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# Polymersomes with high loading capacity prepared by direct self-assembly of block copolymers in drugs

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ABSTRACT

We develop a new method to prepare block copolymer vesicles with high loading capacity of drugs which can then be released in a controlled manner. The block copolymers, including PS-*b*-PAA, PS-*b*-PEO, and biocompatible PCL-*b*-PEO, can directly self-assemble into vesicles in aspirin and encapsulate aspirin by solvent annealing with ethanol which imparts mobility to the originally solid block copolymers and aspirin molecules. Aspirin associates with the hydrophilic blocks after premixing, firstly leading to the formation of bilayer structures. During solvent annealing, the bilayers are wrapped into vesicles to enclose aspirin that fills the cores of the vesicles. The interactions between block copolymers and aspirin were probed by FT-IR, and the formation of aspirin-loaded vesicles were confirmed by transmission electron microscopy and dynamic light scattering. The loading content of aspirin in the extracted vesicles is 59 ± 5%, higher than that of conventional vesicles formed in liquid systems with dilute drugs. The release rate and final release amount of aspirin from vesicles in aqueous solutions can be controlled by addition of different amount of *n*-dioxane or by changing pH values.



## 1. Introduction

Block copolymers comprised of two or more homopolymers linked by covalent bonds self-assemble into a variety of nanoscale structures in solvents, driven by the interplays between polymer chains and solvents to lower free energy, where solvophilic blocks tend to contact solvents while solvophobic blocks are shielded from the solvents [1–10]. Because the diffusion rate of long polymer chains is rather low, the kinetically formed structures of block copolymers can be more or less locked and therefore more structures other than those commonly seen in surfactant systems have been created [4]. In addition, the strong intermolecular interactions and the entanglements of long polymer chains can greatly enhance the mechanical properties of the structures [11], leading to higher stability and lower permeability that also lack in conventional surfactant systems. The designable molecular architectures and the various, robust self-assembled structures render block copolymers attractive in the field of nanotechnology for exploiting the possibility in applications such as templates for nanomaterial synthesis [12–15] and controlled release [3,16].

Among the self-assembled structures of block copolymers, vesicles (or polymersomes) have gained much attention due to their ability to carry substances [1,2,10]. One of the promising applications of block copolymer vesicles is the encapsulation of guest molecules in the vesicular interior spaces surrounded by the robust bilayers. The guest functional molecules, such as bioactive molecules, drugs, fragrances, dyes, and reactive agents, could potentially be released in a controlled manner. This type of nano-encapsulation technology has been regarded as an opportunity for numerous specialty chemical industries, including biomedicine, personal care, agriculture, food, and resin. The use of vesicles for controlled drug delivery has been particularly focused recently because of the urgent demands in medical therapy, such as cancer treatments, as well as the many advantages the vesicles can offers compared to conventional dosage forms, such as improved efficacy, precise targeting, and reduced toxicity [17–26].

Most micellization of block copolymers were studied in liquid media near room temperature. However, it has been shown that the encapsulation of guest molecules in vesicles in liquid systems is inefficient due to the dilution effect [27]. Also, most block copolymers form vesicles in toxic organic liquids, which thus limits their biomedical applications [28]. Different from the conventional methods, we have previously reported that the block copolymer, poly(styrene-*b*-4-vinylpyridine) (PS-*b*-P4VP), can self-assemble in

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melted deoxycholic acid (DCA) above the melting point of DCA, which is a crystalline solid at room temperature. The structures could be retained in solid state after cooling down to room temperature [29]. In other words, DCA molecules not only provide the driving forces for the formation of vesicles but also are encapsulated in the core of the vesicles. Since DCA molecules inside the vesicles are protected by bilayers while the exterior ones can be washed away by proper solvents, the vesicles filled with DCA molecules can be extracted in an intact form. Through this strategy, the extracted vesicles with enclosed functional molecules can be re-dispersed in desired solvents for controlled releases or in other media where specific functions are needed.

In this study, we extended the idea and directly formed block copolymer vesicles in drugs and encapsulate the drugs in the vesicles. Aspirin, a common anti-inflammatory and anti-platelet agent, was used as the model drug. The carboxylic acid and ester groups on aspirin can interact with polymers bearing -OH, -COOH, and -O- groups [30]. Poly(styrene-*b*-acrylic acid) (PS-*b*-PAA) was chosen as the model block copolymer [31]. It was expected that the melted aspirin can work as a selective solvent to PAA block and induces the self-assembly of PS-*b*-PAA. However, the melting point of aspirin is as high as ~135 °C. One major issue encountered when melting aspirin is that at high temperature, the chemical structure of aspirin molecules has been found to change [32]. To prevent the unwanted reaction, we adopted the solvent annealing method at low temperature to replace thermal annealing for preparing aspirin-loaded vesicles. During solvent annealing with ethanol, the solvent vapors diffuse into samples and swell aspirin and the block copolymer. The molecules can then move toward thermodynamically equilibrium states and form vesicles with aspirin-filled cores.

The extracted vesicles show a high loading content of aspirin and the release behaviors of aspirin can be tuned by addition of good solvents or by changing the pH-value of the aqueous solutions [33]. Other amphiphilic block copolymers, including poly(styrene-*b*-ethylene oxide) (PS-*b*-PEO) [34], and biocompatible poly($\epsilon$-caprolactone-*b*-ethylene oxide) (PCL-*b*-PEO) [35], were also successfully used to form aspirin-loaded vesicles. This study provides a facile route to prepare biocompatible vesicles that can carry high amounts of drugs, an idea option for controlled drug release, especially for the long-term usage due to the low permeability of the vesicular bilayers.

