

# Synthesis and Engineering of Polymersomes for Treatment of Lysosomal Storage Disease

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**Statement of Purpose:** Delivery of therapeutics to the brain through noninvasive administration is a difficult task due to the presence of the blood-brain barrier (BBB). Molecules of less than 500 Daltons and with less than 8 hydrogen bonds, only encompassing two percent of small therapeutic molecules, are transported through the brain endothelium [1, 2]. We are designing and characterizing self-assembling polymersomes due to their high physiological stability and tunable properties, for the purpose of encapsulating and delivering treatment for GM1 gangliosidosis through intravenous methods. When coupled with an osmotic diuretic or brain endothelium specific receptor, delivery through the BBB and into the lysosome of neural cells is expected. Long term stability is an important consideration with bio-therapeutic delivery and the use of potential lyoprotectants, including mannitol, trehalose, and inulin, to sequester water and maintain particle size distribution (PSD) was studied.

**Methods:** With distilled water as a solvent, polyethylene glycol-b-poly(lactic acid) copolymer was allowed to self-assemble. PSD was determined using intensity-weighted dynamic light scattering techniques using a Malvern Zetasizer Nano, with a varying laser position based upon sample concentration. The use of 0.45 and 0.80  $\mu\text{m}$  microporous membranes was employed to provide a more complete size distribution in lower diameter ranges. Transmission electron microscopy was used to confirm structure appearance. Varying temperature gradients were studied in the lyophilization process without lyoprotectants. Initial studies were done using 2 wt%/v mannitol in particle formation. The effect of mannitol, inulin, and trehalose on the lyophilization process was studied at concentrations of 2, 5, and 8 wt%/v.

**Results:** Polyethylene glycol-b-poly(lactic acid) copolymer has been proven to self-assemble, forming vesicle structures at an average minimum peak size of  $216.2 \text{ nm} \pm 12.9 \text{ nm}$  when at a concentration of 0.1 - 0.2 wt%. The structure formed during self-assembly was confirmed using transmission electron microscopy. After this lyoprotectant-free lyophilization, the polymersomes maintain the integrity of their hydrophobic membrane if freeze-dried immediately following cryogenic freezing of the solution. Without the presence of a lyoprotectant, the polymersomes lose their size properties in freeze drying. Using an ideal procedure, a pre-membrane diameter of polymersomes pre-lyophilization was found to be  $1113 \pm 18 \text{ nm}$ . After lyophilized and reconstituted at the same concentration in solvent, the size distribution showed an average diameter of  $9368 \pm 33.11 \text{ nm}$ . This measurement allows for the maintenance of properties and feasibility of long-term storage to be determined [3]. Due to the discrepancy in size consistency, mannitol was added to the sample formation, believed to sequester water during lyophilization due to the presence of hydroxyl groups.

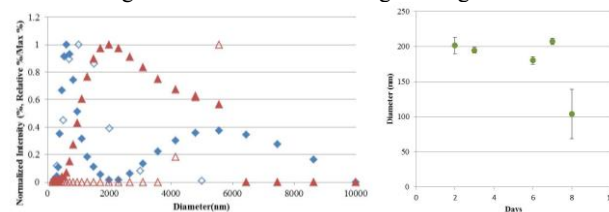


Figure 1. Normalized Particle Size Distribution of Pre and Post Lyophilized Particles and Post Membrane Data. The data presented on the left is before the application of a membrane. Pre-lyophilization ( $\diamond$ ) and post-lyophilization ( $\blacktriangle$ ) data is presented both without lyoprotectants (control) and with lyoprotectant (mannitol). The right is the average diameter of particles formed over an 8 day period with 2wt%/v mannitol after the use of a 0.45  $\mu\text{m}$  membrane ( $\bullet$ )

Initial studies using mannitol at 2 wt%/v show an increased volume of the lyophilized cake of these nano-sized polymersomes, showing increased particle stability. Pre-lyophilization ( $\diamond$ ) without microporous membranes gave an overall average diameter of  $1113 \pm 18 \text{ nm}$  without mannitol and  $832 \pm 20 \text{ nm}$  with mannitol, suggesting that the PSD is not negatively affected using this lyoprotectant (Figure 1). Also, after reconstituting the lyophilized sample into solution ( $\blacktriangle$ ) at the same concentration, an overall average diameter of  $5153 \pm 28 \text{ nm}$  was found in the control while the sample with mannitol had an average diameter of  $1943 \pm 144 \text{ nm}$ . Although at 2 wt%/v the exact distribution as original was not found, it provides proof that lyoprotectants can help the polymersome maintain shape when lyophilized. The size range after a 0.45 microporous membrane with 2 wt%/v mannitol was a diameter of  $104 \pm 35 \text{ nm}$

**Conclusions:** The use of microporous membranes demonstrates the presence of therapeutically deliverable sized vesicles being formed. The post-membrane size falls into the size range used in some in vitro blood-brain barrier models with brain endothelium receptors, like transferrin [4, 5]. The use of mannitol at 2 wt%/v does not show a negative effect on particle sizing and the effect of trehalose and inulin on sizing will continue to be studied. Preliminary data suggests that long term stability of these lyophilized polymersomes is possible with the use of lyoprotectants. Further studies will be done to determine the ideal molecule and concentration for this purpose. Future work includes a full analysis of the mechanism of action of these lyoprotectants and proof of the hypothesis that they prevent water from escaping the polymersome core, allowing the maintenance of size.

**References:** [1] Tsuji A. *Introduction to the Blood-Brain Barrier: Methodology, Biology, and Pathology*. 2006; 238-245 [2] Khawli L. *Mol Pharm*. 2013; 10:1471-1472 [3] Ayen W. *Eur J Pharm Sci*. 2012; 46:405-414 [4] Gan C. *Biomaterials*. 2010; 31:7748-7757 [5] Ulbrich K. *Eur J Pharm Biopharm*. 2009; 71:251-256

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