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Toward Photocontrolled Release Using Light-Dissociable Block Copolymer Micelles

Jinqiang Jiang,[†] Xia Tong,[†] Denis Morris,[‡] and Yue Zhao*,[†]

Département de chimie and Département de Physique,
Université de Sherbrooke, Sherbrooke,
Québec, Canada J1K 2R1

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1. Introduction

Micellar aggregates (core−shell micelles, vesicles, etc.) formed by amphiphilic block copolymers (BCPs) are being actively investigated as a nanocarrier system for controlled delivery of drugs and other biological substances.[1] Generally speaking, a good nanocarrier of drugs would be (1) stable during the circulation in the blood, allowing no release of encapsulated hydrophobic drugs, (2) able to accumulate specifically in the target site (e.g., tumors), and (3) once on target, able to release the carried drug quickly or in a controlled fashion. Each of these requirements has prompted a great deal of research efforts in the design, synthesis and exploitation of new BCPs. As far as the control of release is concerned, BCP micelles that can be disrupted by external stimuli, thus triggering the release of entrapped agents, are of particular interest. The stimuli studied so far not only include changes that BCP micelles in the body can experience, such as increase or decrease in pH,[2] in temperature[3] and exposure to oxidation reaction,[4] but also include sound[5] and light[6−8] that can be applied from outside of the body for remote activation of the disruption of micelles.

If the interaction of BCP micelles with light lead to their dissociation, photocontrolled release of encapsulated agents is possible. One obvious, and attractive, feature of stable, light-responsive polymer micelles is that the release of the carried guest (not limited to drugs) could be started at required time (when light is applied) and at required location (where light exposure is directed to). This offers new perspectives in controlled release that would be difficult to realize using other stimuli. To obtain usable BCP-based nanocarriers for photocontrolled delivery applications, the development of effective designs of BCP structures and fundamental studies of their photoinduced dissociation and release processes are necessary. There are many studies of light-responsive micellar aggregates of small-molecule surfactants,[9−16] but very few reports on light-dissociable polymer micelles.[6−8] We first studied amphiphilic BCPs whose hydrophobic block is an azobenzene-containing side-chain liquid crystalline polymer (Azo-SCLCP).[6,7] We found that their core−shell micelles and vesicles in solution can be dissociated by UV light irradiation and re-formed upon visible light exposure, as a result of the reversible trans−cis photoisomerization of azobenzene side groups of the hydrophobic block. From a study of the mechanism of the reversible micellar dissociation, a general design principle for azobenzene-based light-dissociable BCP micelles has been suggested.[7] The azobenzene moiety should have a small (near zero) dipole moment in the trans form and a high dipole moment in the cis form (determined by the substituents on the azobenzene unit); when this is conjugated with a weakly hydrophilic block (determined by the chemical nature and the length of the block), the increase in polarity of the azobenzene polymer block under UV light (trans−cis isomerization taking place) can alter the hydrophilic/hydrophobic balance which results in dissociation of micelles, while the decrease in polarity upon visible light irradiation (reverse cis−trans isomerization occurring) shifts the hydrophilic/hydrophobic balance in the opposite direction which brings back the micelles. More importantly, these studies suggest that any chromophores whose photoinduced structural rearrangements gives rise to a significant change in dipole moment could be used in designing light-dissociable BCP micelles based on the same principle. If the photoinduced process is reversible, the micellar dissociation is reversible.

More recently, we have demonstrated the efficiency of a different, and more general, strategy for designing BCPs whose micelles can undergo irreversible light-induced dissociation.[8] We synthesized an amphiphilic BCP whose hydrophilic block is poly(ethylene oxide) (PEO) and whose hydrophobic block is a polymethacrylate bearing pyrene moiety in the side group. Upon UV light irradiation of the micellar solution, the photosolvolysis of pyrenylmethyl esters cleaves the pyrene moiety from the polymer which converts the hydrophobic block into hydrophilic poly(methacrylic acid) (PMA) and, consequently, leads to straightforward dissociation of BCP micelles. That study further confirmed that a variety of chromophores or dyes could be incorporated in the structures of BCPs to design light-dissociable polymer micelles. What is needed is that the interaction of the chromophore with light results in a structural change that alters the hydrophilic/hydrophobic balance toward the direction unfavorable to the micellar association. However, in the previous works,[6−8] the photocontrolled release of guests has not been studied.

In this paper, using a new dye-containing amphiphilic BCP, we report, to our knowledge, the first detailed study on photocontrolled release of a model hydrophobic guest from light-dissociable polymer micelles. While keeping PEO as the hydrophilic block, the hydrophobic block of the new BCP is poly(2-nitrobenzyl methacrylate) (Figure 1). The simple chromophore is a photolabile protecting group utilized in many applications.[17] In this diblock copolymer, the photolysis of 2-nitrobenzyl moieties, via either one-photon UV (365 nm) or two-photon near-infrared (700 nm) absorption, detaches the chromophore from the polymer and transforms the hydrophobic block into hydrophilic PMA. Unlike pyrenylmethyl esters whose photosolvolysis needs the presence of a nucleophilic solvent,[18] the photolysis of 2-nitrobenzyl is an intramolecular rearrangement process that can readily occur both in solution and in the solid state.[19] Using Nile Red (NR) as a model hydrophobic guest for encapsulation by the photolabile polymer micelles and investigation of the photocontrolled release (Figure 1), the effects of a number of variables on the process were studied, which includes the intensity of UV irradiation, the relative length of the 2-nitrobenzyl polymer block, and partial cross-linking of micellar aggregates. The results show the interest of developing light-dissociable polymer micelles for photocontrolled delivery applications.

2. Experimental Section

2.1. Synthesis. Materials. THF were purified by distillation from sodium with benzophenone. Catalyst Cu^{I}Br was washed repeatedly

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* Corresponding author: yue.zhao@usherbrooke.
[†] Département de chimie, Université de Sherbrooke.
[‡] Département de Physique, Université de Sherbrooke.

