Thermoresponsive Polymeric Micelles with Controlled Instability Based on Hydrolytically Sensitive *N*-Isopropylacrylamide Copolymers

D. Neradovic, C. F. van Nostrum,* and W. E. Hennink

Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Faculty of Pharmacy, Utrecht University, P.O. Box 80.082, 3508 TB Utrecht, The Netherlands

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Introduction. In recent years polymeric micelles are under investigation as carriers for poorly water-soluble drugs.[1] Amphiphilic A−B block copolymers self-assemble in dilute aqueous solutions in core−shell micellar structures. The hydrophilic shell is responsible for the stabilization of the micelle, and the hydrophobic core can play a role as a reservoir for poorly water-soluble drugs. A great variety of block copolymers were designed and evaluated for micelle formation. In most of these systems poly(ethylene glycol) (PEG) is used as hydrophilic block, whereas for example poly(L-lactic acid), poly(ϵ-caprolactone), or poly(β-benzyl L-aspartate) was used as hydrophobic block.[2] Because of their small size (30−100 nm) and their hydrophilic surface, block copolymer micelles show prolonged blood circulation times,[3] and they might end up in for example tumor tissue by the so-called EPR (enhanced permeation and retention) effect.[4,5] Recently, block copolymers containing thermosensitive elements such as poly(*N*-isopropylacrylamide) (PNIPAAm) have been used in polymeric micelles.[6] It is well-known that PNIPAAm exhibits a reversible thermoresponsive phase transition in aqueous solutions: it is highly soluble in aqueous solutions below its cloud point (CP) but precipitates above the CP.[7] It was shown that the block copolymers of PNIPAAm with PEG exhibit thermoresponsive solubility in aqueous solutions.[8] Above the CP, the thermosensitive PNIPAAm block precipitates, and the diblock copolymers organize in polymeric micelles, which consist of a PNIPAAm core and hydrophilic shell of PEG.

In our previous paper we reported the synthesis of a new type of thermosensitive NIPAAm-based polymer (poly(NIPAAm-*co*-HEMA-lactate)) with hydrolytically sensitive lactate ester side groups.[9] During the incubation of this random copolymer in aqueous solution the CP of the polymer increased due to the hydrolysis of the hydrophobic lactate ester side group, which yielded poly(NIPAAm-*co*-HEMA) with an increased overall hydrophilicity and a CP, independent of the copolymer composition, of 31 °C. On the basis of this concept, we now designed a novel type of polymeric micelles from block copolymers containing a temperature-sensitive block, which upon hydrolysis converts to a polymer having the CP above 37 °C. We anticipate that this new concept will lead to polymeric micelles to be applied as a new drug delivery systems in the future.

Experimental Section. *Synthesis of Macroinitiator PEG_2-ABCPA.* 2 g (0.4 mmol) of poly(ethylene glycol) monomethyl ether (PEG, number-average molar mass M_n = 5000 g mol^{-1}), 0.056 g (0.2 mmol) of 4,4-azobis-(4-cyanopentanoic acid) (ABCPA), 0.0189 g (0.06 mmol) of 4-(dimethylamino)pyridinium-4-toluenesulfonate (DPTS),[10] and 0.125 g (0.6 mmol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) were dissolved in 1:1 mixture of dichloromethane and dry *N*,*N*-dimethylformamide. The mixture was stirred at room temperature for 24 h. After purification by extraction and filtration, the product was obtained in a high yield (∼ 80%) and characterized by ^{1}H NMR and gel permeation chromatography (GPC).

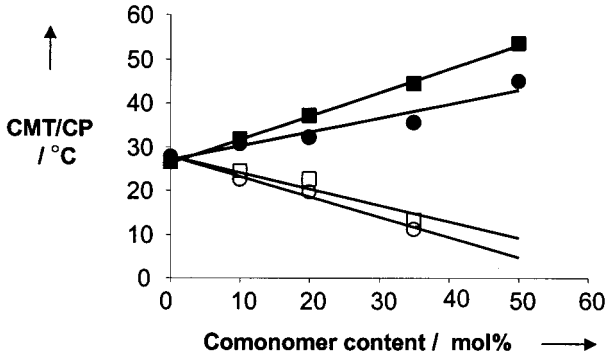

Figure 1. Onsets of cmt's and CPs determined by static light scattering as a function of the HPMAm-lactate or HPMAm comonomer fraction in PNIPAAm random copolymers (circles) and corresponding block copolymers with PEG (squares): □, poly(NIPAAm-*co*-HPMAm-lactate)-*b*-PEG; ■, poly(NIPAAm-*co*-HPMAm)-*b*-PEG; ○, poly(NIPAAm-*co*-HPMAm-lactate); ●, poly(NIPAAm-*co*-HPMAm).

