Original Research Article

Preparation of poly(NIPAAm)-Pluronic F68 as a thermosensitive surfactant for a controlled drug release

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Abstract

This paper describes the synthesis of thermosensitive surfactants by polymerizing *N*-isopropylacrylamide (NIPAAm) into the Pluronic F68 surfactant and their application for a controlled drug release. Poly(NIPAAm)-Pluronic surfactants with different lengths of the NIPAAm block were synthesized by activating two hydroxyl groups of poly(ethylene oxide) (PEO) at the end of Pluronic F68 using cerium ammonium nitrate (CAN, redox initiator), followed by adding the NIPAAm monomer into a reactor. The resultant poly(NIPAAm)-Pluronic surfactants were characterized by FT-IR and gel filtration chromatography (GPC). It was observed that their critical micellar concentrations increased with an increase in the length of the poly(NIPAAm) block. In addition, poly(D,L-lactide-co-glycolide) (PLGA) microparticles was prepared by an oil-in-water emulsion and solvent evaporation method using the poly(NIPAAm)-Pluronic surfactants in an aqueous continuous phase. At 37°C, nile red (model dye) was released from the PLGA microparticles in a more sustained manner when the length of poly(NIPAAm) was longer due to a thicker layer of shrunken poly(NIPAAm) at the surface of the microparticles.

Key words: Controlled drug release, *N*-isopropylacrylamide (NIPAAm), Pluronic F68, redox polymerization, thermosensitive surfactant

INTRODUCTION

Intelligent drug carriers have attracted much attention in biomedical applications due to their size, core-shell structure, decoration with targeting ligands, and stimuli-responsive properties.[1,2] Recently, pH and/or temperature-sensitive micro/nano-particles have been intensively investigated for controlled drug delivery systems.[3-7] Among the various temperature-sensitive polymers, poly(*N*-isopropylacrylamide) [poly(NIPAAm)] is the most often employed material as a core or shell in the forms of particle and gel.[8-10] Generally, poly(NIPAAm) exhibits a hairy structure below the lower critical solution temperature (LCST) and undergoes hydrophobic aggregation above the LCST due to a change in hydrogen bonding between water and amide groups of poly(NIPAAm).[11] Therefore, particles consisting of poly(NIPAAm) show a unique conformational behavior with respect to environmental temperature, eventually allowing for the thermosensitive drug release.

Pluronic surfactants, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO), are known triblock copolymers with biocompatible properties. To extend their application, a few researchers had synthesized functional copolymers based on Pluronic surfactants by polymerizing hydrophilic or hydrophobic segments at the ends of the PEO blocks. Wang *et al.* polymerized hydrophobic segments at the ends of a Pluronic surfactant to improve the hydrophobic property for the better encapsulation of liphophilic drugs.[12] Mei *et al.* synthesized pentablock terpolymers based on poly(NIPAAm) and a Pluronic surfactant by atomic transfer radical polymerization and characterized their micelle formation at different temperatures.[13] However, the fabrication of microparticles using a poly(NIPAAm)-Pluronic surfactant and their application for controlled drug delivery have not been reported.

In this study, thermosensitive poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) were synthesized by activating two hydroxyl groups at the ends of Pluronic F68 using redox initiator[14-16] and subsequently adding a NIPAAm monomer. Poly(d,l-lactide-co-glycolide) (PLGA) microparticles were produced by the typical oil-in-water emulsification method using poly(NIPAAm)-Pluronic surfactants in an aqueous continuous phase. The rationale for our approach is as follows: at room temperature (below the LCST),

