Thermosensitive cross-linked polymer vesicles for controlled release system

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Thermosensitive cross-linked polymer vesicles were formed by self-assembly of block copolymer poly(2-cinnamoylethyl methacrylate)-*block*-poly(*N*-isopropylacrylamide) ($PCEMA_{61}$-*b*-$PNIPAM_{22}$) and subsequent photo-cross-linking PCEMA shells. The ability of these polymer vesicles to load and release 4-aminopyridine (Apy), a model compound, is discussed. It is found that the polymer vesicles can load a large amount of Apy and release the compound at a tunable rate depending on the temperature. The polymer vesicles also show satisfactory reversible and reproducible thermosensitive release characteristics.

1. Introduction

Micelles formed from amphiphilic block copolymers have been extensively explored as drug delivery and separation systems over the past decades.[1–4] But their cargo space is very limited, maximally to a core volume. Based on this consideration, polymer vesicles were developed to use as potential drug carriers in very recent years.[5,6] Compared with micellar carriers, polymer vesicles have the following advantages. They not only encapsulate soluble species in their cavities but also accumulate insoluble species in their shells. Furthermore, their cavities can load larger and more species. Unfortunately, the effect of the morphology of the carriers on their efficiency as drug carriers has remained virtually unexplored.

On the other hand, controllable and targeted release is a key issue for a drug carrier in order to improve the effectiveness of drug therapy. It is well established that regulation of permeability can be controlled by temperature using the thermosensitive PNIPAM segment. For example, PS-*b*-PNIPAM and PBMA-*b*-PNIPAM spherical micelles have been widely applied for drug delivery.[7–10] But there have been no groups reporting polymer vesicles with thermosensitive properties. So we recently devoted ourselves to the preparation of the thermosensitive poly(2-cinnamoylethyl methacrylate) (PCEMA)-cross-linked vesicles from PCEMA-*b*-PNIPAM.[11,12] In the vesicular aggregates, PCEMA chains form a hydrophobic shell and help to "lock in" the structure of the aggregates by photo-cross-linking, while PNIPAM chains stretch into the aqueous phase from both the outer and inner surface of the hydrophobic shell in order to stabilize the vesicles. More important is that PNIPAM chains can exhibit a reversible thermo-responsive phase transition at LCST in aqueous solution, which may provide "on-off" switches for the vesicles. Therefore, the vesicles can be expected to be applicable in a controlled release and separation system. In this paper, we focus on the ability of the thermosensitive cross-linked vesicles to load and release 4-aminopyridine (Apy), a model compound, and discuss the effects of the morphology of the carriers, degree of cross-linking of the PCEMA chains, and the temperature on the loading and releasing behavior of the polymer vesicles.

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2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAM, Aldrich, USA) was purified by recrystallization in hexane and toluene and dried *in vacuo* at room temperature. 2-Hydroxyethyl methacrylate (HEMA, Tokyo Kaisei, Japan) was used as received, 2-aminoethanethiol hydrochloride (AESH, ABCR GmbH & Co. KG) was sublimed at 85 °C under reduced pressure just before use. 3-Mercaptopropionic acid (MPA, ABCR GmbH & Co. KG) was distilled under reduced pressure. Cinnamoyl chloride (ABCR GmbH & Co. KG) was used as received, *N*,*N*′-azoisobutyronitrile (AIBN) was recrystallized from methanol. Solvents and all other reagents were of reagent grade or better and were used without further purification.

2.2. Preparation of block copolymer PNIPAM-*b*-PCEMA

Amino-terminated PNIPAM (PNIPAM-NH_2) was synthesized by telomerization using AESH as a chain transfer agent. NIPAM (50 mmol), AESH (5.7 mmol) and AIBN (0.5 mmol) were dissolved in CH_3OH (20 ml). The solution was bubbled with nitrogen and sealed in an ampoule. The reaction mixture was stirred at 65 °C for 22 h. After reaction, KOH–methanol was added to remove HCl from AESH. The reaction mixture was filtrated and precipitated into an excess of diethyl ether. The product was purified by reprecipitation three times and dried *in vacuo*. Molecular weight was determined by conductometric titration with 0.01 mol L^{-1} HCl, which detects an amino group at the polymer end.