## 2. Experimental section

### 2.1. Materials

PS(70000)-*b*-PAA(13000) (PDI = 1.10) and acetylsalicylic acid, i.e. Aspirin, (≥99.0% purity), were purchased from Sigma-Aldrich. PS(42000)-*b*-PAA(4500) (PDI = 1.18), PS(20500)-*b*-PAA(2600) (PDI = 1.10), PS(58000)-*b*-PEO(8200) (PDI = 1.05), and PCL(32500)-*b*-PEO(5000) (PDI = 1.3) were purchased from Polymer Source. The numbers in the parentheses of the block copolymers are the number-average molecular weights in g/mol. The solvents, tetrahydrofuran (THF), ethanol, and *n*-dioxane, were purchased from Macron Chemicals, Sigma-Aldrich, and J. T. Baker, respectively. Borate buffer (pH = 10) and phosphate buffer (pH = 7) were purchased from Fisher Scientific UK. Acetate buffer (pH = 5) was purchased from ACROS. All the chemicals and solvents were used as received.

### 2.2. Sample preparation

The block copolymers were first dried in vacuum oven at 100 °C for 1 day. For preparation of aspirin-loaded vesicles, 5 mg block copolymers and 45 mg aspirin were dissolved in 2 ml THF, followed by stirring for 2 days at room temperature. After well mixed, the polymer/aspirin solutions were placed in Teflon beakers covered by an inverted dish for the solvent to evaporate slowly at room temperature for several days. The dried block copolymer/aspirin samples were placed in an oven along with a cup of ethanol at 45 °C for solvent annealing. After annealing, the samples were removed from the oven and ethanol was evaporated. The solvent-annealed solid samples were re-dispersed in THF, ethanol, or water for further characterization and release tests.

### 2.3. Drug release tests

Solvent annealed solid samples were dispersed in 5 ml ethanol. The dispersion was then centrifuged at 11000 rpm for 15 min and the sediments of aspirin-loaded vesicles were collected and air-dried. The release behaviors of aspirin were studied in water/*n*-dioxane cosolvents with varying volume ratios and in water with varying pH values, respectively. In the tests of water/*n*-dioxane cosolvents, the collected dried aspirin-loaded vesicles were dispersed in 4 ml cosolvents with volume ratio of water/*n*-dioxane = 50%/50%, 75%/25%, and 100%/0%. The solutions were then transferred into dialysis bags with a cut-off molecular weight of 1000 g/mol. The bags were placed in 40 ml cosolvents with the same water/*n*-dioxane ratio as that inside the dialysis bags. To determine the release amount of aspirin, 3 ml of solution was taken out and was measured with a JASCO V-650 UV–Vis spectrophotometer at the wavelength of 296 nm which is the characteristic absorbance of aspirin. After the measurement, the sampling solution was added back to solutions to maintain the total volume of the solution. The tests of the release in water of different pH-values were conducted in a similar manner. The aspirin-loaded vesicles were dispersed in 5 ml buffer solutions of pH = 5, 7, and 10. The dialysis bags were placed in 35 ml the same buffer solutions. The aspirin contents in the buffer solutions were determined by the absorptions at 296 nm from UV–vis measurements according to the calibration curves shown in Fig. S9 of the Supplementary Materials.

### 2.4. Characterization

To take the transmission electron microscopy (TEM) images of the microphase separated structures of block copolymers with and without aspirin, samples were embedded in resin (Araldite 502) and cured at 60 °C overnight and were then sectioned into ultrathin films with a thickness ~ 80 nm using a diamond knife. The thin sections were transferred to copper grids and were exposed to iodine or $RuO_4$ vapor that selectively stains the PAA or PEO block, respectively, to enhance the contrast. To image the extracted structures, the annealed dried block copolymer/aspirin samples were ultrasonically re-dispersed in 5 ml ethanol or THF and then the solutions were dropped onto carbon-coated copper grids, followed by air-drying. TEM images were collected on a JEOL JEM-1230 transmission electron microscope at an accelerating voltage of 100 kV. Fourier transform infrared (FT-IR) spectra were recorded at room temperature by a Jasco Model FTIR 4100 spectrometer. For the dynamic light scattering (DLS) measurements, the annealed dried block copolymer/aspirin samples were re-dispersed in ethanol at a concentration of 0.1 wt%. The hydrodynamic diameters of the particles were determined by a Brookhaven 90 Plus light scattering instrument at 25 °C.

