

Spontaneous Vesicle Formation of Poly(ethylene oxide)–Poly(propylene oxide)–Poly(ethylene oxide) Triblock Copolymer

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A novel method has been developed to prepare vesicles from aqueous solutions of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer, by adding anionic surfactant sodium dodecyl sulfate (SDS) and inorganic salt NaF. As determined by TEM and dynamic light scattering (DLS) measurements, the average diameter of vesicles is about 800 nm having 50 nm outer shell thickness. Identifying hydrophobic interactions between the block copolymers and the microenvironments around the vesicles using FTIR, ^1H NMR, and fluorescence spectroscopy techniques revealed the vesicle formation mechanism. The spontaneously formed vesicles were further cross-linked by converting the terminal hydroxyl groups of block copolymers into aldehydes, and then chemically bridging the polymer chains by the reaction between aldehydes and diamine compounds. The cross-linked vesicles are proved much more stable than free vesicles even at higher dilutions. The obtained vesicles with good stability and biocompatibility are promising candidates for widespread applications.

1. Introduction

Vesicles are hollow microscopic sacs that are composed of a thin molecular membrane enclosing a pool of solvent. Such vesicle structures have attracted large interest due to their potential applications in drug delivery, gene therapy, and nanomaterials, etc.^{1,2} Usually, lipids or low molecular weight surfactants are used to construct vesicles. Recent developments show that amphiphilic block copolymer vesicles are more promising not only in terms of their stability and toughness, but they also offer numerous possibilities of tailoring the properties by adjusting block lengths or chemical structure of the block copolymers.^{3–5}

Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO-PPO-PEO, Pluronic) triblock copolymer, which consists of hydrophilic PEO segments and hydrophobic PPO segments, is an important class of water-soluble amphiphilic block copolymer.^{6,7} It is well-established that PEO-PPO-PEO block copolymers would self-assemble into several mesostructures in selective solvents, for example, micelles; reverse micelles; and lamellar, cubic, and hexagonal lyotropic liquid crystalline. The self-assembly behavior of PEO-PPO-PEO block copolymer depends upon the temperature, relative EO/PO block length, and addition of various additives.⁸ We have studied the micellization of PEO-PPO-PEO block copolymer by several spectroscopic techniques including FTIR, FT-Raman, fluorescence spectroscopy, and NMR.^{9–20} The characteristic bands of the copolymer were very sensitive to the conformation and local microenvironments of the polymer chains; hence, they may serve as an indicator of the self-assembly process. The self-assembled structures of Pluronic copolymers could meet the specific requirements of many application fields, for example, drug delivery carrier and template for synthesis of nanomaterials.^{21,22} Recently, we have also developed methods of modifying or

functionalizing inorganic nanoparticles toward defined properties by using the self-assembly properties of Pluronic copolymers.^{23,24}

To date, there are very limited reports on the formation of vesicles in PEO-PPO-PEO block copolymer system. Bryskhe and co-workers developed vesicles from the aqueous solution of the most hydrophobic Pluronic, (EO)₅-(PO)₆₈-(EO)₅ (L121), by extruding the polymer solution through a porous membrane or heated across the phase boundary between isotropic polymer solution and lamellar phase.^{25,26} Richtering and co-workers reported a shear-induced vesicle formation from a ternary system of PEO-PPO-PEO block copolymer ((EO)₂₀-(PO)₇₀-(EO)₂₀, P123 or (EO)₁₀₀-(PO)₇₀-(EO)₁₀₀, F127), water, and butanol.^{27,28} In these methods, external energy input by extrusion, heat, or shear is normally required to facilitate the transition from lamellar structures to vesicles. The formed vesicles of PEO-PPO-PEO block copolymer are sometimes too delicate with lower stability and, therefore, may easily disintegrate by dilution, which limits their applications.^{21,25–29}

Herein, we presented the spontaneous vesicle formation in an aqueous solution of PEO-PPO-PEO block copolymer, (EO)₂₀-(PO)₇₀-(EO)₂₀ (P123), by simply adding anionic surfactant sodium dodecyl sulfate (SDS) and inorganic salt NaF. The formed vesicles were further cross-linked by first chemically converting the terminal hydroxyl groups of PEO-PPO-PEO block copolymer into aldehydes and then allowing the terminal aldehydes to react with diamine compounds. The cross-linked vesicles were proved very stable against critical dilutions. This method is simple, economic, and provides a new avenue to prepare vesicles from PEO-PPO-PEO copolymers. The obtained Pluronic vesicles have the following advantages: (a) good biocompatibility and stability; (b) provide an aqueous environment inside which is very suitable for encapsulation of biomolecules; and (c) the properties might be well-controlled by variation of the composition of Pluronic copolymer.

