Thermosensitive Micelle-Forming Block Copolymers of Poly(ethylene glycol) and Poly(*N*-isopropylacrylamide)

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Introduction. Micelles formed from block copolymeric surfactants have recently attracted a lot of attention as drug delivery systems. When hydrophobic drugs are loaded into the core of the micelles, small reservoirs can be formed with virus-like dimensions. A few of the recently reported block copolymers for this application have a hydrophilic block consisting of poly(ethylene glycol) (PEG) and a hydrophobic block that is biodegradable, like poly(lactic acid)[1−3] or poly(β-benzyl L-aspartate).[4−7] In another approach, polymeric micelles have been constructed from a hydrophilic block of poly(*N*-isopropylacrylamide) (PNIPAAm) and a hydrophobic block of polystyrene.[8] PNIPAAm is well-known for its thermosensitive properties in water and is therefore widely applied. Below 30.9 °C, its lower critical solution temperature (LCST) in water, the polymer is soluble, whereas phase separation takes place when the temperature is raised above the LCST. The LCST of PNIPAAm can be adjusted by copolymerizing NIPAAm with acrylamide or other hydrophilic comonomers. Through copolymerization of NIPAAm with acrylic cross-linkers, hydrogels have been prepared that may be applied in the biomedical field.[9−10]

We here report on the synthesis of block copolymers composed of PEG−PNIPAAm as well as their micellization behavior. The design of these copolymers is based on the hydrophobic character of PNIPAAm above its LCST in water. Combined with the hydrophilic properties of PEG, thermosensitive micelles may be obtained. Such micelles may dissolve due to the loss of the micellization capacity when the temperature is decreased below the LCST of the PNIPAAm in the block copolymer. After administration and arrival of the micelles at the target in the body, for instance a tumor, a burst of a loaded drug could be achieved by local hypothermia.

Results and Discussion. Block copolymers of PNIPAAm and PEG were synthesized according to the method shown in Scheme 1. A ceric ion redox system[11−14] was applied for the formation of radicals at the terminal carbons of PEG. These radicals are used for the polymerization of NIPAAm, providing a direct route to A−B−A block copolymers using HO-PEG-OH and A-B block copolymers if CH_3O-PEG-OH is applied. These block copolymers differ from PNIPAAm−PEG graft copolymers, which have been synthesized using an amido condensation reaction between P(NIPAAm-*co*-(acryloxy)succinimide) and diamino-PEG.[15] These graft copolymers showed thermoreversible gelation at 35 °C.

To prepare the AB and ABA block copolymers, a solution of ammonium cerium(IV) nitrate in 4 mL of 1 N nitric acid was added to an aqueous solution of PEG and NIPAAm at 50 °C at an initial molar ratio of $[Ce^{IV}]_0$/[HO end groups] of 1.2/1, to yield block copolymers with a narrow molecular weight distribution (Table 1).[16]

Termination of the polymerization occurs through reaction with another ceric ion. Radical recombination has not been observed.[14]

Aqueous solutions of PNIPAAm oligomers of at least three repeating units already exhibit a LCST, which implies that the polymer will phase separate in water above 30.9 °C. This phenomenon could be observed almost immediately after the addition of the ceric solution to the reaction mixture. The mixture turned from clear to milky, indicating the rapid formation of micelles by the growing block copolymers. The polymerization is therefore thought to occur in four stages. During the first stage a radical is formed through oxidation at the PEG-OH end group by the ceric ion and the ceric ion is reduced. In the second stage the polymerization of NIPAAm starts, whereafter in the third stage the block copolymers acquire the micellization capacity and start to form micelles. In the last stage the polymerization proceeds within the core of the micelles.

To verify the occurrence of in situ emulsion polymerization, a small amount of the cross-linker ethylene glycol dimethacrylate (EGDMA) was added after 5 min reaction time. In this stage of the polymerization, phase separation has taken place, indicating that the micelles are already formed. If the propagation takes place outside the core of the micelles, the addition of a cross-linker to the reaction mixture should result in a hydrogel, whereas polymerization in the core of the micelles should result in cross-linked nanospheres.

