Direct observation of time and temperature dependent transition from spherical micelles to vesicles†

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An interesting transition from spherical micelles to vesicles, which was time and temperature dependent, was observed for the first time; it is tentatively attributed to the thermal hysteresis of temperature-responsive poly(*N*-isopropylacrylamide).

Micelles and vesicles represent the two most important classes of self-assembled structures that can be formed by amphiphiles in selective solvents.[1] Unlike micelles with a core–shell structure, vesicles are hollow spheres enclosed by a bilayer of the amphiphiles.[2]

To our best knowledge, vesicles are commonly prepared in mixed solvents by controlling the mixing ratio of water and organic solvent for dissolving the block copolymer.[3] In this study, a time and temperature dependent transition from spherical micelles to vesicles based on poly(*N*-isopropylacrylamide-*co*-3-(trimethoxysilyl)propyl methacrylate)-*b*-poly(ethylene glycol) (P(NIPAAm-*co*-MPMA)-*b*-PEG) was observed for the first time and the transition is attributed to the thermal hysteresis property[4] of PNIPAAm. To date, the fast response of temperature-responsive polymers has found applications in many fields,[5] and our findings provide new applications of the thermal hysteresis of temperature-responsive polymers besides their use as memory function materials by taking advantage of the hysteresis time, therefore expanding the potential of the temperature-responsive polymers. In addition, based on our very recent work,[6] an inorganic silica-based cross-linking strategy was also employed in this report to fix the vesicle structure by using the hydrolysis and condensation of trimethoxysilyl ($Si(OCH_3)_3$) functions from MPMA unit.

P(NIPAAm-*co*-MPMA)-COOH (M_n = 13 600 Da, M_w/M_n = 1.4) was synthesized by free radical copolymerization using 3-mercaptopropionic acid (MPA) as a chain transfer agent. P(NIPAAm-*co*-MPMA)-*b*-PEG block copolymer (M_n = 17 500 Da, M_w/M_n = 1.5, W_{EO}‡ = 0.16) was obtained by a condensation reaction between the amino group of CH_3O-PEG-NH_2 (M_n = 2600 Da, M_w/M_n = 1.1) and the activated ester terminal group of P(NIPAAm-*co*-MPMA). The yield was *ca.* 40%. The block coupling was confirmed by molecular weights determined by SEC-MALLS (Fig. S1†) and the ^{1}H NMR spectrum of P(NIPAAm-*co*-MPMA)-*b*-PEG in $CDCl_3$ (Fig. S2†), which reveals the presence of signals from both P(NIPAAm-*co*-MPMA) and PEG blocks.

From our experiments, we found that the micelle–vesicle transition process is time dependent due to the thermal hysteresis of PNIPAAm chains. The micelle suspensions were prepared and kept at 60 °C for different time periods of 3 days, 2 days and 1 day to study the effect of thermal hysteresis time on the micelle–vesicle transition process. Here, the vesicles formed by P(NIPAAm-*co*-MPMA)-*b*-PEG under different conditions are coded as vesicle (*m*-*n*) for simplicity, where *m* denotes the thermostatted time of micelle suspension at 60 °C and *n* refers to the temperature of dialysis. For example, vesicle (2 d-20 °C) indicates the vesicle is obtained under the condition of being thermostatted at 60 °C for 2 days followed by dialysis at 20 °C.

To form vesicle (3 d-20 °C), P(NIPAAm-*co*-MPMA)-*b*-PEG (50 mg) was dissolved in DMF (5 mL), and the solution was then poured into distilled water (45 mL) thermostatted at 60 °C with stirring to form micelles. When the DMF solution was added into the hot water, a white turbidity was observed immediately because PNIPAAm segments became hydrophobic above the lower critical solution temperature (LCST). The suspension was stirred at 60 °C for 3 days, and then put into a dialysis tube and dialyzed against 5 L of distilled water renewed every day for 2 weeks at 20 °C. It should be pointed out that the micelle suspension was kept at 60 °C for 3 days in order to make use of the hysteresis phenomenon of PNIPAAm. It is well known that PNIPAAm exhibits a reversible conformational transition at around 32–33 °C, the LCST. When PNIPAAm is subjected to fast heating and cooling cycles through the LCST, no obvious hysteresis is observed. But when PNIPAAm is kept for a relatively long time above the LCST, significant hysteresis is observed due to the strong intra- and intermolecular interactions (hydrogen bonding and hydrophobic interactions) among amide groups when dehydrated. Herein, the hysteresis property is utilized for the formation of vesicles since it is hard for PNIPAAm segments to become hydrophilic in a short time after being kept at 60 °C (above the LCST) for 3 days. In other words, it takes PNIPAAm segments a much longer time to become totally hydrophilic when the temperature is cooled down to 20 °C (below the LCST). As expected, the suspension did not become transparent instantly when immersed into the distilled water at 20 °C. Instead, it remained turbid for quite a long time (two weeks at least based on the experiments), and became transparent very slowly, indicating obvious hysteresis and a rather slow transition of PNIPAAm segments from hydrophobic to hydrophilic phase.