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PLGA microparticles prepared using poly(NIPAAm)-Pluronic surfactants exhibit appropriate colloidal stability due to the hairy structure of poly(NIPAAm) at the surface of the microparticles. When the PLGA microparticles are injected into the human body, the poly(NIPAAm) block shrinks and forms a hydrophobic layer at the surface of the microparticles, eventually resulting in the sustained release of the drug. The ultimate goal of this work is to evaluate the feasibility of the poly(NIPAAm)-Pluronic copolymer as a surfactant and demonstrate its application for a controlled drug delivery.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide (NIPAAm), ceric ammonium nitrate (CAN), nitric acid, nile-red (NR), ethyl acetate, and poly(D,L-lactide-co-glycolide) (PLGA, 75:25, $M_w \approx$ 66,000–107,000) were purchased from Sigma-Aldrich (USA). Pluronic F68 was obtained from BASF (Germany). Distilled water (DI) from a Milli-Q water purification system (Direct-Q 3; Millipore, Bedford, MD, USA) with a resistance of 18 $M\Omega cm^{-1}$ was used. All organic solvents were either HPLC grade or American Chemical Society analytical grade reagents.

Synthesis and characterization of poly(NIPAAm)-Pluronic surfactant

Poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) were synthesized by the polymerization of NIPAAm at the ends of the hydroxyl group of Pluronic F68 using CAN as a redox initiator. DI water (150 g) was poured into a double-jacket reactor equipped with a mechanical stirrer, nitrogen inlet apparatus, and a reflux condenser. Pluronic F68 (3 g) was poured into the reactor with stirring at 200 rpm at 65°C. The solution of CAN (0.1 g) and NIPAAM (0.1, 0.2, and 0.25 g) in 5 ml of nitric acid (0.2 mol/l) was added and then polymerization progressed for 4 h under a nitrogen atmosphere. The resultant was sufficiently cleaned using a dialysis membrane (MWCO 6000–8000; Membrane Filtration Products, Inc., TX, USA) and then dried at 25°C for 12 h in a vacuum oven.

The prepared poly(NIPAAm)-Pluronic surfactants were characterized by FT-IR (TENSOR27, BRUKER, The Netherlands) and gel filtration chromatography, GPC, (Waters Breeze System; Waters Co., USA). The GPC column was a series of Styragel® columns (HR5, HR4, HR1, and HR5E) and THF was used as an eluent at a flow rate of 1 ml/min and 1×10^3 Pa pressure. Polystyrene samples (Sigma-Aldrich) with different molecular weights were used for the standard in GPC analysis. To measure the critical micellar concentration (CMC), aqueous solutions with different concentrations of the poly(NIPAAm)-Pluronic surfactant (0.01, 0.025, 0.05, 0.1, 0.25, and 0.5 wt.%) were prepared and the surface tensions of the samples were measured using a semiautomatic tension meter (Surface Tensiomat 21, Fisher Scientific, USA). From the plot of surface tension versus concentration, the CMC value could be obtained as the intersect point of two regressed lines.

Preparation and characterization of PLGA microparticles using the poly(NIPAAm)-Pluronic surfactant

PLGA microparticles were prepared using an oil-in-water emulsion and solvent evaporation method at room temperature. In brief, an oil phase containing PLGA (0.2 g) and NR (1 mg) in ethyl acetate (10 g) was poured into an aqueous phase (50 g) containing a poly(NIPAAm)-Pluronic surfactant (0.5 g) and subsequently emulsified for 7 min with a high-speed homogenizer at 10,000 rpm (Omni International, Waterbury, CT, USA), followed by stirring for 6 h for solvent evaporation. After placing the samples in the water bath at the different temperatures at 15°C and 37°C for 1 h, the average size of the microparticles was determined by the dynamic light scattering method (DLS, ZetaPlus; Brookhaven Inst. Co., NY, USA).

Drug release

After fabricating the PLGA microparticles containing nile red, the encapsulation efficiency was measured by the centrifugation of the dispersion containing the PLGA microspheres and then analyzing the amount of nile red in the aqueous continuous phase using a UV spectrophotometer (UV-1601, Shimadzu, Japan) at 460 nm. In all the cases, the encapsulation efficiencies were found to be 88.2 ± 3.4%. The cumulative release of the dye was calculated on the base of the total encapsulated amount of the dye.

