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Nanoparticles with Tunable Internal Structure from Triblock Copolymers of PAA-*b*-PMA-*b*-PS

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ABSTRACT

Polymer nanoparticles containing their own inherent nanostructure (lamellar, bicontinuous-like, or porous) were formed via the self-assembly of a triblock copolymer complexed with a multiamine counterion in mixed solvents. The internal nanostructure is due to local phase separation of the block copolymers and can be tuned by varying the solvent composition, the relative block composition, and the valency of the organic counterion.

It is well-known that lipid amphiphiles can self-assemble into a variety of liquid-crystalline phases, including lamellar, reversed hexagonal, and bicontinuous cubic, when dispersed in aqueous media. Over the past two decades, much work has focused on making lipid particles from the nanometer to micrometer length scales that exhibit interior liquid-crystalline order (cubosomes, hexosomes, etc.) for potential use in drug delivery.[1–4] Furthermore, recent efforts have focused on controlling the size and internal structure of these lipid nanoparticles.[5–9] In most cases, the bulk liquid crystalline phase has to be dispersed into particles via high-energy methods such as sonication or microfluidization and/or a heat treatment.[10,11] Protein macromolecules have also been used to destabilize bulk cubic phases, resulting in three-dimensional nanostructured proteocubosomes (protein-loaded cubosomes).[12,13] More recently, block copolymer nanoparticles with tunable internal structure have been investigated.[14–18]

The advantages of using amphiphilic block copolymers for the formation of nanoparticles with internal structure, relative to lipid and surfactant systems, are their mechanical stability, versatility in terms of chemical composition, molecular architecture, and the availability of selective solvents used for assembly and processing.[19,20] Amphiphilic block copolymers that can be used for assembly into particles with internal structure have been studied for various technological applications due to, for example, the ability to control inorganic particle arrangement on the micro- and nanoscales.[21,22] Recently, there have been advances in the use of block copolymers, as well as homopolymers, for particle formation. Shimomura et al. showed diblock copolymers could be used to form spherical particles exhibiting internal lamellar phase separation by evaporation of a good solvent for both blocks.[14] Okubo et al. used seeded dispersion polymerization to produce micrometer-sized polymer particles with surface pores.[15] In other work, Okubo and coworkers also used seeded dispersion polymerization to produce "onionlike" multilayered poly(styrene)-*block*-poly(methyl methacrylate) particles.[16,17] In addition, Zhao et al. used the reprecipitation method to make polyimide nanoparticles of large size dispersity with surface pores.[18] In this Letter, we demonstrate that by varying the solvent composition, molecular architecture of charged, amphiphilic triblock copolymer, and the valency of an added organic counterion, polymer nanoparticles with either a lamellar, bicontinuous-like, or porous internal nanophase separated structure could be formed simply by self-assembly in solution. Transmission electron microscopy (TEM) was used to confirm the structures of cast samples and cryoTEM was used to characterize these copolymer nanoparticles in situ while in suspension.

We start with the triblock copolymer poly(acrylic acid)-*block*-poly(methyl acrylate)-*block*-polystyrene (PAA-*b*-PMA-*b*-PS) and di- or triamine organic counterions (Scheme 1) in neat tetrahydrofuran (THF), which is a selective solvent for the polystyrene block and produces conditions similar

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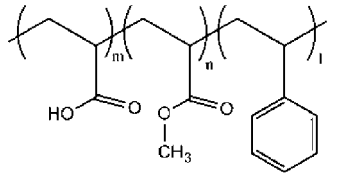
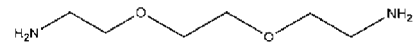
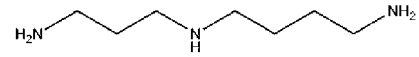
Scheme 1. Chemical Compositions of the PAA-*b*-PMA-*b*-PS Triblock Copolymers and Amine Counterions Used in This Study



	m	n	l	PDI
S66:	99	73	66	1.05
S203:	99	73	203	1.09
S188:	94	101	188	1.13



2,2-(ethylenedioxy)diethylamine (EDDA)



N-(3-Aminopropyl)-1,4-diaminobutane (Spermidine)

to those employed for the reprecipitation method that is commonly used to form polymer nanoparticles.

