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Multiresponsive Micellar Systems from Photocleavable Block Copolymers

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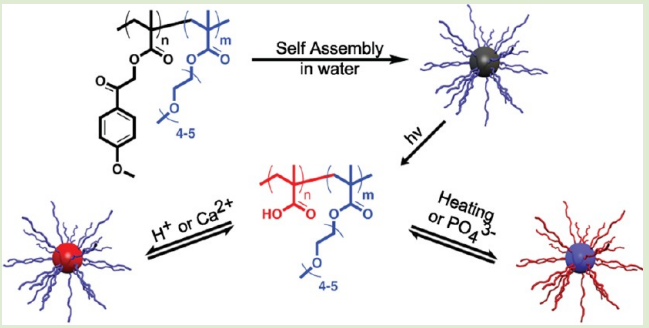
Supporting Information

ABSTRACT: This contribution describes the synthesis and associating behavior in water of a multiresponsive amphiphilic diblock copolymer. This copolymer is composed of an hydrophobic photocleavable poly(*para*-methoxyphenacyl methacrylate) block (PMPMA) and a hydrophilic thermosensitive poly[(oligo ethylene glycol)methacrylate] block (POEGMA). The PMPMA-*b*-POEGMA copolymer forms micelles with a PMPMA core and a POEGMA corona in water. Light irradiation leads to the transformation of PMPMA into poly(methacrylic acid) (PMAA) and to the disruption of the initial micelles. The response of the accordingly obtained PMAA-*b*-POEGMA copolymer to pH, temperature, calcium (Ca^{2+}), and phosphate (PO_4^{3-}) ions is demonstrated.



For many years, the ability of block copolymers to self-assemble into micelles of precise size and shape in a selective solvent for one of the blocks has been the focus of intense research.[1−4] Moreover, the development of "smart" block copolymers in the mid 90s has considerably increased this interest because of the possibility to finely tune their behavior in solution and, thus, to broaden their scope of application.[5−8] Typically, such smart amphiphilic block copolymers, when submitted to the adequate stimulus, can be transformed into a "double-hydrophilic" copolymer and inversely. This transition thus allows either stimuli-induced micellization when the copolymer is shifted from the double-hydrophilic structure to the amphiphilic one, or micelle disruption when the amphiphilic block copolymer is transformed into a double-hydrophilic one, the latter transition being particularly interesting for controlled drug delivery applications. Among all the available stimuli, the responses to a variation of pH,[9,10] temperature,[11−13] salt concentration,[10,14] and light irradiation[12,15,16] have been widely studied. The concept of stimuli-responsive amphiphilic block copolymers has been complemented in 1998 by Armes and co-workers by introducing the so-called "schizophrenic copolymer" that can exist in three different states in solution: unimers, micelles, and inverse micelles in which the core−corona structure is inverted compared to the regular micelles.[17] In those schizophrenic block copolymers, each of the constituent blocks of the copolymer can be transformed via an adequate stimulus from a soluble to an insoluble state and, inversely, independently from the other blocks. Since the introduction of this concept, numerous examples of schizophrenic copolymers have been studied with different combinations of stimuli: for example, salt−salt,[18] salt−pH,[19,20] pH−pH[21] pH−temperature,[22−28] temperature−temperature,[29] temperature−light,[30] and temperature−salt.[14,31] This variety of behaviors stimulates the growing interest in schizophrenic copolymers and, more generally, in multiresponsive block copolymers.[32−36]

In this contribution, the synthesis and behavior in aqueous solution of a multiresponsive diblock copolymer is presented. The investigated diblock copolymer is composed of a poly(*para*-methoxyphenacyl methacrylate) sequence (PMPMA) and of a poly[oligo(ethylene glycol) methacrylate] sequence (POEGMA). This block copolymer self-assembles in water to form micelles with a PMPMA core and a POEGMA corona. The photosensitive character of the PMPMA sequence, which is transformed into poly(methacrylic acid) (PMAA) after light irradiation, is used to induce the disruption of the micelles (Figure 1). Indeed, *p*-methoxyphenacyl esters are well-known photolabile protecting groups of carboxylic acids and caged phosphates.[37−39] The accordingly obtained PMAA-*b*-POEGMA double hydrophilic diblock copolymer displays a rich stimuli-responsive behavior in solution, including schizophrenic behavior.[40−43] Indeed, the formation of micelles with a POEGMA core and a PMAA corona is induced in this work by a thermal stimulus and by the addition of PO_4^{3-} anions to the solution. On the other hand, the formation of the inverse micelles with a PMAA core and a POEGMA corona is promoted by a variation of pH and by the addition of Ca^{2+} cations into the solution.