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2. Experimental Section

Materials. The PEO-PPO-PEO block copolymer P123 ((EO)₂₀(PO)₇₀(EO)₂₀, MW = 5750) were kindly donated by BASF. SDS and NaF were purchased from Beijing Chemical Reagents Company. Dess-Martin periodinane, 1,4-diaminobutane(DAB), and 2-dimethyl-2-silapentane-5-sulfonate sodium salt (>97%, DSS) was purchased from Sigma-Aldrich Chemical Co. D₂O (>99.9 atom % 2H) were purchased from CIL Corp. (Andover, MA). Pyrene (99%) was purchased from Sigma and recrystallized twice from ethanol.

Vesicle Preparation. The vesicle aqueous solutions with different concentrations (weight percent, wt %) were obtained by subsequently dissolving a given amount of triblock copolymer P123, SDS, and NaF salt in distilled water. The mixed solutions were agitated gently to achieve better dissolution and then were stored in a refrigerator.

To prepare cross-linked vesicles, a similar protocol that Sung and co-workers recently developed was employed in this study.³⁰ Briefly, the terminal hydroxyl groups of P123 copolymer chains were first chemically converted to aldehydes (P123-CHO) using the Dess-Martin periodinane as an oxidized catalyst. A 1.0 mM portion of P123 and 0.5 mM of Dess-Martin were reacted in methylene chloride for 24 h at room temperature. Afterward, the P123-CHO was isolated by filtration of the organic extracts (0.45 μ m) through hexane, and the solvent was evaporated at reduced pressure. Similar to the procedure of free P123 vesicles described above, aqueous solution of P123-CHO vesicles could be prepared. 1,4-Diaminobutane (DAB) was added to the vesicle solutions dropwise to react with the terminal aldehydes on P123-CHO, to form covalent bridges between the block copolymer chains. According to the studies on cross-linking reaction conditions by Sung and co-workers, the molar ratio of $-NH_2$ to $-CHO$ was adjusted to 5. These mixtures were stirred continuously for 30 min, at pH 4.5 and 25 $^{\circ}$ C, to maximize the degree of cross-linking.

Characterizations. TEM Measurement. The morphologies of vesicles were observed by transmission electron microscopy (TEM) operated at 100 keV (FEI TECNAI 20). The TEM sample was prepared by placing a drop of the vesicle solution onto a 400 mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with a filter paper to remove surface water and negative stained using a 2 wt % phosphor tungsten acid solution.

DLS Measurement. The hydrodynamic diameters of vesicles in water were monitored by dynamic light scattering (DLS) method at a 90 $^{\circ}$ detection angle at room temperature (25 $^{\circ}$ C) (Brookhaven Instruments, 90Plus; laser wavelength is 659.0 nm). The intensity average diameters were used for data analysis and comparison.

FTIR Measurement. FTIR spectra of P123, P123-CHO, and aqueous vesicle solutions were recorded on a Bruker Vector 22 FTIR spectrometer. Temperature-dependent FTIR spectra of aqueous vesicle sample were measured by a thermocouple inserted in a stainless steel block that functions as a sample cell. Equilibration time for each temperature was 2 min.^{9,10}

Fluorescence Spectroscopy Measurement. A stock solution of 1 mM pyrene in methanol was prepared as probe for fluorescence measurement. One microliter of pyrene was injected into 5 mL of aqueous polymer solution, and the solution was stirred using a magnetic stirrer. The steady-state fluorescence of pyrene in water was measured using a Perkin-Elmer LS-55 luminescence spectrometer, which is equipped with a Biokinetic accessory. Pyrene was excited at a wavelength of 335 nm, and the emission intensity from 350 to 500 nm was

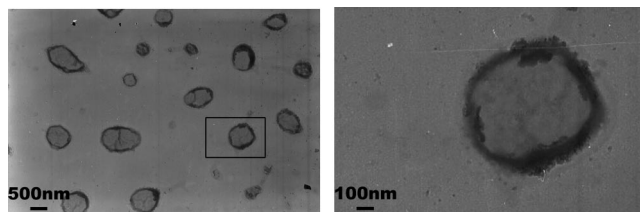


Figure 1. TEM images of vesicles in aqueous solution of 5% P123/0.5% SDS/0.5% NaF (left), and the local magnification of the black rectangle area (right).

