

Thermosensitive nanosized micelles from cholesteryl-modified hydroxypropyl cellulose as a novel carrier of hydrophobic drugs

Massoumeh Bagheri · Shaghayegh Shateri

Received: 19 November 2011 / Accepted: 14 March 2012 / Published online: 22 March 2012
© Iran Polymer and Petrochemical Institute 2012

Abstract The thermosensitive micelles based on the two series of cholesteryl-modified hydroxypropyl cellulose (series **1** and **2**, respectively) were used as a promising drug carrier. The polymers **1a** and **2a** with side chain substitution degrees $D_{\text{Chol}} = 0.7$ and $2.1 \text{ mol}\%$ were selected for micelle preparation, respectively. Polymeric micelles were prepared by the co-solvent evaporation method. The aqueous self-assembly of the polymers was studied using fluorescence analysis and transmission electron microscopy (TEM). The critical micelle concentrations (CMCs) values of the various D_{Chol} of polymers were evaluated in the range of ca. $0.13\text{--}0.29 \text{ g/L}$ which decreased with the increase of D_{Chol} in both series. Furthermore, the CMC values displayed a downtrend profile, with increasing the temperature. The polymer **1a** with less D_{Chol} had lower CMC than that of polymer **2a**. By using the naproxen as a hydrophobic model drug, the drug-loaded micelles were prepared. The TEM image of naproxen-loaded micelles of polymer **1a** with 40% drug-loading efficiency and 8% loading capacity showed that micelles were regularly spherical in shape with a mean diameter of 70 nm . The unmodified HPC exhibited a lower critical solution temperature (LCST) of more than $41 \text{ }^\circ\text{C}$ in water, while polymeric micelles in aqueous solution presented an LCST of $38.7 \text{ }^\circ\text{C}$. A drug release study was performed by dialysis method in phosphate-buffered solution at $25, 37$ and $40 \text{ }^\circ\text{C}$, respectively. The release kinetics of naproxen from the polymeric micelles revealed a thermosensitivity, since its release rate was higher at $40 \text{ }^\circ\text{C}$ than at $25 \text{ }^\circ\text{C}$.

Keywords Hydroxypropyl cellulose · Cholesterol-modified · Thermoresponsive polymeric micelles · Drug-delivery system · Nano-carrier

Introduction

Polymeric micelles formed by self-assembly of amphiphilic block copolymers exhibit many merits, such as nanoscale size, core-shell structure, relatively high stability due to low critical micelle concentration (CMC) and prolonged circulation owing to their high water solubility [1]. Many investigations [2–4] have reported that these self-assemblies, used as drug-delivery systems, can reduce the unwanted toxic side effects, increase solubility of poorly water soluble drugs within the hydrophobic core of the micelles, prolong the circulation time, reduce the uptake by the reticuloendothelial system (RES) and enhance the therapeutic index of drugs. Polymeric amphiphiles form micelles which consist of an inner core of hydrophobic segments and an outer shell of hydrophilic segments. The hydrophobic inner core is surrounded and stabilized by the hydrophilic outer shell. The hydrophilic outer shell enhances dispersion, inhibits intermicellar aggregation and interactions with other hydrophobic components irrespective of the high inner core hydrophobicity. Drugs to be delivered can be covalently attached prior to micellization or vesicle formation, or physically entrapped into the micellar core or polymersome by the use of various techniques like solution/precipitation, salting-out process and solvent evaporation method [5, 6].

Recently, interest has grown in amphiphilic polysaccharide derivatives (APDs) which have been used in the formulation of micelles used as hydrophobic drug-delivery vehicles [7, 8]. APD micelles are self-assemblies composed

M. Bagheri (✉) · S. Shateri
Chemistry Department, Science Faculty,
Azarbaijan University of Tarbiat Moallem,
P.O. Box: 53714-161, 5375171379 Tabriz, Iran
e-mail: massoumehbagheri@yahoo.com;
m.bagheri@azaruniv.edu



of hydrophobic microdomains and amphiphilic shells of polysaccharides in aqueous media. Controlled release of the loaded drug can be achieved through diffusion from the micelles or by biodegradation of APDs.

Furthermore, by the use of stimuli-sensitive polymers (temperature, pH, etc.) as a part of the amphiphilic copolymers, it is possible to achieve a controlled release of encapsulated drugs from micelles in response to environmental changes [9–12]. In the case of thermosensitive polymers, the lower critical solution temperature (LCST) in aqueous solution is particularly attractive for the applications in drug delivery, smart bioactive surfaces and molecular recognition agents [13–15]. Thermosensitivity of these block copolymer micelles could be modulated by the alteration of the concentration of the aqueous solution and/or the composition of the copolymers.

Among the thermosensitive polymers, hydroxypropyl cellulose (HPC) is attractive due to its wide and tunable LCST, which is in the range of 41–45 °C in water [16] and can be adjusted by grafting HPC with a more hydrophobic side chain. HPC is non-toxic, biodegradable and biocompatible; hence, it has a wide range of applications in food, agriculture, cosmetics, textiles, nanotechnology, water engineering and medicine (as a drug carrier) [17].

In our previous work, synthesis and characterization of liquid crystalline amphiphilic cholesteryl-modified HPC derivatives (CHDs, Fig. 1) with different side chain content

were described [18]. The main purpose of this study is to produce a new micellar vehicle for the delivery of hydrophobic drug naproxen which is prepared from the above-mentioned CHDs. It is expected that CHD micelles would have good loading capacity and controlled-release property in addition to their good biocompatibility, because of the strong self-assembly capacity of the cholesteryl groups [19]. In this paper, the effects of the cholesteryl side chain structure and the substitution degree on the characteristics of the CHD micelles were studied. In particular, thermosensitive properties of the CHD micelles in aqueous solution were investigated.