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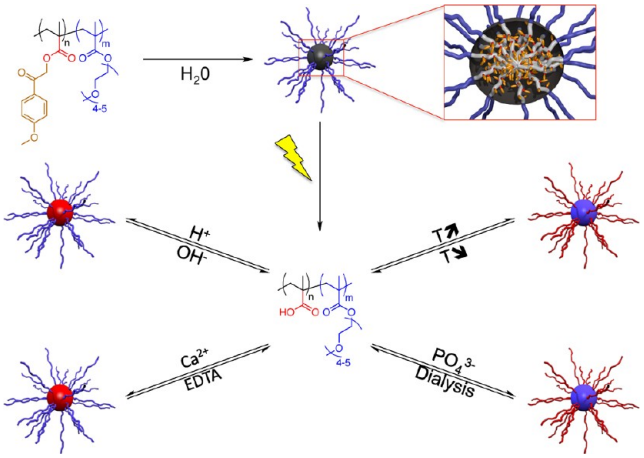

Figure 1. Multistimuli responsive behavior of the PMPMA-*b*-POEGMA copolymer in water.

The investigated $PMPMA_{99}$-*b*-$POEGMA_{48}$ photocleavable diblock copolymer was synthesized by atom transfer radical polymerization (the numbers in subscript represent the average degree of polymerization of each block, M_w (GPC-MALLS) = 35500 g/mol, polydispersity index (PDI) = 1.21 (see Supporting Information (SI) for synthesis and characterization). The micellization of this copolymer was realized by the cosolvent method from a dimethylformamide solution to which buffered water was added dropwise (pH = 8 buffer solution of borax = 2.5 mM, see SI for details). The final micellar concentration was fixed to 0.1 g/L. The apparent hydrodynamic radius ($R_{h,app}$) of the micelles determined by dynamic light scattering (DLS, see SI for further details) is equal to 69 nm (PDI = 0.028), while the radius of the dried micelles (R_{TEM}) determined by transmission electron microscopy (TEM) is 22 nm (Figure 2a, Table 1).

The disruption of the $PMPMA_{99}$-*b*-$POEGMA_{48}$ micelles was promoted by light irradiation at λ = 300 nm and was followed by DLS (Figure 3a) while the concomitant photocleavage of the PMPMA sequence was monitored by UV−vis spectroscopy (Figure 3b). The evolution of the normalized scattered intensity, I/I_0, with irradiation time was followed by DLS. A sharp decrease in the I/I_0 ratio was measured as a function of irradiation time, indicating the disruption of the initial micelles. In UV−vis spectroscopy, a decrease of the band at 270 nm was observed with irradiation time indicating the photocleavage of the *p*-methoxyphenacyl ester functions into carboxylic acid groups.[38,39] In addition, TEM images confirmed the disappearance of the micelles in solution after irradiation since a nonstructured polymer film was observed but no micelles (Figure 2b).