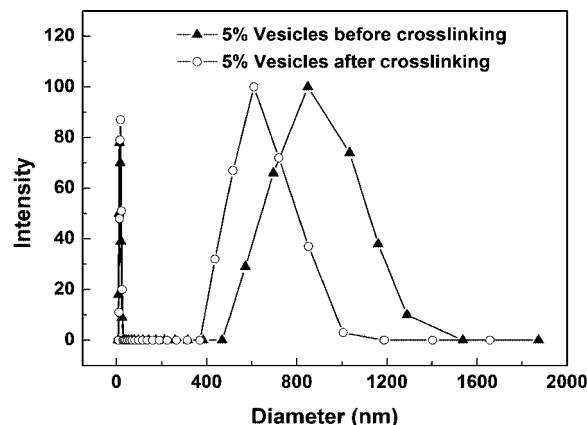


Figure 2. Hydrodynamic diameter distribution of vesicles in an aqueous solution of 5% P123/0.5% SDS/0.5% NaF before and after cross-linking.

recorded. The scan speed was set at 600 nm/min. Circulating water through an internal passage in the cell holder can control the temperature of the sample, and all measurements were performed at 25 $^{\circ}$ C.

1H NMR Measurement. All NMR experiments were conducted on a Bruker Avance 600 spectrometer at a Larmor frequency of 600.13 MHz for protons. The spectrometer was equipped with a microprocessor-controlled gradient unit and an inversedetection multinuclear BBI probe with an actively shielded z-gradient coil. DSS was added to the vesicle solution as an internal reference to eliminate temperature-induced shifts. The vesicle samples were prepared in D₂O, and the temperature was kept constant at 25 $^{\circ}$ C using a Bruker BCU-05 temperature control unit.^{16,18}

3. Results and Discussions

Morphology of the Vesicles. The morphology of vesicles in aqueous solution was first observed by TEM. Figure 1 shows the representative TEM images of 5% P123, 0.5% SDS, and 0.5% NaF system with either low (left) or relatively high (right) magnification. There is a remarkable contrast between the central areas and the black periphery of the block copolymer aggregates, which confirms that they are hollow. The black ring indicates the uniform membrane of the vesicle, and the membrane thickness is about 50 nm. The average diameter of the vesicles is about 800 nm, which is smaller than that measured by the DLS because of the shrinkage that arises from evaporation of the water in TEM measurements (Figure 2). In addition, the DLS results indicate that the sample mainly consists of vesicles with the hydrodynamic diameters in the range of 600–1000 nm, where a lesser amount of micelles with 30 nm diameters coexist.

Cross-linking of Pluronic Vesicles. One of the approaches to increase the stability of the polymeric mesostructures is to

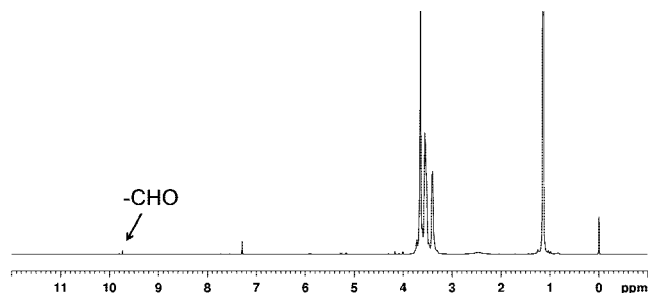


Figure 3. ^1H NMR spectrum of P123-CHO sample from the chemical conversion of the terminal hydroxyl groups of P123 into aldehydes.

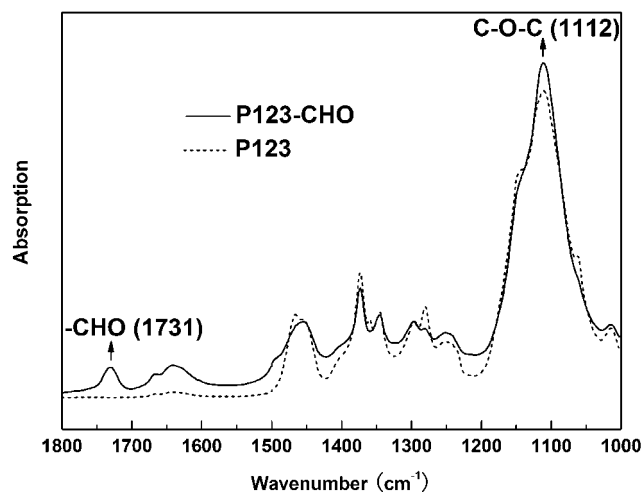


Figure 4. FTIR spectrum of pure P123 sample and P123-CHO sample from the chemical conversion of the terminal hydroxyl groups of P123 into aldehydes in the wavenumber range of 1000–1800 cm^{-1} .

covalently cross-link the polymer chains after their formation.^{31–33} In this study, the spontaneously formed vesicles were cross-linked by first converting the terminal hydroxyl groups of block copolymers into aldehydes using Dess-Martin periodinane as an oxidized catalyst and later chemically bridging of the polymer chains by the reaction between aldehydes and diamine compounds. Figures 3 and 4 show the ^1H NMR spectrum and FTIR spectra of the obtained P123-CHO sample, respectively. The peak at around 9.7 ppm in NMR represents the chemical shift of the hydrogen in aldehydes. The broad peak in FTIR between 1200 and 1000 cm^{-1} is due to the C–O stretching vibration, which is a characteristic band of the PEO-PPO-PEO block copolymer. After the terminal hydroxyl groups of P123 copolymer were converted to aldehydes, a new peak appears at 1731 cm^{-1} , that arises from the C–H bonds of aldehydes. Consequently, the terminal hydroxyl groups on P123 were successfully converted into aldehydes.