However, instead of adding a large amount of nonsolvent to the copolymer/counterion/THF solution all at once, water, a selective solvent for acrylic acid, is titrated in at a rate of 15 mL/h while stirring. Then, small aliquots are taken from the solution at different stages of the water addition, resulting in polymer particle suspensions with a final THF/water volume ratio of 1:0 < x < 1:4. The THF is not evaporated off, so the assemblies remain in mixed THF/water solutions. It is important to mention that only a small amount of water (∼2%) in THF is needed for PS to bulk phase separate.[23] In addition, the PAA is strongly complexed with the multiamines.[24] Therefore, the block copolymer−multiamine complex effectively phase separates into polymer nanoparticles. The exact nanophase separated structure within the particles is then dictated by both the relative block lengths and the different solvent compositions that swell the polymer blocks to differing extents.

The use of the counterion is critical for the formation of nanophase-separated particles or micelles as demonstrated in previous work by the Pochan and Wooley laboratories; micellar assemblies of PAA-*b*-PMA-*b*-PS triblock copolymers could be manipulated by varying the amount and type of multiamine added.[25,26] For example, it was found that diamines interact with PAA blocks primarily through inter-PAA block complexation and change the occupied corona volume, thus, changing the interfacial curvature of assembled micelle structures.[26] Important to the work presented here, the multiamine counterion opens a solvent composition window in which bulk phase separation into polymer-rich droplets occurs. The exact solvent compositions for nanostructured polymer particle formation depend on the polymer and counterion being used. The counterion is also important because it can undergo reaction to covalently cross-link these structures within the PAA domains. Additionally, the assembled structures can be kinetically trapped by quick evaporation of the solvent or by shocking the samples with a large amount of water (a nonsolvent for PS), thus vitrifying the high glass transition PS blocks.

The morphologies of these particles were investigated via TEM. TEM micrographs (Figure 1) show that 150−600 nm sized nanoparticles with internal lamellar or disordered, bicontinuous-like nanophase separation were formed from **S66** and **S203**. All of these samples are stained for better contrast; the dark regions in the micrographs are PAA since uranyl acetate preferentially stains PAA while the light regions are composed of PMA and PS.

These samples had an amine-to-acid molar functionality of 1:1 using EDDA as the counterion. Figure 1A shows spherical nanoparticles of relatively uniform size (∼200 nm) formed from triblock copolymer **S66** at a THF:water volume ratio of 1:0.4. These particles exhibit internal lamellar phase separation. This represents the majority structure, although a minority of particles with internal hexagonal phase and individual cylindrical micelles were also seen. Panels B and C of Figure 1 show particles that were made from **S203** using EDDA exhibiting a disordered, bicontinuous-like internal phase and a lamellar phase, respectively, depending on the THF:water volume ratio. These particles bulk phase separate

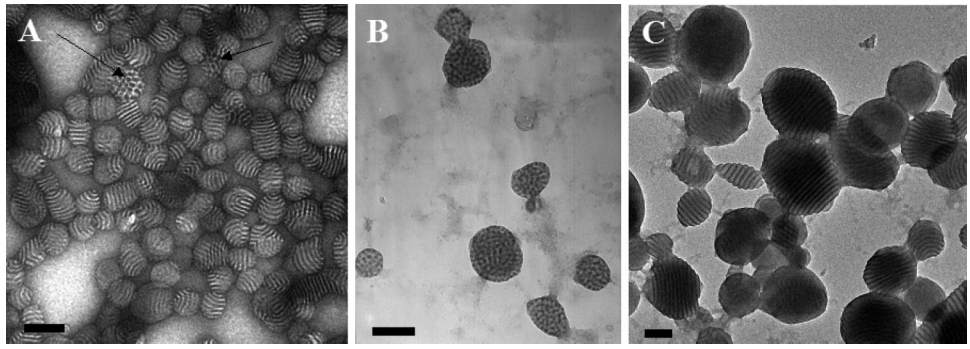


Figure 1. TEM images showing nanoparticles with phase-separated structures formed via the self-assembly of the triblock copolymer complexed with the EDDA counterion in mixed solvents: (A) **S66** at 1:0.4 THF:water volume ratio showing lamellar phase separated particles and a few with a hexagonal phase; **S203** at THF:water volume ratios of (B) 1:0.2 showing particles with a bicontinuous-like phase and (C) 1:0.8 showing lamellar phase separated particles. Each sample was stained with uranyl acetate and the scale bars are 200 nm.