Carboxyl-terminated PHEMA (PHEMA-COOH) was prepared by radical polymerization in the presence of the chain transfer agent MPA. HEMA (70 mmol), MPA (0.68 mmol) and AIBN (0.38 mmol) were dissolved in CH_3OH (63 ml). The

solution was bubbled with nitrogen and sealed in an ampoule. Polymerization was carried out at 65 °C for 24 h. The reaction mixture was reduced and precipitated into an excess of diethyl ether. The product was purified by reprecipitation three times and dried *in vacuo*. Molecular weight was determined by end group titration with 0.01 mol L^{-1} NaOH solution.

PNIPAM-NH_2 (concentration of terminal NH_2 groups = 1.04 mmol), PHEMA-COOH (concentration of terminal COOH groups = 0.43 mmol) and *N*-hydroxysuccinimide (8.6 mmol) were dissolved in DMF (5 ml) and dioxane (20 ml). Dicyclohexylcarbodiimide (DCC, 8.6 mmol) was added to the polymer solution at 10 °C under nitrogen atmosphere. After 24 h reaction at room temperature, the reaction mixture was filtrated and precipitated in a large excess of diethyl ether. The product PNIPAM-*b*-PHEMA was extracted with acetone in order to remove the free PNIPAM-NH_2. The required block copolymer PNIPAM-*b*-PCEMA was prepared by reacting the above block copolymer PNIPAM-*b*-PHEMA with excess cinnamoyl chloride in pyridine at room temperature. The resulting copolymer was characterized by FT-IR, ^{1}H-NMR spectroscopy, and GPC.

FTIR (KBr pellets): 3417 cm^{-1} (ν_{N-H} from PNIPAM block), 1716 cm^{-1} ($\nu_{C=O}$ from PCEMA block), 1670 cm^{-1} ($\nu_{C=C}$ from PCEMA block), 1598 cm^{-1}, 1497 cm^{-1} and 1450 cm^{-1} (ν_{phenyl} from PCEMA block), 1386 cm^{-1} and 1367 cm^{-1} (δ_{CH_3} from PNIPAM block), 1166 cm^{-1} (ν_{C-O} from PCEMA block).

^{1}H-NMR (acetone-d_6, 300 MHz): 7.40–7.70 ppm (phenyl from PCEMA block), 6.51–6.60 ppm (CH_2= from PCEMA block), 4.33–4.21 ppm (–CH_2–O– from PCEMA block), 4.00 ppm (>N–CH< from PNIPAM block), 2.89 ppm (–O=C–NH– from PNIPAM block), 1.02–1.13 ppm (–CH_3 and –CH_2– from PNIPAM block and PCEMA block).

The copolymer was denoted as PNIPAM$_{22}$-*b*-PCEMA$_{61}$, where 22 and 61 stand for the number average degrees of polymerization of PNIPAM and PCEMA blocks, respectively, according to the above end group titration.

2.3. Micelle formation

To prepare micellar solutions, deionized water, as a precipitant, was added at a rate of 0.3 wt.% per 10 s with vigorous stirring to the copolymer solutions in different common solvents, acetone or THF. The initial copolymer concentration was 0.5 wt.%. More water was added until the water content reached *ca.* 50 wt.%. Finally, the common solvents were removed by dialysis of the micellar solutions against pure water for 3 days.

The above micelle solutions (2.5 mg ml^{-1}, 30 ml) were irradiated with UV light (8 W, λ = 254 nm) under stirring. Samples (2 ml) were taken at different irradiation times and diluted with THF. Then they were used for absorbance analysis at 274 nm to determine the conversion of the aliphatic double bonds of CEMA.[13]

2.4. Loading and releasing 4-aminopyridine

The model compound, Apy, and the cross-linked micelle solution (1.2 mg ml^{-1}, 25 ml) were mixed and stirred. At different times, the concentration of Apy remaining in the supernatant was determined by an UV-Vis spectrophotometer at 278 nm to calculate the amount of Apy loaded after being separated by centrifugation.