After the formation of vesicles in aqueous solution of P123-CHO, SDS, and NaF, the 1,4-diaminobutane (DAB) was used to react with the converted aldehyde termini on P123-CHO in order to form imines with carbon–nitrogen double bonds. The P123 chains were bridged together within the formed vesicles. The reaction conditions were selected according to previous studies by Sung and co-workers to maximize the degree of cross-linking.³⁰

Figure 5 shows the representative TEM images of 5% cross-linked P123 vesicles with both low (left) and relatively high (right) magnification. It is obvious that the cross-linking reaction did not destroy the vesicle structures. Compared with the irregular edges of the uncross-linked vesicles in Figure 1, the edges of the cross-linked vesicles become smoother, which

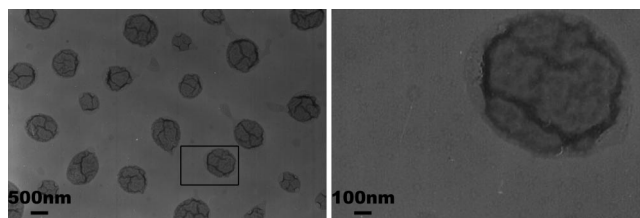


Figure 5. TEM images of cross-linked vesicles in aqueous solution of 5% P123/0.5% SDS/0.5% NaF (left), and the local magnification of the black rectangle area (right).

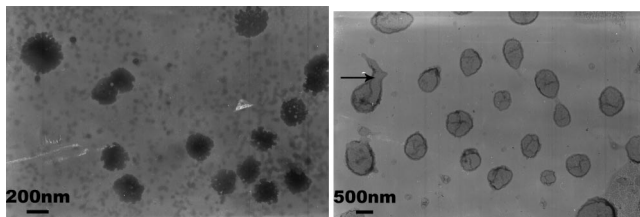


Figure 6. TEM images of the diluted samples of uncross-linked vesicles (left) and cross-linked vesicles (right). The formulation of diluted samples is 0.1% P123, 0.01% SDS, and 0.01% NaF.

provides further evidence for the cross-linking reaction. Several black strips appear inside the central area, which might be originated from the cross-linking on the spherical surface of the vesicles. As determined by TEM (Figure 5) and DLS (Figure 2), the diameters of the vesicles decreased to the range of 500–900 nm after the cross-linking.

The Stability of Vesicles. The stability tests of the formed Pluronic vesicles were performed by allowing the vesicle samples to be extremely diluted against water. Both free vesicles and cross-linked vesicles experienced a 50-fold dilution and were observed by TEM and DLS at first. Figure 6(left) shows the TEM observations of the noncross-linked vesicle sample after dilution to 0.1% P123, 0.01% SDS, and 0.01% NaF. Only micelles (20–30 nm) and some large aggregates (200–300 nm) were observed, and the vesicles with hollow structures disappeared due to the collapse during dilution. However, as shown in Figure 6(right), a large number of vesicles still exist in the diluted sample of cross-linked vesicles. Compared with the nondiluted sample (Figure 5), no obvious changes in the morphologies and sizes of the cross-linked vesicles was observed except the closed membrane of some vesicles opened after dilution (indicated by the black arrow). As shown in Figure 7, the DLS results further indicate that the majority of the diluted cross-linked sample is in vesicle form, having hydrodynamic diameter ranging from 500 to 900 nm. In contrast, micelles with about 30 nm diameters take a major fraction of the diluted uncross-linked sample, which confirms the dissociation of the noncross-linked vesicles. As a result, the stability of P123 vesicles was greatly improved by cross-linking.

Mechanism of Vesicle Formation. In aqueous solutions, the PEO-PPO-PEO block copolymers usually form micelles at concentrations above their critical micelle concentration (CMC) or at a temperature above their critical micelle temperature (CMT). The micelles are in spherical shape with a hydrophobic core of PO blocks and an outer shell of hydrated EO blocks.^{6–8} In the past decades, the interactions between PEO-PPO-PEO block copolymers and anionic surfactants, especially SDS, have been extensively studied by several techniques, for example, small-angle neutron scattering (SANS), surface tension, differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), and electromotive force measurement.^{34–42}