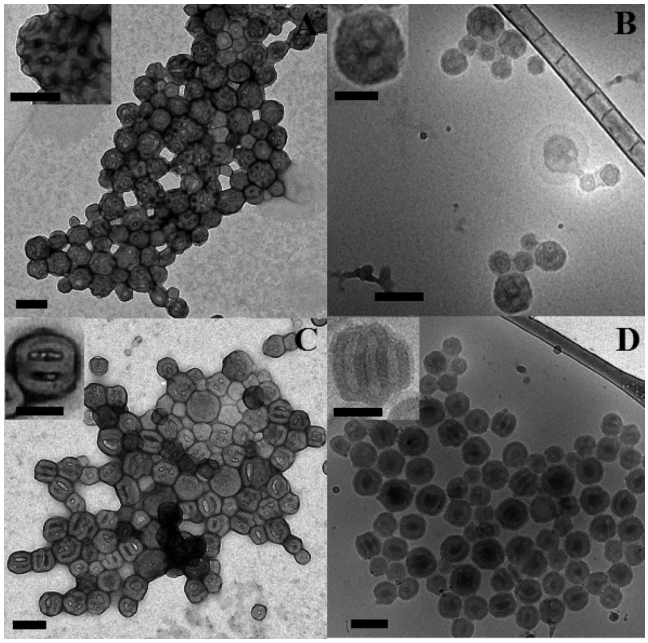


Figure 2. TEM micrographs of porous and channel-like particles of **S188** and spermidine. Stained, cast TEM images and cryoTEM images at THF:water volume ratios of (A, B) 1:0.8 and (C, D) 1:1.7. Scale bars are 200 nm for the large images and 100 nm for the insets.

from solution into polymer/amine droplets and further nanophase separate internally into different structures depending on the block length and solution conditions. For **S203**, parts B and C of Figure 1 illustrate that as the water content increases from a THF:water volume ratio of 1:0.2 to 1:0.8, the PAA chains become more swollen with water and create flatter interfaces within the particles resulting in an internal lamellar phase separation, as opposed to residing on a concave interface at lower water content as seen in Figure 1B. With an increase in the length of PS from 66 to 203, the lamellar spacing within the particles could be increased from ~20 to ~44 nm, respectively, between panels A and C of Figure 1. These values were calculated from the TEM images.

Unique, porous nanoparticles were seen with **S188** at a THF:water volume ratio of 1:0.8 and an amine:acid molar functionality of 1:1 using trivalent spermidine as the counterion (Figures 2A,B). On the basis of the cryoTEM data, solvent-rich pores continue through the interior of the particles and are not simply on the surface (Figure 2B). Panels C and D of Figure 2 show that by increasing the water content of the **S188/** spermidine solution to a THF:water ratio of 1:1.7, the sizes of the particles remain constant (~150 nm), but the internal structure changes from a porous structure, similar to a bicontinuous emulsion phase, to a more channel-like phase. Therefore, one can manipulate the internal structure of these triblock copolymer nanoparticles by varying the solution conditions. At the solvent compositions studied here, the overall particle size for a single polymer/spermidine combination is primarily determined by the stirring process when the polymer first precipitates from pure THF on water titration. Therefore, the particle size stays relatively constant for a single block copolymer at different THF:water ratios such as in Figure 2. In the unstained, cryoTEM micrographs (Figure 2B,D), the dark regions represent the hydrophobic PS and PMA blocks, surrounded by the pores which are lighter because they are filled with solvent. The PAA cannot be seen in the cryoTEM as it is confined to the edges of the solvent-filled channels and highly swollen with solvent. However, the pores or channels appear smaller in the cast particles since the PAA blocks are no longer swollen with solvent, are now visible, and are occupying some of the pore or channel volume. Figure 3 shows a cartoon schematic of the location of the polymer domains in these nanoparticles.

The mechanism for the formation of these unique nanoparticles may be similar to that proposed for bowl-shaped micelles[27,28] or porous polyamide nanoparticles.[18] According to these reported, proposed mechanisms, nanophase separation occurs within the triblock copolymer nanoparticle upon the addition of water, resulting in polymer-rich and solvent-rich regions. The bubbles of the solvent-rich phase can become trapped, leading to a porous structure. As more water