The Apy-loaded micelle powder (30 mg) was put into a dialysis membrane. Then the dialysis membrane was introduced into a vial with deionized water (10 ml), and the media was stirred at 25 or 50 °C. At specific time intervals, 3 ml medium was replaced with the same volume of fresh deionized water. The concentration of Apy released from the micelles was determined by an UV-Vis spectrophotometer at 278 nm to calculate the amount of Apy released.

2.5. Characterization

Transmission electron microscopy experiments were carried out on a JEM-100CX instrument operating at an acceleration voltage of 80 kV. TEM samples were prepared by placing an aqueous sample onto a copper EM grid. A few minutes after the deposition, the aqueous solution was blotted away with a strip of filter paper and stained with 2wt.% phosphotungstic acid aqueous solution, then dried in air for a few hours.

The hydrodynamic diameters and diameter distribution of the micelles were measured with a Zeta Sizer (Nano-ZS90) at a wavelength of 532 nm, a temperature of 25 °C and an angular range of 90°.

3. Results and discussion

3.1. Vesicle formation

In general, vesicles based on amphiphilic block copolymers are formed by first dissolving the copolymers in a common solvent for both blocks. Water, which is a poor solvent for the hydrophobic block, is then added dropwise to induce self-assembly. The self-assembly mechanism of such vesicles has been studied in detail by A. Eisenberg's group.[14–17] It is found that the formation of various aggregate morphologies, including vesicles, is determined by a balance among three main forces: core-chain stretching, corona-chain repulsion, and interfacial tension. Therefore, factors that affect the above balance, including the copolymer composition, the nature of common solvent, the initial copolymer concentration, and the water content, can be used to control the morphologies of block copolymer aggregates. According to the above method and principle, we have successfully prepared various aggregates of thermosensitive block copolymer PNIPAM-*b*-PCEMA, under different self-assembly conditions.[12] When acetone and THF were used as the common solvents and the initial copolymer concentration was 0.5 wt.%, spherical and vesicular aggregates were obtained, respectively, as shown in Fig. 1. It is obvious that the aggregates formed in THF possess a hollow structure compared with ones formed in acetone. In the vesicular aggregates, the PCEMA blocks form an essentially water-free shell, while the PNIPAM blocks face inner and outer water, helping to delimit the two interfaces of the shell.

Aggregate formation was also confirmed by diameters analysis, as seen in Fig. 2. In the aqueous solution, the hydrodynamic diameters of the aggregates formed in THF and acetone are 194 nm and 180 nm, respectively. These values

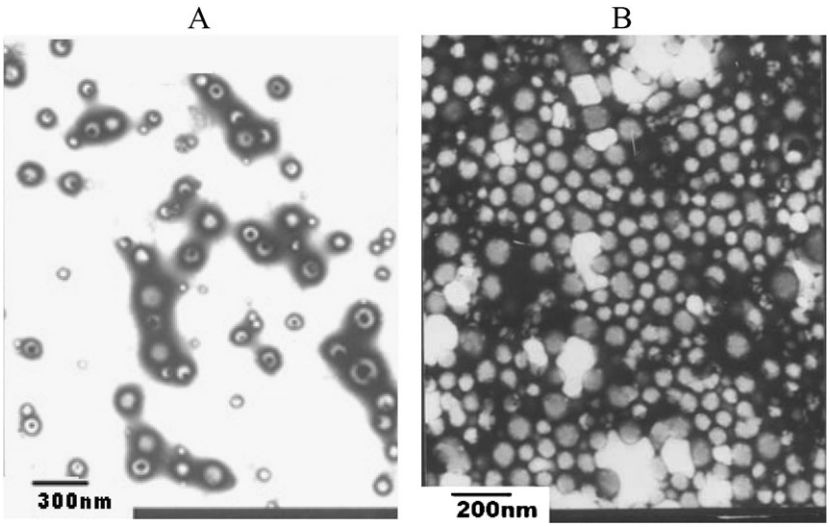

Fig. 1 TEM image of PNIPAM-*b*-PCEMA vesicles (A) and spheres (B) formed by self-assembly of block copolymer PNIPAM-*b*-PCEMA under the common solvents, THF and acetone, respectively.

are larger than the TEM radii, because the aggregates are swollen in the aqueous solution.