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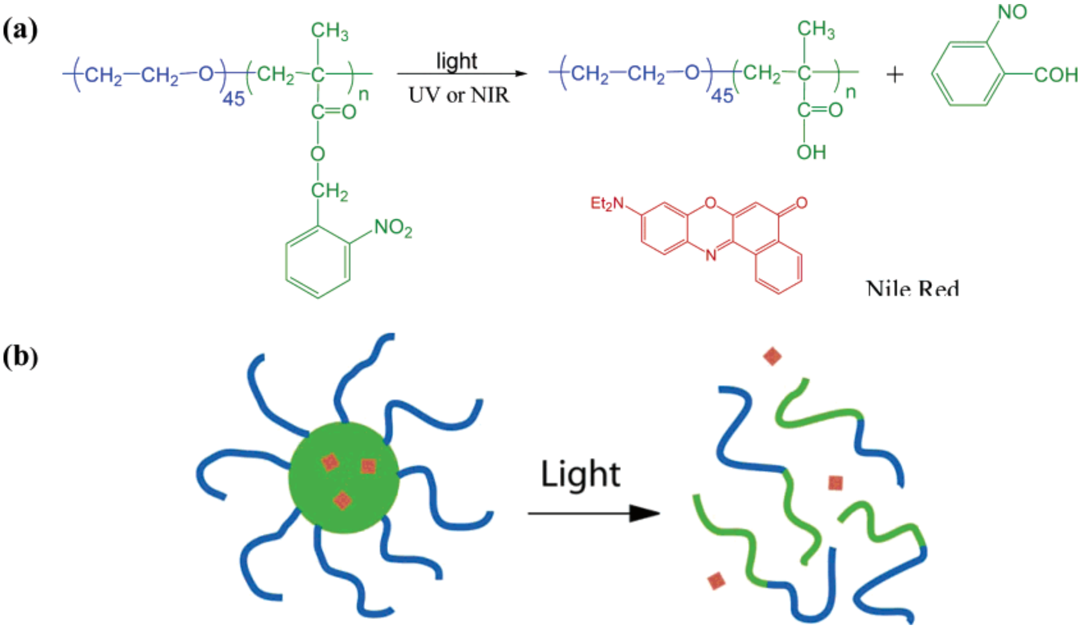
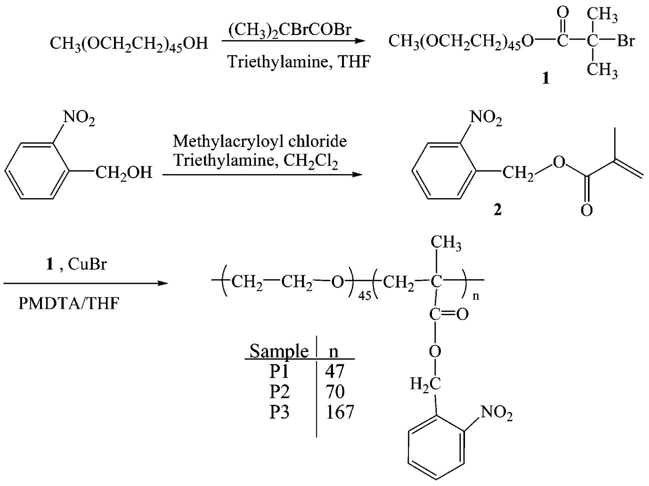


Figure 1. (a) Chemical structure and the photolysis of the 2-nitrobenzyl-containing amphiphilic block copolymer; and chemical structure of Nile Red. (b) Schematic illustration of the photocontrolled release of an encapsulated agent as a result of the photoinduced dissociation of the polymer micelle.

Scheme 1. Synthetic Route to Diblock Copolymers of PEO-*b*-PNBM



with acetic acid and ether, then dried and stored under nitrogen. 2-Bromo-2-methylpropionyl bromide, methylacryloyl chloride, 2-nitrobenyl alcohol, *N*,*N*,*N*′,N′,*N*″-pentamethyldiethylenetriamine (PMDETA), triethylamine, Nile Red, and poly(ethylene glycol) methyl ether were purchased from Aldrich and used without further purification.

Synthesis of Diblock Copolymers. Scheme 1 shows the synthetic route to the photoactive amphiphilic diblock copolymer composed of PEO and poly(2-nitrobenzyl methacrylate) (PNBM). Both PEO macroinitiator **1** and the 2-nitrobenzyl monomer **2** were prepared following literature methods;[20,21] no details on their syntheses are given here. The PEO macroinitiator was obtained from poly(ethylene glycol) methyl ether with a number-average molecular weight of 2000 g mol^{-1}. Atom transfer radical polymerization (ATRP) was utilized to synthesize PEO-*b*-PNBM. An example of such a reaction is detailed below.

Cu^{I}Br (29 mg, 0.2 mmol), macroinitiator **1** (200 mg, 0.1 mmol), and monomer **2** (1 g, 4.5 mmol) were mixed in a 5 mL ampule, degassed, and filled with nitrogen. Then, PMDETA (35 mg, 0.2 mmol) dissolved in THF (3 mL) was added through a syringe. The mixture was degassed three times using the freeze−pump−thaw procedure and sealed under vacuum. After 30 min of stirring at room temperature, the ampule was placed in a preheated oil bath (60 °C) for 14 h. The solution was passed through a neutral Al_2O_3 column with THF as eluent to remove the catalyst. The light blue filtrate was concentrated under reduced pressure and reprecipitated twice into water and washed with hexane. The obtained diblock copolymer, after being collected by filtration and dried under vacuum, was found to contain about 167 NBM units based on the ^{1}H NMR analysis. As indicated in Scheme 1, three samples with different lengths of PNBM block were synthesized, which are denoted as p1 (PEO$_{45}$-*b*-PNB$_{47}$), p2 (PEO$_{45}$-*b*-PNB$_{70}$), and p3 (PEO$_{45}$-*b*-PNB$_{167}$). We note that GPC measurements using polystyrene standards yielded much smaller molecular weights presumably due to interaction between the chromophore-containing polymers and the columns.[22] The polydispersity revealed by GPC is about 1.8 for all three samples.