The micelle–vesicle transition process was monitored by TEM observation. It was found that there are three stages, *i.e.* the

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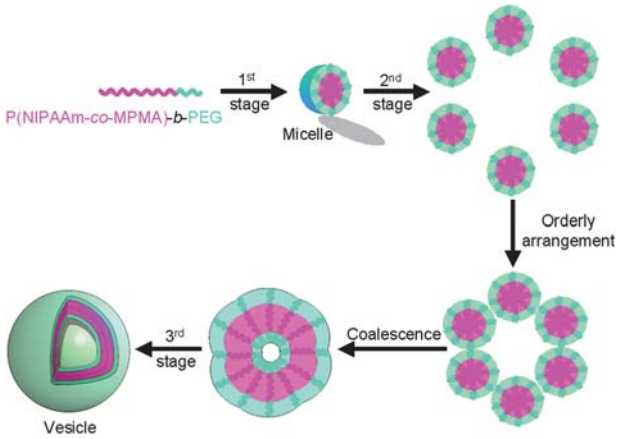

Scheme 1 Schematic representation of the process for the formation of vesicles. The self-assembly of P(NIPAAm-*co*-MPMA)-*b*-PEG to form spherical micelles (1st stage), the orderly arrangement, coalescence and fusion of the micelles (2nd stage), and finally the formation of vesicles (3rd stage). The illustrations are not drawn to scale.

self-assembly of P(NIPAAm-*co*-MPMA)-*b*-PEG to form spherical micelles, the orderly arrangement, coalescence and fusion of the micelles, and finally the formation of vesicles, in the transition process as schematically illustrated in Scheme 1. In the first day of dialysis, small spherical micelles with shrunk hydrophobic P(NIPAAm-*co*-MPMA) chains as the core and hydrophilic PEG chains as the shell are obtained, as shown in Fig. 1a; well-dispersed micelles with a regular spherical shape and a diameter of around 70 nm could be observed.

In the second stage, probably because the shrunk PNIPAAm chains become stretched slowly, and the hydrophobic core becomes increasingly large correspondingly, the hydrophilic shell formed by short PEG chains cannot stabilize the enlarged core any longer, which changes the preferred self-assembly morphology towards that with lower curvature, namely, from spherical micelle to vesicle. One can see from Fig. 1b that the small nanoparticles gather in an orderly way to form a ring in the fifth day of dialysis. In the seventh day (Fig. 1c), the neighboring nanoparticles associate with each other and further fuse to form the wall of the vesicle, and individual nanoparticles with a clear border disappear. It appears that the small spherical micelle in the first stage is no longer thermodynamically stable, and the system seeks to evolve towards a new equilibrium state of P(NIPAAm-*co*-MPMA)-*b*-PEG assemblies dispersed in water. The orderly arrangement and fusion of spherical micelles occur in the second stage. As compared with the regular nanoparticles in the previous stage, the morphology in this stage is something like an intermediate state, described as the rudiments of the vesicle. Cross-linked vesicles are obtained in the twelfth day of dialysis, which is designated as the third stage. As exhibited in Fig. 1d, the vesicle bears a well-defined spherical shape with outside and inside radius of around 170 nm and 110 nm, respectively. Furthermore, the bilayer structure of the vesicle could be seen clearly. The thickness of the wall is around 60 nm, which is close to the diameter (70 nm) of the spherical micelles observed in the former two stages. This result is considered as further evidence of formation of vesicles resulting from the coalescence of the spherical micelles. It is worth pointing out that the suspension in the dialysis tube still exhibits white turbidity in the last stage, although the turbidity is obviously fainter than that in the first stage, indicating the remaining hydrophobic nature of PNIPAAm even after dialysis for twelve days at 20 °C. Hence, it appears that the state of PNIPAAm is mainly hydrophobic in the whole course of transition due to the rather serious thermal hysteresis of PNIPAAm chains.