Since the irradiation led to a double hydrophilic PMAA-*b*-POEGMA copolymer, existing as unimers in water buffered at pH = 8, several other stimuli were applied to this solution in order to induce further micellization. In this respect, micelles with a POEGMA core were obtained either by the increase of temperature or by the addition of phosphate anions in the aqueous solution buffered at pH = 8. In the case of the thermally induced micellization the driving force is the destabilization of the H-bonds between oligo(ethylene glycol) side groups and surrounding water molecules, resulting in the observation of a lower critical solubility temperature (LCST) for the POEGMA block. The cloud point of the PMAA-*b*-POEGMA diblock was determined by measuring the scattered

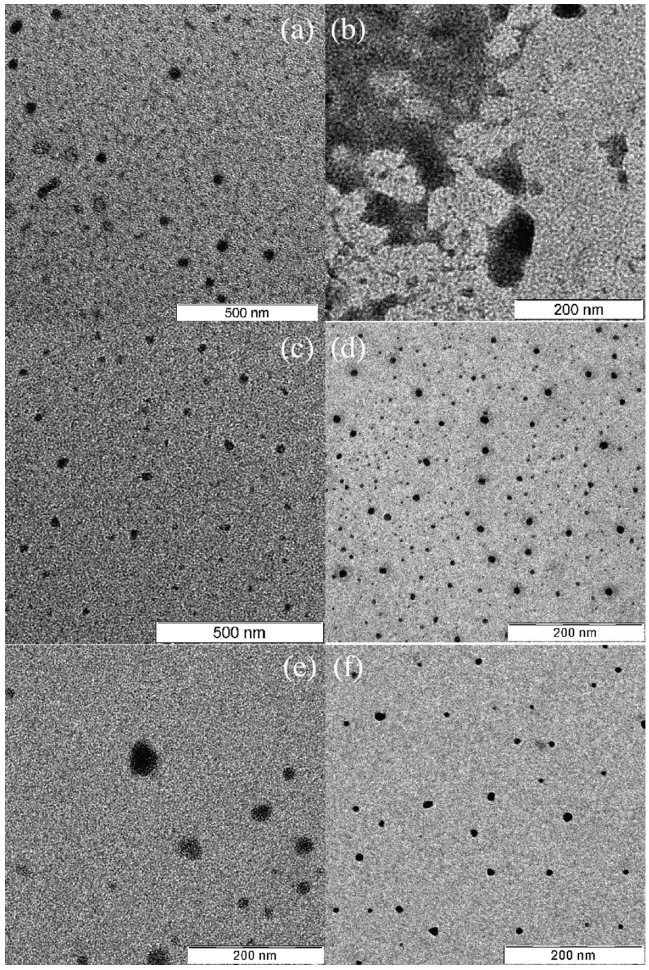

Figure 2. TEM images of the different micelles, stained with RuO_4 vapors, investigated in this study: (a) PMPMA-*b*-POEGMA starting micelles, (b) double hydrophilic PMAA-*b*-POEGMA copolymer obtained after irradiation, (c) micelles obtained by heating the PMAA-*b*-POEGMA solution at 65 °C, (d) addition of 4.4×10^{-4} mol of K_3PO_4 to the PMAA-*b*-POEGMA solution, (e) acidification of the PMAA-*b*-POEGMA solution to pH = 3, and (f) addition of 3.3×10^{-4} mol of $CaCl_2$ to the PMAA-*b*-POEGMA solution.

Table 1. Typical sizes of the different micelles investigated in this study, as determined by DLS and TEM[a]

	$R_{h,app}$ (nm)	PDI	R_{TEM} (nm)
starting micelles	69	0.028	22 ± 3.9
T = 65 °C	370	0.100	16 ± 2.6
addition of 4.4×10^{-4} mol of K_3PO_4	182	0.038	6 ± 0.9
pH = 3	50	0.209	12 ± 4.9
addition of 3.3×10^{-4} mol of $CaCl_2$	151	0.326	6 ± 1.2

[a]Starting micelles are prepared from the $PMPMA_{99}$-*b*-$POEGMA_{48}$ copolymers; all the other results are measured on the $PMAA_{99}$-*b*-$POEGMA_{48}$ system.

intensity by DLS as a function of temperature (Figure 4) and was found to be located at 57 °C, in good agreement with the reported LCST of POEGMA homopolymer.[44] At 65 °C, the micelles were characterized by a $R_{h,app}$ of 370 nm and a R_{TEM} of 16 nm. The value obtained for R_{TEM} is indeed in agreement with the formation of spherical micelles from diblock copolymers with characteristic features similar to the ones of the samples investigated here.[1,3] However, the $R_{h,app}$ measured

