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Morphogenic Effect of Added Ions on Crew-Cut Aggregates of Polystyrene-*b*-poly(acrylic acid) Block Copolymers in Solutions

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ABSTRACT: The morphology of crew-cut aggregates of amphiphilic block copolymers in dilute solutions can be controlled by the addition of ions in micromolar (HCl, NaOH, $CaCl_2$, $Ca(Ac)_2$) or millimolar (NaCl) concentrations. The copolymers are highly asymmetric polystyrene-*b*-poly(acrylic acid) diblocks, PS-*b*-PAA, in which the lengths of the insoluble PS blocks are much longer than those of the soluble PAA blocks. In addition to spherical, rodlike, and univesicular or lamellar aggregates, large compound vesicles (LCVs), a new morphology, can be obtained from a single block copolymer. The morphogenic effect of different added ions on the crew-cut aggregates can be ascribed to the changed repulsive interactions among the hydrophilic PAA segments, due to neutralization by NaOH, protonation by HCl, ion-binding or bridging by Ca^{2+}, and electrostatic screening by NaCl, respectively. The formation of the LCVs may involve a secondary aggregation of individual vesicles and a subsequent fusion process. Some features of the spontaneously formed LCVs may make them especially useful as drug delivering vehicles, and as models of biological cells.

1. Introduction

The self-assembly of highly asymmetric amphiphilic block copolymers in solutions has been receiving much attention in this laboratory[1−5] and in others.[6,7] The aggregates are "crew-cut" because their cores are made from long core-forming blocks, attached to much shorter corona forming blocks.[8] For polystyrene (PS) based copolymers,[1−5] aggregates of this type were prepared by dissolving copolymers in a common solvent for both of the blocks, then adding water to induce the aggregation of the PS blocks. The aggregates were isolated into water by dialyzing the resulting solution. Due to the large fraction of the insoluble PS blocks in the copolymers and the resulting extremely low critical micellization concentration (cmc) as well as the glassy nature of the PS cores at room temperature, the structures of the aggregates are kinetically frozen and observable directly by electron microscopy. For polystyrene-*b*-poly(acrylic acid), PS-*b*-PAA, diblocks, it was found that in dilute solution aggregates of various morphologies can be made depending on the copolymer composition.[3] Specifically, as the length of the PAA block decreased, the aggregate morphology changed from spherical to rodlike, to lamellar or vesicular, and finally to large compound micelles (LCMs) consisting of an assembly of inverted spherical micelles surrounded by a hydrophilic surface. The morphology of the aggregates is undoubtedly a result of the interplay of several different factors during the formation of the aggregates. Most important is the force balance involving three factors, i.e. the deformation (stretching) of the PS blocks in the core, the surface tension between the core and the solvent, and the interactions among the corona chains. It was also shown that polystyrene-*b*-poly(ethylene oxide) diblocks, PS-*b*-PEO, in dilute solution, can also form aggregates of different morphologies from block copolymers of different compositions.[5]

Very recently, in a short report,[4] we described the phenomenon of added-ion-induced morphological changes in crew-cut aggregates of amphiphilic block copolymers. It was shown that the morphology of the aggregates made from an identical block copolymer can be controlled by the addition of ions in micromolar ($CaCl_2$ or HCl) or millimolar (NaCl) concentrations to the original solutions. Morphological changes at such low added ion contents are significant. The morphologies are the same as those produced by changing the copolymer composition in the absence of added ions. For PS-*b*-PAA copolymers, the morphogenic effect of added ions on the aggregates is probably a result of decreased repulsion among the PAA segments due to protonation (by HCl) or ion-binding or bridging (by Ca^{2+}). However, the added ion effect seems to be general because it has also been observed in systems of block copolymers in which the hydrophilic blocks are ionic (P4VP·MeI) or nonionic (PEO). That report also described aggregates of a new morphology, i.e. large compound vesicles. These may be especially useful as possible drug delivery vehicles, and as models of stable microstructured biomaterials. In addition, an ion-induced gelation phenomenon was demonstrated in which spherical micelles in aqueous solution form a cross-linked "pearl necklace" structure in the presence of 20 mM HCl.

In this paper, we present a detailed study of the morphogenic effects of added ions on the crew-cut aggregates of polystyrene-*b*-poly(acrylic acid) diblocks in solutions of low molecular weight solvents.

2. Experimental Section

The copolymers used in this study are polystyrene-*b*-poly(acrylic acid) diblocks, PS-*b*-PAA. The anionic polymerization method used to synthesize the PS-*b*-PAA copolymers has been described previously in detail.[3] In the text, the copolymer samples are differentiated by the previously developed notation, i.e. PS(410)-*b*-PAA(25) represents a copolymer containing 410 styrene units and 25 acrylic acid units. All copolymers had a relatively narrow distribution of molecular weights, with a polydispersity index (M_w/M_n), estimated by gel permeation chromatography (GPC), in the range of 1.04−1.06.

To prepare aqueous solutions of the crew-cut aggregates, the copolymers were first dissolved in *N*,*N*-dimethylformamide (DMF), a common solvent for both PS and PAA blocks, to give a stock solution. The stock solution was divided into several portions. Different amounts of salt, acid, or base were added

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(as aqueous solutions) to each of the containers. Subsequently, deionized water was added dropwise to the copolymer solutions, with stirring. The increment of water content for each step of the water addition was 0.3 wt % per 10 s. As the addition of water progressed, the quality of the solvent for the PS block decreased gradually. Micellization took place in the range of ca. 4−6 wt % water in solution, as indicated by the appearance of turbidity. However, the addition of water was continued until 25 wt % of water had been added, i.e. well after the aggregation occurred in order to make sure that the structure of the formed aggregates is kinetically frozen.[9] The aggregates were isolated into water by dialyzing the resulting solutions to remove the DMF. In the present studies, the initial polymer concentration in DMF (before the addition of water) was 1 wt % for most of the studies unless specified otherwise.

