Micelles and Reverse Micelles with a Photo and Thermo Double-Responsive Block Copolymer

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ABSTRACT: A novel photo and thermo double-responsive block copolymer was developed to fabricate micelles and reverse micelles in aqueous solution. The block copolymer was synthesized by ATRP block copolymerization of a spiropyran-containing methacrylate (SPMA) with di(ethylene glycol) methyl ether methacrylate (DEGMMA). By facile control of the photo irradiation and solution temperature, PSPMA-core and PDEGMMA-core micelles can be obtained, respectively. The thermo- and photo-responsive micelles were used as smart polymeric nanocarriers for controlled encapsulation, triggered release, and re-encapsulation of model drug coumarin 102. The double-responsive self-assembly and disassembly were tracked by dynamic light scattering, transmission electron microscopy, and fluorescence spectroscopy. © 2010 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 48: 2855–2861, 2010

KEYWORDS: block copolymers; micelle; microencapsulation; nanocarrier; nanoparticles; photo responsive; reverse micelle; stimuli-sensitive polymers

INTRODUCTION The stimuli-responsive block copolymers are of great interest for controlled encapsulation and triggered release of substances in many technological applications including targeted drug delivery and nanomedicine. This kind of block copolymers usually have one hydrophilic block and the other stimuli-responsive block. The combination of two different stimuli-responsive blocks in one block copolymer provides more possibilities to tune the morphology of aggregates. The individual block can be independently tuned to become either hydrophilic or hydrophobic depending on the presence of the stimulus. The copolymers can consequently self-assemble in aqueous media to form both micelles and reverse micelles.

Among various chemical and physical stimuli, photo is an especially attractive external stimulus and has been used in various areas. By taking advantage of the noncontact mode, as well as utilizing the optimal, precisely controlled wavelength, direction, illuminated area, and intensity, the triggered release of encapsulated substances can be controlled at a specific time and location. Compared to other stimuli, the use of photo to trigger a desired reaction of polymer micelles enables more temporal and positional control. Compared to irreversible photo-induced transition, efficient photo-reversible micellization is extremely promising since reversible control of encapsulation and release of hydrophobic substrates can be achieved by photo irradiation. Among the reversible photo switching, photochromic spiro-pyran (SP) molecule is of great interest because of the spiro-pyran-merocyanine (SP–MC) chemistry. The SP molecule is colorless, nonpolar, hydrophobic, and in a “closed” form under visible light irradiation. Exposure of the SP molecule to UV (365 nm) irradiation induces ring-opening isomerization, giving the colored, polar, hydrophilic, and zwitterionic MC form. Lee et al. synthesized a diblock copolymer of poly(ethylene glycol) and poly(spiropyran-containing methacrylate). The SP units respond to light and undergo a reversible isomerization between hydrophobic SP and hydrophilic MC form. This micelle system was successfully applied to the efficient encapsulation, release, and partial re-encapsulation of hydrophobic dye.

Herein, we presented a photo and thermo double-responsive block copolymer that was synthesized by ATRP block copolymerization of a spiropyran-containing methacrylate (SPMA) with di(ethylene glycol) methyl ether methacrylate (DEGMMA) (Scheme 1). Because of the photo-switchable PSPMA block and the thermo-responsive PDEGMMA block, both PSPMA-core and PDEGMMA-core micelles can be obtained just by adjustment of the solution temperature and photo irradiation (Scheme 2). As far as we are aware, there
is no report in literature about photo-switchable "schizophrenic" micelles. The exploration of the photo and thermo
double-responsive micellization as nanocarrier for controlled
release and re-encapsulation will be very interesting for bio-
medical applications.

EXPERIMENTAL

Materials

\(N,N',N',N'\text{-pentamethyldiethylenetriamine (PMDETA, 99%}
\) Aldrich), ethyl 2-bromoisobutyrate (EBiB, 98%, Aldrich), and
coumarin 102 (97%, Fluka) were used without further puri-

fication. SPMA was prepared as previously reported\(^\text{33,34}\)

DEGMMA (95%, Aldrich) was passed through a basic alu-

mina column to remove inhibitor and then stored in a refrig-

erator before use.

