Cryogenic Transmission Electron Microscopy Imaging of Vesicles Formed by a Polystyrene–Polyisoprene Diblock Copolymer

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Amphiphilic molecules, including block copolymers, surfactants, and lipids, are well-known to self-assemble in solution, typically forming spherical micelles, thread-like micelles, or vesicles. The last structure has received a great deal of recent attention, especially in terms of the property advantages that might accrue from the use of block copolymers vs lipids, i.e., “polymersomes” compared to “liposomes”. As noted in a recent comprehensive review, polymer vesicles have been formed by a wide variety of ionic and nonionic block copolymers, but almost exclusively in aqueous solution. Exceptions include vesicles from polystyrene (PS)—poly(phenylenoxide) rod–coil diblocks in TFA/methylene chloride mixtures, polyisoprene (PI)—poly(2-cinnamoylethyl methacrylate) in THF/hexane mixtures, PS—poly(4-vinylpyridine) complexed with acids in chloroform, and cyclic PS—PI diblocks in heptane and decane. Given that the selection of the bilayer packing motif, relative to the more highly curved interfaces in cylindrical and spherical aggregates, is presumably dictated by a universal balance of interfacial tension and spontaneous curvature, it is perhaps curious that vesicles have not yet been reported for the most extensively studied copolymer systems such as PS—PI, PS—polybutadiene, or PS—poly(methyl methacrylate) in organic solvents. (In the exception noted above, that of a cyclic PS—PI diblock copolymer, the constraints of the cyclic architecture presumably favor the flatter interface.) It is the purpose of this communication to report the formation of well-defined vesicles by a linear PS—PI diblock copolymer in dialkyl phthalate solvents. A second important aspect of this work is to demonstrate the successful use of cryogenic transmission electron microscopy (cryo-TEM) to image block copolymer vesicles in these nonaqueous systems. Cryo-TEM has emerged in recent years as the method of choice for imaging aqueous assemblies of amphiphilic molecules, as the rapid vitrification process allows examination of the delicate micellar structures without potentially disruptive processes such as staining, microtoming, and solvent evaporation. Some previous examples of cryo-TEM of micelles in organic media have been reported. Finally, we propose a simple explanation for the relative rarity of vesicles in the aforementioned organic block copolymer systems compared to their aqueous counterparts.

We have previously characterized the phase behavior of a series of PS–PI diblocks in dialkyl phthalate solvents in detail, as a function of temperature, concentration, copolymer composition and molecular weight, and solvent composition. The last aspect is particularly interesting, in that the sequence dioctyl, dibutyl, diethyl, and dimethyl phthalates (DOP, DBP, DEP, and DMP, respectively) and their mixtures allows a continuous variation from neutral (nonselective, DOP) to strongly PS-selective (DMP) solvent conditions. For the current study a highly asymmetric diblock, PS–PI 13–71 was selected, where the numerical designation refers to the block molecular weights in kDa. The polymer was prepared by standard living anionic polymerization protocols, as described elsewhere, and has a polydispersity index of 1.02. Solutions were prepared gravimetrically by codissolution in dichloromethane, which was subsequently removed under vacuum over the course of a few days.

Thin specimens (useful areas thinner than 300 nm) were prepared for cryo-TEM, as previously described for aqueous11 and nonaqueous8 systems, by applying a small (ca. 3 μL) drop on a perforated film supported on a copper TEM grid held by the tweezers of the controlled environment vitrification system (CEVS).11 The chamber was kept at 25 °C; because of the low volatility of the phthalates, we did not need to saturate the atmosphere in the CEVS with phthalate vapors. Following blotting of the drop into a thin film, the specimen was plunged into liquid nitrogen. This is a sufficiently good cryogen to vitrify these phthalates. Ethane at its freezing point, our cryogen of choice, dissolves phthalates and many other organic materials and thus cannot be used. The vitrified specimen was loaded into either a Gatan 626 or an Oxford CT-3500 cryo-holder and was examined in an FEI T12 G2 dedicated cryogenic temperature transmission electron microscope. Images were recorded with a Gatan UltrScan 1000 bottom-mounted high-resolution cooled CCD camera, using the Gatan DigitalMicrograph software package.