For the release profile, 20 ml of a microparticle suspension (4 mg/ml) was sealed in a dialysis tube (MWCO 6000-8000), and then placed in 100 ml of the PBS medium (pH 7.4) with gentle shaking at 37°C. At predetermined time intervals, 4 ml of the solution was collected from the released media and replaced with fresh PBS. The released nile red was analyzed by a UV/VIS spectrophotometer at different time points.

RESULTS AND DISCUSSION

Figure 1a is a schematic diagram showing the synthesis of a poly(NIPAAm)-Pluronic surfactant, where Pluronic F68 was used as a macroinitiator.[13] Hydroxyl groups at the ends of Pluronic F68 were radicalized by CAN and reacted with a NIPAAm monomer, followed by the propagation of the monomers. There could be little possibility for two or more Pluronic F68 molecules to be synthesized together serving NIPPAm as a linker, because the reaction was conducted in a quite diluted solution and the resulting poly(NIPAAm)-Pluronic surfactants exhibited fairy uniform molecular weights. FT-IR analysis showed that a broad peak at around 3400 nm corresponding to the hydroxyl group in the Pluronic F68 disappeared after the polymerization, indicating the synthesis of the poly(NIPAAm)-Pluronic surfactant [Figure 1b]. The poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) were synthesized by varying the amounts of the NIPAAm monomer (0.1, 0.2, and 0.25 g) with other conditions kept same (namely, NP-1, NP-2, and NP-3) Table 1 shows the properties of the resultant surfactants, obtained from GPC analysis. An increase in the amount of NIPAAm monomer led to an increase in the molecular weight of the resulting surfactant, suggesting an increase in the unit number of poly(NIPAAm) because the molecular weight of Pluronic F68 was not changed through polymerization. Although nuclear magnetic resonance (NMR) or elementary analysis is

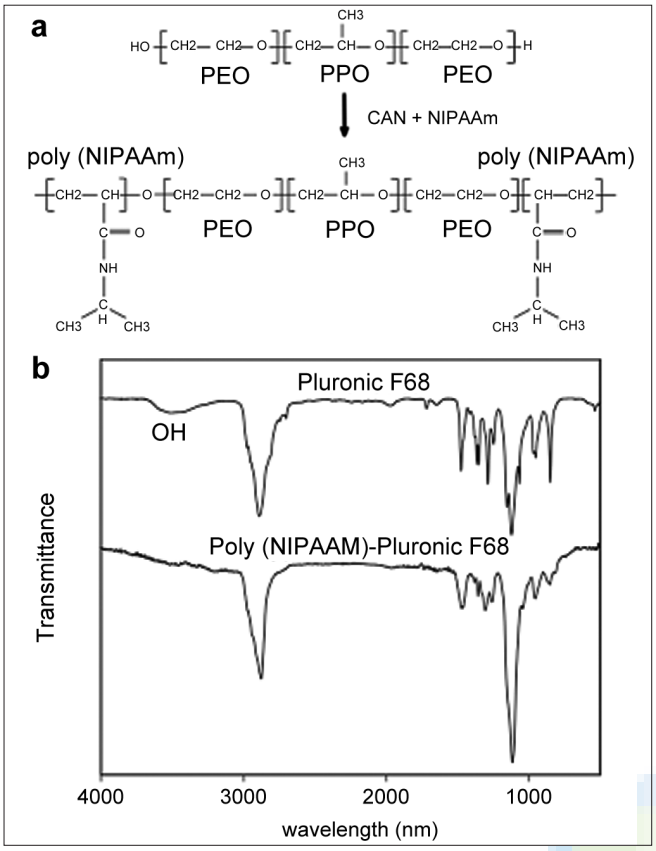

Figure 1: (a) A schematic diagram of the synthesis of a poly(NIPAAm)-Pluronic surfactant and (b) FT-IR spectra of Pluronic F68 and poly(NIPAAm)-Pluronic surfactants

Table 1: Properties of poly(NIPAAm)-Pluronic surfactants

	NIPAAm (g)	$\overline{M}_w$	$\overline{M}_n$	PDI
Pluronic F68	0	6886	7382	1.07
NP-1	1.0	10403	10681	1.02
NP-2	2.0	12036	14845	1.11
NP-3	2.5	17211	17362	1.01