Experimental

Materials

Cholesteryl-modified hydroxypropyl cellulose derivatives with different cholesteryl side chains (HPC-G1-Chol **1a** and HPC-Chol **2a**), in which HPCs number-average molecular weight (M_n) of 100,000 g/mol according to manufacturer ($M_n = 150,000$ g/mol obtained using SEC with THF as mobile phase) were previously synthesized in our laboratory (Table 1) [18]. The chemical structures of CHDs used in this study are shown in Scheme 1. Naproxen and dialysis membrane (MWCO = 10,000) were purchased from

Fig. 1 ^1H NMR spectra of polymer **1a**: **a** dissolved in CDCl_3 and **b** aqueous micellar solution obtained in D_2O

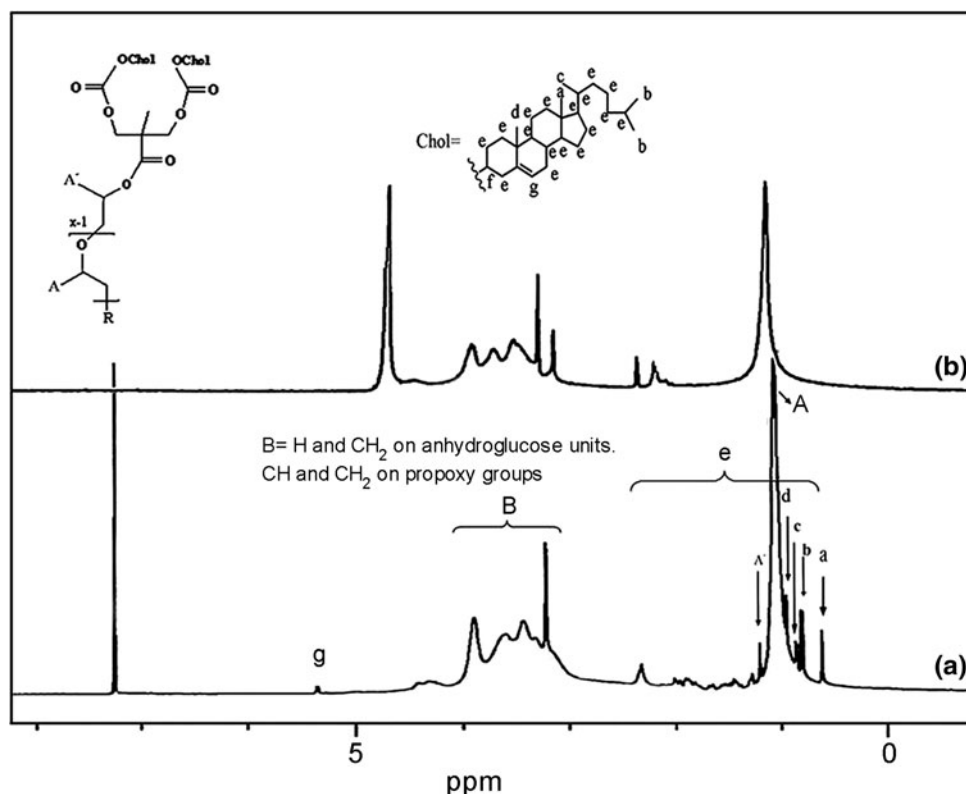
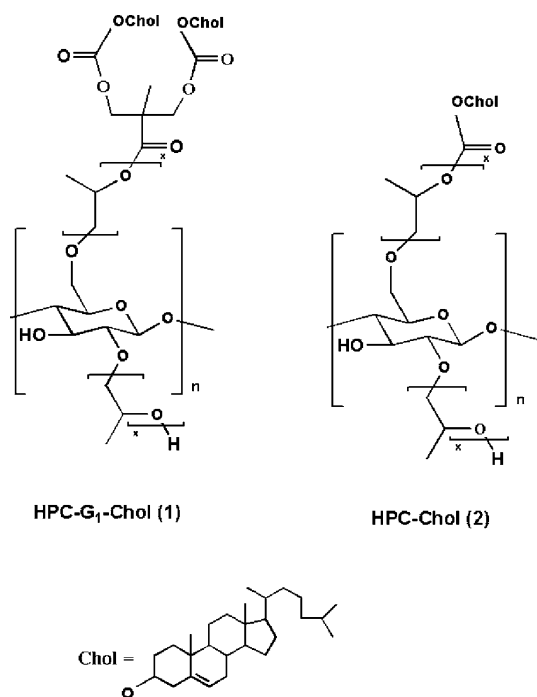


Table 1 Polymerization, substitution degree and critical micelle concentration of the CHDs

Sample	Molar ratio of cholesteryl moiety per hydroxyl groups	No. of cholesteryl moiety/anhydroglucose unit ^a	Critical micelle concentration (g/L)
HPC-G1-Chol (1a)	0.05	0.007	0.2
HPC-G1-Chol (1b)	0.1	0.018	0.13
HPC-G1-Chol (1c) ^b	0.2	–	–
HPC-Chol (2a)	0.1	0.021	0.29
HPC-Chol (2b)	0.2	0.040	0.22
HPC-Chol (2c) ^b	0.4	–	–

^a Calculated from ¹H NMR spectroscopy

^b Partially insoluble in chloroform

**Scheme 1** Structures of cholesteryl-modified HPC derivatives (CHDs)

Sigma-Aldrich (USA). Pyrene, potassium dihydrogen phosphate and sodium hydrogen phosphate were purchased from Merck (Germany). THF and acetone were purified by distillation and all other chemicals were used as received.

