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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(ϵ-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.