## 3. Results and discussion

### 3.1. Interaction between aspirin and PAA

We first utilized FT-IR to investigate the interaction between PS-*b*-PAA and aspirin. Fig. 1 shows the FT-IR spectra of PS-*b*-PAAs with varying molecular weights of blocks, aspirin, and the mixtures of PS-*b*-PAAs and aspirin. The mixture is denoted as PS-*b*-PAA(aspirin)$_x$ where $x$ is the molar ratio of aspirin to acrylic acid (AA) unit. Pure aspirin solid shows absorption bands at 1750 and 1680 cm$^{-1}$ that are contributed from carbonyl C=O groups on ester and carboxylic acid while C=O groups on PAA solid shows a broad band at 1712 cm$^{-1}$. Note that aspirin and PAA respectively form intermolecular hydrogen bonds in solid state and therefore, the absorptions of pure aspirin and pure PS-*b*-PAA shown in Fig. 1 are the bands that have been affected by the hydrogen bonding interaction. When aspirin and PS-*b*-PAA are mixed, new bands at 1724 and 1689 cm$^{-1}$ are observed in addition to the original ones. It is suggested that aspirin and PAA can intermix in molecular level so that the original hydrogen bonds of the same species in aspirin and PAA are partly replaced by aspirin-AA hydrogen bonds, which thus causes the new bands. The 1689 cm$^{-1}$ band is particularly prominent for PS-*b*-PAA(aspirin)$_{0.5}$, indicating that a small amount of aspirin can uniformly distribute in PAA blocks. When more aspirin is added ($x \geq 1$), excess aspirin aggregates by itself and the original bands at 1680 cm$^{-1}$ become more pronounced. The intermixing of aspirin and PAA can also be revealed by the slight blueshift of the aspirin 1750 cm$^{-1}$ band in the mixtures. Such a change suggests that the aspirin-aspirin interaction is weakened in the presence of PS-*b*-PAA. The block copolymers with different molecular weights but similar fractions of PS and PAA blocks show similar changes of the absorption bands, implying that the interaction between the PAA blocks and aspirin is independent of molecular weight.

The interaction between aspirin and PAA blocks can further be evidenced by the change of PS-*b*-PAA phase-separated microstructures. Fig. 2 shows the TEM images of PS(42000)-*b*-PAA(4500) and PS(42000)-*b*-PAA(4500)(aspirin)$_1$ where PAA blocks were stained with iodine to enhance the electron density [36]. For pristine PS(42000)-*b*-PAA(4500) shown in Fig. 2a, PAA blocks form spherical microdomains (dark regions) in PS matrix due to a small volume fraction of PAA block. When mixed with aspirin at $x = 1$, instead of the spherical microdomains, cylinder-like structures are observed as shown in Fig. 2b. A similar transition is clearly observed in PS(70000)-*b*-PAA(13000)-based samples as shown in Fig. S1 of the Supplementary Materials. The results confirm that aspirin associates with PAA blocks to increase the volume fraction of PAA(aspirin), thus leading to the structural transition from spheres to cylinders, similar to the widely studied block copolymer-based supramolecular systems [37–40].

### 3.2. Aspirin-loaded vesicles

Having known that there are strong interactions between aspirin and PAA blocks, we then examined the self-assembly of PS-*b*-PAA in a great amount of aspirin that works as a "selective solvent." PS(42000)-*b*-PAA(4500) and aspirin at a weight ratio of 1:9, i.e. 10 wt% of PS-*b*-PAA in aspirin, was firstly dissolved in THF and then THF was slowly evaporated to form premixed samples. Because aspirin is a crystalline solid at room temperature, the premixed samples with kinetically frozen structure after THF evaporation require further annealing to reach the equilibrium state. To prevent the chemical reaction of aspirin at a high temperature [32], we adopted the solvent annealing method at a low temperature of 45 °C, instead of the conventional thermal annealing generally at a temperature higher than the glass transition or melting point [29]. The dried premixed samples were annealed with ethanol vapor. During the process of solvent annealing, ethanol diffuses into samples and imparts mobility to PS-*b*-PAA and aspirin. Mobile aspirin molecules work as a selective solvent to PAA blocks and allow PS-*b*-PAA to self-assemble into a thermodynamically more stable structure. After annealing, the structures were extracted by rinsing the samples in ethanol to remove free aspirin.

The TEM images of the dried extracted structures from different annealing time are shown in Fig. 3. For the as-cast premixed sample from THF solution without solvent annealing, thin flat sheets with a size of several micrometers are formed, as shown in Fig. 3a. The flat sheets are supposed to be the bilayers formed by PS-*b*-PAA where PAA blocks are even more swollen in a great amount of aspirin compared to the case of PS(42000)-*b*-PAA(4500)(aspirin)$_1$ which

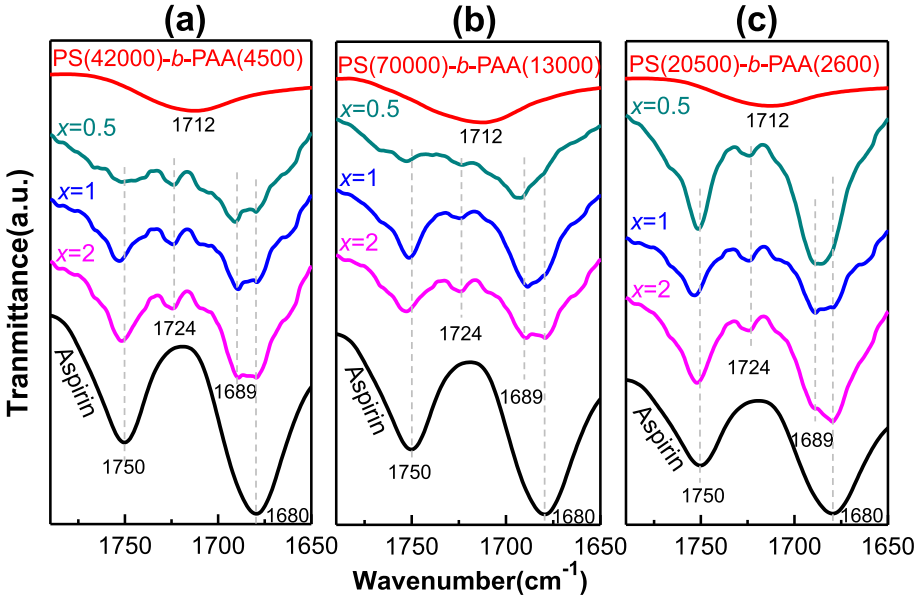


**Fig. 1.** FT-IR spectra of PS-*b*-PAAs with different molecular weights of blocks, aspirin, and their corresponding PS-*b*-PAA(aspirin)$_x$ mixtures.