Transmission electron microscopy (TEM) was performed on a Phillips EM410 microscope operating at an acceleration voltage of 80 kV. To prepare TEM samples for the study of the size and the morphology of the aggregates, a drop of the dilute aqueous solution (about 1 mg/mL) was deposited onto a copper EM grid, which had been precoated with a thin film of Formvar and then coated with carbon. Two minutes after the deposition, the aqueous solution was blotted away with a strip of filter paper. Finally, the grids were shadowed with a palladium/platinum alloy.

Proton NMR spectra in solution were recorded on a Varian XL-200 spectrometer. Deuterated *N*,*N*-dimethylformamide (DMF-d_7, 99.5% D; from Aldrich) was used as the solvent. Water was added directly to the polymer/DMF solutions in the NMR sample tubes with a microsyringe. Vigorous shaking homogenized the solution. All the experiments were performed at 18 ± 0.2 °C. Tetramethylsilane served as the internal reference for the zero point of chemical shift, and the residual proton resonance of the solvent served as another internal reference, its chemical shift being set to 8.01 ppm. The chemical shift of the water protons was an average over the different types of water protons because of the fast exchange.

3. Results and Discussion

3.1. Effect of Added Acid on the Aggregate Morphology. Figure 1 presents a set of TEM pictures which show aggregates of various morphologies prepared from the same diblock copolymer but with different amounts of added HCl. The copolymer is PS(410)-*b*-PAA(13) at an initial concentration 1 wt % in DMF. Without the added acid, this polymer yielded small spherical micelles of low polydispersity with an average diameter of 31 nm (Figure 1A). When the added HCl concentration was 113 μM (or a molar ratio, R, of added HCl to acrylic acid repeat units of 0.04), the aggregates were still spherical (Figure 1B), but with an average diameter of 37 nm. However, as the HCl concentration was increased to 141 μM ($R = 0.05$), vesicular aggregates started to form; most of the aggregates were still spherical (Figure 1C). As the added acid concentration was increased further to 155 μM ($R = 0.055$), most of the aggregates became vesicular (Figure 1D). When the HCl concentration reached 170 μM ($R = 0.06$), the aggregates were still vesicular, but the average size increased (Figure 1E). When the HCl concentration was 200 μM ($R = 0.07$), most of the aggregates became tubular or elongated vesicles (Figure 1F), with many small vesicles seen in the background. At an HCl concentration of 225 μM ($R = 0.08$), large compound vesicles (LCVs) were formed (Figure 1G). The same morphology was observed at an HCl content of 253 μM ($R = 0.09$), but the aggregates became more spherical, the average size decreased, and the structure became more dense. A schematic cross-section of these multivesicular aggregates is shown in Figure 1I.

3.2. Effect of Added NaOH on the Aggregate Morphology. Addition of NaOH can also induce morphological changes. Figure 2 shows the TEM pictures of the aggregates prepared without and with the addition of different amounts of NaOH. The copolymer is still PS(410)-*b*-PAA(13), but at a 2 wt % concentration in DMF. As shown in Figure 2A, at that concentration and without any added NaOH, the copolymer yielded vesicular aggregates. However, when the NaOH concentration was 28 μM ($R = 0.005$), Figure 2B shows that most of the aggregates became spherical with an average diameter 38 nm, while vesicles were only observed occasionally. As the NaOH concentration was increased to 56 μM ($R = 0.01$), the aggregates were spherical with an average diameter of 35 nm (Figure 2C). At a NaOH concentration of 115 μM ($R = 0.02$), the average diameter decreased further to 33 nm.

3.3. Effect of Added Salt on the Aggregate Morphology. The aggregate morphology can also be changed by the addition of salt. It was found that the effect of divalent ions, e.g. Ca^{2+}, is much stronger than that of univalent ions, e.g. Na^+. Figure 3 shows that the morphology changes progressively as a function of added NaCl concentration. The copolymer is PS(410)-*b*-PAA(25) with an initial concentration of 1 wt % in DMF. Specifically, without added salt, the copolymer formed spherical aggregates with an average diameter of 29 nm (Figure 3A). When the concentration of added NaCl was 1.1 mM ($R = 0.20$), the aggregates were spherical with an average diameter of 33 nm (Figure 3B). At a NaCl concentration of 2.1 mM ($R = 0.40$), the average diameter of the aggregates increased further to 37 nm (Figure 3C). When the NaCl concentration was increased to 3.2 mM ($R = 0.60$), the aggregates became cylindrical (Figure 3D). In the concentration range of 4.0 to 10.6 mM NaCl ($R = 0.80-2.0$), cylindrical and vesicular aggregates coexisted. However, as the NaCl concentration was increased, the majority of the aggregates shifted from cylindrical to vesicular and the overall size of the vesicles increased progressively (Figure 3E−G). When the NaCl concentration was increased to 16 mM ($R = 3.0$), the aggregates became large vesicles with diameters up to 400 nm, while rodlike aggregates were observed only rarely (Figure 3H). Finally, LCVs were formed when the NaCl concentration was above 21 mM ($R = 4.0$) (Figure 3I). Table 1 summarizes the given dominant morphologies of the aggregates made from different copolymers vs the added NaCl concentration.