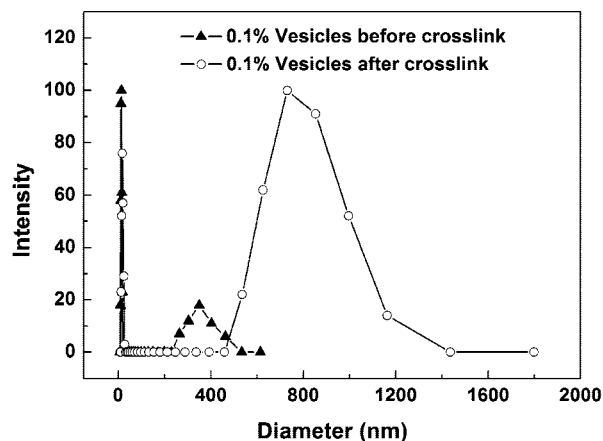


Figure 7. Hydrodynamic diameter distributions of the diluted samples of free vesicles (A) and cross-linked vesicles (B). The formulation of the diluted samples is 0.1% P123, 0.01% SDS, and 0.01% NaF.

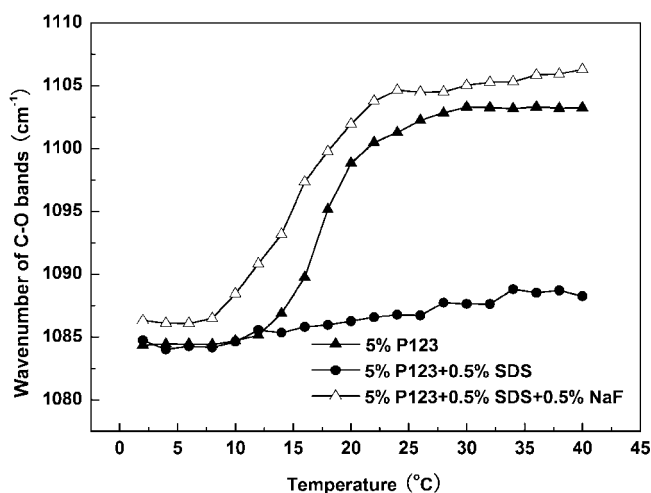


Figure 8. The temperature-dependent evolutions of C–O stretching band in FTIR spectra of aqueous solutions of 5% P123, 5% P123/0.5%SDS, and 5% P123/0.5% SDS/ 0.5% NaF.

Depending on the concentration and temperature, the interactions may lead to the formation of either mixed micelles or different kinds of mixed aggregates in the aqueous mixtures of PEO-PPO-PEO block copolymers and anionic surfactants. It is generally concluded that, when SDS is gradually added to the solution, binding of SDS to the Pluronic micelles takes place. The interaction between the SDS and PPO blocks is significantly stronger than that between SDS and PEO blocks, and thus, SDS micelles preferentially bind to PPO blocks followed by binding to PEO.^{34,39,40} As the SDS concentration increases, the hydration degree of Pluronic micelles also increases that may destroy micelles, and a necklace-like complex of unassociated Pluronic unimers and SDS micelles would form.^{36–38,41} Here, we provided further evidence on the binding process by using a FTIR spectroscopic technique. Our previous studies^{9–14} showed that the frequency of the C–O–C stretching band in FTIR spectra of PEO-PPO-PEO block copolymer was very sensitive to the local polarity and conformation of block copolymer chains. As a result, its variations could be an indicator of the self-assembly process. Figure 8 shows the temperature-dependent evolution of C–O stretching band in FTIR spectra of 5% P123 aqueous solution. As the temperature increases, the C–O stretching band shifted to a higher wavenumber (1105 cm^{−1}), which indicates a dehydration process of the block copolymer chains during the formation of micelles. The CMT value was

determined as 10 °C by the first inflection point of the sigmoid plots according to our previous studies.^{9–14} In contrast, after the addition of 0.5% SDS, the C–O stretching band remained steady at lower wavenumber (1085 cm^{−1}) in the temperature range of 2–40 °C, which suggests that the copolymers existed as unimers and that the micellization was totally suppressed by binding of SDS. The Pluronic copolymer chains were greatly stretched by the bound SDS micelles because of the ionic repulsion between SDS micelles.