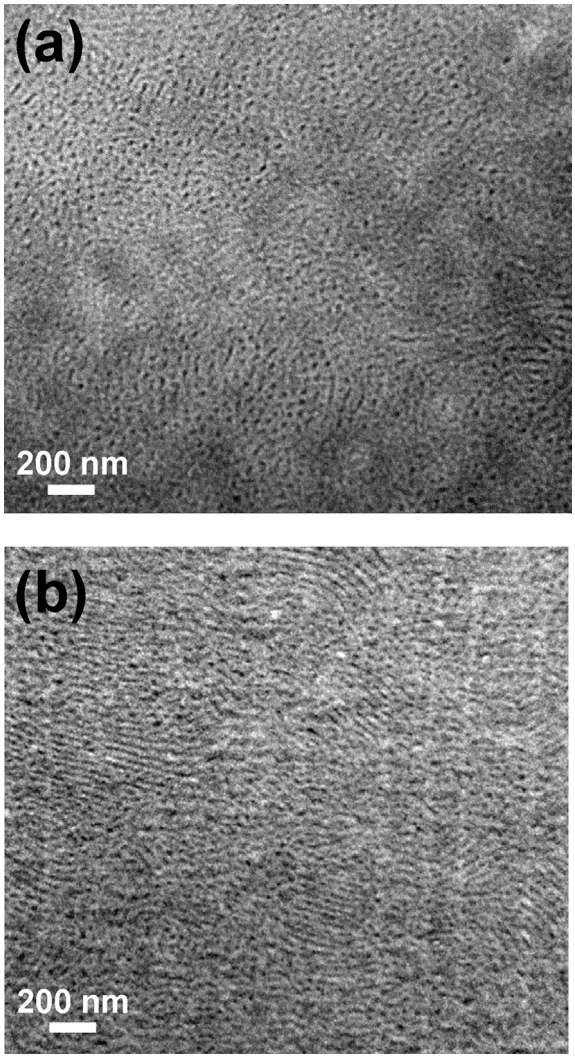


**Fig. 2.** TEM images of (a) PS(42000)-*b*-PAA(4500) and (b) PS(42000)-*b*-PAA(4500)(aspirin)$_1$.

forms cylinder-like structures (Fig. 2b). After annealing for 1 h, the bilayers are gradually bent toward spherical structures, i.e. vesicles, as shown in Fig. 3b where a coexistence of bilayers and vesicles is observed. Because the vesicles are directly formed in aspirin, the vesicles are filled with aspirin, shown as the dark cores due to the higher electron density of crystalline aspirin. When the annealing time is increased to 5 h, most of the bilayers are converted into aspirin-loaded vesicles, as shown in Fig. 3c. Note that the aspirin molecules associated with the bilayers may further crystallize after solvent annealing, which reduces the contrast of the electron density between cores and shells. This explains why the particles in Fig. 3c show micelle-like structure rather than the core-shell structure normally seen for vesicles. Similar structure can be observed for 60-h annealing, implying that the vesicles are the thermodynamically stable structure. The size of the particles estimated from the TEM images is polydisperse, ranging from tens to hundreds of nanometers. Dynamic light scattering (DLS) was used to more accurately measure the size of the extracted particles dispersed in ethanol. The data in Fig. 4 show two distributions of diameters, the major one around 280 nm and the minor one around 80 nm. The larger size should be assigned to the vesicles while the smaller one may result from other minor self-assembled structures, most probably micelles that coexist with the vesicles.

