetic resonance (NMR) spectroscopy was performed using a Varian VXR-Unity NMR spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as the solvent. The transmittance of the aqueous solution was acquired on a Turbiscan LAB-expert and measured at a wavelength of 880 nm. The morphologies of the copolymer micelles were investigated using a JEM-2100F transmission electron microscope operating at an accelerating voltage of 120 kV. A drop of aqueous PEG-*b*-P(HEMA-*co*-MMA) diblock copolymer solution (0.5 mg/mL) was deposited onto a 200 mesh copper grid that had been coated with carbon. The size and shape of the micelles were directly determined using transmission electron microscopy (TEM).

3. Results and discussion

ATRP is one of the most successful controlled radical polymerization techniques for the preparation of polymers having controlled MWs and MW distributions with well-defined architectures [21]. This ATRP technique has also been used for the preparation of various copolymers with controlled MWs and MW distributions [22–25]. The PEG-*b*-P(HEMA-*co*-MMA) diblock copolymer was synthesized via ATRP using a PEG macroinitiator at 85 °C. The molecular characteristics of PEG-*b*-P(HEMA-*co*-MMA) were determined using ^{1}H NMR spectroscopy and GPC. Fig. 1 shows the ^{1}H NMR spectrum of PEG-*b*-P(HEMA-*co*-MMA) ($M_n = 7200$, $M_w/M_n = 1.26$). Its chemical composition was evaluated from the relative intensities of ^{1}H NMR peaks at 1.8 and 3.9 ppm, which were attributed to the methylene groups in the backbone of the P(HEMA-*co*-MMA) block, and to the PHEMA methylene group in α-position to the ester oxygen, respectively. The numbers of HEMA and MMA units in PEG-*b*-P(HEMA-*co*-MMA) were determined to be 14 and 46, respectively, on the basis of the PEG block. Its M_n was calculated to be 8250 g/mol. There was a slight deviation between the monomer feed composition and the average polymer composition. The monomer feed composition of $[HEMA]_0/[MMA]_0$ was 10/40, indicating that the addition of HEMA was favored over that of MMA to a growing polymer chain. Fig. 2 shows the GPC traces of the PEG

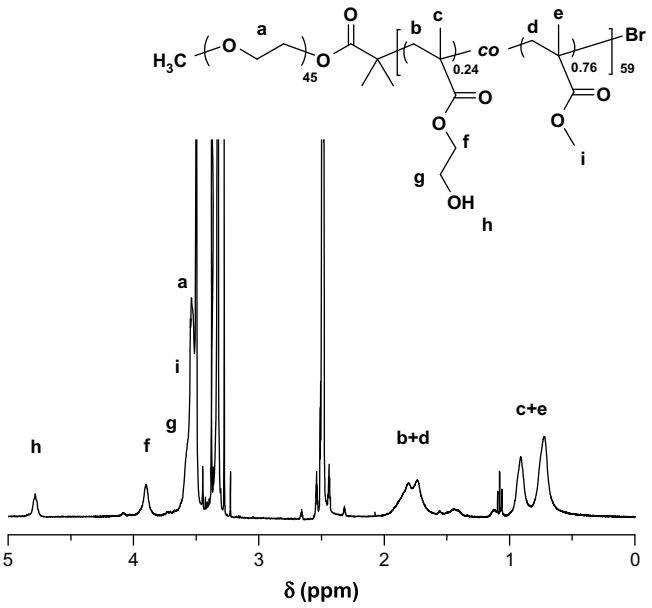


Fig. 1. ^{1}H NMR spectrum of PEG-*b*-P(HEMA-*co*-MMA).

macroinitiator and PEG-*b*-P(HEMA-*co*-MMA) diblock copolymer. The GPC trace of PEG-*b*-P(HEMA-*co*-MMA) was monomodal without any tailing caused by the residual PEG macroinitiator, suggesting high initiation efficiency.

Scheme 1 shows the cross-linked chemical structure in the PEG-*b*-P(HEMA-*co*-MMA) micelles by BPD. BP is a type-II initiator that can be excited photo-chemically with UV irradiation. The essential feature of type-II initiation involves abstracting a labile hydrogen atom from a suitable hydrogen donor group, such as alcohol, ether, thiol, amine, and hydrocarbon, to create radicals [26]. When BPD is exposed to UV, the carbonyl group in the BP moiety is excited to a triplet excited state, and abstracts a hydrogen atom from the hydroxyl group in the HEMA unit. This reaction produces radicals, and the radical–radical combination results in subsequent chain cross-linking [27–30].