Preparation of polymeric micelles

The blank polymeric micelles were prepared using a co-solvent evaporation method. In brief, modified polymer **1a** (or **2a**) (20 mg) was dissolved in 2 mL of acetone in a 50 mL flask, and then the solution of the polymer was added dropwise into 12 mL of deionized water under high-speed stirring. Finally, the mixed solution was devolved to a beaker and slowly stirred for 4 h at room temperature to facilitate the removal of acetone. Polymeric micelles were lyophilized to obtain a white powder of micelles.

Drug encapsulation

Low aqueous solubility drug naproxen was used as a model drug for investigating the loading and release properties of drug in the polymer carrier. The naproxen-loaded polymeric micelles were prepared as follows. The polymer **1a** (20 mg) and naproxen (4 mg) were dissolved in 2 mL of acetone, and the solution was added into deionized water (12 mL) and stirred for 4 h. After the acetone was removed by evaporation, micelle-dispersed solution was obtained. The obtained suspension was centrifuged at 4,000 rpm for 10 min, and then the supernatant containing naproxen-loaded micelles was obtained. Drug-loaded polymeric micelles were lyophilized. The precipitate containing unloaded drug was dissolved in 50 % ethanol solution, and its amount was analyzed by UV–visible spectrophotometry at 330 nm. The standard solutions were prepared at concentrations ranging from 0.001 to 0.1 g/L. The correlation coefficient (R^2) value was at least 0.999.

It was found that ~40 wt% of the free drug naproxen was loaded into polymeric micelles ($W_{\text{total}} = 4$ mg). Drug-loading efficiency (40 %) and drug-loading capacity (8 %) were calculated as follows:

$$\text{Loading capacity (\%)} = (A - B)/C \times 100 \quad (1)$$

$$\text{Loading efficiency (\%)} = (A - B)/A \times 100 \quad (2)$$

where A is the total weight of naproxen used, B is the weight of unloaded naproxen in the precipitate after centrifugation and C is the weight of CHD.

In vitro drug release test

In vitro release of naproxen from the micelle solution was determined using the dialysis membrane diffusion technique. Three milliliter of drug-loaded micelle solution was transferred into a dialysis tube (MWCO = 10,000) and immersed into 30 mL of release media [pH 7.4 phosphate-buffered solution (PBS)] at the given temperature (25, 37 and 40 °C) and stirred at 250 rpm. At predetermined intervals, 3 mL of the medium was taken and 3 mL of fresh PBS was added after each removal. Concentration of the drug released was determined by using a UV–visible



spectrophotometer at 330 nm, and all experiments were carried out in triplicate. The standard aqueous solutions were prepared at concentrations ranging from 0.001 to 0.01 g/L. The correlation coefficient (R^2) value was at least 0.996. The release percentage of naproxen was calculated from the following equation:

$$\% \text{Release} = (W_t)/(W_{\text{total}}) \times 100 \quad (3)$$

where W_t is the weight of released naproxen at time t and W_{total} is the total absorbed naproxen in the polymeric micelle structure. W_{total} was calculated by the free drug amount, i.e., the total drug amount used in this work; here it is 4 mg (A) minus the amount of unloaded drug (B).

Micelle characterization

The polymeric micelles were analyzed by transmission electron microscopy (TEM). TEM observation was performed with a PHILIPS SM10 TEM (The Netherlands) and EPSON HP8300 Photo flat-bed scanner operated at an accelerating voltage of 150 keV. TEM sample was prepared by placing a drop of micelle dispersion on a copper grid, with carbon film and staining with 2 % (w/v) phosphotungstic acid aqueous solution.

The average size and size distribution of polymeric micelles were determined by dynamic light scattering (DLS) using a light scattering spectrometer (Sematech, SEM-633, France) at 25 °C. The CMCs of CHD micelles were determined using pyrene as a fluorescence probe. Polymeric micelles with the concentrations of CHD polymers ranging from 1.0×10^{-3} to 10 mg/mL were equilibrated with a saturated aqueous solution of pyrene for 24 h under shielded light to load pyrene molecules into the polymeric micelle core. The excitation spectrum of pyrene for each sample was obtained at room temperature using a FP-6200 spectrophotometer (JASCO Corporation, Tokyo, Japan) at an excitation wavelength of 335 nm and an emission wavelength of 392 nm.

Temperature-responsive behavior of amphiphilic CHD micelles was measured by a turbidity method. The aqueous polymer solutions (1 g/L of polymers) at various temperatures were measured at 400 nm by a UV–vis spectrometer (PG Instrument T80, England). The sample was thermostated in a refrigerated circulator baths at a different temperature from 28 to 48 °C. At least 20 min was allowed for the temperature equilibration of the sample. Values of the LCSTs of polymer solutions were determined at a temperature showing the onset of turbidity.

^1H NMR spectra of polymer **1a** were taken on a 300 MHz-Brucker SP-300 AVANC spectrometer (Germany) by CDCl_3 and D_2O as solvent with tetramethylsilane as internal standard.