3.2. Cross-linking of vesicles

In order to obtain polymer vesicles with enough mechanical stability, the polymer vesicles were cross-linked through PCEMA functional groups with UV light at a wavelength of 254 nm. The cross-linking of the polymer vesicles is based on dimerization between CEMA groups of different chains of the same vesicles. Due to the special structure of the polymer vesicles, the probability of bond formation between CEMA groups from different vesicles is small. TEM images confirm that most of the cross-linked polymer vesicles are individual particles and their morphology is similar with the structure before irradiation.[11] The conversion of PCEMA can be obtained by comparing the change of carbon–carbon double bond peak intensity in UV spectrum at different irradiation times. Fig. 3 is the disappearance rate of CEMA groups under UV light. The conversion of CEMA gradually increases with increasing irradiation time, resulting in an increase in the degree of cross-linking of the polymer vesicles.

3.3. Thermosensitive property of the cross-linked polymer vesicles

The thermosensitive property of the cross-linked polymer vesicles is based on coil–globule transition of PNIPAM blocks on both the outer and inner surfaces of PCEMA shell. The structural transition of the vesicles was investigated by measuring the change of the vesicle diameter with temperature, shown in Fig. 4. It is obvious that the change of the vesicle diameter takes place at about 32 °C, which is consistent with the LCST of PNIPAM. With temperature further increasing, the vesicle diameter becomes smaller. This suggests the cross-linked polymer vesicles have a good thermosensitive property.

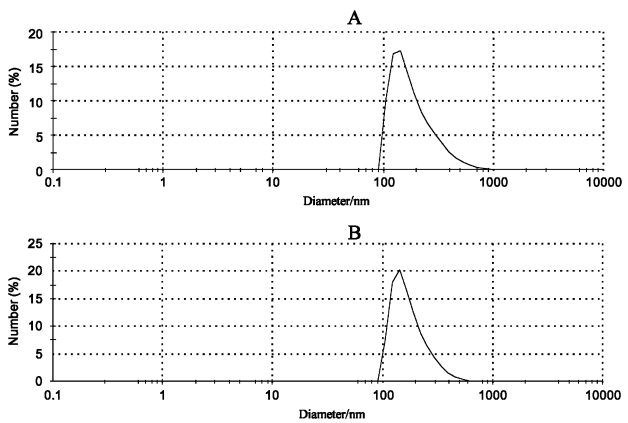

Fig. 2 Size and size distribution of PNIPAM-*b*-PCEMA vesicles (A) and spheres (B) formed by self-assembly of block copolymer PNIPAM-*b*-PCEMA under the common solvents, THF and acetone, respectively.

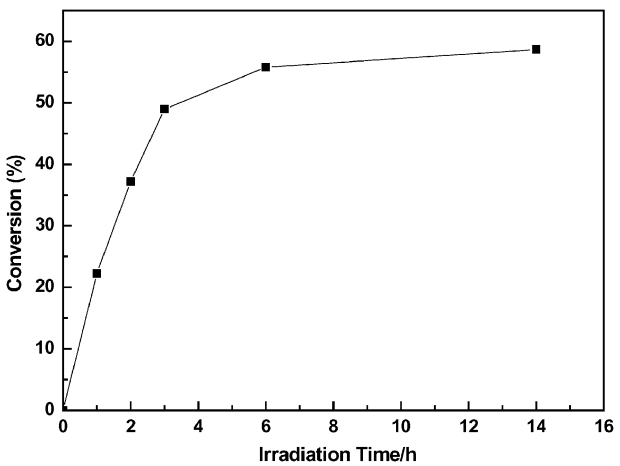

Fig. 3 PCEMA conversion as a function of the irradiation time for PNIPAM-*b*-PCEMA vesicles. The conversions are calculated from $[A(0) - A(t)]/A(0)$, where $A(0)$ and $A(t)$ denote the absorbance of PCEMA at 274 nm at time zero and t, respectively.