2.2. Preparation of Polymer Micelles and Encapsulation of Nile Red. Three types of micellar solutions were prepared and utilized in this study. First, polymer micelles without Nile Red (NR) were obtained in a mixed solvent of THF/water and used for all static light scattering experiments and related scanning electron microscopy observations. In this case, a sample of PEO-*b*-PNBM was dissolved in THF, which is a good solvent for the two blocks; with a concentration of, for example, 0.2 mg mL^{-1}; water was then added slowly to induce the aggregation of PNBM forming the micelle core. Second, polymer micelles with encapsulated NR in aqueous solution were prepared for the fluorescence measurements used to monitor the photocontrolled release. Here, the polymer and NR were first dissolved in THF at a concentration of 0.2 and 0.04 mg mL^{-1}, respectively; water was then added to induce the formation of micelles and the simultaneous encapsulation of NR by the hydrophobic core of PNBM. After 2 h of stirring, a 4-fold volume of water was added for quenching of micelles and precipitation of unloaded NR. Precipitated NR was removed by filtration through 0.45 μm membrane, while THF and most of water evaporated at room temperature until the solution regained the initial polymer concentration of 0.2 mg mL^{-1}. This procedure gave rise to NR-containing polymer micelles dispersed in aqueous solution.

The third preparation procedure was used to obtain core-cross-linked photolabile polymer micelles. Detailed conditions are as follows. The sample p3 (6 mg) was dissolved in THF (2 mL), and the solution was exposed to UV light to photolyze a small portion of 2-nitrobenzyl moieties introducing carboxylic acid groups on the polymer (Figure 1). Afterward, the polymer solution was added slowly into the premixed NR (1 mg), 2,2′-(ethylenedioxy)bis(ethylamine) (8 mg, cross-linker) and 1-(3′-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (30 mg, catalyst) in a solution of 20 mL of water and 10 mL of THF. The formation of polymer micelles and encapsulation of NR took place quickly, but the micellar solution was stirred for 2 days at room temperature for the cross-linking reaction inside the hydrophobic core of PNBM.[23] The solution was finally transferred to a dialysis bag, and dialyzed against distilled water for 3 days to remove THF and all small-molecule byproducts; precipitated NR was removed by filtration. This procedure yielded partially cross-linked, NR-loaded micellar aggregates. All micellar solutions used for fluorescence measurements were kept at a polymer concentration of 0.2 mg mL^{-1}.

2.3. Characterizations. ^{1}H NMR spectra were obtained with a Bruker spectrometer (300 MHz, AC 300). UV−vis spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Fluorescence emission spectra were recorded on a SPEX 1680 double-monochromator spectrophotometer. Gel permeation chromatography (GPC) measurements were performed using a Waters system equipped with a refractive index and a photodiode array detector; THF being used as eluent (elution rate, 0.5 mL min^{-1}) and polystyrene standards used for calibration. Micellar aggregates were examined using a Hitachi S-4700 Field-Emission-Gun SEM operating at 3 kV. The samples were prepared by casting a drop of micellar solution, either before or after irradiation, on a silicon wafer, followed by drying at room temperature. Static light scattering (SLS) measurements were carried out using a DAWN-EOS instrument (Wyatt Technology), with the 30 mW linearly polarized GaAs laser tuned at 690 nm. All solutions were filtered through 0.4 μm filter before the measurement.

To study the photoinduced dissociation of polymer micelles and the release of encapsulated NR, UV irradiation of variable intensities was obtained from a UV−vis spot curing system (Novacure) combined with a 365 nm filter. This wavelength is far from the absorption maximum of 2-nitrobenzyl below 300 nm, which means that more efficient photolysis, thus faster dissociation of micelles and release of encapsulated agents, can be obtained using shorter UV wavelengths. Unless otherwise specified, 0.7 mL of micellar solution was placed in a standard cuvette, while the solution was exposed to UV light under stirring, having a spot size of ∼ 1 cm^2, applied vertically from the top of the cuvette. In the case of two-photon absorption of near-infrared light, the second harmonic of the signal output of an optical parametric amplifier pumped by a regenerative Ti:sapphire amplifier was utilized to produce ∼80 fs pulses at 700 nm and at a repetition rate of 1 kHz. The excitation spot diameter was about 50 μm and nearly constant over the length of the microcuvette, in which 0.3 mL of micellar solution was placed. The pulse energy was fixed at 1 μJ, which corresponds to an energy density of 50 mJ cm^{-2}. After each irradiation, the solution was repeatedly taken out of and then reintroduced into the cuvette to be homogenized before the fluorescence measurement.

3. Results and Discussion

3.1. Photoinduced Dissociation of Micelles in THF/Water Solutions. Since the photocleaved 2-nitrosobenzaldehye (Figure 1) is insoluble in water, but soluble in the mixed solvent of THF/water used to induce the micellization, the micelle formation and the dissociation upon UV light irradiation of the three BCP samples in THF/water were first investigated using SLS and SEM. As mentioned earlier, adding water in THF solution of the polymer induces the formation of BCP micelles; the critical water content for micellization (CWCM) could be revealed through the SLS measurement. Figure 2 shows the plots of scattered light intensity (measured at 90°) vs water content (vol. % with respect to THF) for p3 at three initial concentrations and for p2 and p1 at one concentration. The formation of micelles is indicated by an abrupt rise of scattered intensity. On one hand, from the results obtained with p3, it is clear that CWCM is lowered as the amount of polymer dissolved in THF increases, decreasing from about 22% for the BCP concentration of 0.2 mg mL^{-1} to 17% for the BCP concentration of 5 mg mL^{-1}. On the other hand, the effect of the length of the hydrophobic PNBM block on CWCM is evidenced from the results of the three samples at the same concentration of 0.2 mg mL^{-1}. As expected, more water is needed to induce the micellization when the length of PNBM is shorter. These results indicate the formation of BCP micelles in the THF/water mixture and show that changes in scattered light intensity, with respect to the scattering by the polymer solution before the micelle formation, are indicative of the state of BCP aggregation.