Results and discussion

Preparation and characterization of hydrophobically modified HPC

In our previous work, the cholesteryl-modified HPC polymer (**1a**) was prepared through one-step reaction between O–H groups of HPC and carboxylic acid group of performed first-generation dendron having cholesteryl groups using DCC/DPTS coupling. The modified polymer (**2a**) was obtained through the reaction of the hydroxyl groups of HPC with cholesteryl chloroformate in the presence of pyridine and DMAP as catalyst [18]. The detailed chemical structure of obtained polymers is shown in Scheme 1. Different samples with different feeding ratios were synthesized and characterized by FTIR and ^1H NMR spectroscopy methods. The degree of substitution (D_{Chol}), which can be defined as the number of cholesteryl moieties per anhydroglucose unit of HPC, was evaluated by ^1H NMR spectroscopy methods according to previously reported method (Table 1) [18]. The samples **1** and **2** with $D_{\text{Chol}} = 0.7$ and 2.1 mol% were selected for this study, respectively.

Formation of polymeric micelles

Herein, the solvent evaporation method was used for the preparation of micelles. As most of the micelles cases, the main attractive driving force for the CHDs micellization is hydrophobic interaction and entropy driven. Typically, the ionic (or hydrophilic) head groups are exposed to the bulk aqueous solution, while the hydrophobic hydrocarbon tail groups form the interior of the micelle. CHDs form cholesteryl core-micelle in an aqueous solution. As an evidence, the micelle solution was prepared by dialysis method and its structure was confirmed using ^1H NMR. Figure 1 illustrates the ^1H NMR spectra of the selected polymer **1a**, measured in pure CDCl_3 and pure D_2O to observe the influence of the solvent polarity on the structure in the solution. As indicated in Fig. 1, in CDCl_3 , the specific peaks of HPC and cholesterol are visible (spectrum a) [18]. In D_2O , including the formation of micelles, the specific peaks arising from cholesterol is not observable, while the peaks of HPC are still shown. These results proved that cholesterol moieties are aggregated into the compact micelle core to minimize their interaction with D_2O and the HPC formed the hydrated outer shell (spectrum b). In a good solvent (CDCl_3) for both the hydrophilic and hydrophobic part, both parts stick out into the solution, whereas in a poor solvent (D_2O) the hydrophobic parts would prompt these groups to agglomerate into micellar domains. This trend in the ^1H NMR spectra is also consistent with other polymeric amphiphiles that formed micelles or aggregates in the aqueous phase [20, 21].



Fluorescence studies

The CMC of polymeric micelles can be determined by fluorescence spectroscopy using pyrene. Pyrene is a hydrophobic fluorescence probe that preferentially partitions into the hydrophobic core of the micelle. Below the CMC, pyrene is solubilized in water, and shows only small fluorescence intensity due to its self-quenching. Above the CMC, it strongly emits radiation because the pyrene molecules prefer to be inside the hydrophobic core of the polymeric micelles. This provides a measure for both the presence of such regions and their hydrophobicities [22, 23].

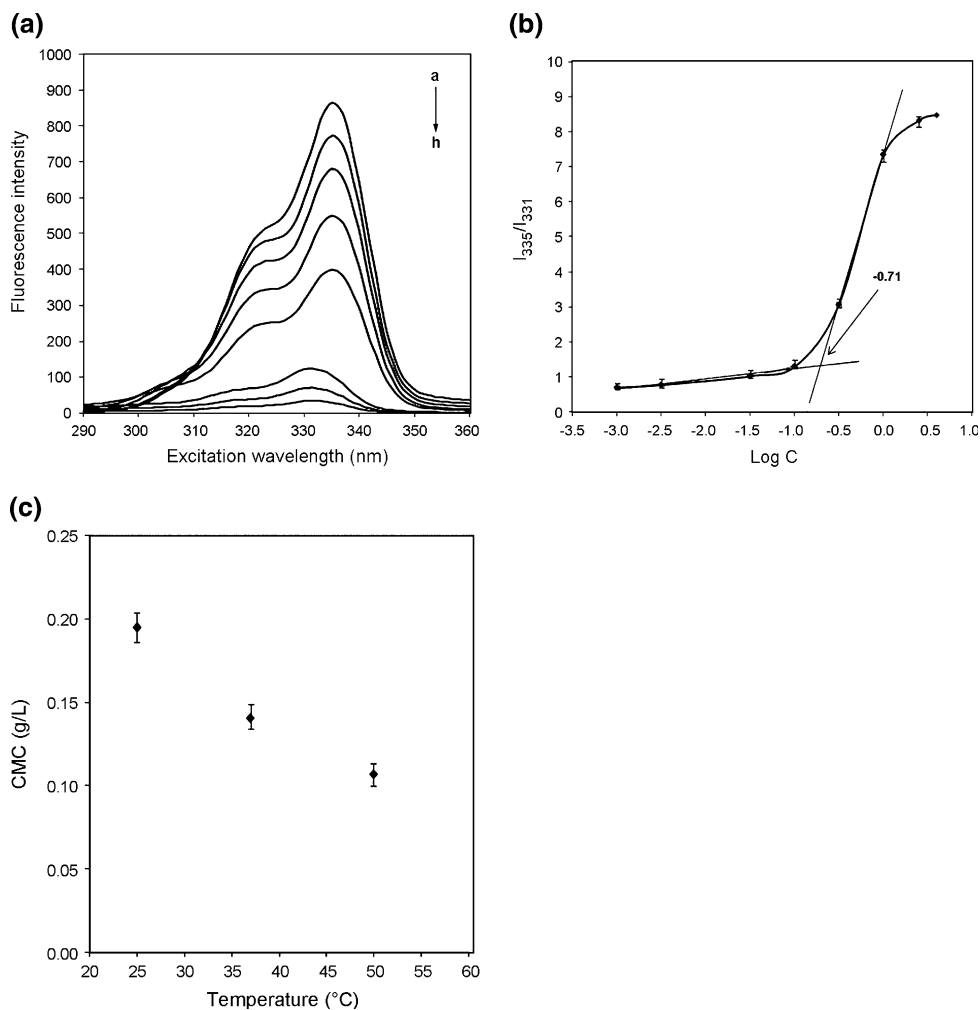
Figure 2a shows the excitation spectra of pyrene as a function of polymer **1a** concentration in water. A plot of I_{331}/I_{335} , where I_{331} and I_{335} are the pyrene fluorescence intensities excited at 331 and 335 nm, respectively, versus the logarithm of the polymer **1a** concentration which is shown in Fig. 2b. As shown in Fig. 2b, the intersection point at $C = 0.1999$ g/L is estimated to be the CMC of the polymer **1a** at 25 °C. Table 1 summarizes the CMC values

