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The effect of formation and modification methods on the physical properties of polymersomes is critical for their use in applications relying on their ability to mimic functional properties of biological membranes. In this study, we compared two formation methods for polymersomes made from polybutadiene-polyethylene oxide diblock copolymers: detergent-mediated film rehydration (DFR) and solvent evaporation (SE). DFR-prepared polymersomes showed a three times higher permeability compared to SE-prepared polymersomes as revealed by stopped-flow light scattering. SE-prepared polymersomes broke down faster to structures <50 nm diameter when processed with extrusion, which was more pronounced at 5 mg mL−1, compared to 10, 20, and 25 mg mL−1. Our results indicate that the bilayer of SE-prepared polymersomes has a lower apparent fluidity. We also investigated the role of n-octyl-β-D-glucopyranoside (OG), a detergent typically used for reconstitution of membrane proteins into lipid bilayers. Specifically, we compared dialysis and biobeads for OG removal to investigate the influence of these methods on bilayer conformation and polymer rearrangement following detergent removal. There was no significant difference found between method, temperature, or time within each method. Our findings provide insight on how biocompatible polymersome production affects the physical properties of the resulting polymersomes.