It is interesting that the temperature-dependent curve of C–O stretching bands of 5% P123, 0.5% SDS, and 0.5% NaF aqueous solution become sigmoid shape once again, which is similar to that of 5% P123 aqueous solution where the block copolymers formed micelles (Figure 8). Addition of NaF salt induced the necklace-like complex of block copolymer unimers and SDS micelles to further self-assemble and form mixed aggregates, which are actually vesicles according to TEM observations in Figure 1. The salt-induced vesicle formation has been reported in many literatures, where normally anionic surfactants or cationic surfactants or their mixtures were used as building blocks.^{43–47} The vesicle formation and stability depend strongly on the electrostatic conditions of the corresponding systems.⁴⁸ The formation mechanism could be well-explained according to the theory of Israelachvili for the aggregation of amphiphilics in aqueous solutions.⁴⁹ The aggregate morphology depends on the packing parameter P : $P = V/LocA_0$, where V and Loc are the volume and chain length of the hydrophobic group, respectively, and A_0 is the cross sectional area of the head groups that is related to the electrostatic repulsion between adjacent head groups in the associates. In systems of ionic surfactants, addition of salt will reduce the electrostatic repulsion between adjacent head groups, and the head groups will be compressed.⁴³ Therefore, the value of P will increase and once it reaches to the range of 0.5–1, vesicle structure may be formed. Although the PEO-PPO-PEO block copolymer is known as nonionic, it is conceivable that strong binding of anionic SDS to PPO blocks of the stretched PEO-PPO-PEO block copolymers will result in a “complex ionic surfactant”. As a result, addition of NaF salt will lead to the screening of the electrostatic repulsion between SDS and will, therefore, induce the necklace-like complex transform into vesicles.

According to previous studies, the hydrophobic interactions and microenvironments between the block copolymers have been proved to be very crucial in the self-assembly of PEO-PPO-PEO block copolymer,^{6–8} which can be detected by several spectroscopic techniques.^{9–20} To get deep insights into the underlying mechanism that controls the vesicle properties, ¹H NMR, FTIR, and fluorescence spectra of the vesicle samples were monitored during their formation and dissociation.

Figure 9 presents the evolution of the chemical shift of PO-CH₃ protons in ¹H NMR during the vesicle formation, cross-linking, and dilution. Figure 9A gives the chemical shift of PO-CH₃ protons in ¹H NMR of 5% P123 and 0.5% SDS aqueous solution. Compared with the chemical shifts of PO-CH₃ protons during the micellization of pure Pluronic solutions, as we reported previously (1.12–1.16 ppm in the temperature range of 2–40 °C),¹⁵ the chemical shifts experienced a significant upfield shift toward 1.08 ppm after addition of SDS. It has been mentioned in various studies that the formation of C–H...O hydrogen bonds enhanced the deshield effect of protons and led to ¹H downfield chemical shifts.^{50,51} Therefore, the upfield chemical shift of C–H protons might be related to the breakdown of the intermolecular hydrogen bonds between the C–H protons in PO segments and water oxygen atoms. The

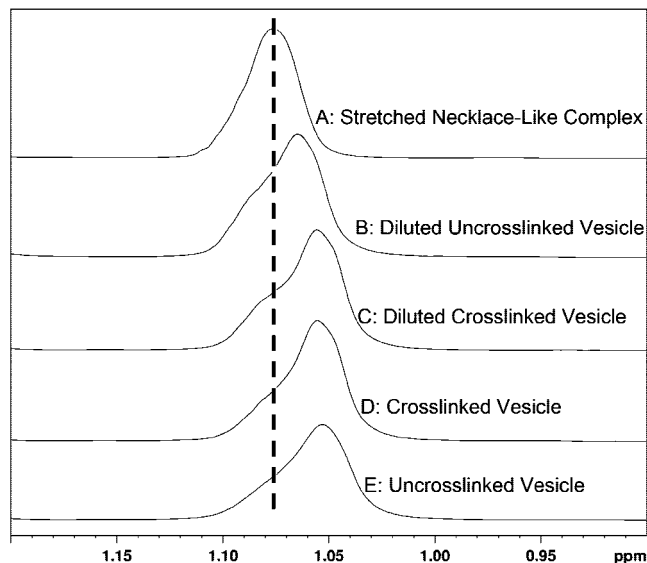


Figure 9. The chemical shifts of PO-CH₃ protons in ¹H NMR of aqueous solutions of necklace-like complex in 5% P123/0.5% SDS (A), uncross-linked vesicles in 5% P123/0.5% SDS/0.5% NaF (E), cross-linked vesicles in 5% P123/0.5% SDS/0.5% NaF (D), noncross-linked vesicles in 0.1% P123/0.01% SDS/0.01% NaF (B), and cross-linked vesicles in 0.1% P123/0.01% SDS/0.01% NaF (C).

environment of the PO groups becomes more hydrophobic after addition of SDS, which confirms that SDS preferentially bind to the PO blocks.^{16,18} Adding 0.5% NaF to the aqueous solution of 5% P123 and 0.5% SDS further change the chemical shift of PO-CH₃ protons to upfield at ~1.05 ppm (Figure 9E), which reflects a more hydrophobic environment around the PO blocks inside vesicles. Folding of the necklace-like complex resulted in stronger interactions between PPO blocks, which may help the PPO blocks away from the water outside and inside the vesicle. Consequently, the vesicle consists of a PPO inner layer surrounded by PEO layers on each side, and a water pool was enclosed inside. A 50-fold dilution of the free vesicles led to an obvious downfield chemical shift of PO-CH₃ protons (Figure 9B), the value of which is almost same as for P123/SDS necklace-like complex where the block copolymers were stretched and exist as unimers (Figure 9A). In contrast, the chemical shift of PO-CH₃ protons in cross-linked vesicles remains at around 1.05 ppm before and after dilution (Figure 9D and 9C). Consequently, the hydrophobic interaction between PPO blocks is actually the driving force to assemble the block copolymers into vesicles.