of the various substitution degrees of CHDs in the range of ca. 0.13–0.29 g/L in water.

It could be deduced from the comparison between polymers with different cholesteryl side chain content (Table 1) that the aggregation of CHD molecules in aqueous media was due to the hydrophobic interactions of cholesterol moieties. The CMC value decreased with the increase of D_{Chol} in both series of polymers (for example, comparison of polymer **1a** with **1b**). However, it could be seen in Table 1 that CMC of polymer **1a** is lower than polymer **2a** despite having less D_{Chol} . It appears that the main reason is due to differences in the structure of side chains as polymer **1a** has bifunctional side groups based on cholesterol. Therefore, polymer **1a** has stronger hydrophobicity than that of polymer **2a** despite its lower degree of substitution.

Recently, developing thermoresponsive polymeric micelles as intelligent drug carriers has attracted much attention which react by a sharp change of properties in response to a small change of temperature [24]. These synthesized CHDs are also thermoresponsive. The CMC

Fig. 2 **a** Excitation spectra of pyrene as a function of polymer **1a** concentration in water, *a* 5, *b* 2.5, *c* 2, *d* 1, *e* 0.5, *f* 10^{-2} , *g* 3×10^{-3} g/L; **b** CMC of the polymer **1a** at 25 °C; and **c** CMC dependence on the structure of the polymer **1a** and its corresponding aqueous solutions at different temperatures (25, 37 and 50 °C)



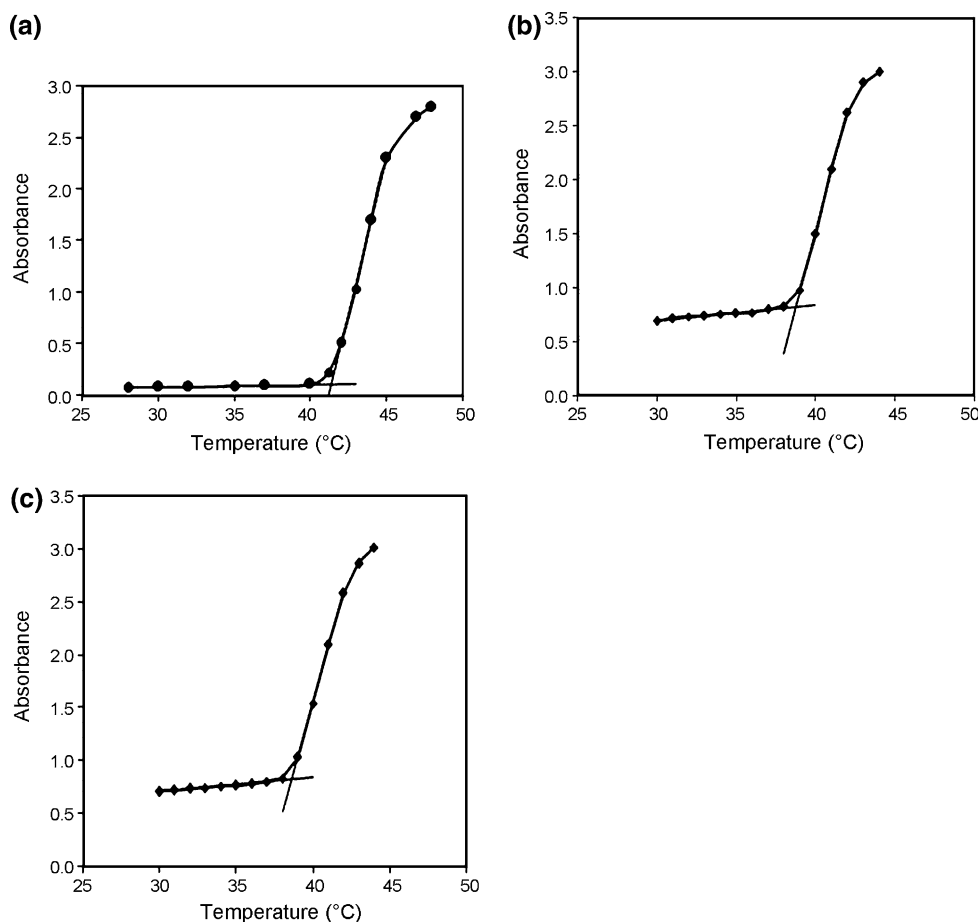
value of the polymer **1a** at 37 °C is obviously lower than that at 25 °C (Fig. 2c). This could be attributed to an increase in hydrophobicity at elevated temperatures, thus leading to dehydration of HPC chains and a subsequent decrease in the CMC. Furthermore, the CMC values display a downtrend profile with the temperature increasing as shown in Fig. 2c.