Because the mixture did not afford a hydrogel, a sample of the cross-linked micelles in the reaction mixture was taken for TEM analysis and dynamic light scattering. TEM showed separate micelles with a spherical shape of approximately 120 nm (Figure 1),[17] thereby proving that the propagation takes place in the core of the micelles. When the same samples were analyzed with dynamic light scattering at 37 °C in water, particle sizes were found to be 89.0 ± 1.1 nm, whereas at 25 °C the size of the particles was 367.7 ± 10.2 nm. This expansion of the cross-linked micelles to 4 times their size when cooled below the LCST is a good indication of the thermosensitive behavior of the PNIPAAm core and shows its expansion and shrinkage according to a change in temperature. On the basis of the results of the TEM pictures, we assume that the non-cross-linked micelles will be spherical in shape as well.

All block copolymers show a low critical micelle concentration (CMC) (Table 2)[18] in water. No large differences were found between the CMC's of the diblock copolymers. For the triblock copolymers a somewhat lower CMC value is observed. The presence of a small amount of diblock copolymer may account for a lower CMC value.[19] The micelles formed from polymers with a $M_{n,PNIPAAm}/M_{n,PEG}$ ratio of 1/3 exhibit reversible thermosensitive behavior (Table 1). This means that above the LCST micelles form while below the LCST the polymers completely dissolve. A similar behavior was observed for the sol−gel transition of PEG grafted copolymers described earlier by Yoshioka et al.[15] Dynamic light scattering measurements revealed no ag-

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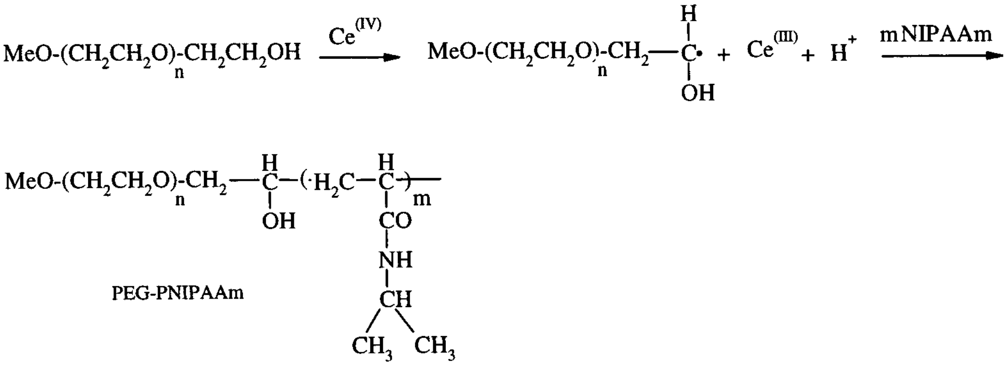
Scheme 1



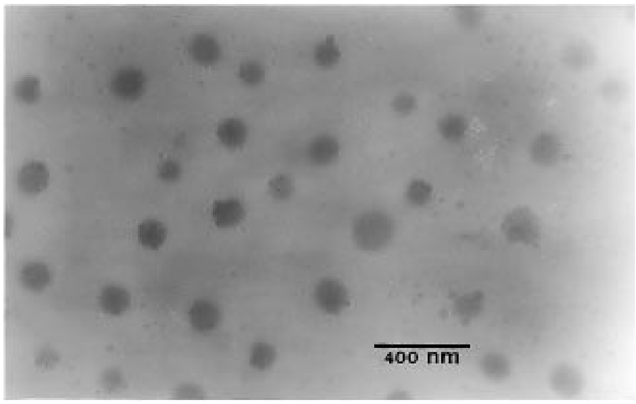


Figure 1. TEM picture of cross-linked micelles.