Synthesis of Macroinitiator PSPMA-Br

A typical procedure for the synthesis of macroinitiator

PSPMA-Br using EBiB as initiator was described below. SPMA

(0.9 g, 2 mmol), EBiB (0.1 mmol), PMDETA (0.1 mmol, 21 \(\mu\)L),

5 mL tetrahydrofuran (THF), and 1 mL methanol were added

into a three-necked flask. The mixture was stirred under \(N_2\)

atmosphere and degassed by three freeze-pump-thaw cycles.

The degassed CuBr (14.4 mg, 0.1 mmol) was then added

under nitrogen blanket to start the polymerization at 25 \(^\circ\)C. Af-

After 3 h, the polymerization was quenched by exposing the

reaction mixture to air. The solution was diluted with THF and

passed through a neutral \(\text{Al}_2\text{O}_3\) column with THF as eluent to

remove the catalyst. The obtained polymer solution was con-

centrated and precipitated in methanol. The polymer was then

dissolved in THF and precipitated again in methanol. The polymer

was then dried in high vacuum at 40 \(^\circ\)C.

Synthesis of Block Copolymer PSPMA-PDEGMMA

PSPMA-Br (0.1 mmol), DEGMMA (3.5 mmol), PMDETA (0.1

mmol), 5 mL THF, and 1 mL methanol were added into a

three-necked flask. The mixture was stirred under \(N_2\) atmos-

phere and degassed by three freeze-pump-thaw cycles. The
degassed CuBr (14.4 mg, 0.1 mmol) was then added under

nitrogen blanket to start the polymerization at 25 \(^\circ\)C. After

24 h, the polymerization was quenched by exposing the reac-
tion mixture to air. The solution was diluted with THF and
passed through a neutral Al2O3 column with THF as eluent to remove the catalyst. The obtained polymer solution was concentrated and precipitated in hexane. The polymer was then dissolved in THF and precipitated again in hexane. The polymer was then dried in high vacuum at 40 °C.

Preparation of PSPMA-b-PDEGMMA Micelle
A micellar solution of PSPMA-PDEGMMA in water was prepared by a typical cosolvent approach at 15 °C [below the lower critical solution temperature (LCST) of PDEGMMA]. PSPMA-PDEGMMA (1.0 mg) was dissolved in 1 mL of THF and then 10 mL of water was added dropwise. THF was completely removed by dialysis against water. The solution was filtered through a 0.22-μm filter before use.

Encapsulation of Coumarin 102
At 15 °C, 2.0 mL coumarin 102 solution in acetone (0.05 mg/mL) was added dropwise into 10 mL blank PSPMA-PDEGMMA micellar solution under stirring. After ultrasonication for 15 min, the solution was stirred for 24 h in dark.

Characterization
Gel permeation chromatography (GPC) was conducted in THF at 40 °C with a flow rate of 1.0 mL/min. Calibration was carried out using a series of low polydispersity polystyrene standards. 1H NMR spectra was recorded on a Bruker DMX500 instrument and scanned in the range 0–15 ppm. UV-vis spectra were carried out with a UV-vis Shimadzu UV-2505 spectrometer using 1-cm-path length quartz cuvettes. Spectra were collected within a range of 400–800 nm. Transmission Electron Microscopy (TEM) analysis was performed on a JEM-1200EX TEM operating at 80 kV in bright field mode. Samples were prepared by placing a drop of the solutions on a 400-mesh carbon-coated copper grid. For the determination of particle size, over 100 particles were counted in multiple pictures from different areas of the TEM grid. Fluorescence emission spectra were recorded from Perkin-Elmer LS 55 fluorescence spectrometer. The excitation wavelength was 420 nm, and the fluorescence emission spectra were recorded from 450 to 700 nm.