The temperature-dependent evolution of the C—O stretching bands in FTIR spectra of different aqueous vesicle samples was monitored to underline the temperature effects on the vesicle structures. As shown in Figure 10, the cross-linking reaction slightly shifted the critical aggregation temperature (CAT) to a lower value. After the vesicles were diluted, the wavenumber at each temperature all move to relatively lower value, which indicates stronger interactions between the copolymers and water molecules. The CAT value of cross-linked sample almost did not change before and after dilution (10 °C). However, no aggregation behavior occurred in the diluted noncross-linked sample while increasing the temperature from 2 to 40 °C, as the wavenumber remained unchanged at about 1075 cm⁻¹, which represents the hydrated state of the copolymers when they are not self-assembled.

Pyrene is a well-characterized spectroscopic probe that exhibits a fluorescence emission spectrum, where the peaks

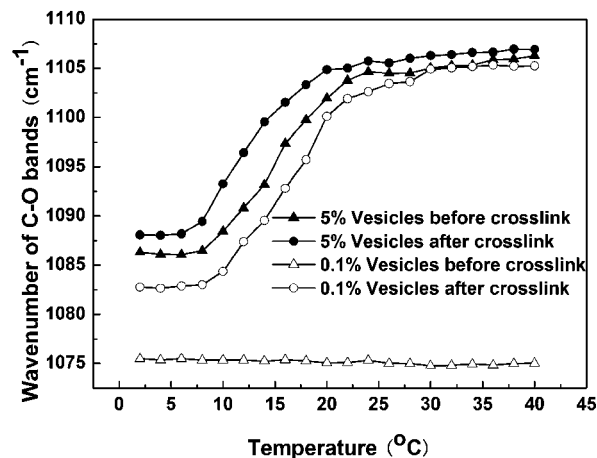


Figure 10. The temperature-dependent evolutions of C—O stretching bands in aqueous solutions of free vesicles in 5% P123/0.5% SDS/0.5% NaF, cross-linked vesicles in 5% P123/0.5% SDS/0.5% NaF, free vesicles in 0.1% P123/0.01% SDS/0.01% NaF, and cross-linked vesicles in 0.1% P123/0.01% SDS/0.01% NaF.

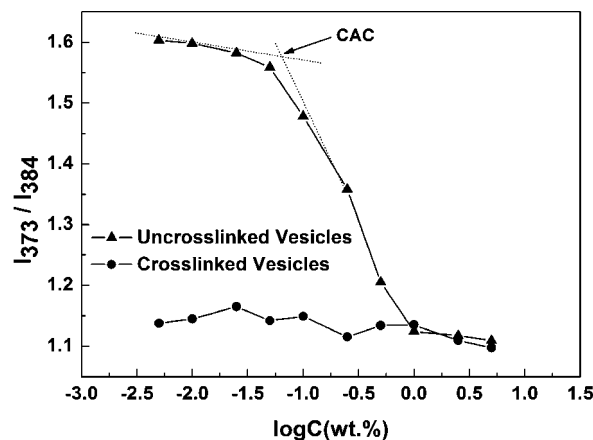


Figure 11. Plot of the ratio of intensities (I_{373}/I_{384}) in fluorescence spectra of cross-linked and uncross-linked vesicle aqueous solutions against their concentration, respectively.

depend strongly on the polarity of the surrounding environment of the probe.^{32,33,51} The intensity ratio of the excitation at 373 and 384 nm (I_{373}/I_{384}) is particularly an excellent index of the polarity around the self-assemblies of PEO-PPO-PEO block copolymers: the larger the ratio, the more polar the medium.¹⁴ As shown in Figure 11, the ratios of the intensities (I_{373}/I_{384}) of the cross-linked vesicle samples or the noncross-linked vesicle samples were plotted against their concentrations at 25 °C. At high concentrations (log C from 0 to 1), the ratios were relatively low whether the vesicles were cross-linked or not, which indicates a nonpolar microenvironment around the vesicles. As the vesicles became gradually diluted, the intensity ratios of the cross-linked samples still remained at a low value, whereas that for noncross-linked samples increased to about 1.60. The increased ratio of I_{373}/I_{384} indicates that the pyrene experienced a change from nonpolar environment to polar environment, which is due to the stronger interactions between block copolymers and water molecules during the dissociation of noncross-linked vesicles. The critical aggregation concentration (CAC) was determined to be 0.08 wt % for the noncross-linked P123/SDS/NaF system from the intersection point of the curve. Therefore, the vesicles of PEO-PPO-PEO block copolymer, SDS, and NaF prefer a more nonpolar medium environment to keep their stability. Besides, all the NMR, FTIR, and fluorescence spectroscopic results again confirm that free vesicles from