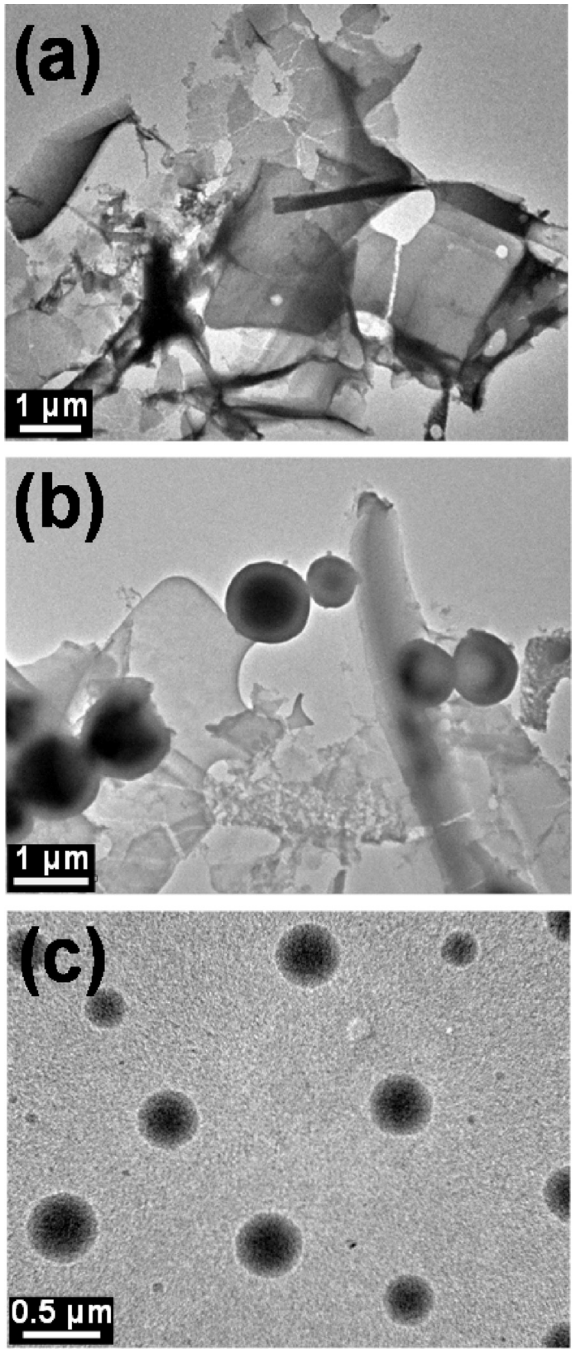


**Fig. 3.** TEM images of the structures formed by 10 wt% of PS(42000)-*b*-PAA(4500) in aspirin before solvent annealing (a) and after annealing for (b) 1 h and (c) 5 h.

PS-*b*-PAAs with different molecular weights but similar fractions of blocks can also be used to prepare aspirin-loaded vesicles by solvent annealing. The structures of lower molecular weight PS(20500)-*b*-PAA(2600) prepared following the same procedure are shown in Fig. S2. Before solvent annealing, the self-assembled structures are flat bilayers. The bilayers are mostly transformed to aspirin-loaded vesicles after solvent annealing for 3 h, which is shorter than the time required for the same stage in PS(42000)-*b*-PAA(4500) case. This is because the diffusion rate of shorter PS(20500)-*b*-PAA(2600) is higher and the chains can move faster under annealing to reach equilibrium. As expected, the block copolymer with higher molecular weight, PS(70000)-*b*-

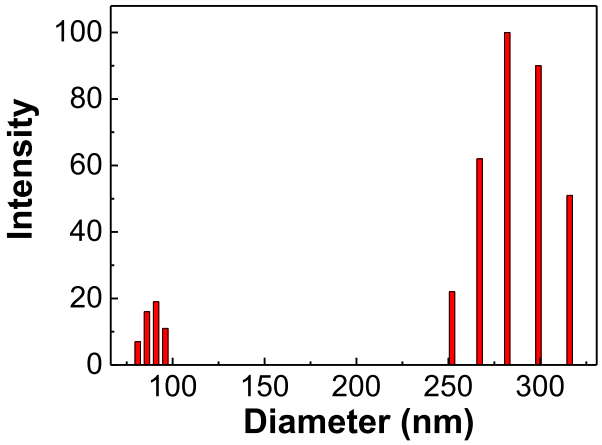


**Fig. 4.** Size distribution of vesicles formed by PS(42000)-*b*-PAA(4500) in aspirin after solvent annealing for 5 h.

PAA(13000), takes a much longer annealing time, ~60 h, to dominantly form aspirin-loaded vesicles, as shown in Fig. S3, apparently due to the lower diffusion rate.

To further confirm the formation of vesicles, the aspirin-loaded vesicles formed by PS(42000)-*b*-PAA(4500) after annealing for 5 h were gently re-dispersed in THF that is a good solvent for both blocks and aspirin. When the aspirin-loaded vesicles are dispersed in THF for 1 day, the structures of vesicles are unchanged, still full of aspirin in the core as shown in Fig. 5a. The vesicles are rather stable for a short time in the good solvent due to the low mobility of long, entangled polymer chains and the high crystallinity of aspirin. After 3 days, the aspirin molecules that attached to the PAA blocks started to diffuse into the solvent, resulting in the roughened interfaces of the vesicles as shown in Fig. 5b. It is interesting that the cores of the vesicles turned to be empty after 5 days and the bilayer walls of the vesicles could be clearly seen as shown in Fig. 5c, implying the release of most aspirin molecules from the cores through the swollen polymer chains of the bilayers. Another test was done by firstly dispersing the aspirin-loaded vesicles in ethanol, followed by addition of equal amount of THF into the solution. The TEM image is shown in Fig. S4. After 1 day, most aspirin molecules on the bilayers are removed and the walls of the vesicles become clear. The results confirm the formation of PS-*b*-PAA vesicles with aspirin enclosed in the core.

THF was also used to extract aspirin-loaded vesicles formed by PS(20500)-*b*-PAA(2600). When aspirin-loaded vesicles are dispersed in THF for 1 day, the cores of most vesicles becomes empty due to the diffusion of aspirin out of the vesicles, as shown in Fig. S5. When we compared aspirin-loaded vesicles formed by PS(20500)-*b*-PAA(2600) with those formed by PS(42000)-*b*-PAA(4500) which requires 5 days to reach the same state (Fig. 5c), aspirin can diffuse out of the vesicles more rapidly through the bilayers composed of shorter polymer chains. The faster release behavior of the vesicles formed by PS(20500)-*b*-PAA(2600) is caused by the high permeability resulting from the lower entanglement and higher diffusion rate of shorter polymer chains, in agreement with the faster structural transition found for the copolymer with lower molecular weight under solvent annealing process as described above.