PDI = $\overline{M}_w/\overline{M}_n$ (determined by GPC)

needed to exactly determine the unit number of poly(NIPAAm) in the surfactants, a simple calculation using data (M_n) from GPC could provide the approximate unit number of poly(NIPAAm), where the results showed that the NP1, NP2, and NP3 had 16, 23, and 46 units of NIPAAm, respectively. Moreover, the CMCs of the surfactants were evaluated using a surface tension meter. As shown in Figure 2, the poly(NIPAAm)-Pluronic surfactants with a longer length of poly(NIPPAm) exhibited a higher CMC value at room temperature, which is due to the increased hydrophilicity and steric hindrance of the poly(NIPAAm) block.[17]

To evaluate the effect of the length of poly(NIPAAm) on the average particle size and release profile from microparticles, four different kinds of PLGA microparticles were prepared by a typical emulsification and solvent evaporation method using

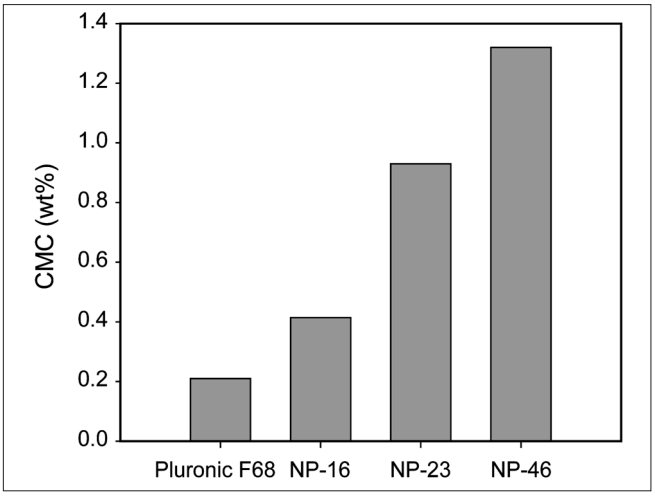

Figure 2: CMCs of the Pluronic F68 and poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) at room temperature

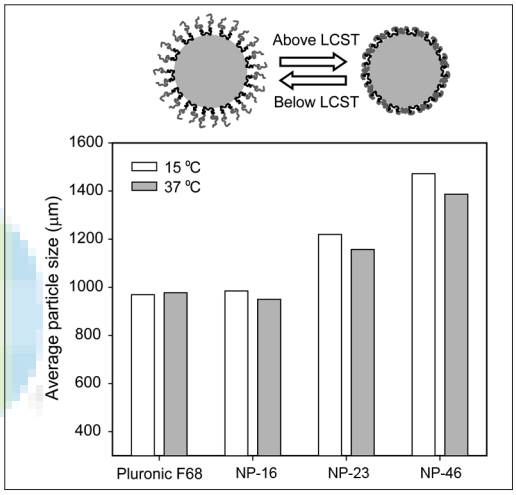

Figure 3: Variation in the average size of the PLGA microparticles at 15°C and 37°C (measured by DLS)

Pluronic F68 and three kinds of poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) in an aqueous phase. The encapsulation efficiency of nile red was found to be around 88.2 ± 3.4% in all the samples without any influence of the length of poly(NIPAAm) block. Figure 3 shows the variation in the average size of the PLGA microparticles, which was measured by DLS at 15°C and 37°C. There was no difference in the average size of the microparticles prepared using Pluronic F68 at different temperatures. In contrast, there was a clear tendency that the microparticles prepared using poly(NIPAAm)-Pluronic surfactants exhibited the smaller average particle size at 37°C than that at 15°C.[13] It is mainly due to the fact that the poly(NIPAAm) block stretches into the aqueous phase at 15°C, whereas PPO and poly(NIPAAm) blocks were insoluble in an aqueous solution at 37°C, resulting in the formation of a dense shell layer of PPO and poly(NIPAAm) at the surface of the microparticles. The dependence of the average particle size on environmental temperature can be a direct evidence of the thermosensitive property of the poly(NIPAAm)

block in the surfactants.