Fig. 3 shows the turbidities of the CCL PEG-*b*-P(HEMA-*co*-MMA) micelles containing different amounts of BPD in THF. All of the PEG-*b*-P(HEMA-*co*-MMA) micelles (0.2 mg/mL) were UV irradiated in aqueous solution for 1 h. The micelles were then recovered and redissolved in THF (0.2 mg/mL). The effectiveness of the cross-linking reaction was judged from the stability of the cross-linked PEG-*b*-P(HEMA-*co*-MMA) micelles in THF. When the core domains of the micelles were sufficiently cross-linked, they remained intact in THF, resulting in a very low transmittance. However, when the micelles were not sufficiently cross-linked due to the lack of BPD, their solution was transparent. As the molar ratio of BPD to the HEMA units in PEG-*b*-P(HEMA-*co*-MMA) ([BPD]/[HEMA]) increased, the micelle solutions became opalescent, meaning that their micellar structures were stabilized by core cross-linking. From a molar ratio of [BPD]/[HEMA] = 0.5, the turbidity reached a constant value, indicating that the CCL PEG-*b*-P(HEMA-*co*-MMA) micelles can be successfully prepared at an equivalent ratio of the BP group to the HEMA units.

Fig. 4 shows changes in the turbidity of the CCL PEG-*b*-P(HEMA-*co*-MMA) micelles in THF as a function of the UV irradiation time. The molar ratio of [BPD]/[HEMA] was fixed at 0.6. The BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles (0.2 mg/mL) were UV

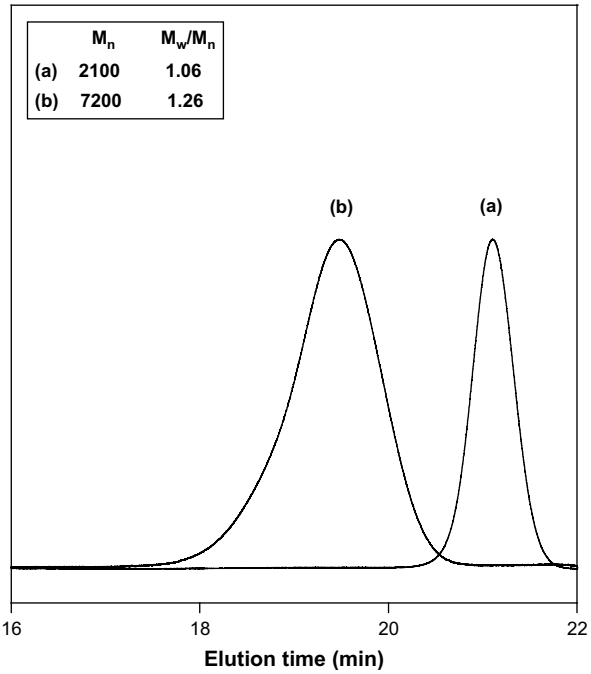
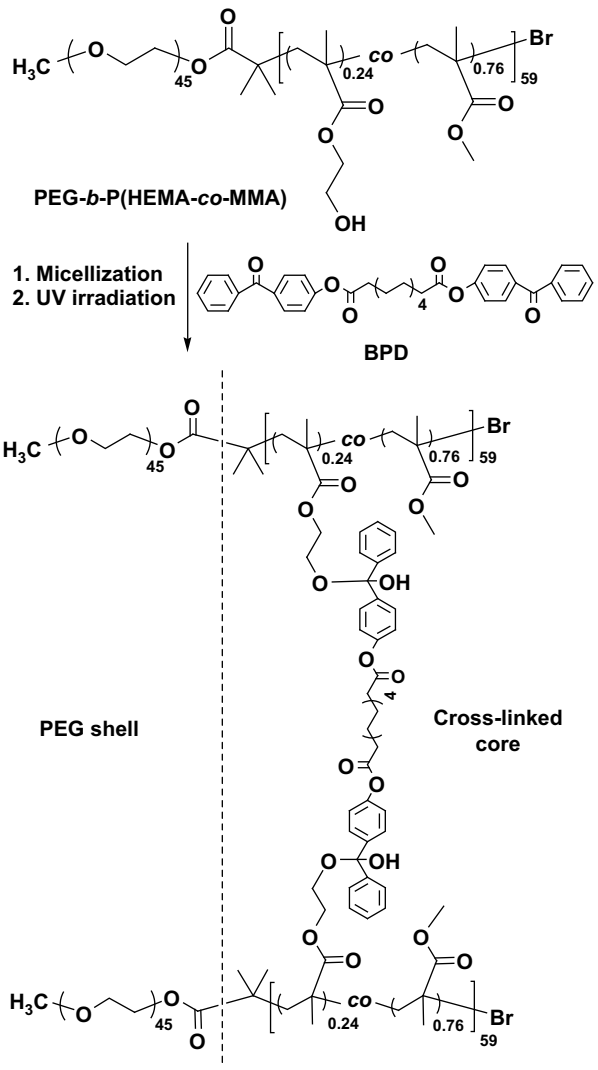