In addition to PS-*b*-PAA, other amphiphilic block copolymers, including PS(58000)-*b*-PEO(8200) and PCL(32500)-*b*-PEO(5000), can form aspirin-loaded vesicles as well. The microphase-separated structure of the stoichiometrically prepared PS(58000)-*b*-PEO(8200)(aspirin)$_1$ is changed to cylinders from the spheres of

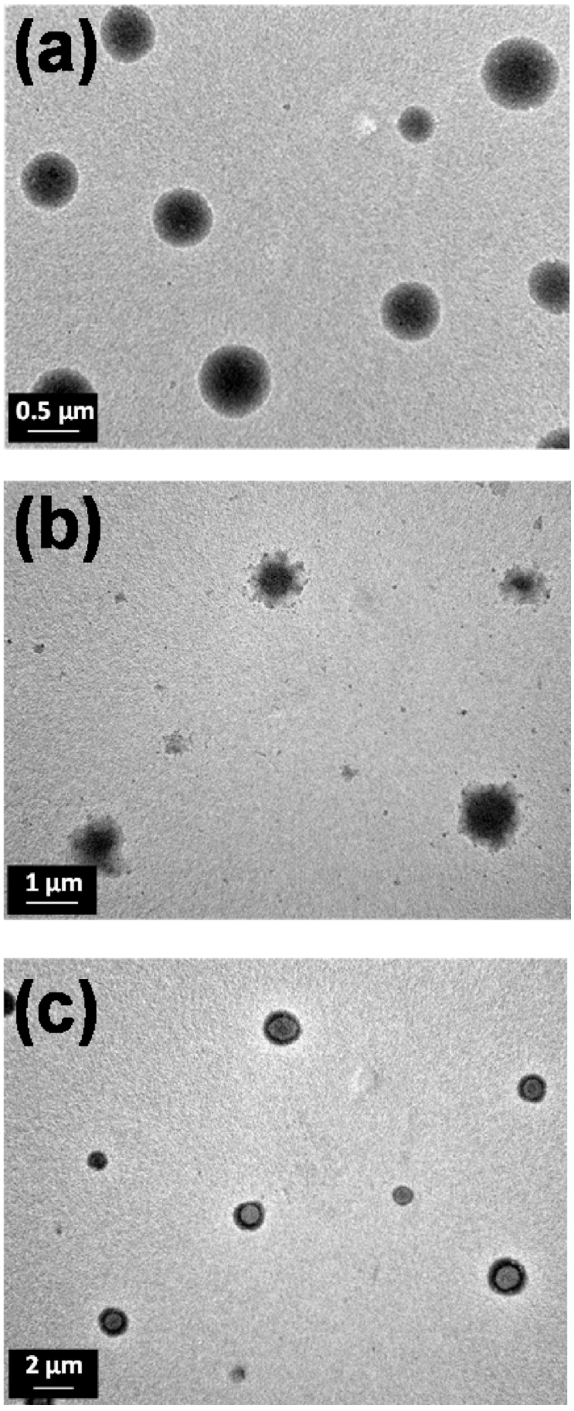


**Fig. 5.** TEM images of the aspirin-loaded vesicles formed by PS(42000)-*b*-PAA(4500) after solvent annealing for 5 h and then re-dispersed in THF for (a) 1 day, (b) 3 days, and (c) 5 days.

pure PS-*b*-PEO without aspirin, as shown in Fig. S6, implying the association between aspirin molecules and PEO blocks through hydrogen bonding interactions. For the sample of 10 wt% of PS-*b*-PEO in aspirin after solvent annealing with ethanol for 24 h, aspirin-loaded vesicles are formed following the same steps described above, as shown in Fig. 6a. In the case of PCL-*b*-PEO, although the carboxylic acid groups on aspirin can interact with both the ether groups on PEO and the carbonyl groups on PCL, the hydrogen bonding interactions between PEO and aspirin should be

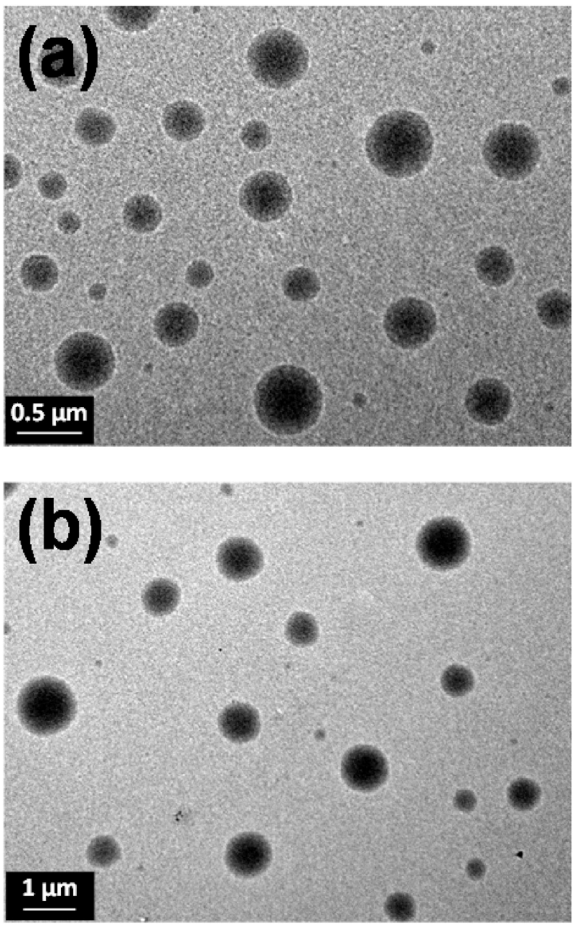


**Fig. 6.** TEM images of aspirin-loaded vesicles formed by (a) PS(58000)-*b*-PEO(8200) and (b) PCL(32500)-*b*-PEO(5000) after solvent annealing for 24 h.

stronger than those between PCL and aspirin [41]. Therefore, aspirin can also work as a selective solvent to PCL-*b*-PEO. Fig. 6b shows the aspirin-loaded vesicles formed by 10 wt% of PCL-*b*-PEO in aspirin after solvent annealing for 24 h. These results confirm that the method developed in this work can be generally applied to amphiphilic block copolymers. Particularly, PCL-*b*-PEO is a fully biocompatible block copolymer and is suitable for in vivo drug delivery [42,43].