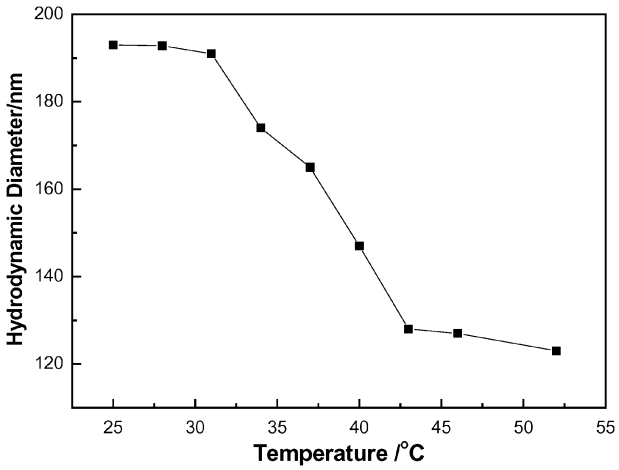

Fig. 4 Hydrodynamic diameter of PNIPAM-*b*-PCEMA vesicles irradiated for 4 h as a function of temperature.

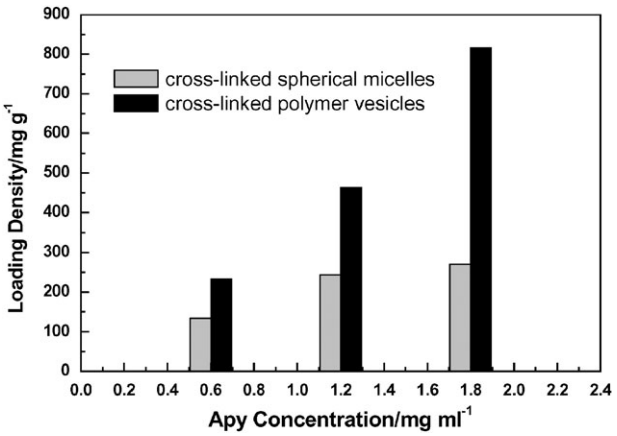

Fig. 6 Change in the loading density of PNIPAM-*b*-PCEMA vesicles and spheres irradiated for 4 h as a function of the Apy concentration.

More important is that the structural changes of the vesicles are repeatable and reproducible, and that precipitates of the vesicles aren't observed in the heating and cooling cycle. The result is seen in Fig. 5, suggesting that the PNIPAM blocks retain their thermal swelling/shrinking and hydrophilic/hydrophobic properties intact, even though they undergo repeated temperature changes across the LCST.

3.4. Loading Apy

Shown in Fig. 6 are the loading densities obtained from the vesicles and spherical micelles, respectively, after the two aggregates were equilibrated with Apy solution at different concentrations and 25 °C for 3 days. As seen in the figure, the loading densities of the vesicles are larger than those of the spherical micelles. This suggests that the polar compound Apy can not only associate with the hydrophobic shells but can also be entrapped in the cavities. For the spherical micelles, when their cargo space is completely occupied, their load increases only very slightly with increasing Apy concentration. For the vesicles, however, the loading densities increase linearly with the Apy concentration. This is expected because we suppose the cavities of the vesicles to be eventually filled with an Apy solution at the approximately the same concentration as that outside the vesicles.

Fig. 7 illustrates the kinetics curves of Apy incorporation into the vesicles at 25 and 50 °C. At 25 °C, the Apy load increases linearly with the loading time up to 12 h, after which time it tends to a limit. This indicates the equilibrium loading time of the vesicles is around 12 h at 25 °C. At 50 °C, however, the loading amount of Apy does almost not increase with the loading time. This result may be explained by Fig. 8. At temperatures below the LCST of PNIPAM, the PNIPAM chains in the outer and inner surface of the vesicles are swollen, which allows substance to diffuse freely between the inside of the vesicles and the medium. When the temperature is increased above the LCST, PNIPAM chains shrink and form a compact film covering the surface of the vesicles, consequently preventing the loading of Apy. A similar phenomenon was also found in thermo-responsive microcapsules with a high graft density PNIPAM.[18]