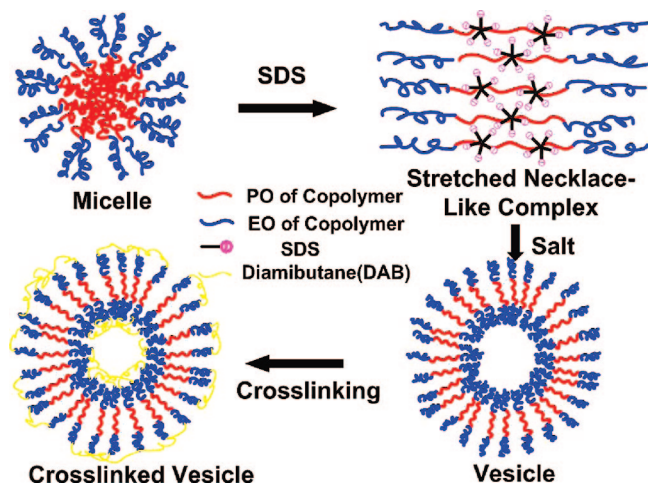


Figure 12. Schematic illustration of the vesicle formation process in a system of PEO-PPO-PEO block copolymer, SDS, and NaF salt. The micelles of block copolymers were first destroyed by binding SDS micelles, and necklace-like complexes of stretched block copolymer unimers and SDS micelles formed. The complexes further folded into vesicles after addition of NaF salt. Finally, the terminal groups of EO blocks were chemically cross-linked to increase the stability of the formed vesicles.

P123/SDS/NaF are easily to dissociate and their stability could be greatly improved by cross-linking.

Considering the previous findings, the vesicle formation mechanism in aqueous solutions of PEO-PPO-PEO block copolymer, SDS, and NaF could be explained and is schematically depicted in Figure 12. The micelles of block copolymers were first destroyed by binding of SDS micelles, and the stretched block copolymer unimers and SDS micelles form necklace-like complexes. The necklace-like complexes results in a “complex ionic surfactant”, and therefore addition of NaF salt leads to the screening of the electrostatic repulsion between SDS, which induces the necklace-like complex transform into vesicles. Hydrophobic interactions between PPO blocks are a driving force to assemble the block copolymers together into vesicle, and therefore, the vesicle consists of a PPO inner layer surrounded by PEO layers on each side, and a water pool was enclosed inside (NaF and water are not shown). A nonpolar medium environment is favorable for the vesicle stability. To improve their stability, the spontaneously formed vesicles are further cross-linked by the chemical reaction between diamino butane (DAB) and converted aldehyde termini of PEO-PPO-PEO block copolymers.

4. Conclusions

In this paper, we have investigated the spontaneous vesicle formation of PEO-PPO-PEO triblock copolymer P123 aqueous solutions by adding anionic surfactant SDS and NaF salt. As observed by TEM, the prepared vesicles are spherical in shape with a hollow structure. The average diameter of vesicles is about 800 nm, having membrane thickness of about 50 nm. The spontaneously formed vesicles are somewhat easily to dissociate. To increase their stability, the terminal hydroxyl groups of block copolymers were first converted into aldehydes and then reacted with diamine compounds to chemically bridge the polymer chains of vesicles. The cross-linked vesicles proved to be very stable even at higher dilutions, and no obvious changes in the morphology and size of vesicles were observed after dilution.

The mechanism of vesicle formation was explained by identifying the hydrophobic interactions between PPO blocks

using ^1H NMR, the temperature effects on the vesicle properties using FTIR, and the hydrophobic microenvironments around the vesicles using fluorescence spectra. The micelles of block copolymers were first destroyed by binding SDS micelles. The block copolymer chains were stretched as unimers and further formed necklace-like complexes with SDS micelles. Induced by addition of NaF salt, the complex further transformed into vesicle because of the salt compression on the “ionic” necklace-like complex. Hydrophobic interactions between PPO blocks are driving force to assemble the block copolymers together into vesicle. A nonpolar medium environment favors the stability of vesicles. All the NMR, FTIR, and fluorescence spectroscopic results further confirm that the vesicles become more stable by cross-linking.

The method we developed makes it much easier to prepare vesicles from PEO-PPO-PEO block copolymers without any input of external energy such as heat and shear. In addition to potential applications in nanoreactors and nanomaterials, the obtained stable vesicles are particularly promising in biomedicine or biotechnology applications such as drug delivery, gene therapy, and artificial cells because of the great biocompatibility of PEO-PPO-PEO block copolymer.

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