The formation mechanism of the vesicles can be explained as follows. In a great amount of aspirin, like 90 wt% in this work, the hydrogen bonding interaction between aspirin and the hydrophilic blocks allows the association of aspirin to increase the volume fractions and results in the formation of isolated bilayer structures after slow evaporation of THF as shown in Fig. 3a. The hydrophilic blocks form the outer layers to contact aspirin while the hydrophobic blocks are buried as the inner layers. During solvent annealing process, ethanol provides mobility to the polymers and aspirin. The block copolymers turn into vesicles because the transformation of large flat bilayers into smaller vesicles can gain translational entropy, and also, ethanol and mobile aspirin are non-solvents to the inner hydrophobic layers so that the edges of the bilayers tend to join together to prevent the inner layers from contacting ethanol and aspirin.

### 3.3. Release behaviors

We have demonstrated that the block copolymers can form vesicles directly in aspirin and encapsulate a significant amount of aspirin in the cores. The release behaviors of aspirin from the vesicles were then investigated. Two methods were adopted: (1) release in aqueous solution with different concentration of *n*-dioxane, a high boiling point solvent that can dissolve both PS and PAA, and (2) release in aqueous solutions with different pH values that can affect the degree of swelling for the pH-sensitive polymers, such as PAA, or can affect the degradation rate of biocompatible polymers, such as PCL. In order to remove the aspirin which is not encapsulated or associated in vesicles, the samples were dissolved in ethanol and centrifuged, and the sediments that contain aspirin-loaded vesicles were collected. The dried aspirin-loaded vesicles were placed in aqueous solutions for release tests. The TEM image of the sediment of PS(42000)-*b*-PAA(4500) vesicles after centrifuged and re-dispersion in ethanol was shown in Fig. S7. The vesicles were still filled with aspirin in the cores.

The drug loading content of polymeric vesicles is calculated by the following equation [44]:

$$Loading\ content\ (\%) = \frac{Weight\ of\ aspirin\ in\ vesicles}{Weight\ of\ vesicles} \times 100\%$$

The aspirin loading content for PS(42000)-*b*-PAA(4500) vesicles is 59 ± 5%, which is higher than that of conventional vesicles formed in liquid systems (30–40%) [45]. The drug loading is efficient because the vesicles are formed directly in aspirin and a great amount of aspirin can be confined in the core of vesicles. Also, the interactions between the PAA blocks and aspirin allow the association of aspirin with PAA, which further enhances the loading capacity.

It has been demonstrated that the permeability of a vesicle wall made by polystyrene can be enhanced by addition of *n*-dioxane [46]. *n*-Dioxane is a good solvent for both PS and PAA blocks and can swell polymer chains to release drugs from vesicles. Fig. 7a shows the accumulated release of aspirin from PS(42000)-*b*-PAA(4500) vesicles as a function of time in *n*-dioxane/water cosolvents at three different volume ratios. The accumulative release is calculated by the following equation [44]:

$$Accumulative\ release\ (\%) = \frac{Weight\ of\ asprin\ released\ in\ medium}{Initial\ weight\ of\ aspirin\ in\ vesicles} \times 100\%$$

Because the block copolymer vesicles are robust and aspirin is crystallized, aspirin is released at a rather slow rate in this system, requiring at least 15 days to reach the maximum release even for the 50/50 cosolvent. The release rates in the early 5 days is not significantly different for the three cosolvents because the major aspirin released at this state should be from those that originally associate with the outer PAA layers. After 5 days, the vesicle walls swell and the aspirin molecules in the core of vesicles diffuse through the walls into the solvents. As expected, the final release amount is increased with increasing fraction of *n*-dioxane. The TEM image of the vesicles after release in 50/50 cosolvent for 20 days is shown in Fig. S8 as well as compared with the image before release in the insets of Fig. 7a. The originally filled vesicles turn to be hollow ones, implying that most aspirin molecules are released out of the vesicles.

The pendant carboxylic acidic groups on the PAA blocks is pH-sensitive. The electrostatic repulsion between the negatively charged carboxylic acidic groups in aqueous solution is increased when the deprotonation occurs at high pH values, which will facilitate the release of aspirin from the PS-*b*-PAA vesicles through the more swollen walls. On the contrary, at low pH values, the carboxylic acids groups are protonated and the repulsion is