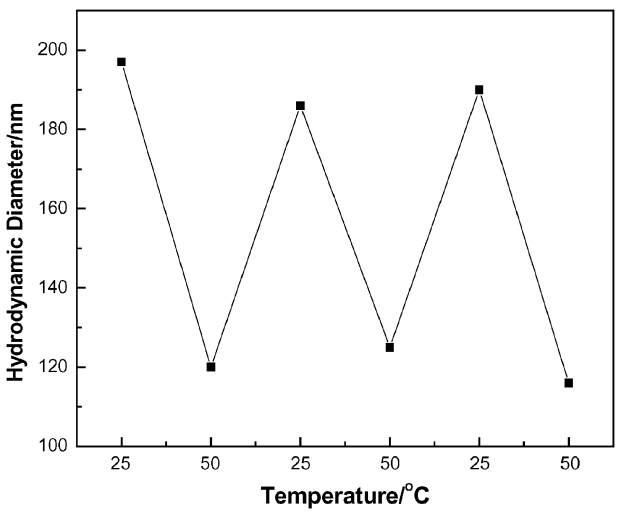

Fig. 5 Hydrodynamic diameter changes of PNIPAM-*b*-PCEMA vesicles irradiated for 4 h with temperature fluctuation.

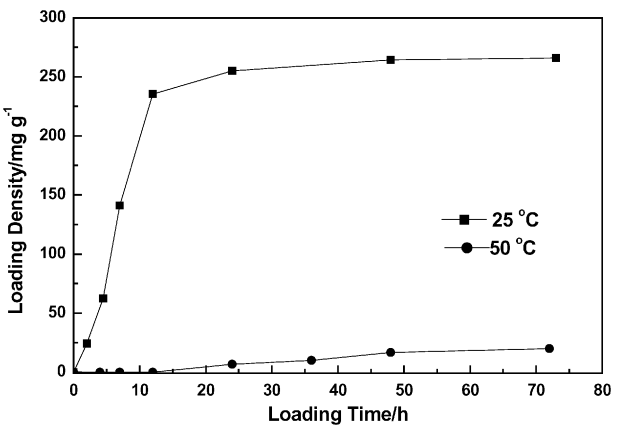

Fig. 7 Increase in Apy loading density as a function of the loading time of PNIPAM-*b*-PCEMA vesicles irradiated for 4 h at 25 and 50 °C, respectively.

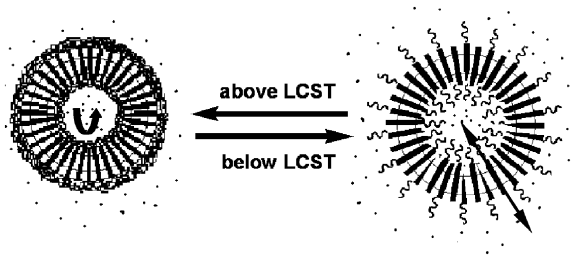

Fig. 8 Schematic illustration of the thermosensitive loading and release principle of the vesicle.

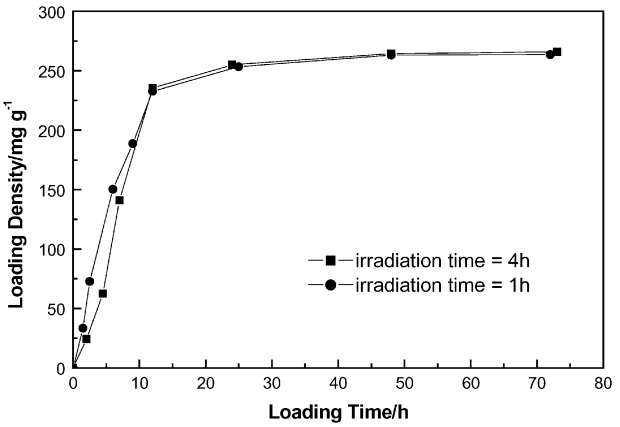

Fig. 9 Increase in Apy loading density as a function of the loading time of PNIPAM-*b*-PCEMA vesicles undergoing different irradiation time at 25 °C.

Shown in Fig. 9 is the comparison between the loading kinetics of vesicles formed through different irradiation times at 25 °C. The degree of cross-linking of the vesicles has no effect on the loading rate of Apy. That is to say, the permeability rate of Apy can't be controlled by the degree of cross-linking of the vesicles.

