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Lyoprotectants modify and stabilize self-assembly of polymersomes

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ABSTRACT

Polymersome formation in water was confirmed with average polymersome diameters of 237.2 ± 66.5 nm over 150 min. Empty polymersomes created by dissolving poly(ethylene glycol)-b-poly(lactic acid) in water increased to 4.63 ± 0.01 times their size after lyophilization, showing lack of long-term stability. The use of lyoprotectants, mannitol and inulin, to maintain particle size distribution (PSD) was studied. The incorporation of both molecules was confirmed and showed no detrimental effects on PSD during formation. Differences in moisture content were found after lyophilization between samples incorporating inulin and mannitol. After lyophilization, PSD of polymersomes lyophilized with inulin was maintained. Polymersomes lyophilized with mannitol showed a significant decrease in diameter, a potential advantage for delivery of therapeutics. It was hypothesized that lyoprotectants replaced water, maintaining polymersome structure under stressful processing conditions. The ability to reconstitute polymersome drug delivery carriers without altering size distribution is paramount to the creation of effective and efficient drug delivery systems.

1. Introduction

Polymersomes, or polymeric vesicles, are made up of two or more amphiphilic block copolymers and can encapsulate both hydrophilic and hydrophobic molecules [1]. Block copolymers will self-assemble into a multitude of different structures based upon their hydrophilic/hydrophobic mass ratio and the overall molecular weight upon introduction to a solvent [2]. A hydrophilic fraction between approximately 25 and 40% by mass, determined using polyethylene glycol (PEG) as a hydrophilic block, have been shown to form vesicular structures in aqueous solvent [2]. The thickness of the membrane and diameter of the vesicle interior increase with increasing molecular weight of the hydrophilic component [3]. Polymersomes, by nature, are tunable on the molecular level [4] and may take advantage of many different release mechanisms including pH, temperature, redox-potential, light, hydrogen bonding, electrostatic forces, magnetic field, ionic strength, concentration of analytes, and other external stimuli [1,5]. The ability to control the release mechanism and size of specific carrier regions through material selection makes polymersomes desirable platforms for drug delivery, as they show potential for delivery of a wide variety of therapeutics in various applications.

This study involves the use of poly(ethylene glycol)-b-poly(lactic acid) (PEG-b-PLA) to create therapeutically deliverable polymersomes that could be produced and stabilized over a long period of time, allowing for more efficient intravenous delivery of both hydrophobic and hydrophilic therapeutics. Both components of this block copolymer are approved by the Food and Drug Administration (FDA) for use in humans [6]. PEG is approved for 17 different routes of administration and polylactide (PLA) is approved for both intramuscular and periodontal routes [7]. PLA is biodegradable and has been shown to cause release based upon pH-triggered hydrolysis [1,8,9]. As with other carriers and drug delivery systems [10], the incorporation of a PEG block may be used to increase the stability of polymersomes and decrease uptake by the reticuloendothelial system due to its resistance to protein

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adsorption and cell adhesion [1].

Although manipulation of block co-polymer composition and molecular weight can aid in control of polymersome size and shape, there is still a large variation in the final hydrodynamic size distribution. Formation methods exploited can lead to polydisperse samples. Particle size diameter of the nanoparticle system being used is an important consideration based upon the necessity of cellular uptake and required surface area to attach targeting or imaging moieties. In general, particle size diameters of drug delivery vehicles should be around 200 nm or less in order to allow for transcytosis through the cell membrane [11]. The nature of self-assembly leads to size variation and necessitates separation techniques.

Extrusion techniques are used, pushing polymersome solutions through membranes of various sizes under high pressure [5] to yield a defined size distribution of polymersomes. This can be time consuming and costly, while still not leading to a sharp enough size distribution for therapeutic delivery. By using lyoprotectant molecules during polymersome formation, further control over polymersome size can be achieved, reducing the need for costly and time consuming separation techniques and creating polymersomes that maintain their size and structure during long-term storage.