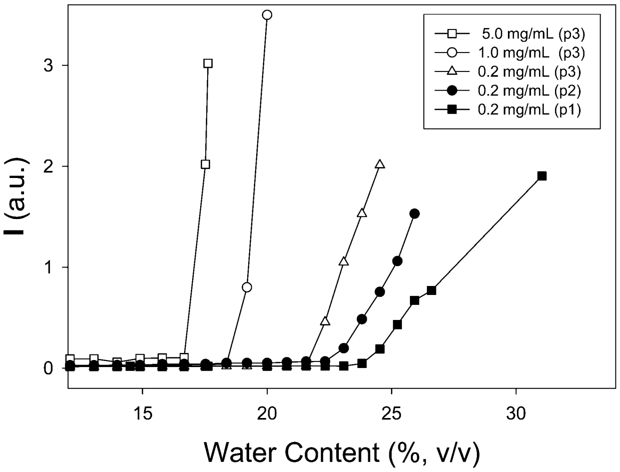


Figure 2. Plots of light scattering intensity (measured at 90°) vs amount of water added to THF solutions of p3 at various initial polymer concentrations, and of p2 and p1 at one concentration, showing the effects of polymer concentration and of the length of hydrophobic block on the critical water content for micellization.

The three BCP samples differ in the length of PNBM block; the size of the photolabile hydrophobic core of micelle should be larger for sample with a longer PNBM block. We wanted to know if the micelle core size might have an effect on the rate of photoinduced dissociation of the micelles. For comparison purposes, in addition to keeping all experimental conditions the same, micelles of the three samples were prepared by adding an amount of water that exceeds the corresponding CWCM by 10% (Figure 2). Since the dissociation of polymer micelles upon UV irradiation results in decrease in turbidity of the micellar solution, changes in light scattering intensity at 90° (as an example) were recorded to monitor the dissociation kinetics. Figure 3 plots the variation of the normalized scattering intensity, with respect to the initial intensity prior to irradiation, as a function of irradiation time for the micellar solutions of the three polymers (8 mL solution exposed to 2000 mW cm^{-1}). In all cases, the turbidity drops within the first 2−3 min of irradiation, then changes only slightly at longer times. Even though the length of PNBM block has an effect on the remaining scattering level that is higher for p3 than p2 than p1, the rate of dissociation seems to be similar for the micelles of the three samples. The results thus suggest that the length of the photoactive PNBM block, which is correlated to the size of the hydrophobic core of micelle, had no noticeable effect on the photoinduced dissociation process. This observation can be explained by the photolysis of 2-nitrobenzyl. In contrast with photosolvolysis that requires the contact of the chromophore with solvent molecules, the photolysis of 2-nitrobenzyl is an intramolecular process and occurs both in solution and in the solid state. This means that regardless of the location of the chromophore moieties in the micelle (deep in the core or at the vicinity of the core−shell interface), they basically have the same probability to absorb UV light and to undergo the ester cleavage reaction.

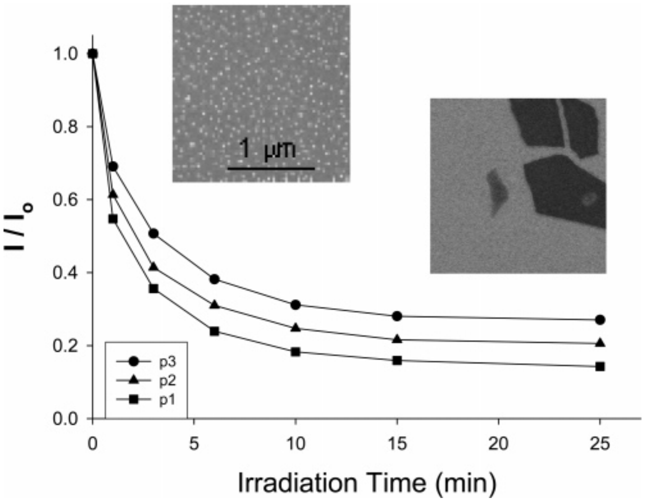


Figure 3. Plots of normalized light scattering intensity (measured at 90°) vs time of UV light irradiation (8 mL solution, 2000 mW cm^{-2}) for three polymer micellar solutions, I_o being the scattering intensity before irradiation. SEM images show the core−shell micelles in the solution of p3 before irradiation and the disappearance of the micelles after 20 min irradiation.

It should be emphasized that the decrease in turbidity as measured by SLS may arise from either a decrease in number of aggregates or a decrease in size of the aggregates, or a combination of both. Data in Figure 3 do not indicate how the dissociation of polymer micelles develops. SEM observations fund that the number of aggregates decreased upon UV irradiation, but changes in average size of micelles cannot be rule out due to the limitation of the techniques available to us. Under the used conditions most micelles were dissociated after about 15 min UV irradiation. Examples of SEM images are also shown in Figure 3 confirming light-induced dissociation of the polymer micelles. Before UV irradiation, core−shell micelles of p3 (average diameter ∼ 20 nm) are formed in the THF/water solution; while after 20 min of irradiation of the micellar solution, micelles are disappeared and only polymer film is seen. Another question is how the micelle dissociation is related to the degree of photocleavage of the chromophore. Direct ^{1}H NMR measurements on micellar solutions with deuterated solvents proved to be unsuccessful due to the low polymer concentration and the broadened signals from the compact micelle core. In an effort to make an estimate, using the same conditions as in Figure 3, three aliquots of the micellar solution of p3 were exposed to UV irradiation for 1, 5, and 20 min, respectively. Irradiated solutions were then dried completely and the residual substances were dissolved in deuterated DMSO for taking ^{1}H NMR spectra. From the change of the peak of methylene group in the block of poly(2-nitrobenzyl methacrylate) with respect to the peak of PEO (see Supporting Information), it was found that about 3, 25 and 100% of 2-nitrobenzyl groups were photocleaved after 1, 5, and 20 min UV irradiation, respectively. On the basis of the mechanism of photoinduced dissociation (Figure 1), it is likely that the micelles start to fall apart when the number of acid groups formed as a result of the continuous removal of 2-nitrobenzyl chromophores upon UV irradiation is important enough to shift the hydrophilic/hydrophobic balance. Combined with the results in Figure 3, it appears that a photocleavage degree higher than 30% would be necessary to give rise to severe dissociation of the micelles. At short irradiation time (1 min), some micelles may already be dissociated, which accounts for the decrease in turbidity, despite the apparently small photocleavage degree that is the average over all 2-nitrobenzyl chromophores. Because of the large volume of micellar solution (8 mL) used for the SLS measurements, only part of the micelles could be excited and photolyzed with a short irradiation time.