LCST of the polymers

The solution behaviors of the thermoresponsive polymers were investigated by an optical method. To determine whether the polymer micelles exhibit a thermal response, first we observed macroscopically that the solution of the polymeric micelles is almost transparent at room temperature and becomes opaque with the increases of the temperature and then precipitates (Fig. 3). Later, the optical absorbance of polymeric micelles in distilled water at 400 nm was measured as a function of temperature. Figure 3 demonstrates the variation in turbidity of HPC, polymer **1a** and **2a** solutions. Values for the LCSTs of polymer solutions were determined at a temperature showing the onset of turbidity.

As shown in Fig. 3, the LCSTs of HPC and polymer **1a** solutions are 41.3 and 38.7 °C, respectively. In these two cases, both solutions show sharp transitions within merely 5 °C. The micelles undergo changes in their structures along with the temperature change. In general, the incorporation of hydrophobic moieties into HPC promoted the LCST shift to a lower temperature than the corresponding pure HPC, because the incorporation of the hydrophobic moieties facilitated chain aggregation [13]. However, there is no obvious change in the absorbance for polymer **1a** and **2a** micelles. Additionally, the CHD polymers exhibit nearly the same LCST as that of pure HPC, i.e., cholesteryl moieties show little hydrophobic contribution to LCST. This indicates that the hydrophobic terminals of the polymers self-assemble into a phase-separated inner core under hydrophobic affinity. Hydrated HPC chains remain dispersed surrounding the aggregated hydrophobic cholesterol moiety inner core. This core-shell micellar structure isolates the hydrophobic inner core from the aqueous media, and therefore does not influence the LCST of the HPC outer shell [25]. HPC exhibits an LCST of more than 41 °C, while CHDs have an LCST of around body temperature. Thus, considering the fact that CHDs are body

Fig. 3 Optical absorbance changes of aqueous solution of **a** HPC, **b** polymer **1a** and **c** polymer **2a** as a function of temperature (concentration 1 g/L and absorbance at 400 nm)



temperature sensitive polymers, the resultant amphiphilic polymers can have large potential application as a drug carrier for thermosensitive drug delivery.

Size and morphology of the CHD polymeric micelles

The aqueous self-assembly of amphiphilic polymer **1a** was monitored by DLS and TEM. The mean diameter and size distribution of the polymer **1a** in water were 239 ± 2.8 nm, and the polydispersity index was 0.244 ± 0.03 , which were measured by DLS method (Fig. 4a).

The observed morphology of polymeric micelles is shown in Fig. 4b. It can be observed that all these polymeric micelles are regularly spherical in shape. Moreover, Fig. 4b displays that the average diameter of the polymer **1a** micelles is about 70 nm. The outer shell of the micelles has indistinct but detectable contrast with the inner core,

which means the darker annulus is flatter than the core part of the micelles.

Drug-loaded polymer **1a** micelles were prepared using naproxen as a hydrophobic model drug. The mean diameter and size distribution of naproxen-loaded polymer **1a** micelles in water were 492 ± 2.93 nm, and the polydispersity index was 0.426 ± 0.0259 , which were measured by DLS method as shown in Fig. 5a. However, compared with blank nanoparticles (Fig. 4a), the drug-loaded polymeric micelles had a larger size and a broader size distribution. The increase in the average diameter of nanoparticles might suggest that naproxen molecules were physically entrapped in the nanoparticles.

The TEM image naproxen-loaded polymer **1a** micelles with 8 % loading capacity showed that micelles were regularly spherical with diameters about 70 nm (Fig. 5b). The observed size of micelles by TEM is under 100 nm,

Fig. 4 **a** Size distribution and **b** TEM image of micelles of polymer **1a** in distilled water