Synthesis of Block Copolymers. Block copolymers of PEG and NIPAAm or NIPAAm−HPMAm(-lactate) were prepared by radical polymerization using PEG_2-ABCPA as initiator (ratio of monomers/ABCPA = 250/1 (mol/mol)). The copolymerization was conducted in 1,4-dioxane at 80 °C for 24 h in a nitrogen atmosphere. The products were purified by precipitation in diethyl ether or dialysis against water and obtained in high yields (83−98%) and characterized by ^{1}H NMR.

Hydrolysis of the Polymers. NIPAAm/HPMAm-lactate copolymers were dissolved in phosphate buffered saline (PBS, pH = 7.2) in concentrations of 5 mg mL^{-1} at a temperature below the CP. The pH of the solutions was adjusted to ≈11 (1 N NaOH) to increase the hydrolysis rate. The NIPAAm/HPMAm-lactate copolymers were incubated at 37 °C for 4−7 days, whereafter the samples were cooled to 5 °C. The polymers were collected after dialysis and freeze-drying. The block copolymers of PEG with NIPAAm/HPMAm-lactate were dissolved in PBS (pH = 7.2), in concentrations of 1 mg mL^{-1} at a temperature below the cmt. The pH of the homogeneous solutions was adjusted to 8.5, and the polymers were incubated at 37 °C. At different time points samples were drown and cooled to 4 °C, and the pH was readjusted to ∼7.

The determination of the CP/cmt of the different polymer solutions was carried out by static light scattering (SLS) essentially as described previously.[9]

Dynamic Light Scattering (DLS) Measurements on Block Copolymer Solutions. The equipment consisted of an Autosizer 4700 spectrometer (Malvern) and an argon ion laser (75 mW, 488 nm, equipped with a model 2500 remote interface controller, Uniphase). The polymers were dissolved in PBS (pH 7.2) in concentrations of 1 mg mL^{-1}. The measurement angle was 90°.

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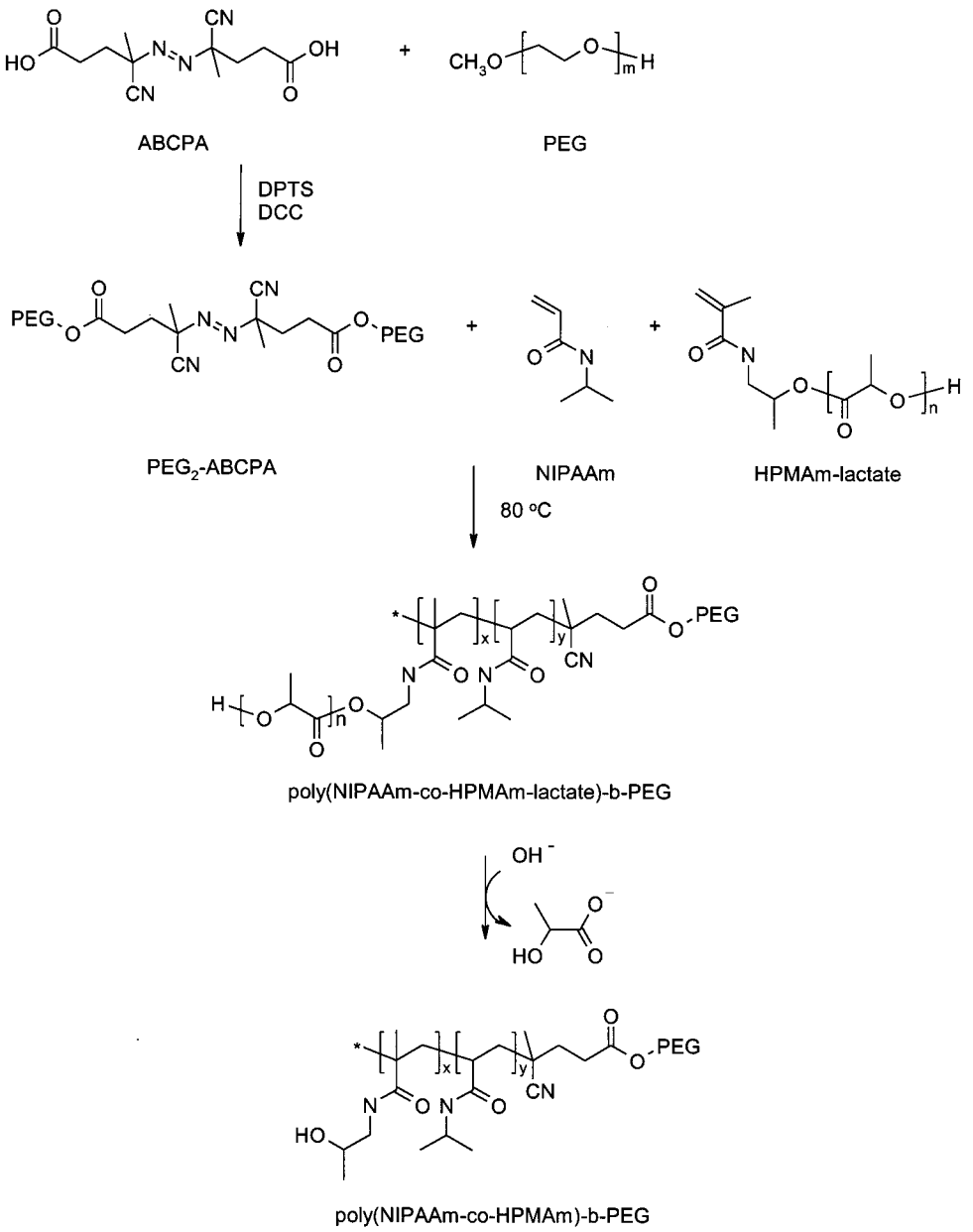
Scheme 1