To form vesicle (2 d-20 °C), after being kept at 60 °C for 2 days, the spherical micelles with mean diameter around 70 nm form in the first day of dialysis at 20 °C (Fig. 2a), the orderly arrangement and coalescence of spherical micelles take place in the fourth day (Fig. 2b), and vesicles with diameter around 110–270 nm appear in the fifth day (Fig. 2c). Dynamic light scattering (DLS) measurements were also performed to further confirm the micelle–vesicle transition. The mean hydrodynamic diameter (D_h) and size distribution of polymer self-assemblies corresponding to the TEM images in the first, fourth, and fifth day of dialysis during the course of transition are displayed in Fig. S3a–c.† It can be seen from Fig. S3a† that the unimodal size distribution corresponds to the micelles observed in the first day, and D_h is determined to be 190 nm (PDI: 0.115). However, a bimodal size distribution with almost equivalent peaks at around 190 nm and 400 nm is recorded in the fourth day (Fig. S3b†), which probably corresponds to the

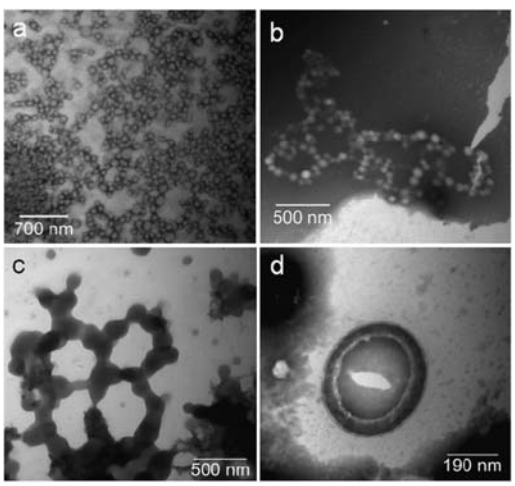

Fig. 1 TEM images of transition from spherical micelle to vesicle (3 d-20 °C) in the (a) first, (b) fifth, (c) seventh, (d) twelfth day of dialysis.

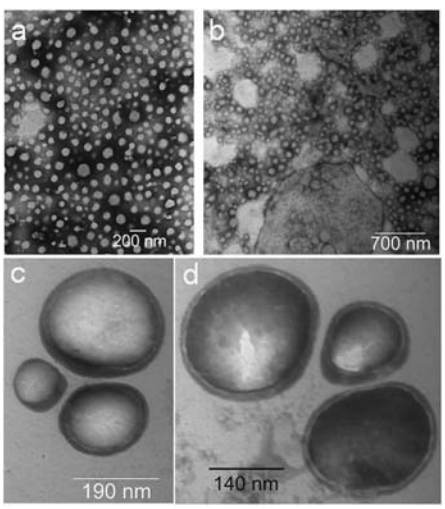

Fig. 2 TEM images of transition from spherical micelle to vesicle (2 d-20 °C) in the (a) first, (b) fourth, (c) fifth day of dialysis and (d) TEM observation of vesicle (2 d-20 °C) after two weeks.

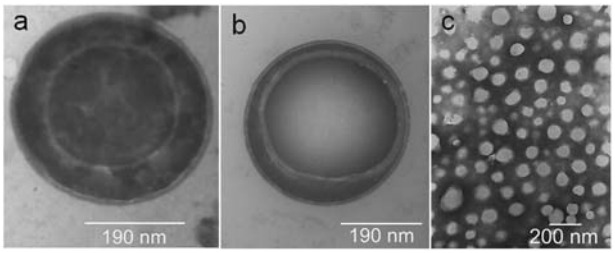

Fig. 3 TEM images of (a) vesicle (1 d-20 °C) forming in the first day of dialysis, (b) vesicle (2 d-0 °C) yielded in the second day of dialysis and (c) spherical micelles obtained after 2 days' stirring at 60 °C followed by 10 days' dialysis at 40 °C.

small micelles and large rings resulting from the orderly arrangement and coalescence of spherical micelles, respectively, and agrees roughly with that visualized by TEM (Fig. 2b). From Fig. S3c† it is apparent that a unimodal size distribution corresponds to the resulting vesicles produced in the fifth day, and D_h is about 260 nm (PDI: 0.294). To sum up, we conclude that the results of DLS are in accord with TEM observations, further confirming the micelle–vesicle transition process.