Pluronic surfactants and poly(NIPAAm) exhibit reversible thermosensitive properties in their aqueous solutions which were attributed to the formation of hydrogen bonds with respect to temperature. The LCSTs of Pluronic surfactants (e.g., F68 and F127) are known to be highly dependent on their concentrations and chemical compositions.[18] Generally, the higher the concentration is, the lower the LCST is.[19] In contrast, the LCST of poly(NIPAAm) is around 32–34°C regardless of its concentration.[20,21] In our approach, despite the difficulty in the determination of the concentration of the poly(NIPAAm)-Pluronic surfactants at the surface of the microparticles, it can be accepted that the poly(NIPAAm)-Pluronic surfactants exhibit two LCSTs less than 37°C in an aqueous solution,[13] still depending on the concentration at the surface of the microspheres.

Nile red was chosen as a model dye to evaluate the release behavior of the microparticles due to its easiness of encapsulation into the organic PLGA phase. Figure 4 shows the release profiles of nile red (model dye) for the PLGA microparticles at 37°C, where the PLGA microparticles prepared using Pluronic F68 were used as a control. Nile red was found to be released from the PLGA microparticles prepared using poly(NIPAAm)-Pluronic surfactants in a sustained manner compared to the control, which is due to the formation of a dense layer of poly(NIPAAm). Moreover, the PLGA microparticles prepared using the poly(NIPAAm)-Pluronic surfactant with a longer length of poly(NIPAAm) exhibited a more sustained pattern of dye release, which is due to the formation of the thicker layer of poly(NIPAAm) at the surface of the microparticles. This result confirmed that the use of a poly(NIPAAm)-Pluronic surfactant can reduce the initial burst release of a drug.

CONCLUSION

We have successfully synthesized poly(NIPAAm)-Pluronic surfactants by polymerizing NIPAAm from the ends of hydroxyl groups of Pluronic F68 using CAN and also demonstrated the reduction in the initial burst release of the drug from the PLGA microparticles prepared using the poly(NIPAAm)-Pluronic surfactants. We believe that the poly(NIPAAm)-Pluronic surfactants with a thermosensitive property can be easily utilized for other drug delivery systems.

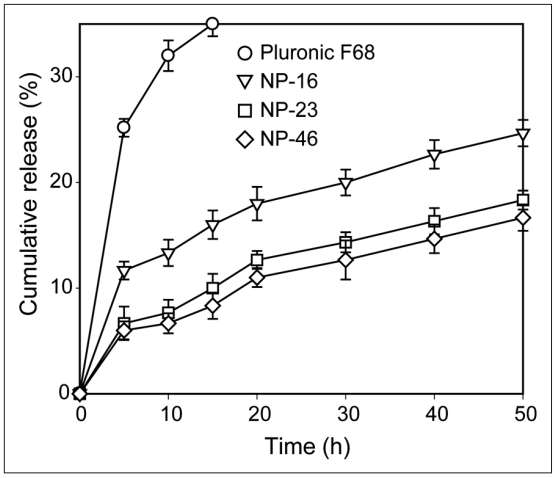

Figure 4: Release profiles of nile red from the PLGA microparticles prepared using Pluronic F68 and poly(NIPAAm)-Pluronic surfactants with different lengths of poly(NIPAAm) at 37°C