Results and Discussion. Novel oligolactate esters of 2-hydroxypropyl methacrylamide (HPMAm-lactate$_n$, n = degree of polymerization of oligolactate) were obtained by ring-opening oligomerization of L-lactide using HPMAm as the initiator and stannous octoate as a catalyst, essentially using procedures described before.[11] Products with low polydispersities were obtained by fractionation by means of column chromatography (silica 60H). Copolymers of NIPAAm with HPMAm or HPMAm-lactate$_3$ ($n_{average} = 3$) were prepared at various monomer ratios by free radical polymerization. The copolymers were obtained in a good yield (~90%), and the comonomer ratios in the polymers corresponded well with the feed ratios. Poly(NIPAAm-*co*-HPMAm) copolymers were also prepared indirectly by hydrolyzing the lactate ester side groups of poly(NIPAAm-*co*-HPMAm-lactate) at 37 °C and pH 11 for 4−7 days. ^{1}H NMR analysis demonstrated that the conversions were complete.

Figure 1 shows that an increasing mole fraction of HPMAm-lactate in the copolymers resulted in a gradual decrease of the CPs, indicating that the incorporation of HPMAm-lactate increased the overall hydrophobicity of the copolymer. Because of hydrolysis of the lactate ester side group, a more hydrophilic copolymer is obtained which is associated with an increase in CP. The CPs of the copolymers after hydrolysis were almost the same as the CPs of the directly synthesized poly(NIPAAm-*co*-HPMAm) copolymers, which demonstrated that, in agreement with ^{1}H NMR data, the hydrolysis of the side groups was complete. Figure 1 shows that poly(NIPAAm-*co*-HPMAm-lactate) copolymers with at least 35% HPMAm-lactate are insoluble in water at human body temperature (CP < 37 °C), while during hydrolysis of the side groups poly(NIPAAm-*co*-HPMAm) copolymers are gradually formed which become soluble at body temperature (CP > 37 °C).