Table 1. Molecular Weights, Molecular Weight Distributions, and LCST of PNIPAAm−PEG and PNIPAAm−PEG-PNIPAAm Block Copolymers[a]

molecular architecture	$10^{-3}M_{n,PEG}$	$10^{-3}M_{n,PNIPAAm}$	$M_w/M_{n,GPC}$	$LCST_{DSC}$ (°C)
A−B	5.0	0.7	1.04	30.9
A−B	5.0	2.4		29.5
A−B	12.0	3.8	1.15	29.7
A−B−A	6.0	1.6		30.9

[a] See ref 16 for detailed information on analytical data.

Table 2. Micellar Characteristics of PNIPAAm−PEG Block Copolymers[a]

$10^{-3}M_n$	CMC (g/L)	micellar size (nm)	poly-dispersity	$10^{-6}M_w$	polymers per micelle
0.7-$PEG_{5.0}$	0.044	117 ± 1.1	0.18	3.3	275
2.4-$PEG_{5.0}$	0.035	66 ± 1.1	0.06		
3.8-$PEG_{12.0}$	0.027	61 ± 0.7	0.07	2.7	170
1.6-$PEG_{6.0}$-1.6	0.0066	72 ± 1.0	0.04		

[a] See ref 18 for detailed information on analytical data.

gregation of these samples. The block copolymers have a narrow molecular weight distribution of 1.04 and 1.15, as determined by GPC measurements. Moreover, this narrow molecular weight distribution leads also to a narrow size distribution of the micelles (Table 2). The narrow molecular weight distribution of these block copolymers allows for the determination of the amount of polymers per micelle with static light scattering measurements and is calculated to be approximately 275 for the block copolymer $PEG_{5.0}$−$PNIPAAm_{0.7}$ and approximately 170 for the block copolymer $PEG_{12.0}$−$PNIPAAm_{3.8}$.

Block copolymers with a $M_{n,PNIPAAm}/M_{n,PEG}$ ratio exceeding 1/3 do also show thermosensitive and micellization behavior. However, dynamic light scattering measurements revealed that these block copolymers still show aggregation in water when the temperature is below the LCST. For this reason these polymers cannot be characterized in the same way as the block copolymers that do not aggregate below the LCST.

In conclusion, the use of a ceric ion redox system for the synthesis of PEG−PNIPAAm block copolymers enables the in situ emulsion block copolymerization, in which the monomers diffuse from the solution to the core of the micelles where the propagation of the growing polymer chains takes place. This type of polymerization yields polymers with a narrow molecular weight distribution that form spherical micelles with a narrow size distribution.

Preliminary results show that copolymerization with varying amounts of acrylamide makes it very well possible to elevate the LCST of the PNIPAAm block close to body temperature. Therefore, a burst of a loaded drug by hypothermia seems to be feasible.

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(16) ^{1}H-NMR spectra were recorded using deuterated chloroform on a Bruker AC 250 operating at 250 MHz. Gel permeation chromatography (GPC) was used to determine molecular weight distributions (M_w/M_n) (Waters 510 pump with TSK G5000PW + G4000PW + G3000PW columns, in water at room temperature at a flow rate of 1.0 mL/min, RI detector Waters 411, and PEG standards). The lower critical solution temperature (LCST) of the PNIPAAm block in water was measured by differential scanning calorimetry (DSC) (Perkin-Elmer, DSC-7 calorimeter) at a concentration of 3 wt %.

(17) For TEM a sample of the reaction mixture containing cross-linked micelles was diluted. The images were taken with a Philips CM30\TWIN TEM. Due to the presence of ceric and cerous ions in the reaction mixture, no futher staining procedure was needed.

(18) The critical micelle concentration (CMC) was determined at 37 °C in deionized water using the Wilhelmy plate method for measuring the surface tension as a function of the concentration (Krüss processor tensiometer K12c). Dynamic light scattering was performed in water at 37 °C (Malvern 4700 system) to determine the micellar size, the analysis being based on accumulation. Static light scattering was performed at 40 °C in water (Dawn DSP-F), the weight average molecular weights (M_w) were established by a Zimm plot, and the scattering angles ranged from 44.5 to 158.3°. The refractive index increments of the polymer were obtained at 40 °C using a double beam differential refractometer (ERMA ERC7510).

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