Rapid Preparation of Polymersomes by a Water Addition/Solvent Evaporation Method

Supporting Information

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Contents

Addition of aqueous phase

During polymersome preparation, the introduction of the aqueous phase is generally the most critical step in the process, being the point at which precipitation of the block copolymer is likely to occur. During the water addition/solvent evaporation method polymersomes form upon PBS addition, and the manner in which samples are treated prior to THF removal may be expected to influence the final structures. Under the standard conditions the aqueous phase is added within 2 s to the organic phase and then gently vortexed for 1 min such that the phases are mixed but not agitated. Simply leaving the samples for 1 min without vortexing also resulted in well-defined final polymersomes, with similar sizes to those prepared with the mixing step (Figure S1). The inevitable mixing upon addition of the aqueous phase and during solvent evaporation is therefore adequate to yield polymersomes with a narrow size range. However, if the THF solution and PBS were mixed with high energy input by vortexing the flask for 1 min such that the solution is strongly agitated, the material precipitated. Therefore within practical limits the structure formation is relatively insensitive to the way in which the organic and aqueous phases are combined. Although the mixing step is not necessary with this block copolymer, the polymersome size distribution was found to be slightly less reproducible without the mixing step for the PBLG-E block copolymer series; therefore it was included in the standard procedure.

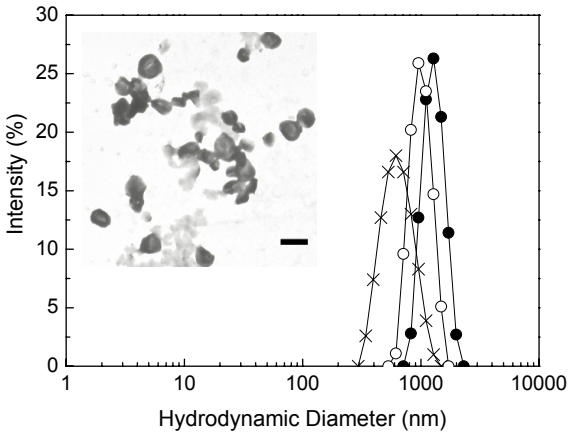

Figure S1. DLS intensity distributions for $PBLG_{50}$-K polymersomes in PBS prepared with 1 min of vortexing at 200 rpm prior to THF evaporation (●), 1 minute without mixing (○), and immediate evaporation (×). Inset: PTA stained $PBLG_{50}$-K polymersomes prepared with immediate evaporation. Scale bar = 500 nm.

In one respect, the WASE method is reminiscent of the preparation of polymersomes by the slow addition of organic solution to an aqueous phase.[1, 2] With both methods there is a rapid decrease in solvent quality for the hydrophobic block of the block copolymer as the organic solution and aqueous phase are mixed.

To compare these two methods, samples were prepared by the slow addition of solvent as follows: $PBLG_{50}$-K (0.02 μmol) was dissolved in 2 mL THF and added dropwise at a rate of 0.2 wt% (4 μL) per min using a syringe pump (NE-300, just infusion, Prosense B.V.) to 3 mL of water or PBS in a 50 mL round bottomed flask while stirring at 700 rpm. THF was removed by dialysis. Dialysis tubing (Spectrum Laboratories, Inc.) with a molecular weight cut-off of 3500 g/mol was thoroughly rinsed with water, and the samples dialyzed against water or PBS at room temperature for at least 24 h with at least 5 changes of buffer.

Slowly adding the organic solution to water results in polymersomes with a similar average diameter to those prepared with the WASE method (d_h ~180 nm, PDI 0.32 and d_h ~210, PDI 0.15 respectively), but the polymersomes take ~100 times longer to prepare, and have a high PDI.

When the organic solution is slowly added to PBS, the samples are initially clear, but as the proportion of THF increases the block copolymer forms insoluble disordered aggregates. In this case the slow solvent addition method is not suitable because the extended time during which the PBLG blocks are mobile and the charges within the corona are screened leads to the growth and collapse of large structures. In contrast, the WASE method is effective because it kinetically traps the polymersomes.

Structure formation in the mixed solvent system

The water addition/solvent evaporation method was modified from a rapid liposome preparation technique.[3] For this method the lipids are also dissolved in an organic solvent, the aqueous phase quickly added, and the organic solvent removed by rotary evaporation under reduced pressure. However, the organic solvent used to rapidly produce liposomes is chloroform, which is immiscible with water, and liposomes form during evaporation. Many block copolymers, including the PBLG-peptide series used in this study, are not soluble in chloroform because of the hydrophilicity of the corona forming block. THF is a polar organic solvent that dissolves a wider range of polymer blocks. As it also has a low boiling point (66 °C), it is suitable for use in a solvent evaporation method. Unlike chloroform, THF is miscible with water; hence the block copolymers are exposed to a high water content upon addition of the aqueous phase, and aggregation induced by the hydrophobic block occurs before solvent evaporation.[4] The block copolymer specific adaptations result in different mechanisms of vesicle formation for the current method and for the rapid preparation of liposomes, hence the two methods are fundamentally divergent.