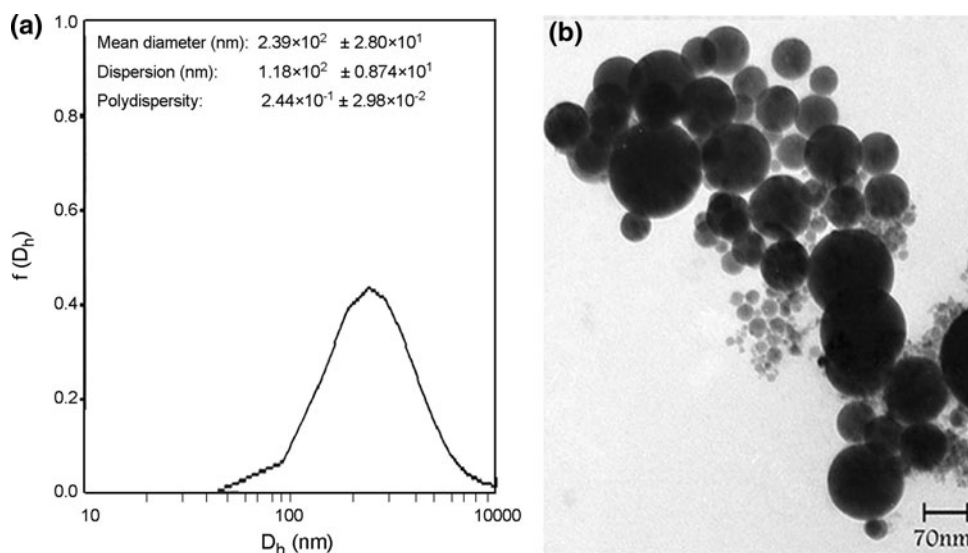
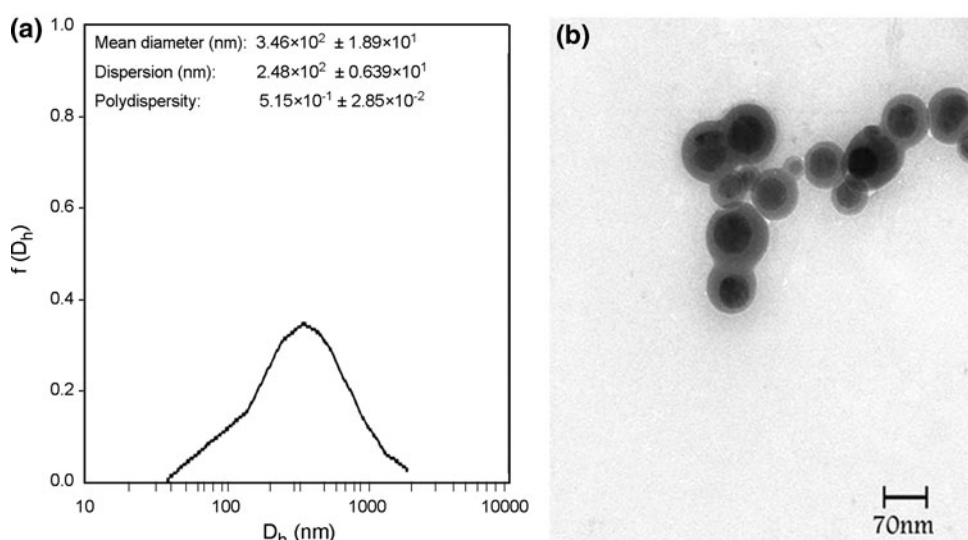


Fig. 5 **a** Size distribution and **b** TEM image of micelles of naproxen-loaded polymer **1a** with drug-loading capacity of 8 % in distilled water



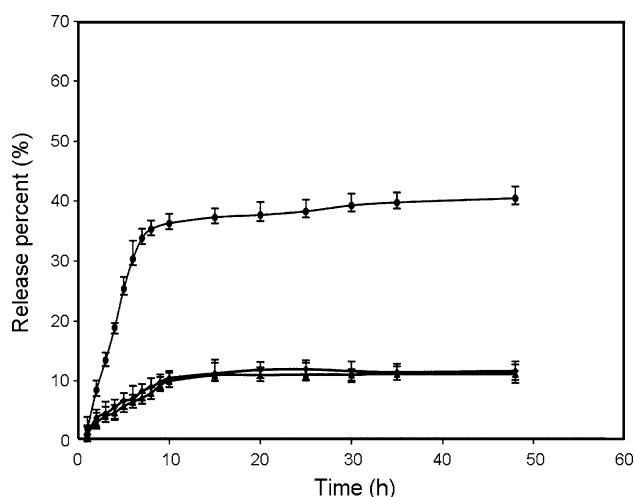


Fig. 6 Release of naproxen from polymer **1a** micelles in PBS at different temperatures [(filled circle) 40 °C; (filled square) 37 °C and (filled triangle) 25 °C]

which is much smaller than the size determined by DLS. This is mainly due to the process involved in the preparation of the sample. TEM depicted the size in the dried state of the sample, whereas the size measured by DLS was a hydrodynamic diameter and had a larger value because of the solvent effect [23].

In vitro drug release study

Naproxen release behavior from polymer **1a** micelles was studied in vitro by the dynamic dialysis method in PBS solution (0.1 M, pH 7.4) at the temperatures of 25, 37 and 40 °C, respectively. Figure 6 presents the in vitro release profiles of naproxen-loaded micelles in PBS. Naproxen was released at a relatively slower rate at 25 °C (below the LCST of **1a**) and only 10 % naproxen was released in 48 h. However, during the same time period, about 40 % naproxen released from polymeric micelles when the temperature was raised above the LCST. The increased release of naproxen might be due to the structural deformation of the polymer **1a** at 40 °C, i.e., above the LCST. The polymeric micelles became hydrophobic above the LCST leading to the deformation and precipitation of the core-shell micelles. As a result, the drug was released quickly from the micelles. Thus, this polymer is an effective drug carrier to control the release amount via changing the temperature.

Conclusion

In conclusion, thermoresponsive self-assembled polymeric micelles composed of different cholesterol-based side chain and HPC were prepared and characterized. The CMC

was estimated from fluorescence spectroscopy. This meant that CHDs were self-associated in water to form core-shell structure micelles. It could be deduced from the comparison between polymers with different mode of functionalization that polymer **1** with bifunctional group based on cholesteryl bis-MPA has CMC values lower than that of polymer **2** with direct functionalization with cholesteryl chloroformate despite having lower degree of substitution. These micelles indicated a thermal transition at 38.7 °C, the LCST of CHDs. From the TEM observations, the micelles were spherically shaped with a mean diameter of 70 nm. Naproxen release from these micelles was thermosensitive as expected. Finally, it is proposed that drug release from thermoresponsive CHDs micelles might be applied to site-specific drug delivery systems by modulating the temperature of the target site.