When $CaCl_2$ was added instead of NaCl, it was found that much lower concentrations of the added salt were needed to induce morphological transitions. Figure 4 shows the morphologies as a function of added $CaCl_2$ concentration. The copolymer is PS(410)-*b*-PAA(13). As mentioned earlier, without added ions, the aggregates were spherical with an average diameter of 31 nm (*cf.* Figure 1A). When the added $CaCl_2$ concentration was 71 μM ($R = 0.025$), the micelles were still filled spheres, but their average size increased to 39 nm (Figure 4A). When the $CaCl_2$ concentration was 85 μM ($R = 0.03$), the copolymers started to form vesicles, while most of the aggregates were still spherical (Figure 4B). When the $CaCl_2$ concentration was between 90 and 115 μM ($R = 0.032$ and 0.04), the copolymer yielded vesicles (Figure 4C−E); however, with increasing $CaCl_2$ concentration, the average size of the vesicles increased rapidly, e.g. with outer diameters up to 300 nm in Figure 4E. Finally, spherical LCVs were formed when

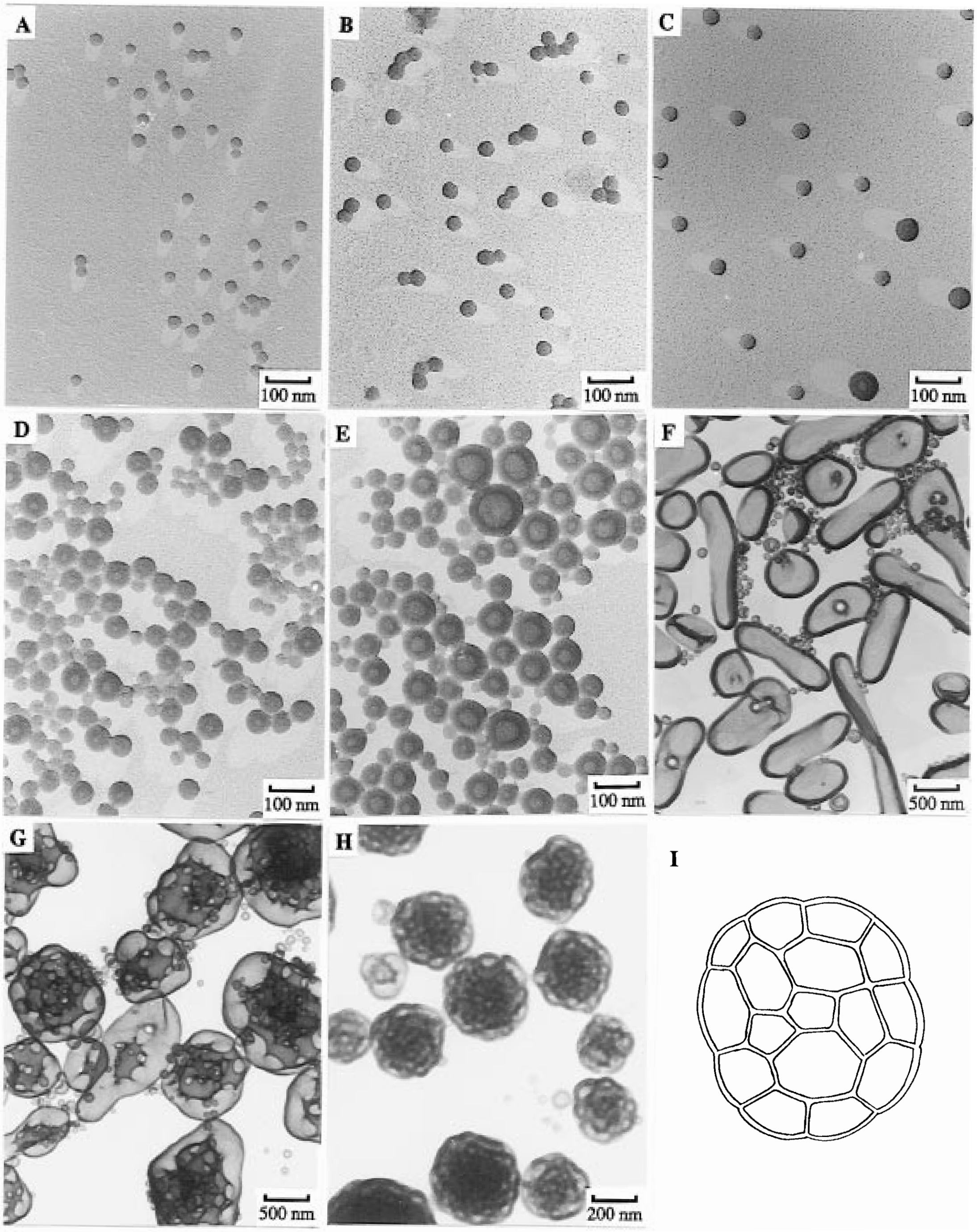

Figure 1. Aggregates from PS(410)-*b*-PAA(13) without any additive (A) and with added HCl to different final concentrations: (B) 113 μM ($R = 0.04$); (C) 141 μM ($R = 0.05$); (D) 155 μM ($R = 0.055$); (E) 170 μM ($R = 0.06$); (F) 200 μM ($R = 0.07$); (G) 225 μM ($R = 0.08$); (H) 253 μM ($R = 0.09$). (I) Schematic picture of the cross-section of an LCV in (H).

the $CaCl_2$ concentration was between 130 and 170 μM ($R = 0.045$ and 0.06). One example is given in Figure 4F, which also shows a range of aggregates, the structures of which are intermediate between univesicular and LCV. For the different copolymers, the observed dominant morphologies of the aggregates at various concentrations of added $CaCl_2$ are summarized in Table 2.