Figure 1 shows vitrified specimens of 1% solutions of PS–PI 13–71 in 2:3 DEP/DMP (A) and 1:1 DEP/DMP mixed solvents (by weight). In those solvents the polymer self-aggregates into well-defined vesicles, as can be seen in Figure 1A,B,D. The vesicles are clearly resolved; the contrast is reversed with respect to images of vesicles in aqueous media, namely, the polymer appears lighter than the solvent. This is due to difference in the electron density between the polymer and the solvent. The vesicle shell thickness can be estimated at about 40 nm, in good agreement with small-angle X-ray scattering (SAXS) data of 38 nm to be presented elsewhere. The vesicle diameters range from about 100 to 200 nm. In the case of the solvent mixture richer in DMP, the system is at the verge of phase separation. Indeed, arrows in Figure 1B,C indicate vesicles in the process of disintegration, as manifested by loss of contrast (in Figure 1B) and in the agglomeration of vesicles (Figure 1C). It was not possible to prepare homogeneous solutions of this polymer in pure DMP.
Vitrified specimens of organic materials are very sensitive to the electron beam, which may alter the specimen even following very small exposures (electrons per unit area). The chemical changes induced by the beam can wipe out fine details, completely alter the observed nanostructure, and, as we have found out for this system, even cause contrast reversal. Figure 2 shows a vitrified specimen of vesicles that are formed in 1% solution of PS–PI 13–71 in pure DEP. The same field of view is shown as it is exposed to increasing electron dose: 8, 120, and 320 electronsÅ², from parts A to C of Figure 2. It is obvious that even at these very small electron doses the contrast changes dramatically: it almost completely disappears (Figure 2B) and then is reversed relative to the original (Figure 2C). Even at those exposures, 4 orders of magnitude smaller than the electron exposures typically used to image “hard materials”, one changes the contrast from light (polymer) on darker background (the phthalate solvent) to dark (the radiolytically altered polymer) on the lighter background of the vitrified solvent. Electron-beam radiation damage has been studied extensively in vitrified and frozen aqueous systems.14,15 No contrast reversal has been observed for aqueous systems, including polymer micellar solutions.16 While we have not yet studied the phenomenon in detail, no doubt the contrast reversal is due to the chemical changes brought about by the radiolytic effect of the electron beam. We have also observed the same effect of contrast reversal in PS–PI 13–71 solutions containing threadlike micelles and spherical micelles in different mixtures of phthalates.12,13

The images in Figure 1 demonstrate conclusively the ability of linear PS–PI diblock copolymers to form vesicles in organic solvents. As the solvent selectivity is reduced, i.e., made more neutral by increasing the length of the alkyl substituents on the phthalate residue, the same polymer forms threadlike micelles and then spherical micelles.12,13 A thorough study of this morphological progression, including both cryo-TEM and detailed SAXS analysis, will be presented elsewhere.13 The crucial point to emphasize here is that this progression is exactly that seen in other studies of vesicle-forming copolymer systems, such as PS–poly(acrylic acid).12,14 Where increasing the water content in solvent mixtures has been shown to induce the vesicle/thread/sphere sequence. Thus, these results for PS–PI confirm the universality of the overall phenomenon; i.e., a systematic variation of interfacial tension can access the three canonical micellar forms. We propose that the relative rarity of vesicles in nonaqueous systems is simply that it is difficult to make the interfacial tension sufficiently high to favor a flat interface. In the case of PS–PI, for example, the polymers themselves are not particularly strongly segregated; on a scale of cohesive energy density, the two polymers are not that far apart. It is therefore unusual to find a solvent that is sufficiently selective for one block over the other. This is clearly not the case in aqueous systems, where hydrocarbon polymers have very large interfacial tensions against aqueous solutions of either ionic or nonionic water-soluble blocks. In the current system it was also necessary to have rather asymmetric copolymers, with a larger solvophobic block, to achieve a flat interface.

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References and Notes

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