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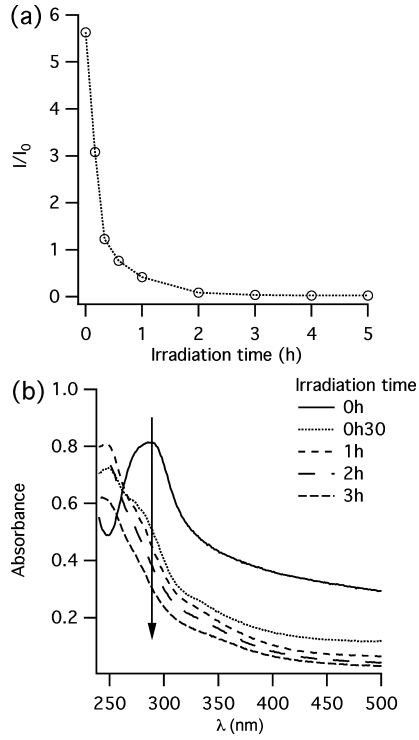

Figure 3. Monitoring the photocleavage of PMPMA-*b*-POEGMA micelles. (a) Normalized scattered intensity I/I_0, as measured by DLS. (b) UV−vis spectroscopy (C = 0.1 g/L).

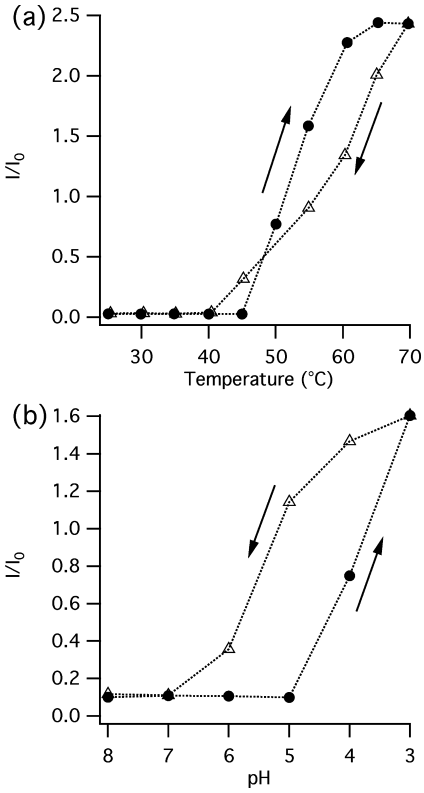

Figure 4. Reversibility of the temperature (a) and pH (b) induced transitions checked by monitoring the evolution of the normalized scattered intensity while increasing and decreasing these two stimuli.

by DLS is much too large to fit to those spherical micelles. The discrepancy observed for $R_{h,app}$ and R_{TEM} values, and especially the high $R_{h,app}$, is a consequence of the presence, at pH = 8, of the negatively charged carboxylates in the micellar corona, which induces the so-called polyelectrolyte effect. Indeed, electrostatic interactions in polyelectrolyte micelles create an electrostatic field, which fluctuates with the Brownian motion of the micelles and reversely influences their motion dynamic by slowing it down. This results in the so-called "slow mode" in the distribution of diffusion coefficients. As a consequence, $R_{h,app}$ is much larger for polyelectrolyte micelles. A straightforward way to evidence the polyelectrolyte effect is to screen electrostatic interactions by adding salts to the micellar solution. In this respect, adding NaCl to reach a concentration of 0.5 mol L^{-1} in the micellar solution at 65 °C resulted in the formation of micelles with a decreased $R_{h,app.}$ Those experiments were not investigated further as the addition of salts may also change the LCST of POEGMA and will therefore not be discussed here. Therefore, the micelles investigated here can be regarded as polyelectrolyte micelles and exhibit the typical behavior of those systems.[45,46]

For the micellization induced by the addition of PO_4^{3-} anions, the driving force is the salting out of the oligo(ethylene glycol) side groups. Phosphate salts were chosen as they have a pronounced salting out effect, in agreement with the Hofmeister series.[47] The micellization was observed after addition of 4.4×10^{-4} mol of K_3PO_4 in the buffered aqueous solution. The results obtained by TEM and DLS show that polyelectrolyte micelles were obtained with a $R_{h,app}$ of 182 nm and a R_{TEM} of 6 nm. The discrepancy between TEM and DLS results can be again understood by a pronounced polyelectrolyte effect due to the charged PMAA corona.