RESULTS AND DISCUSSION
Synthesis of PSPMA-b-PDEGMMA
The synthetic strategy used in our research was schematically illustrated in Scheme 1. The block copolymer PSPMA-b-PDEGMMA was synthesized via sequent ATRP. Macroinitiator PSPMA-Br was polymerized by atom transfer radical polymerization (ATRP) using ethyl 2-bromoisobutyrate as initiator. The synthesized PSPMA-Br was then used as a macroinitiator for block copolymerization with DGEAGMA. The resulting PSPMA-b-PDEGMMA diblock copolymer was characterized by GPC and 1H NMR. The number-average molecular weight ($M_n$) and the molecular weight distributions ($M_w/M_n$) of PSPMA-Br and PSPMA-b-PDEGMMA were determined by GPC using THF as eluent and shown in Figure 1. The typical proton NMR spectra of PSPMA-Br and PSPMA-b-PDEGMMA scattering measurements were performed on the samples using a Brookhaven 90Plus size analyzer at the 90° scattering angle. The 365-nm UV light source was a 200-W high-power mercury-arc lamp. The 620 nm visible light source was a 100-W lamp.
were shown in Figure 2 with the relevant signals labeled and all peaks can be well assigned with their chemical structures. By comparing the well-defined peak integrals of the SPMA block with that of the ATRP initiator fragment in Figure 2(a), the degrees of polymerization (DP) of the SPMA block was 9. By comparing the peak integrals of SPMA and DEGMMA blocks in Figure 2(b), the DP of DEGMMA block was 28. Thus, the obtained polymer was denoted as PSPMA$_9$-b-PDEGMMA$_{28}$.

**Formation of Double-Responsive Micelles and Reverse Micelles**

The PSPMA-b-PDEGMMA block copolymer was expected to self-assemble in response to photo irradiation or changes in solution temperature. PDEGMMA is a new class of thermo-responsive polymer with excellent antifouling/stealth behavior. The LCST of PDEGMMA was around 26 °C. Below the LCST, PDEGMMA is molecularly dissolved in water, but it becomes hydrophobic when the temperature is above the LCST. PSPMA is hydrophobic under visible light irradiation, but it turns to hydrophilic after irradiating with UV light. At 15 °C, 0.1 mg/mL PSPMA-PDEGMMA aqueous solution was exposed to visible light (620 nm) for 1 h to make sure that the ring-closed SP groups were dominant. PSPMA-core micelle was formed at 15 °C since PSPMA block was hydrophobic and PDEGMMA block was hydrophilic. On the basis of chemical intuition, PSPMA block formed the inner core, and the hydrophilic PDEGMMA formed the outer shell of the micelle. The average hydrodynamic diameter ($D_h$) of the micelle was 79.3 nm and the polydispersity index (PDI) was 0.124 [Fig. 3(a)]. Then, the micelle solution was exposed to UV light (365 nm) at 15 °C. The reversible photochemical isomerization from hydrophobic SP to hydrophilic MC was trailed by UV–vis spectroscopy. After the solution was irradiated with UV light, a strong absorption band at 565 nm which was the characteristic peak of the MC form appeared [Fig. 4(a)]. During the UV irradiation process, the absorption peak at 565 nm increased dramatically and reached a plateau after 90 min. It showed that hydrophobic SP group underwent ring-opening isomerization, giving the hydrophilic MC form. The average hydrodynamic diameter ($D_h$) was only 7.2 nm [Fig. 3(c)], indicating that the block copolymer was molecularly dissolved in water at 15 °C under UV light (365 nm) irradiation. As a result, the PSPMA-core micelles were dissociated into unimers under UV light (365 nm) irradiation at 15 °C. The photochemical isomerization of SP to MC was reversible. After it was exposed to UV light for 90 min, if the solution was alternately irradiated with visible light (620 nm), MC was isomerized back to the SP form, as was confirmed by the UV–vis spectra. The absorption peak at 565 nm was gradually decreased during irradiation and reached a plateau after 180 min [Fig. 4(b)].

As mentioned above, PSPMA-core micelle was formed at 15 °C and under visible light irradiation. After irradiation with UV light at 15 °C, both PSPMA block and PDEGMMA block became hydrophilic. If the solution temperature was then increased to 30 °C, which was much higher than the LCST of PDEGMMA, dehydration and interchain aggregation...
of PDEGMMA occurred. The PDEGMMA block became hydrophobic. In this situation, PDEGMMA-core micelle was formed with average hydrodynamic diameter of 24.1 nm and the PDI was 0.241 [Fig. 3(b)]. TEM images of PSPMA-core (15 °C, vis irradiation) and PDEGMMA-core (30 °C, UV irradiation) micelles revealed the presence of presumably spherical morphologies. The mean number-average diameters were about 59.5 ± 7.3 and 16.5 ± 4.6 nm for PSPMA-core and PDEGMMA-core micelles, respectively (Fig. 5). Taking into account both the degree of hydration and polydispersity effects, the mean diameter determined by TEM compared reasonably well to the average hydrodynamic diameter.