The effect of added $Ca(Ac)_2$ on the aggregate morphology was also explored. For the case of PS(410)-*b*-PAA(25) copolymers, it was found that when the concentration of added $Ca(Ac)_2$ was 650 μM ($R = 0.12$), the aggregates were still spherical with a slightly increased average diameter 30 nm (compared to an average diameter 29 nm of the micelles prepared without added ions). However, when the $Ca(Ac)_2$ concentration was 810 μM ($R = 0.15$), the aggregates became much more polydisperse and contained many particles with diameters over 100 nm. As the added $Ca(Ac)_2$ concentration was increased further, the aggregates became highly polydisperse, and spheres of several hundred nanometers in diameter were formed. These large spheres cannot possibly be primary spherical micelles. They appear to be large compound micelles (LCM) consisting of many inverted spherical micelles surrounded by a hydrophilic surface. The results of added $Ca(Ac)_2$ are also summarized in Table 2 for different copolymers.

3.4. Comparison of the Morphogenic Effect of Added Ions in Amphiphilic Diblocks to Those in Small Molecule Surfactants. It is known that one can change the morphologies of aggregates of small molecule surfactants by adding electrolytes.[10,11] In

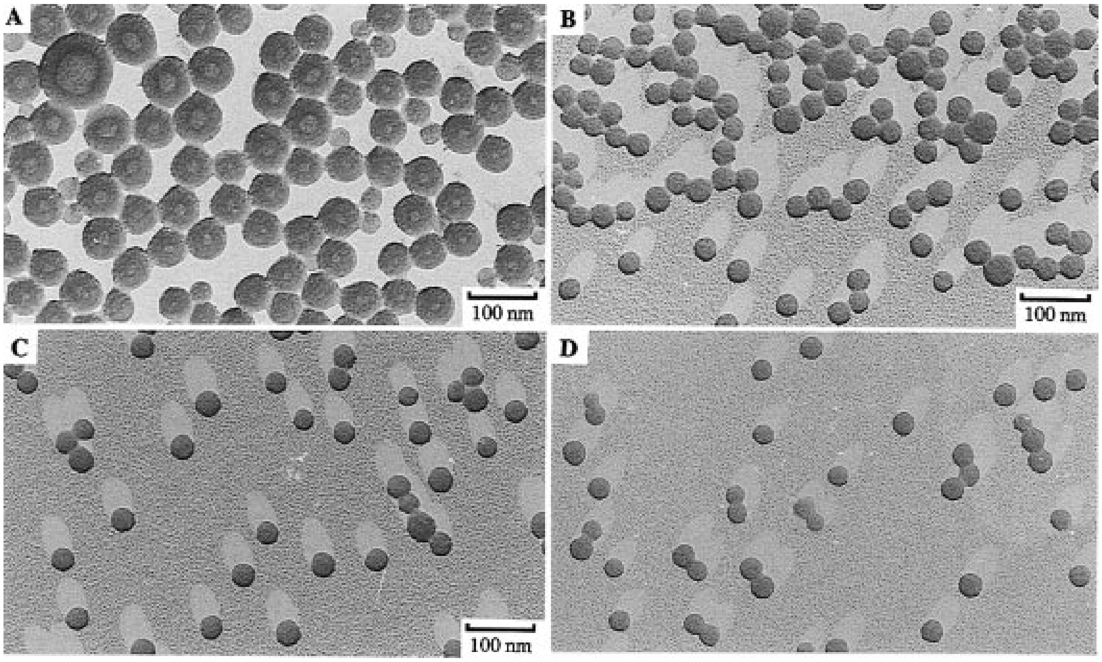

Figure 2. Aggregates from PS(410)-*b*-PAA(13) without any additive (A) and with added NaOH to different final concentrations: (B) 28 μM ($R = 0.005$); (C) 56 μM ($R = 0.01$); (D) 155 μM ($R = 0.02$).

ionic[12−14] and nonionic[15,16] surfactants, it was reported that the addition of salt, e.g. alkali metal chloride, can change the aggregate morphology from spheres to rods, or from rods to vesicles, etc. The changes of aggregate morphology are a result of reduced electrostatic repulsion among charged headgroups due to counterion binding. In those surfactant systems, the added monovalent salt concentration is usually in the range of 10^{-1} mol/L to several moles per liter. The effect of added electrolytes also depends on the structure of the surfactant headgroup, the length of the hydrophobic chain, etc. Usually, the strength of the added salt effect increases as the valence of the salt ions increases, but decreases with increasing length of the hydrophobic chain.[17−19] The importance of electrostatic interactions among headgroups on the phase behavior of surfactant system has been demonstrated in studies of the aggregate structures made from mixtures of anionic and cationic surfactants.[20,21]

Similarly, changing the pH of the solution can also affect the aggregation behavior. In the monoalkyl disodium phosphates,[22] thermally stable unilamellar vesicles with diameters between 25 and 60 nm were formed at room temperature. Lowering the pH leads to partial protonation of the phosphate headgroup and to a decrease of the electrostatic repulsion among the headgroups. This results in large vesicle diameters (50−100 nm). The effect of added $CaCl_2$ was also described.[22] It was found that a large increase in the tendency of the vesicles to aggregate was induced when the added $CaCl_2$ concentration was >0.5 mM.