In addition to the "regular" micelles with a POEGMA core and a deprotonated PMAA corona, "inverse" micelles with a PMAA core and a POEGMA corona were generated by acidification of the solution or addition of calcium ions. The pH-induced micellization is triggered by the protonation at low pH values of the PMAA blocks that lowers considerably their solubility in aqueous medium and induces their aggregation into micellar cores. The pH-induced micellization was carried out after a dialysis step in order to remove all traces of the original buffer salt. Figure 4 shows the evolution of the normalized scattered intensity of the solution versus pH. A sharp increase in the I/I_0 ratio is observed at pH values under 4 indicating the formation of micellar structures. This critical pH is in agreement with the pK_a value of PMAA, indicating that the protonated PMAA blocks are able to aggregate into micellar cores. At pH = 3, micelles with a $R_{h,app}$ of 50 nm and a R_{TEM} of 12 nm are observed. Although TEM and DLS experiments do not lead to the same values, the previously observed polyelectrolyte effect is no longer observed at pH = 3 confirming the protonation of the PMAA blocks. The discrepancy between $R_{h,app}$ and R_{TEM} is no longer due to a polyelectrolyte effect here as no polyelectrolyte chains are present, but to some aggregated micelles, as observed in the TEM picture shown in Figure 2e, and in the rather large value of the polydispersity index (PDI = 0.206) determined by DLS.

The micellization of the PMAA-*b*-POEGMA copolymer was also triggered by the addition of a multivalent cation (Ca^{2+}). The driving force in this case is the complexation of calcium ions with two carboxylate functions.[48] For our system, micellization occurred after addition of 3.3×10^{-4} mol of $CaCl_2$ in the aqueous solution at pH = 8. The accordingly obtained micelles were characterized by a $R_{h,app}$ of 151 nm and a R_{TEM} of 6 nm. The discrepancy between DLS and TEM

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results could be again explained by the formation of a small amount of aggregated micelles.

Finally, the reversibility of the different micellization processes performed in this study was checked. Figure 4 presents the evolution of the normalized scattered intensity of the PMAA-*b*-POEGMA solution while first increasing pH and temperature and second decreasing these two variables. Although a clear hysteresis is observed for both pH and temperature curves, the values of I/I_0 at, before, and after the transition (pH = 8 → 3 → 8 and T = 25 → 70 → 25) are almost the same. The same results were observed for the micellization induced by PO_4^{3-} anions and for the micellization with Ca^{2+} cations. In that case, the removal of Ca^{2+} ions from PMAA was achieved by complexation with a strong competing ligand, that is, ethylenediamine tetracetic acid (EDTA).

In conclusion, we have described the behavior in aqueous media of a multistimuli responsive diblock copolymer. The starting photo- and thermo- responsive PMPMA-*b*-POEGMA copolymer was synthesized by ATRP. The copolymer was self-assembled in water to form micelles with a photocleavable PMPMA core. Light irradiation was used to disrupt the micelles and resulted in PMAA-*b*-POEGMA unimers in solution. The multischizophrenic character of this copolymer was then studied by the application of various stimuli including temperature, pH, calcium cations, and phosphate anions. The number and diversity of the applied stimuli make our system unique compared to previously described stimuli-responsive micelles. Several applications of our system are worth exploring in the future: for example, the light-induced disruption of the micelles could be applied to the release of hydrophobic molecules in water and the schizophrenic character of the PMAA-*b*-POEGMA obtained after photocleavage would be suitable for selective encapsulation.

■ ASSOCIATED CONTENT

Supporting Information

Block copolymer synthesis, size distributions diagrams, and complete experimental procedures are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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