3.2. Photocontrolled Release upon UV Irradiation. In a recent report,[16] by monitoring the change in fluorescence of Nile Red (NR), Frechet and co-workers studied near-infrared light (NIR)-induced release of the dye into aqueous solution from micelles formed by a PEO-lipid amphiphile carrying a chromophore on the end of the hydrophobic tail, which is different from our BCPs.[6−8] This characterization method is based on the fact that the fluorescence emission of NR is low in water due to a very low solubility, but becomes much more intense and blue shifted when solubilized in hydrophobic micelle core. Even though the change in fluorescence intensity gives no quantitative data on the amount of dye released, it is a convenient tool to investigate the effects of variables on the kinetics of release, particularly for dilute micellar solutions, which are required for irradiation studies, for which the very small amount of dye makes the use of other techniques difficult.

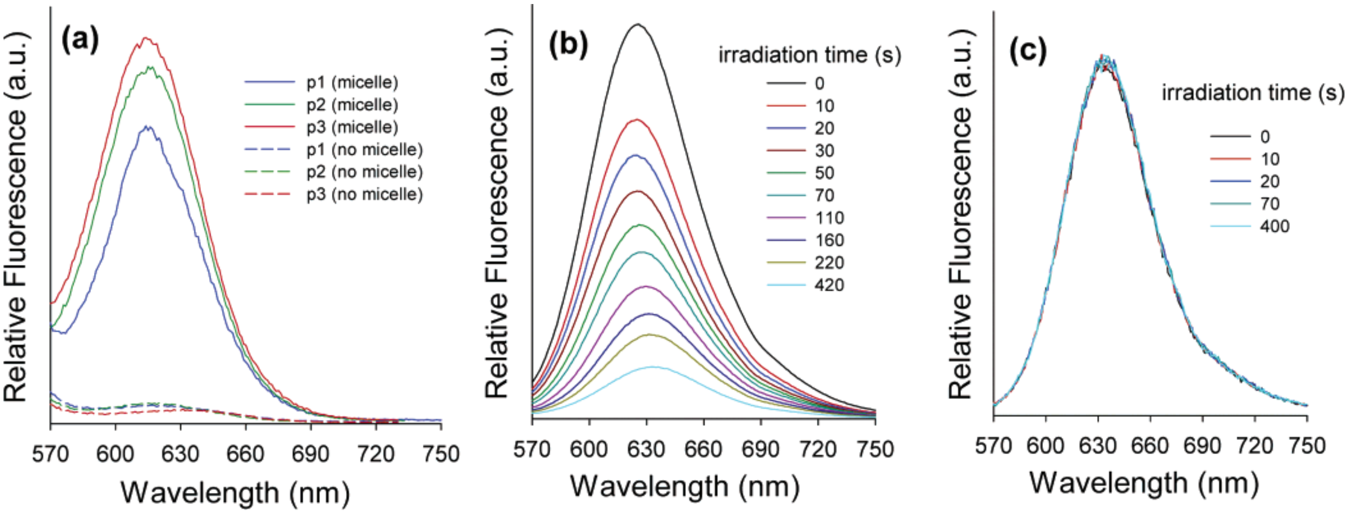


Figure 4. (a) Fluorescence emission spectra of Nile Red (NR) (λ_{ex} = 550 nm) in aqueous polymer solutions with and without the formation of micelles, showing the encapsulation of NR by the polymer micelles. (b) Fluorescence spectra of NR-loaded micelles of p3 in aqueous solution upon UV light irradiation (145 mW cm^{-2}, 0.7 mL solution), showing the decrease and red-shift of fluorescence emission due to exposure (release) of NR molecules to water. (c) Fluorescence spectra of NR-loaded micelles of p3 in THF/water (2:1, v/v) solution upon UV light irradiation (145 mW cm^{-2}, 0.7 mL solution); the release of NR in the mixed solvent, in which NR is soluble, results in no changes in fluorescence emission.

Therefore, we have used the same fluorescence approach to investigate the photocontrollability on the release of NR from our BCP micelles. The three sets of fluorescence emission spectra of NR (excitation at 550 nm) in Figure 4 are meant to show the encapsulation of NR by the photolabile BCP micelles and the release of NR in aqueous micellar solution upon UV light irradiation.

First, the loading of NR in the micelles of the three samples was confirmed by carefully performing an experiment, with the same conditions applied to each sample. The polymer and NR were first dissolved in THF at concentrations of 0.2 and 0.04 mg mL^{-1}, respectively. In an aliquot of the solution, water was added to reach 10% below CWCM (Figure 2), in which polymer remained in the form of dissolved single chains; while in another aliquot of the solution water was added to the content of 10% above CWCM, in which polymer micelles were formed. Afterward, a 4-fold volume of water was added for dilution; after the removal of THF through evaporation and precipitated NR by filtration, the solutions were characterized. As shown in Figure 4a, the encapsulation of NR by the hydrophobic core of polymer micelles is revealed by the fluorescence emission of NR from the three micellar solutions. By contrast, negligible fluorescence emission was observed from the three corresponding polymer solutions with no micelles. The encapsulation of NR also made detectable the absorption peak of NR in the three micellar solutions, while no absorption peak of NR was observable for the three polymer solutions with no micelles (UV−vis spectra not shown). Since the micellar solutions of the three samples were prepared using the same conditions (the same polymer concentration), the noticeable differences in fluorescence emission of NR suggest that the length of the hydrophobic PNBM block may affect the amount of loaded NR. Understandably, polymer micelles with a larger hydrophobic core appear to solubilize more NR.