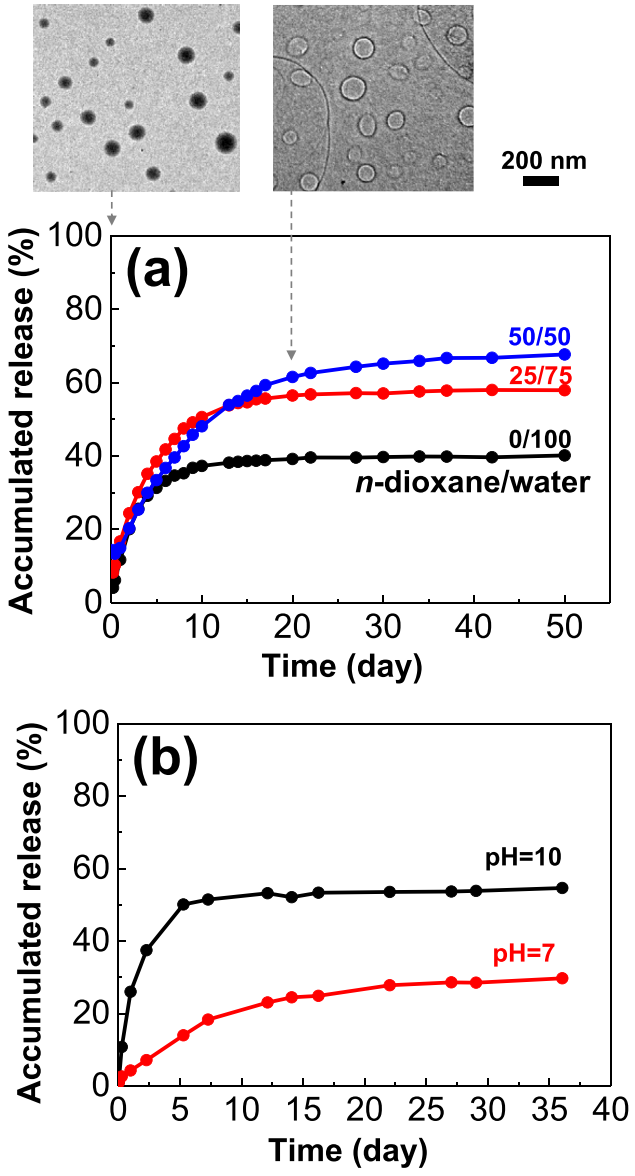


**Fig. 7.** Accumulated releases of aspirin from PS(42000)-*b*-PAA(4500) vesicles as a function of time in (a) *n*-dioxane/water cosolvents with different volume ratios and (b) buffer solutions with different pH values. The insets show the corresponding TEM images before release and after 20-day release for the 50/50 cosolvent.

screened. The release of aspirin should thus be retarded. The borate buffer (pH = 10) and phosphate buffer (pH = 7) were used to test the dependence of aspirin release from PS-*b*-PAA vesicles on pH values. Fig. 7b shows the release profiles of aspirin in these two buffer solutions determined according to the calibration curves shown in Fig. S9. A higher release rate at the initial stage and a larger accumulated release at final stage were observed at pH = 10, consistent with the expectation. The final release at pH = 10 is ~54%, less than that triggered by *n*-dioxane (~68% for the 50/50 cosolvent). It is possible that the release of aspirin at high pH values is hindered by the pH-insensitive PS layers though the conformation of PS chains may be affected by the swollen PAA chains in a basic environment.

In addition to PS-*b*-PAA vesicles, we also tested the release of aspirin-loaded vesicles formed by PCL(32500)-*b*-PEO(5000) in buffer solutions with different pH values. It has been known that PCL hydrolyzes through the breaking of the ester linkages in an

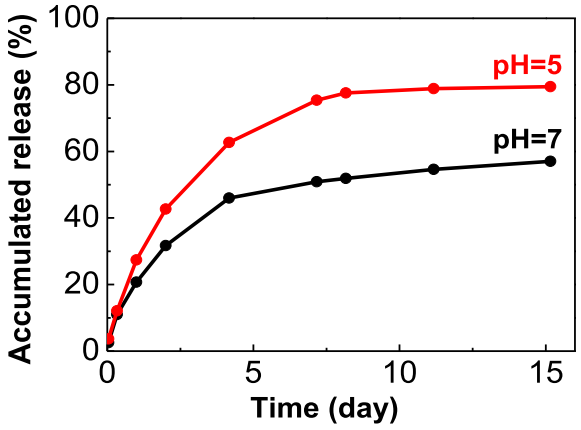


**Fig. 8.** Release behaviors of aspirin-loaded PCL(32500)-*b*-PEO(5000) vesicles in buffer solution with different pH values.

acidic environment [47]. In other words, we can tune the pH values to manipulate the stability of the inner PCL layers in vesicle walls and thus control the release behaviors of the aspirin-loaded vesicles [42]. The release curves in acetate buffer (pH = 5) and phosphate buffer (pH = 7) are shown in Fig. 8. At pH = 5, a higher release rate and release amount can be observed compared to those at pH = 7, confirming the faster degradation of PCL at low pH values. The results show that the new method for preparing vesicles with high loading capacity aiming for controlled release can be applied to biocompatible block copolymers.

## 4. Conclusions

We have demonstrated that block copolymers can successfully self-assemble into vesicles in aspirin and efficiently encapsulate aspirin by solvent annealing with ethanol at low temperature. Ethanol imparts mobility to aspirin which alone is a crystalline solid at low temperature. Aspirin form hydrogen bonds with the hydrophilic blocks, thus working as a selective solvent to drive the self-assembly of the block copolymer. Aspirin is nearly filled in the entire enclosed portions of vesicles, giving the vesicles a higher encapsulation capacity compared to those formed in liquid media. The vesicles with a high aspirin content are extracted and the encapsulated aspirin can be released at a slow rate for a fairly long time in aqueous solutions due to the robust vesicular walls formed by long copolymer chains. The release behaviors can be tuned by addition of good solvents that dissolve both blocks of the copolymers, such as *n*-dioxane, or by changing pH values that affect the degree of swelling or degradation of the copolymers. The aspirin-loaded block copolymer vesicles developed in this work are potential for long-term application of controlled drug release. This strategy paves a way toward new classes of nanomaterials and may expand the applications of the existed nanotechnologies.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.polymer.2017.11.060.

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