To our knowledge, ion-induced morphological changes have not been reported before in solutions of block copolymer aggregates. For the present system, it has been shown that the addition of electrolytes can change the aggregate morphology progressively from spheres to rods, to vesicles, and to large compound vesicles. The added electrolyte concentrations at the onset of the morphological transitions are only of the order of 10^{-4} mol/L for $CaCl_2$, NaOH, and HCl and 10^{-3} mol/L for NaCl. It should be recalled that once the aggregates are formed, the copolymer chains are no longer uniformly distributed in the solution. Thus, the acrylic acid units are confined to spherical corona shells around the micelle cores in the spherical micelles; similar considerations also apply to the other geometries. In the corona shell, the concentration of acrylic acid repeat units is estimated to be ~1 M. Under these circumstances, if the microions are distributed evenly in the whole solution, the acrylic acid concentration in the corona shell is about 2 orders of magnitude higher than that of the NaCl, and about 3 orders of magnitude higher than that of the $CaCl_2$. Ion-induced morphological changes in the block copolymer aggregates at such low ion contents are significant. As will be shown in the following section, water preferentially accumulates to some extent around the PAA segments during the formation of the aggregates; i.e. the water content in the PAA corona shell is higher than that in the rest regions of the solution. Therefore, there may be some enrichment of the added ions in the corona shell as well, considering that the ions are most likely more soluble in the water rich regions than in the water-depleted regions. The degree of enrichment of the added ions around the PAA segments is difficult to estimate. However, even if all the added ions were to accumulate around the PAA segments, the effect is still very strong, especially in view of the fact that a concentration change by less than 20 μM of $CaCl_2$ or HCl can induce morphological changes, e.g. from spheres to vesicles for the aggregates of PS(410)-*b*-PAA(13) copolymer. In the following section, we will discuss the possible causes of the effect of the addition of different electrolytes on the morphology of the block copolymer aggregates.

3.5. Cause of the Added Ion Effect on the Aggregate Morphology.

The detailed causes of the effect of added ions on the aggregate morphology probably differ for different additives. However, in general, the effect must be related to the influence of the added ions on the force balance during the formation of the aggregates. Although many factors undoubtedly enter into the force balance, the aggregate morphology is mainly controlled by three factors, i.e. the extension (stretching) of the PS blocks in the core, the surface