The release of NR as a result of the dissociation of polymer micelles in aqueous solution upon UV light irradiation was monitored through fluorescence emission measurements. Figure 4b shows an example of spectral changes of a micellar solution of p3 exposed to UV light (145 mW cm^{-2}) for various times. The emission intensity of NR decreases with increasing irradiation time, which should reflect the release of NR from a hydrophobic medium (micelle core) into the aqueous solution. After 420 s irradiation, the emission maximum was red-shifted by about 10 nm (from 624 to 634 nm), which is also indicative of the increasing exposure of NR in aqueous solution.[16] To make sure that the observed decrease in fluorescence emission of NR was not caused by photobleaching of the chromophore upon UV irradiation, measurements were carried out on a micellar solution of p3 in THF/water (2/1, v/v) equilibrated with NR (NR is soluble in the mixed solvent). Figure 4c shows the fluorescence spectra of NR in this micellar solution subjected to the same UV irradiation conditions as in Figure 4b. Up to 400 s of exposure, no change in fluorescence of NR was observed, indicating the absence of photobleaching under the used experimental conditions. The same result was obtained in another control test using a much higher irradiation intensity of 1200 mW cm^{-2}.

The kinetic process of the photoinduced release of NR from the BCP micelles in aqueous solution can be investigated from the spectral changes by plotting the normalized fluorescence intensity vs irradiation time. The results obtained at different irradiation intensities for the micellar solution of p3 are presented in Figure 5 as an example. Under the used polymer (micelle) concentration, in the absence of UV irradiation, no decrease in fluorescence intensity of NR was observed after as long as 2 days, which indicates that there is no release of NR from the micelles before UV irradiation. Upon UV irradiation, the release takes place slowly when low-intensity UV light (50 mW cm^{-2}) is applied. The process becomes faster as the irradiation intensity increases, because high UV intensity speeds up the photolysis of 2-nitrobenzyl and, consequently, the disruption of BCP micelles. In all cases, the remaining fluorescence is about 15−20% of the initial level. At the lowest UV light intensity (50 mW cm^{-2}), it took about 30 min to reduce the fluorescence to 20% of the initial level, while it took about 350 s with an intensity of 145 mW cm^{-2} (data after 140 s irradiation for these two intensities are not shown in Figure 5). The results show the photocontrollability of the release of encapsulated guest by varying the intensity of the UV light. It should be emphasized that the kinetics of release of NR does not reflect necessarily the kinetics of dissociation of the polymer micelles. Upon UV irradiation, the photolysis of 2-nitrobenzyl starts quickly and disrupts the micelles due to the changing hydrophilic/hydrophobic balance, the exposure of entrapped NR molecules to water, which quenches the fluorescence, may occur well before the complete dissociation of the micelles. This may account for the apparently faster release of NR than the change in turbidity of the micellar solution that reflects the decrease in number and average size of micellar aggregates in the solution (Figure 3). Another reason for the apparently slower process of micelle dissociation as compared to the process of release of NR is the much larger volume of micellar solution used in the SLS experiment (8 mL) than in the release experiment (0.7 mL). With a larger volume of solution, it took a longer time of irradiation to have the chromophore photolyzed. To fully understand the mechanism of photocontrolled release, it would be interesting to establish the correlation between the time scales related to release of NR, photocleavage of 2-nitrobenzyl groups, and dissociation of micelles. Unfortunately, with the fluorescence approach employed to monitor the release of the hydrophobic NR dye in aqueous solution, we were unable to follow these processes using ^{1}H NMR and SLS techniques because of the aggregation of the 2-nitrobenzyl block in the micelles before dissociation, and the insolubility of the released NR and photocleaved nitrobenzyl alcohol in aqueous solution associated with the dissociation of the micelles.

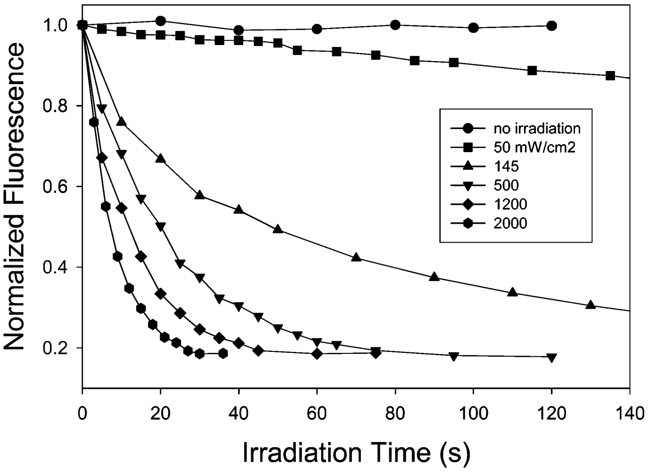


Figure 5. Plots of normalized fluorescence vs irradiation time for NR-loaded micelles of p3 in aqueous solution exposed to UV light of various intensities.