Fig. 2. GPC curves of (a) the PEG macroinitiator and (b) PEG-*b*-P(HEMA-*co*-MMA).



Scheme 1.

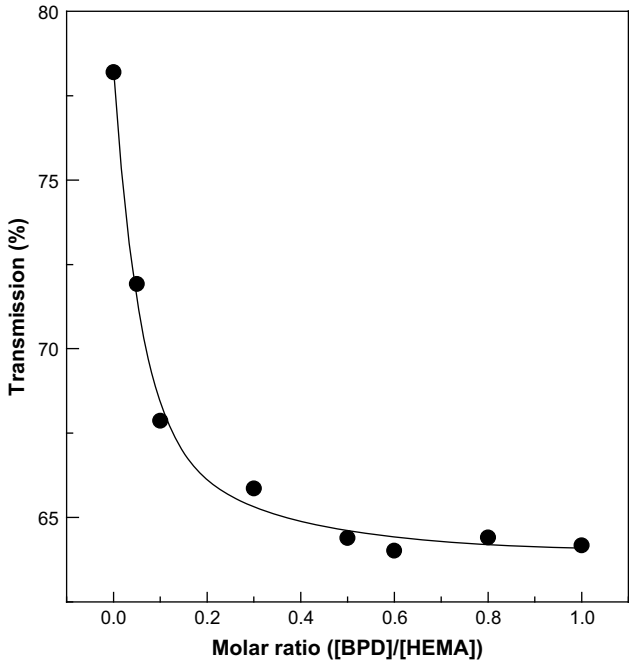


Fig. 3. Turbidity of the CCL PEG-*b*-P(HEMA-*co*-MMA) micelles according to BPD content. The micelles were UV irradiated for 1 h, then dissolved in THF.

irradiated in aqueous solution for different times. The micelles were then recovered and redissolved in THF (0.2 mg/mL). The transmittance decreased rapidly with increasing UV irradiation time, and reached a constant value after 30 min. Fig. 5 shows a TEM image of the BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles ([BPD]/[HEMA] = 0.5). The micelles were UV irradiated for 30 min, then dissolved in THF. The spherical structures of the cross-linked micelles remained intact, indicating that the photo-cross-linking of the PEG-*b*-P(HEMA-*co*-MMA) micelles with BPD was successful by UV irradiation for only 30 min.

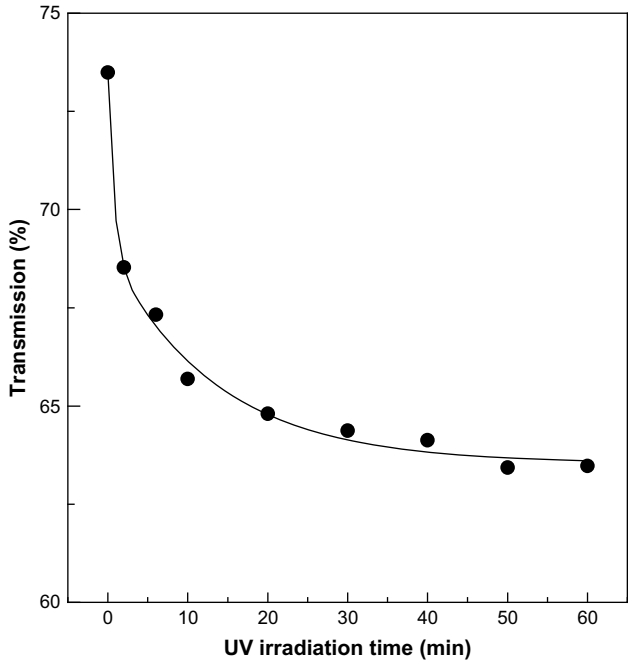


Fig. 4. Changes in turbidity of the CCL PEG-*b*-P(HEMA-*co*-MMA) micelles according to UV irradiation time: Molar ratio of [BPD]/[HEMA] = 0.6.