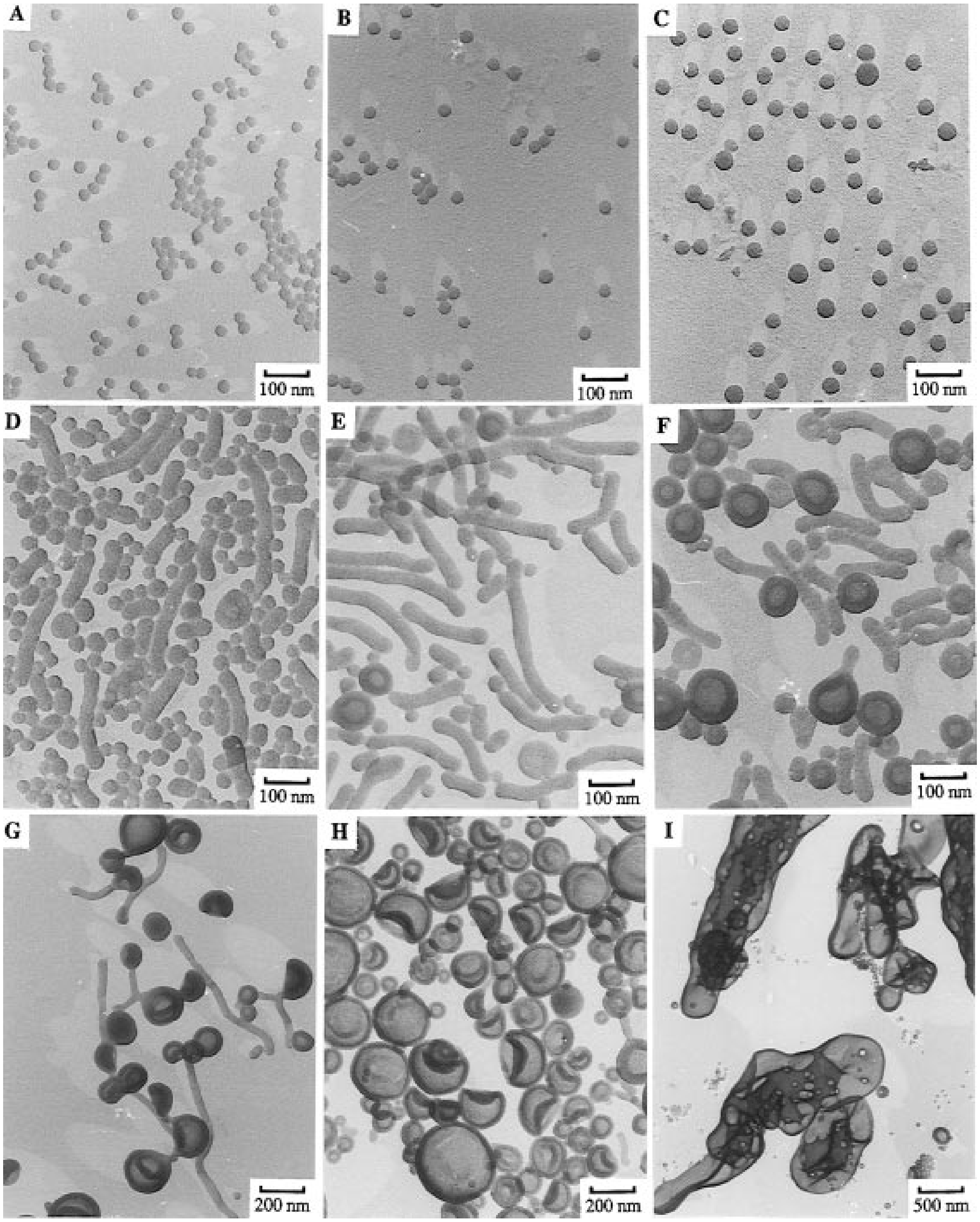

Figure 3. Aggregates from PS(410)-*b*-PAA(25) without any additive (A) and with added NaCl to different final concentrations: (B) 1.1 mM ($R = 0.20$); (C) 2.1 mM ($R = 0.40$); (D) 3.2 mM ($R = 0.60$); (E) 4.3 mM ($R = 0.80$); (F) 5.3 mM ($R = 1.0$); (G) 10.6 mM ($R = 2.0$); (H) 16.0 mM ($R = 3.0$); (I) 21 mM ($R = 4.0$).

Table 1. Summary of the Effect of Added NaCl (M) on the Aggregate Morphology[a]

copolymer	dominant morphology			
PS-*b*-PAA	spheres	rods	vesicles	LCVs
410-*b*-13	$<2.3 \times 10^{-3}$ (0.80)		$(2.8-4.2) \times 10^{-3}$ (1.0−1.5)	5.6×10^{-3} (2.0)
410-*b*-25	$<2.1 \times 10^{-3}$ (<0.40)	$(3.2-5.3) \times 10^{-3}$(0.60−1.0)	$(5.3-16) \times 10^{-3}$ (1.0−3.0)	2.1×10^{-2} (4.0)
660-*b*-24	$<4.8 \times 10^{-3}$ (<1.7)	5.7×10^{-3} (2.0)[b]	$(5.7-8.5) \times 10^{-3}$ (2.0[b]−3.0)	1.1×10^{-2} (4.0)

[a] The initial copolymer concentration in DMF is 1 wt %. The numbers in parentheses represent the molar ratio of added NaCl to acrylic acid. [b] At this NaCl concentration, rodlike micelles and vesicles coexist almost evenly.

tension between the core and the solvent, and the repulsion among the corona chains.[3] Specifically, when the interchain repulsive interactions become weaker, the aggregate size can increase in order to decrease the interfacial energy between the core and the solvent. However, the increase of the core size is restricted by an entropic penalty resulting from the stretching of the PS blocks. At some point, as the strength of the repulsive interactions among corona chains decreases and the stretching of the PS chains increases, the aggregates change their morphology from spherical to rodlike, and from rodlike to bilayer in order to reduce entropic penalty due to PS chain stretching. Therefore, the repulsion among the PAA corona chains, especially

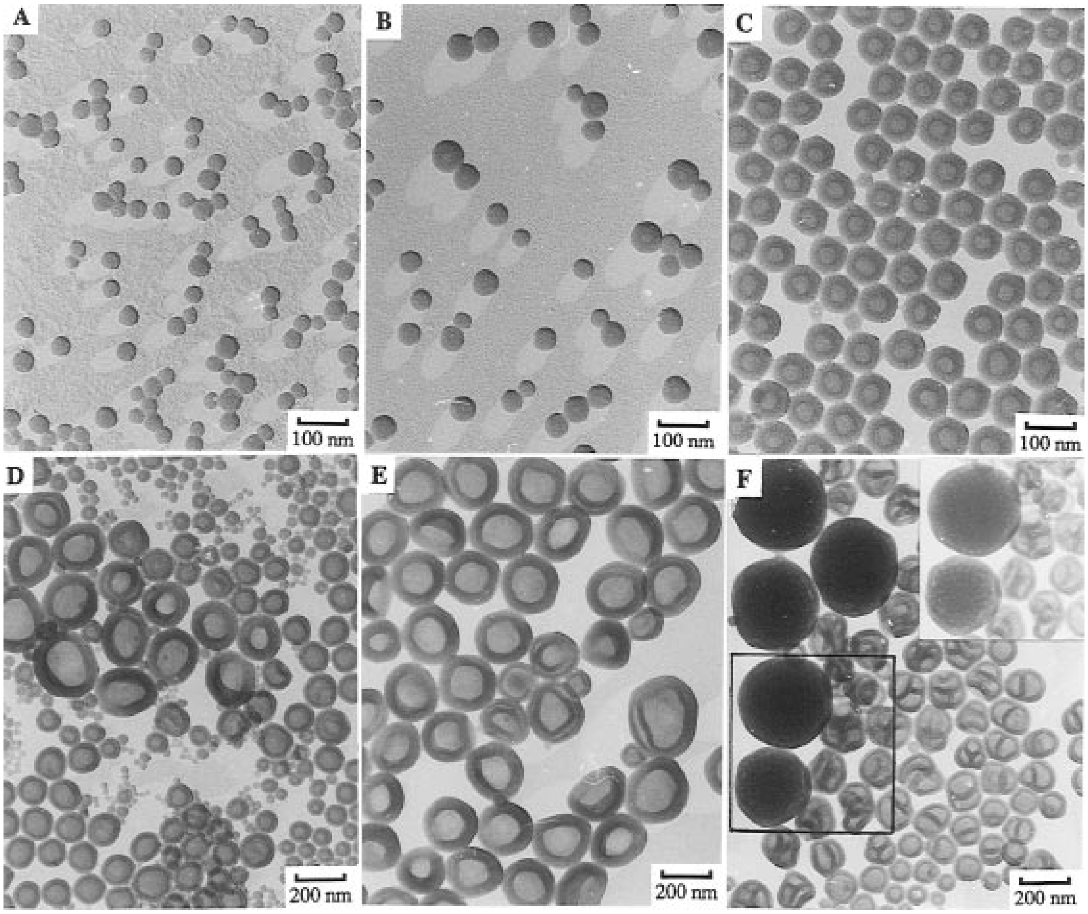

Figure 4. Aggregates from PS(410)-*b*-PAA(13) with added $CaCl_2$ to different final concentrations: (A) 71 μM ($R = 0.025$); (B) 85 μM ($R = 0.03$); (C) 90 μM ($R = 0.032$); (D) 99 μM ($R = 0.035$); (E) 115 μM ($R = 0.04$); (F) 130 μM ($R = 0.045$).