We then investigated the influence of the length of the hydrophobic PNBM block on the photoinduced NR release. Figure 6 shows the results obtained at a given UV light intensity

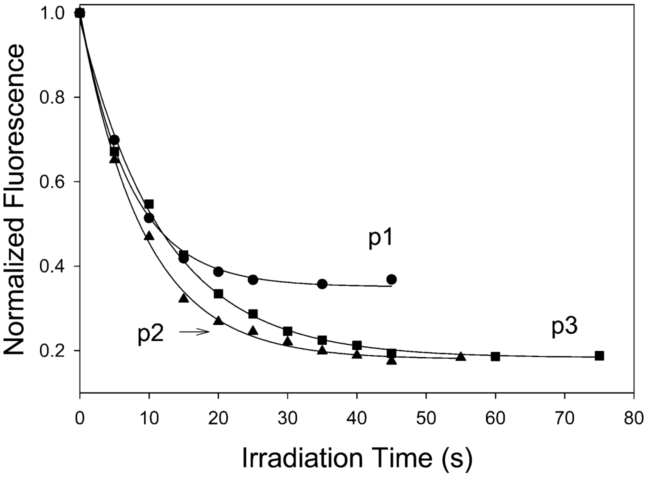


Figure 6. Plots of normalized fluorescence vs irradiation time for NR-loaded micelles of the three polymers in aqueous solution exposed to UV light (1200 mW/cm^2).

(1200 mW cm^{-2}) for micellar solutions of the three BCP samples prepared using the same conditions as described above. In all cases, an exponential change in normalized fluorescence of NR with increasing irradiation time was observed. There are, however, some differences that can be better appreciated by fitting the data through $I/I_o = (I/I_o)_m + [1 - (I/I_o)_m] \exp(-t/\tau)$, where $(I/I_o)_m$ is the achievable minimum of the normalized fluorescence and τ is the characteristic time at which the fluorescence decrease is completed by 63%. The curve fitting results, shown in the figure, yielded a characteristic time τ increasing from 7.2 s for p1 to 9.2 s for p2, and to 12.0 s for p3, the sample having the longest block of PNBM. The results imply that the photoinduced release of NR is slower from polymer micelles with a longer hydrophobic block (larger hydrophobic core). The minimum normalized fluorescence attained at longer irradiation times is the same for p2 and p3, with about 18% of the initial level. Quite surprisingly, this remaining fluorescence of NR is much larger for the micellar solution of p1, with around 38% of the initial level. The use of other UV irradiation intensities gave rise to similar results on the rate of fluorescence decrease and the remaining emission. While the slowing down of the release of NR from polymer micelles with a larger hydrophobic core is understandable, an observation provides a clue to the explanation for the higher level of remaining fluorescence of NR loaded in polymer micelles with the smallest hydrophobic core. It was found that the fluorescence emission of NR in aqueous solution with dissolved PEO (not PEO-*b*-PNBM micelles) is much stronger than that of NR in water alone, suggesting that PEO may improve the solubility of NR in aqueous solution. Of the three BCP samples, p1 has the shortest PNBM block and, consequently, the highest content of PEO; after the photoinduced dissociation of the micelles of p1, it was possible that the polymer solution entrapped more solubilized NR molecules giving rise to greater fluorescence emission of the dye.

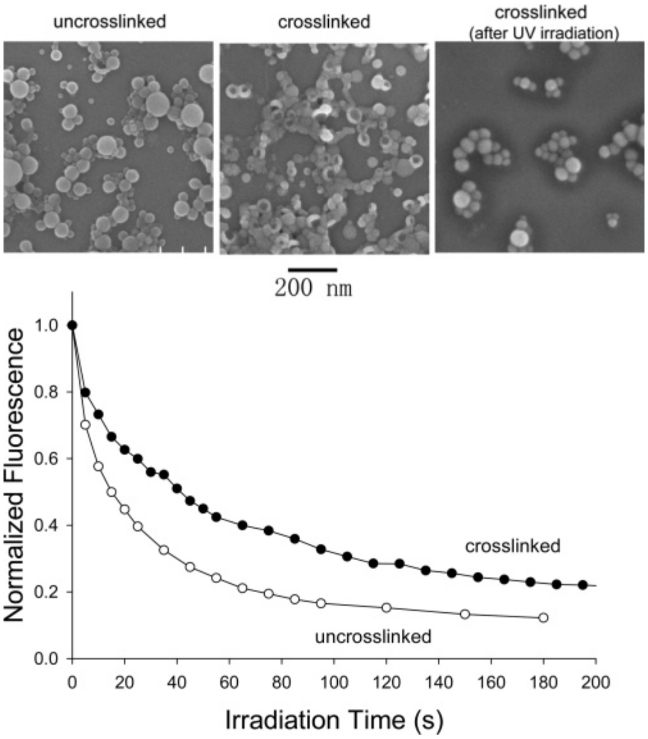


Figure 7. Plots of normalized fluorescence vs irradiation time for cross-linked and un-cross-linked, NR-loaded micellar aggregates of p3 in aqueous solution exposed to UV light (500 mW cm^{-2}). SEM images show the aggregate morphology in un-cross-linked p3 solution before UV irradiation, as well as in cross-linked p3 solution before and after UV irradiation.

3.3. Cross-Linked Photolabile Polymer Micelles. The transformation of the hydrophobic PNBM block to hydrophilic PMA is at the origin of the photoinduced dissociation of polymer micelles; while the micellar disruption associated with the transformation process results in release of the encapsulated guest molecule (Figure 1). If only a small portion of 2-nitrobenzyl moieties is photolyzed, the carboxylic acid groups formed can be used to react with a diamine molecule to cross-link the hydrophobic PNBM block.[23] This creates an interesting situation for the photolabile, NR-loaded polymer micelles. It can be foreseen that upon UV irradiation even the complete conversion of PNBM on PMA can no longer break up the micelles because of the cross-linked micelle core. However, as the polymer becomes increasingly hydrophilic as the photolysis reaction progresses, the micellar aggregates should swell in aqueous solution and still allow the release of encapsulated NR. Using p3, we performed experiments to see the effect of partial cross-linking on the photoinduced release. ^{1}H NMR analysis indicated the presence of about 3 mol % of carboxylic acid groups on PNBM available for cross-linking with the diamine. For comparison purposes, un-cross-linked micelles of p3 were prepared using exactly the same conditions as described in the Experimental Section; that is, the polymer slightly photolyzed was allowed to form micelles, to encapsulate NR and purified through dialysis, only in the absence of the cross-linker and catalyst.