Acknowledgments The authors would like to thank Research Vice Chancellor of Azarbaijan University of Tarbiat Moallem for financially supporting this research. The authors' warm thanks are also extended to Ms. V. Barri whose suggestions on the English language used in parts of the paper were helpful.

References

- Allen TM, Cullis PR (2004) Drug delivery systems: entering the main stream. *Science* 303:1818–1822
- Jones MC, Leroux JC (1999) Polymeric micelles—a new generation of colloidal drug carriers. *Eur J Pharm Biopharm* 48:101–111
- Kwon GS, Kataoka K (1995) Micellar solubilization of drugs. *Adv Drug Deliv Rev* 16:295–309
- Torchilin VP (2001) Polymeric micelles for delivery of poorly soluble drugs: preparation and anticancer activity in vitro of paclitaxel incorporated into mixed micelles based on poly(ethylene glycol)-lipid conjugate and positively charged lipids. *J Control Release* 73:137–172
- Cai SS, Vijayan KS, Cheng D, Lima EM, Discher DE (2007) Micelles of different morphologies advantages of worm-like filomicelles of PEO-PCL in paclitaxel delivery. *Pharm Res* 24:2099–2109
- Martini L, Attwood D, Collett J (2005) The bioadhesive properties of a triblock copolymer of ϵ -caprolactone and ethylene oxide. *Int J Pharm* 113:223–229
- Gref R, Rodrigues J, Couvreur P (2002) Polysaccharide-based nano-particles as drug delivery systems. *Macromolecules* 35:9861–9866
- Akiyoshi K, Sunamoto J (1996) Supramolecular assembly of hydrophobized polysaccharides. *Supramol Sci* 3:157–163
- Bae Y, Fukushima S, Harada A, Kataoka K (2003) Design of environment sensitive supramolecular assemblies for intracellular drug delivery: polymeric micelles that are responsive to intracellular pH change. *Angew Chem Int Ed Engl* 42:4640–4643
- Webber GB, Wanless EJ, Armes SP, Tang YQ, Li YT, Biggs S (2004) Nano-anemones: stimulus-responsive copolymer-micelle surfaces. *Adv Mater* 16:1794–1798
- Liu TY, Hu SH, Liu DM, Chen SY, Chen IW (2009) Review: biomedical nanoparticle carriers with combined thermal and magnetic responses. *Nano Today* 4:52–65

12. Bawa P, Pillay V, Choonara YE, du Toit LC (2009) Topical review: stimuli-responsive polymers and their applications in drug delivery. *Biomed Mater* 4:1–15
13. Li W, Tu W, Cao D (2009) Synthesis of thermoresponsive polymeric micelles of PNIPAAm-b-OMMA as a drug carrier for loading and controlled release of prednisolone. *J Appl Polym Sci* 111:701–708
14. Nath N, Chilkoti A (2002) Creating ‘smart’ surfaces using stimuli responsive polymers. *Adv Mater* 14:1243–1247
15. Yang CC, Tian YQ, Jen AKY, Chen WCJ (2006) New environmentally responsive fluorescent *N*-isopropylacrylamide copolymer and its application to DNA sensing. *J Polym Sci Part A Polym Chem* 44:5495–5504
16. Ichikawa H, Fukumori Y (1999) Negatively thermosensitive release of drug from microcapsules with hydroxypropyl cellulose membranes prepared by the wurster process. *Chem Pharm Bull* 47:1102–1107
17. Zhang Z, Chen L, Zhao C, Bai Y, Deng M, Shan H, Zhuang X, Chen X, Jing X (2011) Thermo- and pH-responsive HPC-g-AA/AA hydrogels for controlled drug delivery applications. *Polymer* 52:676–682
18. Bagheri M, Shateri Sh (2012) Synthesis and characterization of novel liquid crystalline cholesteryl-modified hydroxypropyl cellulose derivatives. *J Polym Res* 19:9842–9844
19. Zhou Y, Briand VA, Sharma N, Ahn S, Kasi RM (2009) Polymers comprising cholesterol: synthesis, self-assembly, and applications. *Materials* 2:636–660
20. Jang M-K, Jeong Y-I, Nah J-W (2010) Characterization and preparation of core-shell type nanoparticle for encapsulation of anticancer drug. *Colloids Surf B* 81:530–536
21. Pelletier M, Babin J, Tremblay L, Zhao Y (2008) Investigation of a new thermosensitive block copolymer micelle: hydrolysis, disruption, and release. *Langmuir* 24:12664–12670
22. Zhao C, Wang Y, Winnik MA, Riess G, Croucher MD (1999) Fluorescence probe technique used to study micelle formation in water-soluble block co-polymer. *Langmuir* 6:514–516
23. Wang Y-S, Liu L-R, Jiang Q, Zhang Q-Q (2007) Self-aggregated nanoparticles of cholesterol-modified chitosan conjugate as a novel carrier of epirubicin. *Euro Polym J* 43:43–51
24. Rapoport N (2007) Physical stimuli-responsive polymeric micelles for anti-cancer drug delivery. *Prog Polym Sci* 32:962–990
25. Wei H, Zhang X-Z, Zhou Y, Cheng S-X, Zhuo R-X (2006) Self-assembled thermoresponsive micelles of poly (*N*-isopropylacrylamide-*b*-methyl methacrylate). *Biomaterials* 27:2028–2034