Table 2. Summary of the Effect of Added $CaCl_2$ and $Ca(Ac)_2$ (M) on the Aggregate Morphology[a]

copolymer PS-*b*-PAA	additive	dominant morphology			
		spheres	vesicles	LCVs	LCMs
410-*b*-13	$CaCl_2$	$<8.5 \times 10^{-5}$ (<0.03)	$(9.0-11) \times 10^{-5}$ (0.032−0.04)	$(1.3-1.7) \times 10^{-4}$ (0.045−0.06)	
	$Ca(Ac)_2$	$<2.8 \times 10^{-4}$ (<0.10)			4.0×10^{-4} (0.14)
410-*b*-25	$CaCl_2$	$<1.2 \times 10^{-4}$ (<0.023)	$(1.4-2.0) \times 10^{-4}$ (0.026−0.038)	$(2.4-4.8) \times 10^{-4}$ (0.045−0.09)	
	$Ca(Ac)_2$	6.5×10^{-4} (<0.12)			8.1×10^{-4} (0.15)

[a] The initial copolymer concentration in DMF is 1 wt %. In the table, numbers in parentheses represent the molar ratio of added $CaCl_2$ or $Ca(Ac)_2$ to acrylic acid.

if they are partially ionized, plays a considerable role in determining the final morphology of the aggregates. Certainly, the added ions may also affect the surface tension between the core and the solvent in the course of aggregate formation. However, the strength and direction of this effect is not clear.

3.5.1. Microenvironments of the PAA Chains during Micellization. Before we discuss the influence of the added ions on the repulsion among the corona chains, it is very helpful to explore qualitatively the change in the microenvironment of the PAA segments in the course of water addition. Proton NMR is used for this purpose. Figure 5 shows three typical NMR spectra of PS(180)-*b*-PAA(28)/DMF solutions. Spectrum A was recorded before the addition of water. All three proton peaks originate from the residual protons in the DMF. From the relative intensities of these peaks, the doublet peaks between 2.5 and 3 ppm can be ascribed to the methyl protons in DMF. The single peak at 8 ppm is due to the single proton bonded to the carbon atom. Spectrum B was recorded when the added water concentration was about 0.6 wt %. Obviously, the new peak at ca. 3.8 ppm is due to the water protons. Spectrum C was obtained from the same solution, but with an added water content of 6 wt %. Compared to spectrum B, the peak of the water protons narrowed and its position shifted from 3.8 to 4.0 ppm. Furthermore, splitting of the lines was observed although the side peaks are very weak relative to the major peak.

The information about the distribution of added water in the solutions can be obtained by analyzing the line widths of the water proton NMR spectra. The study is based on the difference in mobility between the water molecules and the polymer chains in solution. Usually, small molecules show a very narrow resonance peak because of their high mobility, while polymer chains give relatively broad NMR peaks because of their high molecular weight and low mobility. Thus, if the added water molecules bond to (or interact with) polymer chains, their mobility is decreased, and the NMR peak will be broadened as a result of this interaction. If, on the other hand, added water molecules distribute themselves evenly in the solution, their mobility should not be affected at all because the major component of the solution, DMF, is also a small molecule. Figure 6 shows a plot of the full-width at half-height (FWHH) of the

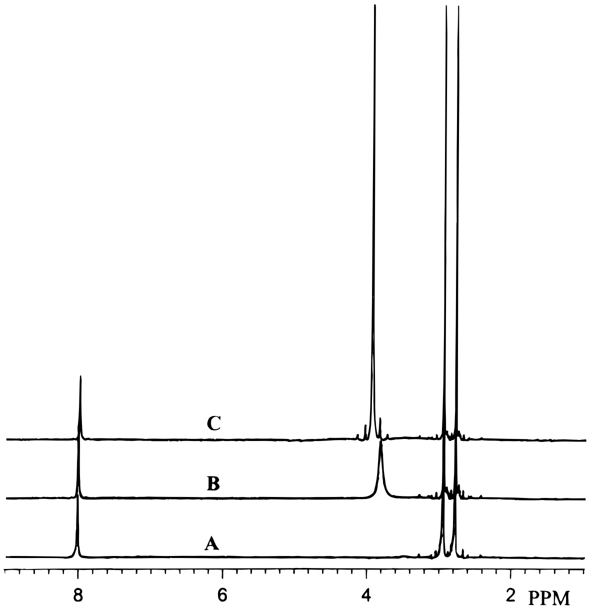

Figure 5. Three typical proton NMR spectra from PS(180)-*b*-PAA(28)/DMF solution before the addition of water (A) and after the addition of water to 0.6 wt % (B) and 6 wt % (C). The copolymer concentration in DMF is 0.1 wt %.