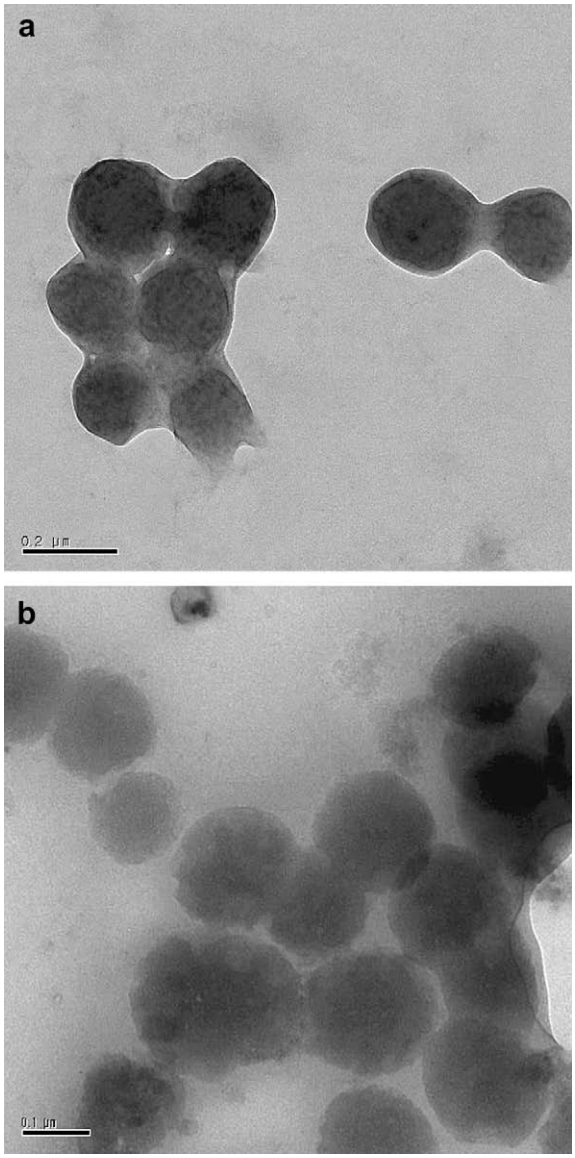


Fig. 5. TEM images of the BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles (a) before and (b) after UV irradiation. The micelles were UV irradiated for 30 min, then dissolved in THF.

In the present study, small amounts of HEMA units, which can act as good hydrogen donors to excited-state BP, were incorporated into the hydrophobic block in PEG-*b*-P(HEMA-*co*-MMA). Consequently, it was found that the photo-cross-linking reaction of PEG-*b*-P(HEMA-*co*-MMA) micelles occurred very fast and efficiently. Various other monomers having suitable hydrogen donor groups can be employed instead of HEMA. This study demonstrated a new concept for the convenient preparation of polymeric CCL micelles using photo-cross-linkable agents. Study on the encapsulation of functional nanomaterials using this concept is under way to improve the efficacy of the nanomaterial encapsulation.

4. Conclusion

The PEG-*b*-P(HEMA-*co*-MMA) diblock copolymer ($M_n = 7200$, $M_w/M_n = 1.26$) was synthesized via ATRP using a PEG macroinitiator at 85 °C. The addition of HEMA was favored over that of MMA to a growing polymer chain. The photo-cross-linking of the

BPD-containing PEG-*b*-P(HEMA-*co*-MMA) micelles was judged from the stability of the cross-linked PEG-*b*-P(HEMA-*co*-MMA) micelles in THF, using turbidity measurement and TEM. The CCL PEG-*b*-P(HEMA-*co*-MMA) micelles were successfully prepared at an equivalent ratio of the BP groups to the HEMA units. The photo-cross-linking of the PEG-*b*-P(HEMA-*co*-MMA) micelles with BPD was successfully achieved by UV irradiation for only 30 min. The micelles exhibited a spherical shape with an average diameter of 200 nm in aqueous solution.

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