When water is added to the polymer solution it is likely that there is a structural evolution that is driven by changes in interfacial tension, core stretching, and repulsion between corona blocks. The most common route is structures of decreasing interfacial curvature, going from spherical micelles to rod-like micelles, lamellar sheets, which close to form polymersomes, and subsequent polymersome growth.[5] Due to the rod-like blocks of $PBLG_{50}$-K the stability of planar interfaces is expected to be quite pronounced,[6] which may alter the route to polymersomes for the PBLG-*b*-coiled-coil-peptide series.[7]

During evaporation of the THF the composition changes within the interior of the polymersomes. The fluorescent dye rhodamine B was added to $PBLG_{50}$-K samples after the PBS and THF had been mixed, but before solvent evaporation. The polymersome membranes were impermeable to rhodamine B, with the interior of the polymersomes remaining dark as observed by fluorescence spectroscopy (Figure S2A). However after solvent evaporation for 2 min at 20 °C the interior of the polymersomes contained the fluorescent dye (Figure S2B). This means that the aggregates are permeable to rhodamine B during solvent evaporation, and it is likely that they are also permeable to THF/PBS.

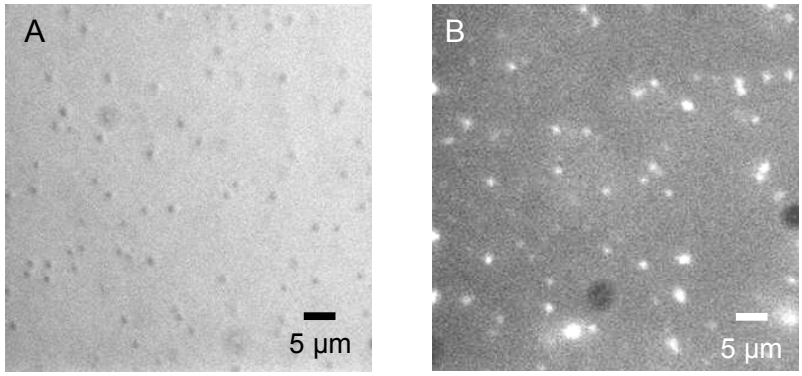

Figure S2. A) Fluorescence microscopy image of $PBLG_{50}$-K polymersomes in the mixed solvent phase after the addition of rhodamine B. The rhodamine B is outside the polymersomes. B) Fluorescence microscopy image of $PBLG_{50}$-K polymersomes in PBS after solvent evaporation, showing that the Rhodamine B is inside the polymersomes. Scale bars = 5 µm.

Circular dichroism spectra of K

CD spectra were obtained using a Jasco J-815 spectropolarimeter equipped with a peltier-controlled thermostatic cell holder. Spectra were recorded from 260 to 200 nm in a 1 mm quartz cuvette at 5 °C intervals from 5 °C to 75 °C. Data were collected at 0.5 nm intervals with a 1 nm bandwidth and 1 s readings. Each spectrum was the average of five scans.

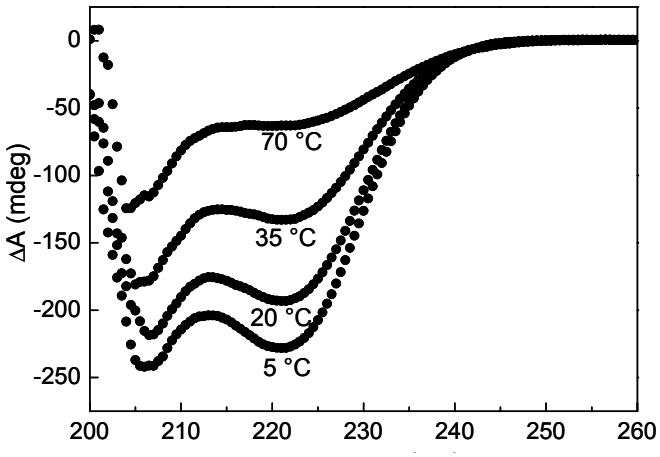

Figure S3. Circular dichroism (CD) spectra of K at increasing temperatures. Conditions: [K] = 2 mg/mL, PBS.

Additional TEM images

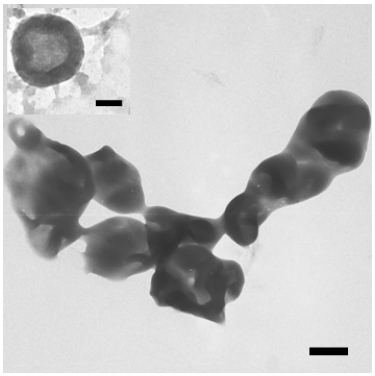

Figure S4. OsO_4 stained TEM image of $PBLG_{50}$-K polymersomes prepared in water by the common slow water addition followed by dialysis method. Inset PTA stained sample. Scale bars = 200 nm.

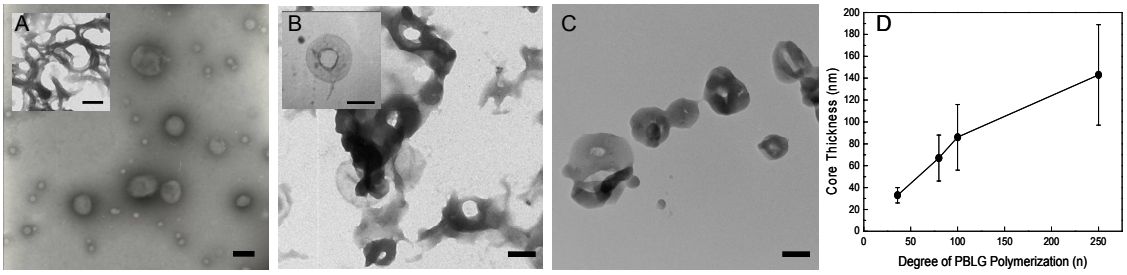

Figure S5. TEM images of polymersomes prepared using A) $PBLG_{36}$-E, B) $PBLG_{100}$-E, and C) $PBLG_{250}$-E. Scale bars = 200 nm. D) Core thicknesses of polymersomes formed from the PBLG-E series (obtained from OsO_4 stained TEM images).

References

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