Micelles and Reverse Micelles as Nanocarriers for Triggered Release of Hydrophobic Dye
Stimuli-responsive micelles are ideal nanocarriers for triggered release of hydrophobic drugs and widely used in drug delivery systems. The hydrophobic fluorescent dye coumarin 102 was then chosen as model hydrophobic drug to be reversibly captured within the photo and thermo double-responsive PSPMA-PDEGMMA system. Coumarin 102 was used as the fluorescence probe since its fluorescence is known to increase substantially in a hydrophobic environment such as the core of micelles. The controlled encapsulation and triggered release of substances by adjusting photo and thermo stimulus was investigated by fluorescence spectroscopy. An excitation wavelength of 420 nm was used, since no photo isomerization between SP and MC occurred at this wavelength. After incorporation of coumarin 102 into micelle solution at 15 °C under visible light irradiation, a strong emission peak of coumarin 102 with maximum at 488 nm appeared, which is the characteristic emission peak of coumarin 102 (Fig. 6). Coumarin 102 was encapsulated into the micelle. However, after the micellar solution was exposed to UV light, the emission intensity of coumarin 102 decreased gradually from fluorescence spectroscopy. Hydrophobic coumarin 102 was released from the micelles, which was attributed to the disruption of PSPMA-core micelle. If visible light was irradiated again under vigorous stirring, MC

![FIGURE 5 TEM imagines of (a) PSPMA-core micelles under visible light (620 nm) irradiation at 15 °C and (b) PDEGMMA-core micelles under UV light (365 nm) irradiation at 30 °C.](image)

![FIGURE 6 Fluorescent emission spectra of micellar solution with encapsulated coumarin 102 at 15 °C. (a) Coumarin 102 was released from disrupted micellar solution after UV (365 nm) irradiation and (b) coumarin 102 was partially re-encapsulated into the reorganized micelles after irradiation with visible light (620 nm).](image)
group turned to SP group. Fluorescent emission intensity of coumarin 102 at 488 nm increased rapidly, which was accompanied by the regeneration of PSPMA-core micelles (Fig. 6). Coumarin 102 was partly re-encapsulated into PSPMA-core micelles again. The incomplete re-encapsulation of coumarin 102 might be due to the relatively high regeneration rate of micelle, which would prevent the released hydrophobic dye molecules from interacting with the hydrophobic block. As a result, it was not sufficient to re-encapsulate all of them.

On the other hand, if the temperature was increased from 15 to 30 °C under UV irradiation, from the fluorescence emission spectra, the emission intensity at 30 °C was much stronger than 15 °C. It showed that coumarin 102 was re-encapsulated into PDEGMMA-core micelles (Fig. 7). Thermo-triggered release and re-encapsulation of coumarin 102 were also realized. During the reversible capture and release of the hydrophobic dye coumarin 102, we also proved the formation of both PSPMA-core and PDEGMMA-core micelles in aqueous media. Because the PSPMA-PDEGMMA copolymer solid powder could not be dissolved in water directly, it was very difficult to obtain conclusive spectroscopic evidence by \(^1\)H NMR that PSPMA-core and PDEGMMA-core micelles were formed. However, from the solubility transition of the PSPMA block and PDEGMMA block after photo and thermo stimulus, we can conclude PSPMA-core and PDEGMMA-core micelles were formed, respectively.

CONCLUSIONS

In summary, we have reported herein a novel photo- and thermo-responsive diblock copolymer PSPMA-PDEGMMA, which can self-assemble to form either micelles (PSPMA-core) or reverse micelles (PDEGMMA-core) in aqueous solution. At 15 °C under visible light irradiation, the block copolymer self-assembled to form PSPMA-core micelles. In contrast, at 30 °C under UV light irradiation, PDEGMMA-core micelles were formed. This double-responsive micelle system was also successfully used as nanocarriers to the efficient encapsulation, triggered release, and partial re-encapsulation of model drug coumarin 102. The thermo- and photo-responsive micelles open the door to develop photo-switchable polymer nanocarriers, which may have potential applications in biomedical areas.

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