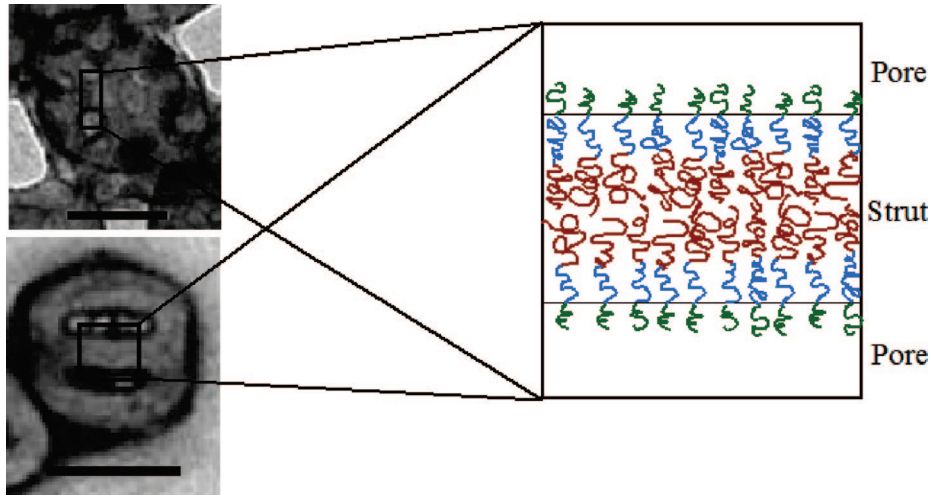


Figure 3. Cartoon schematic of nanophase separated polymer particles showing where the different blocks are located. Red, blue, and green represent PS, PMA, and PAA, respectively. The counterion has been omitted for clarity. Scale bar equals 100 nm.

is added, these bubbles coalesce resulting in the channel-like structures as seen in Figure 2C,D. Another explanation is that these particles may be forming microemulsions similar to those in lipid systems; i.e., a bicontinuous phase consists of a swollen polymer matrix phase with channels full of bulk solvent.

In summary, nanoparticles with different internal structures were formed by PAA-*b*-PMA-*b*-PS triblock copolymers in THF/water mixed solvents in the presence of multivalent organic counterions. The internal lamellar spacing of the particles could be tuned by using triblock copolymers with different block lengths. The triblock copolymer with the longer PS block length, **S203**, exhibited a lamellar spacing of ~44 nm compared to that of 20 nm within the particles made from the triblock with the shorter PS block length, **S66**. In addition, the internal symmetry of the nanoparticles made from **S203** was changed simply by changing the solvent composition. Within another polymer/amine system, **S188** and spermidine, nanoparticles with pores or channels were produced conveniently by manipulation of the water content. Therefore, we have demonstrated that by changing the lengths of the blocks, the solvent composition, and the valency of the counterion one can control the size, shape, and internal structure of triblock copolymer nanoparticles. These unique triblock copolymer nanoparticles could be interesting materials for catalysis, templating, or delivery applications.

Experimental Section. Triblock copolymers of acrylic acid, methyl acrylate, and styrene (Scheme 1) were dissolved in THF forming a 0.1 wt % solution. The synthesis of these triblock copolymers has been described elsewhere.[29] Next, a bifunctional (EDDA) or a trifunctional (spermidine) amine counterion was added so that the molar ratio of amine to acid functionality was 1:1. This solution was allowed to stir overnight. EDDA and spermidine were obtained from Aldrich Chemical Co. and were used as received. For self-assembly, Milli-Q deionized water was added with a syringe pump at a rate of 15 mL/h. Small aliquots of solution were taken from the solution at different stages of water addition.

Transmission electron microscopy was used to examine the polymer nanoparticles. The TEM samples were prepared by dropping 4 μL of polymer solution onto a carbon-coated copper TEM grid and allowing the solution to dry at ambient temperatures (approximately 30 min). Once dry, 4 μL of aqueous uranyl acetate was used to stain the samples. CryoTEM was also used to examine some of the nanoparticles in situ. For cryo-imaging, a thin film of solution was added to lacey carbon TEM grids. The grids were then plunged into liquid ethane at −170 °C using an automated cryo preparation system (FEI Vitrobot). The vitrified samples were transferred to a Gatan 626 cryoholder and cryotransfer stage. During observation of the vitrified samples the cryoholder temperature was maintained at −175 °C to prevent sublimation of vitreous water and/or THF. Images of the stained samples and the vitrified samples were taken in bright-field mode either with a Technai G2 12 transmission electron microscope at 120 kV accelerating voltage or with a JEOL 2000FX transmission electron microscope at 200 kV accelerating voltage.

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