For the synthesis of AB block copolymers composed of PEG (block A, $M_n = 5000$ g mol^{-1}) and a temperature-sensitive block B of PNIPAAm or poly(NIPAAm-*co*-HPMAm(-lactate)), we used free radical polymerization using a monomethoxy-PEG substituted macroinitiator

Table 1. Cmt, Average Particle Size (Z_{ave}), and Polydispersity (PD) Obtained for the Block Copolymers in PBS by DLS

NIPAAm−HPMAm (-lactate) ratio (mol/mol)	poly(NIPAAm-*co*-HPMAm)-*b*-PEG			poly(NIPAAm-*co*-HPMAm-lactate)-*b*-PEG		
	cmt (°C)	Z_{ave} (nm)	PD	cmt (°C)	Z_{ave} (nm)	PD
100/0	27−28	154	0.062			
90/10	32−33	200	0.045	25	175	0.047
80/20	37−38	205	0.129	23	172	0.080
65/35	45	657	0.560	17−19	191	0.064
50/50	54	491	0.715	<12	187	0.117

(PEG_2-ABCPA, Scheme 1).[12] ^{1}H NMR analysis of the macroinitiator showed that two PEG chains were present per ABCPA unit. The M_n of this product was about twice that of the starting PEG according to GPC. Moreover, upon heating the macroinitiator (24 h at 80 °C) in the presence of a radical quencher (4-methoxyphenol), the macroinitiator decomposed and its M_n dropped to almost the M_n of the monomethoxy-PEG, showing that the macroinitiator splits into two radicals.

In the second step of the synthesis of the block copolymers, NIPAAm or mixtures of NIPAAm and HPMAm(-lactate$_n$) ($n_{average}$ = 2.5) were polymerized using PEG_2-ABCPA as initiator (Scheme 1). The block copolymers were obtained in a good yield (~90%). ^{1}H NMR showed that the monomer ratios in the block copolymers were close to the feed ratios. M_n of the polymers were 15−20 kDa (values based on ^{1}H NMR). Static light scattering (SLS) measurements showed that the block copolymers had critical micelle temperatures (cmt's) similar to the CP temperatures for the random copolymers (see Figure 1). Dynamic light scattering (DLS) was used to determine the size of the micelles once formed (Table 1). The size of the micelles is in good agreement with values reported previously.[8] From the low polydispersity values, especially for the block copolymers containing high amounts of NIPAAm, it can be concluded that the micelles had a narrow size distribution. In contrast, when an aqueous solution of a random copolymer (without PEG) of NIPAAm with HPMAm(-lactate) was heated above its CP, rather large particles (>500 nm) with an extremely high polydispersity were detected. This indeed demonstrates that the PEG chains favor the formation of small particles, probably consisting of a hydrophilic shell around a core of collapsed PNIPAAm. When the block copolymer solutions were cooled, the aggregates dissociated, demonstrating the reversible character of the micelles.[6b,13]

When the lactate ester side groups of the poly(NIPAAm-*co*-HPMAm-lactate)-*b*-PEG block copolymers were hydrolyzed (Scheme 1, T = 37 °C, pH 8.5), a gradual increase in cmt was observed (Figure 2). The final cmt values corresponded well with those of the directly prepared HPMAm-containing block copolymers. Interestingly, the cmt of poly(NIPAAm-*co*-HPMAm-lactate)-*b*-PEG block copolymers with 35% and 50% HPMAm-lactate reaches values above 37 °C during incubation. This means that these block copolymers form micelles at physiological temperatures but destabilize once the cloud point temperature of this block passes 37 °C because of the hydrolysis of the side groups. As will be presented in a forthcoming paper, we have shown that the kinetics of hydrolysis can be adjusted by the number and length of the oligolactate grafts in the copolymers, which indicates that the destabilization time of the polymeric micelles can be precisely controlled. This attractive feature for, for example, drug delivery and biotechnological purposes is distinct to presently known degradable micelles, e.g., based on PEG-*b*-PLA block copolymers.

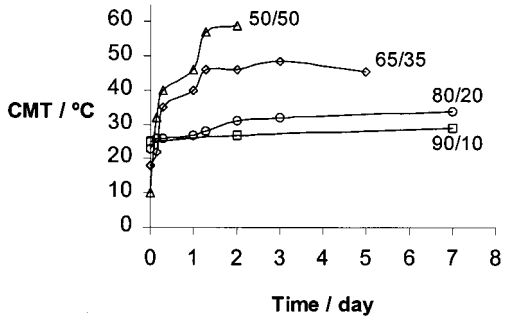


Figure 2. Changes in cmt during hydrolysis of the block copolymers (AB block copolymers composed of PEG (block A) and a temperature-sensitive block B of poly(NIPAAm-*co*-HPMAm-lactate)) in PBS (37 °C, pH 8.5) followed by DLS. 90/10, 80/20 etc. refer to the comonomer content (mol %) of NIPAAM and HPMAm-lactate, respectively.

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