The first finding was that cross-linking apparently could induce morphological changes of the micelles. The SEM image obtained from the solution subjected to the cross-linking reaction (middle image in Figure 7) shows a number of vesicle-like aggregates (deflated capsules with a hole-like central area). This morphology was neither observed for the un-cross-linked polymer (left image, even though the micelles appear larger than those in Figure 3 probably due to the very different preparation conditions) nor observed for the cross-linked polymer after UV irradiation (right image). At this point, we do not know how to explain the apparent morphological changes. We mention, however, that, on the basis of their fluorescence spectra (not shown), the cross-linked aggregates may encapsulate more NR than the un-cross-linked micelles (∼15% increase), and have a more hydrophobic environment since the maximum emission wavelength of NR loaded in cross-linked aggregates displayed a blue shift of ∼8 nm with respect to NR in un-cross-linked micelles. Also shown in Figure 7 are the plots of normalized fluorescence emission of NR as a function of irradiation time

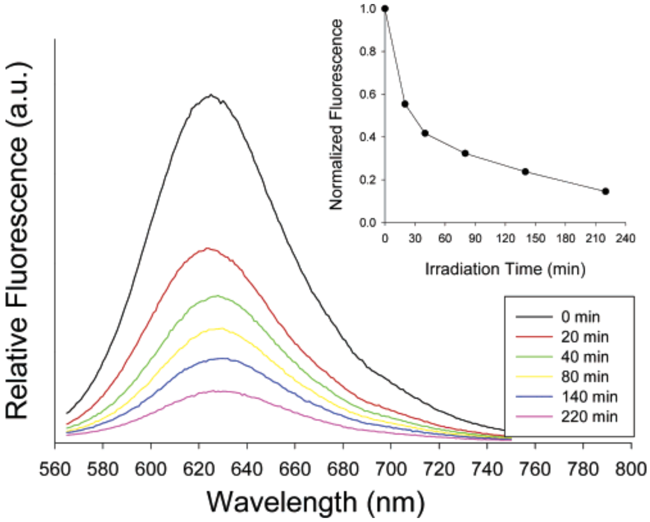


Figure 8. Fluorescence spectra of NR-loaded micelles of p3 in aqueous solution upon near-infrared light irradiation (700 nm), showing the decrease and red-shift of fluorescence emission due to exposure (release) of NR molecules to water. Inset is the plot of normalized fluorescence vs irradiation time.

for the two solutions, with the same UV light intensity of 500 mW cm^{-2}. As expected, the photoinduced release of NR from cross-linked aggregates is significantly slower than from the un-cross-linked micelles. The release process is slowed because the cross-linking prevents the dissociation of the micellar aggregates, as confirmed by SEM observations. These results show that cross-linking can readily be used in photolabile polymer micelles, which provides yet another means to adjust the rate of photocontrolled release of the encapsulated guest.

3.4. Photocontrolled Release upon Two-Photon Absorption of Near Infrared Light. Two-photon absorption of 2-nitrobenzyl-based chromophores has already been used to make light-induced uncaging of bioactive substances.[24] We wanted to know if the polymer micelles of PEO-*b*-PNBM can also be dissociated via two-photon absorption of near-infrared (NIR) light, allowing loaded NR to be released in aqueous solution. As compared with the use of UV light, the possibility to release encapsulated species from polymer micelles upon NIR irradiation (at wavelengths of ∼700−1000 nm) may be particularly attractive for biomedical applications for several reasons including deeper penetration through water and tissues due to diminished absorption and scattering of NIR, as well as being less detrimental to healthy cells.[16] We used a femtosecond IR laser (at 700 nm) to illuminate the same NR-loaded micellar solution of p3 as used in the experiments of one-photon absorption of UV light, and measured the change in fluorescence intensity of NR after various times of NIR irradiation. The fluorescence emission spectra in Figure 8 together with the inset showing the plot of normalized fluorescence intensity vs irradiation time confirm the release of NR upon NIR light irradiation. However, as expected, the irradiation time needed is much longer than in the case of using UV irradiation, due to the much smaller two-photon absorption cross-section as compared with one-photon absorption. Studies on amphiphilic BCPs containing more efficient two-photon absorbing chromophores are underway in our laboratory and the results will be reported in due time.

4. Conclusion

Micelles of the amphiphilic block copolymer comprising hydrophilic PEO and hydrophobic PNBM can be dissociated by light via either one-photon UV (∼ 365 nm) or two-photon near-infrared (∼ 700 nm) absorption of the photolabile 2-nitrobenzyl moieties, the photolysis of which converts the hydrophobic PNBM on hydrophilic PMA. Fluorescence emission measurements show that NR, a hydrophobic dye, encapsulated by the BCP micelles can be released upon irradiation as a result of the disruption of the BCP micelles. The rate of photoinduced release can be controlled by a number of means, particularly by varying the irradiation intensity that determines the rate of the photolysis reaction of 2-nitrobenzyl. It was also found that cross-linking the hydrophobic PNBM block can assist the photoinduced disruption of the micellar aggregates in controlling the rate of release. This first detailed study of photocontrolled release from polymer micelles demonstrates the viability, generality and interest of utilizing light-responsive BCP micellar aggregates (core−shell micelles and vesicles) as nanocarriers for photocontrolled delivery applications. By choosing the photolabile chromophore that interacts with light and changes the hydrophilic/hydrophobic balance, it would be possible to design stable BCP micellar aggregates that can be opened or disrupted by light at a desired range of wavelengths depending upon the application. Given the many advantages of polymer micelles over small-molecule (surfactant) micelles,[1,2] it is believed that light-responsive BCPs represent an attractive alternative in developing materials for controlled delivery applications.

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Supporting Information Available: Figures showing ^{1}H NMR spectra, UV−vis, fluorescence spectra, and GPC curves of the polymers before and after UV irradiation and text discussing the figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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