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REFERENCES

1. Lavasanifar A, Samuel J, Kwon GS. Poly(ethylene oxide)-block-poly(L-amino acid) micelles for drug delivery. Adv Drug Deliv Rev 2002;54:169-90.
2. Motornov M, Roiter Y, Tokarev I, Minko S. Stimuli-responsive nanoparticles, nanogels and capsules for integrated multifunctional intelligent systems. Prog Polym Sci 2010;35:174-211.
3. Topp MD, Dijkstra PJ, Talsma H, Feijen J. Thermosensitive micelle-forming block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide). Macromolecules 1997;30:8518-20.
4. Inoue T, Chen G, Nakamae K, Hoffman AS. An AB block copolymer of oligo(methyl methacrylate) and poly(acrylic acid) for micellar delivery of hydrophobic drugs. J Control Release 1998;51:221-9.
5. Chung JE, Yokoyama M, Okano T. Inner core segment design for drug delivery control of thermo-responsive polymeric micelles. J Control Release 2000;65:93-103.
6. Jones CD, Lyon LA. Synthesis and characterization of multiresponsive core-shell microgels. Macromolecules 2000;33:8301-6.
7. Jeong JH, Kim SW, Park TG. A new antisenseoligonucleotide delivery system based on self-assembled ODN-PEG hybrid conjugate micelles. J Control Release 2002;93:183-91.
8. Shin Y, Chang JH, Liu J, Williford R, Shin YK, Exarhos GJ. Hybrid nanogels for sustainable positive thermosensitive drug release. J Control Release 2001;73:1-6.
9. Gao J, Frisken BJ. Cross-linker-free n-isopropylacrylamide gel nanospheres. Langmuir 2003;19:5212-6.
10. Zschoche S, Rueda J, Boyko V, Krahl F, Arndt KF, Voit B. Thermo-responsive nanogels based on poly[NIPAAm-graft-(2-alkyl-2-oxazoline)]s crosslinked in the micellar state. Macromol Chem Phys 2010;211:1035-42.
11. Zhou YM, Jiang KQ, Song QL, Liu SY. Thermo-induced formation of unimolecular and multimolecular micelles from novel double hydrophilic multiblock copolymers of N,N-dimethylacrylamide and N-isopropylacrylamide. Langmuir 2007;23:13076-84.
12. Wang T, Wu Y, Zeng AJ. Synthesis and characterization of amphiphilic Pluronic (F68)-1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine copolymers and their micelles as a drug

carrier. J Appl Polym Sci 2010;117:604-13.

13. Mei A, Guo X, Ding Y, Zhang X, Xu J, Fan Z, *et al*. PNIPAm-PEO-PPO-PEO-PNIPAm pentablock terpolymer: synthesis and chain behavior in aqueous solution. Macromolecules 2010;43:7312-20.
14. Han TL, Kumar RN, Rozman HD, Noor MA. GMA grafted sago starch as a reactive component in ultra violet radiation curable coatings. Carbohyd Polym 2003;54:509-16.
15. Studer K, Decker C, Beck E, Schwalm R, Gruber N. Redox and photoinitiated crosslinking polymerization: I. Dual-cure isocyanate-acrylate system. Prog Org Coat 2005;53:126-33.
16. Joshi JM, Sinha VK. Ceric ammonium nitrate induced grafting of polyacrylamide onto carboxymethyl Chitosan. Carbohyd Polym 2007;67:427-35.
17. Chen LJ, Lin SY, Huang CC. Effect of hydrophobic chain length of surfactants on enthalpy-entropy compensation of micellization. J Phys Chem B 1998;102:4350-6.
18. Cellesi F, Tirelli N, Hubbell JA. Materials for cell encapsulation via a new tandem approach combining reverse thermal gelation and covalent crosslinking. Macromol Chem Phys 2002;203:1466-72.
19. Alexandridis P, Holzwarth JF, Hatton TA. Micellization of Poly(ethy1ene oxide)-Poly(propy1ene oxide)-Poly(ethy1ene oxide) Triblock Copolymers in Aqueous Solutions: Thermodynamics of Copolymer Association. Macromolecules 1994;27:2414-25.
20. Gao J, Wu C. The coil-to-globule transition of poly(N-isopropylacrylamide) on the surface of a surfactant-free polystyrene nanoparticles. Macromolecules 1997;30:6873-6.
21. Qiu XP, Wu C. Study of the core-shell nanoparticle formed through the "coil-to-globule" transition of poly(N-isopropylacrylamide) grafted with poly(ethylene oxide). Macromolecules 1997;30:7921-6.

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