In order to evaluate the stability of the cross-linked vesicles, the vesicle suspension was preserved at 20 °C after the formation of vesicle (2 d-20 °C). TEM observation (Fig. 2d) of vesicle (2 d-20 °C) after two weeks shows that the vesicle is quite stable with both morphology and structure unaltered, which is probably attributable to the silica cross-linked PNIPAAm wall. Consistent with the TEM observation, it is evident from Fig. S3d† that the D_h (278 nm) and the size distribution (PDI: 0.327) of the vesicles are quite similar to those recorded two weeks previously (Fig. S3c†). The results suggest that the stability of the vesicles would be favorable for biomedical applications.

In contrast to the relatively slower formation of vesicle (2 d-20 °C) and vesicle (3 d-20 °C), after the suspension is kept at 60 °C for 1 day, vesicle (1 d-20 °C) forms in the first day of dialysis (Fig. 3a) since the transition process is very fast.

In a comparison of the three observations (Fig. 1a–d, 2a–c, 3a), it can be concluded that the longer P(NIPAAm-*co*-MPMA)-*b*-PEG is kept at 60 °C (above the LCST), the slower the transition from spherical micelles to vesicles. The reason is probably that the time during which PNIPAAm is thermostatted at a temperature above the LCST would affect the thermal hysteresis time of PNIPAAm chains, *i.e.* the transition rate of PNIPAAm chains from collapsing to extending conformation, and further affect the transition rate from spherical micelles to vesicles. Thermostatting of PNIPAAm chains at a temperature above the LCST for a longer time will lead to enhanced thermal hysteresis of PNIPAAm chains and a longer hysteresis time, resulting in a slower transition from micelles to vesicles. Therefore, the process of formation of vesicles could be controlled by varying the thermostatting time of P(NIPAAm-*co*-MPMA)-*b*-PEG at a temperature above the LCST.

Besides time, temperature is another important factor in controlling the micelle–vesicle transition. To investigate the effect of dialysis temperature on the formation of vesicles, after the micelle suspension was kept at 60 °C for 2 days, the suspension was dialyzed against distilled water at another two different temperatures of 40 °C (above the LCST) and 0 °C. Compared with vesicle (2 d-20 °C) formed in the fifth day as mentioned above, vesicle (2 d-0 °C) forms much faster and appears in the second day of dialysis (Fig. 3b). While at 40 °C, small spherical nanoparticles are observed in the tenth day of dialysis (Fig. 3c) and no vesicle could form with prolonged dialysis time. Thus, two conclusions can be drawn: (1) a temperature above the LCST restricts the transition of PNIPAAm and the formation of vesicle, thus only spherical micelles are obtained, and (2) a temperature below the LCST assists in the formation of vesicle, and the lower the temperature, the faster the vesicle forms. The reason is probably that the lower temperature shortens the time of thermal hysteresis and promotes the transition of PNIPAAm as well as the formation of vesicle from micelle. The results show that in addition to thermostatting time, temperature could also be utilized to control the formation of vesicles.

In addition, calculation of molecular dimensions, control experiments with addition of HCl, and thermoresponsive properties of vesicle (2 d-20 °C) are described in the Supporting Information.†

In summary, based on a new amphiphilic block copolymer P(NIPAAm-*co*-MPMA)-*b*-PEG, a novel concept was demonstrated by changing the treatment time and temperature to prepare vesicles from the micelles, although the essential reason leading to the occurrence of the orderly arrangement and coalescence of the micelles remains unclear and further work is necessary. Vesicles prepared here with a cross-linked thermoresponsive hybrid wall may find applications for the encapsulation of drugs, dyes, catalysts, and other functional species as well as the construction of stimuli-responsive sensors, artificial cells and bioreactors.

Notes and references

‡ For amphiphilic PEG-based block copolymers, their self-assembly structures in water are mainly determined by the weight fraction of the hydrophilic PEO block (W_{EO}) and a lower W_{EO} disfavors spherical micelles and favors structures with lower mean curvature such as wormlike micelles and vesicles. Spherical micelles dominate when $W_{EO} > 0.55$, whereas wormlike micelles form when $W_{EO} = 0.50$–0.55 and vesicles form when W_{EO} is well below 0.5.[7] So the composition of P(NIPAAm-*co*-MPMA)-*b*-PEG ($W_{EO} = 0.16$) favors the formation of vesicles in this study.

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