3.5. Releasing Apy

Apy release kinetics were monitored by calculating the increase of the Apy concentration in the water as a function of time after an Apy-loaded vesicle sample was dialysed with water at 25 and 50 °C, as shown in Fig. 10. At 25 °C, the equilibrium release time of the vesicles is at least 6 h. The amount of Apy released is much higher at 25 than at 50 °C. The result accords with the assumption in Fig. 8.

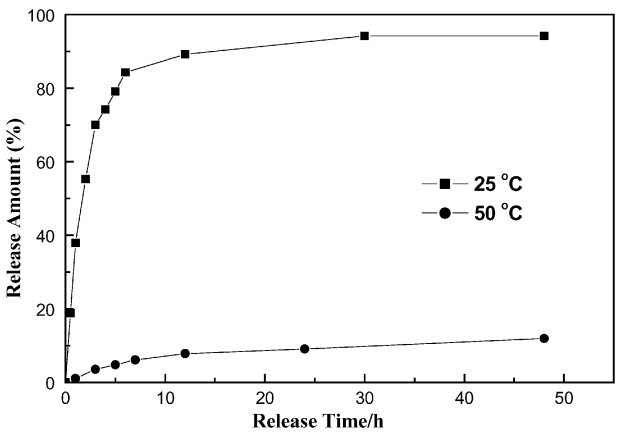

Fig. 10 Release of Apy from PNIPAM-*b*-PCEMA vesicles irradiated 4 h at 25 and 50 °C, respectively.

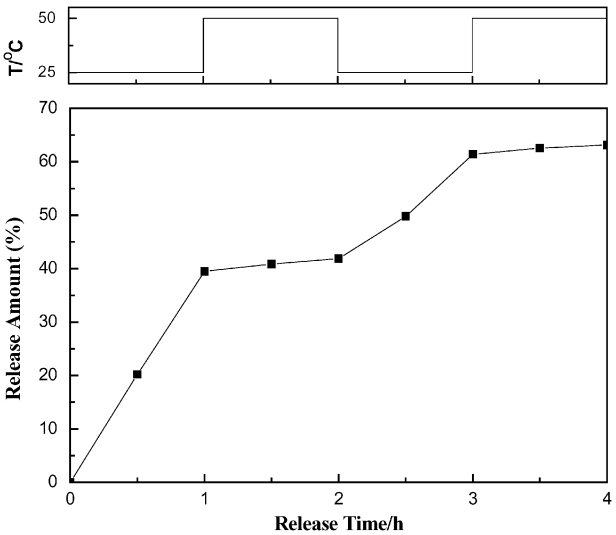

Fig. 11 Thermosensitive release of Apy from PNIPAM-*b*-PCEMA vesicles irradiated 4 h.

To verify the reversible of the PNIPAM "gates" on the surface of the vesicle, release experiments were carried out by alternatively changing the environmental temperature at 25 and 50 °C. The reversible thermosensitive release characteristics of the vesicles are shown in Fig. 11. It is found that the amount of Apy released increases slowly at 50 °C, but rapidly at 25 °C, and the thermosensitive release of the vesicles is reversible and reproducible.

4. Conclusions

Thermosensitive cross-linked polymer vesicles have been prepared by self-assembly of block copolymer PNIPAM-*b*-PCEMA, and subsequent photo-cross-linking of PCEMA chains. The thermosensitive loading and release behavior of the vesicles have been studied. Compared with spherical micelles, the vesicles show a larger loading and their loading density increases linearly with the drug concentration. The cross-linking degree of vesicles has no effect on the loading rate. The loading amount and rate of vesicles are much larger at 25 °C than 50 °C. The thermosensitive release behavior of the vesicles agrees with their loading behavior, that is, the release amount and rate are high at 25 °C, but low at 50 °C, and the vesicles show satisfactory reversible and reproducible thermosensitive release characteristics.

From these results, the cross-linked polymer vesicles is proposed to be a good on–off permeability regulation system. They have many potential applications in controlled release and separation systems. Further studies of the cross-linked polymer vesicles are expected to make a more complete analysis of the thermosensitive release process and mechanism and set up a release model.

Acknowledgements

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