In this study, cryogenic freezing and lyophilization (or freeze-drying) was used to quench self-assembly. Lyophilization has been used to stabilize and preserve biological and pharmaceutical products for extended periods of time, allowing reconstitution into solution when necessary [12]. Reconstitution of polymersomes means the re-introduction of the lyophilized polymersomes into an appropriate solvent for injection. During reconstitution, polymersomes should maintain their average diameter. Both inulin, a polysaccharide, and mannitol, a sugar alcohol, have been previously explored for their hydrophilic nature as lyoprotectant molecules in polymersomes [13]. However, the focus of previous studies was on long-term storage of polymersomes, while our study was focused on controlling the dynamic nature of self-assembled systems needed to form polymersomes while maintaining size distribution. Because of this, the polymersome formulation method used was the most widely applicable method, taking advantage only of the hydrophobic interactions between the block co-polymer and water and allowing our results to be applicable to other polymer systems.

The objective of our study was to determine the effect of incorporating mannitol and inulin on polymersome properties including particle size diameter formed, moisture retention during lyophilization, and maintenance of particle size diameter after processing. We hypothesized that the incorporation of lyoprotectant molecules, inulin and mannitol, would increase the stability and maintain the size distribution of the formed polymersomes after lyophilization.

2. Materials and methods

2.1. Materials

Co-polymer with 17% PEG (molecular weight = 1000 Da) blocked with remainder PLA (molecular weight = 5000 Da) was used in all particle formation studies (Polysciences, Inc). Inulin (Alfa Aesar) and mannitol (Sigma Aldrich) were used as received. Deionized (DI) water was obtained for particle formation using a Millipore water filtration system. Millex syringe filter units of pore sizes 0.80 μm and 0.45 μm were used (Millipore). HYDRANAL Coulomat reagent (VWR) was used in Karl Fischer titration.

2.2. Polymersome formation

Block co-polymer, PEG-b-PLA, was dissolved in DI water at a concentration between 0.7 and 0.8 wt%/v. The solution was mixed for a period of 2.5 h. Samples in which solely block co-polymer were dissolved are indicated as control studies. Inulin and mannitol were dissolved simultaneously in DI water with PEG-b-PLA at concentrations of 2, 5, and 8 weight percent per volume as lyoprotectant molecules in corresponding studies.

2.3. Particle size distribution analysis

During polymersome formation, aliquots were drawn from the vial every half hour for 2.5 h. Each aliquot was diluted to a polymersome concentration of 0.2 wt%/v, which showed consistent DLS measurements. Removal of a small sample size led to minimal changes in the driving force of polymer dissolution, which is proportional to $e^{-\frac{\Delta G}{k_B T}}$ [14]. A total sample volume of 1 mL was pipetted into a plastic cuvette and placed in the Zetasizer to obtain the particle size distribution (PSD) of the sample. The PSD data was obtained prior to the use of both 0.80 μm and 0.45 μm membranes, as well as after the use of both membranes, which allowed for further study of smaller polymersomes. Data was collected and presented using intensity-weighted size distributions and percentage of polymersomes found in specific bins pre-determined by the software.

2.4. Cryogenic freezing

After 2.5 h, aliquots of polymersomes in DI water, with or without mannitol or inulin based upon the study, was vitrified using liquid nitrogen, causing the self-assembly to seize. After cryogenic freezing, the polymersomes suspended in water were placed in a freeze dryer under sublimation conditions (0.040 mbar and −52 °C), allowing the water to evaporate off and leaving behind polymersomes. This lyophilized polymersome sample, in the form of a powdery cake, was used in further analysis.

2.5. Fourier transform- infrared spectroscopy

Identification of the lyophilized polymersome samples with and without lyoprotectant molecules was done using attenuated total reflection coupled with Fourier transform-infrared spectroscopy (ATR FT-IR). This method of analysis allows for solid samples to be examined and has a penetration depth of about 500 nm. Because of this, ATR FT-IR was capable of penetrating the hydrophobic membrane of the polymersomes and analyzing both the surface and interior core of the vesicle.

2.6. Particle reconstitution

Polymersome size maintenance was measured by reconstituting the lyophilized samples into DI water at the same concentration used during initial formation. Therefore, an appropriate mass of powdery sample was added to 1 mL of DI water. Vortexing was applied for 5–10 min depending on the resistance to dissolution of the sample in order to reconstitute as many particles as possible. Then, the intensity-weighted PSD was obtained (Zetasizer Nano, Malvern) and compared to the intensity-weighted PSD found prior to lyophilization. A normalization value was measured by dividing the overall average diameter found post-lyophilization by the overall average diameter found pre-lyophilization. The closeness of this value to one indicates the closeness of the final diameter to the diameter initially studied.