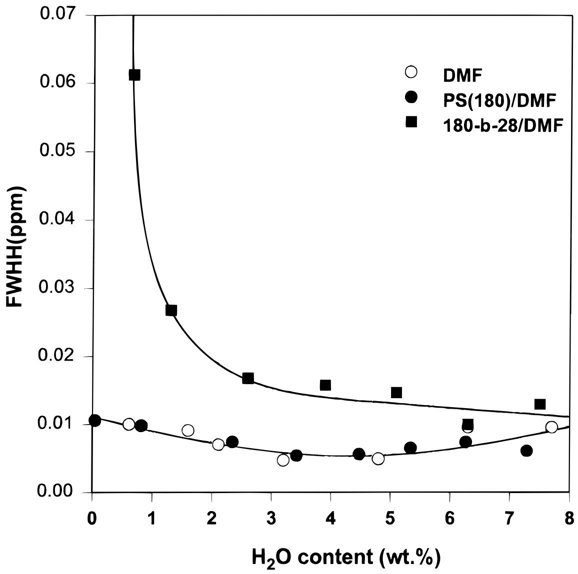

Figure 6. Plot of the full-width at half-height (FWHH) of the water proton NMR peak against the added water content in (○) DMF solvent and in solutions of (●) 1.0 × 10^{-3} (g/g) PS-(180) in DMF and (■) 1.0 × 10^{-3} (g/g) PS(180)-*b*-PAA(28) in DMF. The lines are shown as a guide for the eye.

water proton NMR peak against the added water content in solutions. For both the DMF solvent and the homopolystyrene/DMF solution, as the water content increased, the FWHH of the water proton peak did not change very much, indicating that the water molecules were generally distributed evenly in the solutions. There was evidently no strong interaction between water molecules and homopolystyrene chains. However, for the block copolymer/DMF solution, it was found that the FWHH of the water proton peak was very large at low water contents, and then decreased as the water content increased. The broadened peak of water protons in this case must be a reflection of the interaction between water molecules and the PAA segments as a result of which the mobility of water molecules is reduced. This suggests that, to some extent, the added water accumulated preferentially near the PAA blocks. Therefore, although the micellization usually started at a water content around 5 wt %, the local water concentration around the PAA blocks could actually be higher than the overall water concentration in the solution. On the basis of the above fact, we now address the question of how the added ions affect the repulsion among the PAA blocks (corona chains).

3.5.2. Effect of Added Acid or Base on Inter-Corona-Chain Repulsion. Since the water is preferentially distributed around the PAA segments, the PAA blocks probably behave in a manner similar to that in aqueous solutions, i.e. they are partially ionized. If the acrylic acid were distributed uniformly in the solution, then the concentration of acrylic acid units in a 1 wt % copolymer solution would be about 3−5 mM, depending on the copolymer composition. The degree of the ionization is about 10−13%, as calculated from a pK_a of 4.25 for propionic acid in water. However, we are dealing not with pure water but a DMF/water mixture. Also, the PAA blocks are confined to the coronal shells. Thus, the dissociation of the PAA may be different and is most likely lower. In any case, because HCl is a strong acid and the acrylic acid is a weak acid, the addition of HCl protonates the ionized carboxylic acidic units of the PAA blocks, leading to a still lower degree of ionization. As a result, the charge density of the PAA blocks decreases, and the overall (both electrostatic and steric) repulsion among the corona chains decreases as well. When acetic acid was added instead of HCl, it showed only a very weak morphogenic effect on the aggregates. For PS-(410)-*b*-PAA(25) copolymer, an addition of 210 μM HCl (R = 0.04) changed the aggregate morphology from spherical to rodlike. However, when acetic acid was added, the aggregates were still spherical even when the concentration of added acetic acid was increased to 4 mM (R = 0.8). This may be due to the fact that acetic acid is a weak acid (pK_a = 4.74), and its effect on the ionization of the PAA blocks is very weak.

When NaOH was used as an additive, the morphology was found to change in an opposite direction, i.e. from vesicles to spheres, and then to smaller spheres with increasing NaOH concentration. This effect is not surprising, since the added NaOH neutralizes the PAA segments. As a result, the degree of ionization and the coil dimension of the PAA blocks and the repulsion among the PAA corona chains are increased.

The effect of added HCl on the aggregate morphology was further explored by using PS(410)-*b*-PAA(25) copolymers in which the PAA segments had been preneutralized to a degree of 5% by using NaOH. To neutralize the PAA blocks, the copolymers were dissolved in tetrahydrofuran (THF), and a NaOH/methanol solution was added. The copolymers were then precipitated into methanol and dried under vacuum at 60 °C. The dominant morphologies of the aggregates for various added HCl concentrations are summarized in Table 3. Because neutralization increases the degree of ionization of the PAA segments, a higher concentration of added HCl was needed. One can see that the preneutralization of the PAA blocks by NaOH only shifts the morphological transitions to higher HCl contents but that the overall amount of HCl needed to change the morphology from spheres to LCVs remains practically the same.

3.5.3. Effect of Added Salt on Inter-Corona-Chain Repulsion. The effect of added $CaCl_2$ on the aggregate morphology is remarkably strong. From

Table 3. Summary of the Effect of Added HCl (M) on the Aggregate Morphology of a PS(410)-*b*-PAA(25) Diblock Copolymer with and without Preneutralization[a]

	dominant morphology			
M_{NaOH}/M_{PAA}	spheres	rods	vesicles	LCVs
0	1.9×10^{-4} (0.035)	2.1×10^{-4} (0.040)	2.4×10^{-4} (0.045)	2.7×10^{-4} (0.050)
0.05	4.5×10^{-4} (0.085)	4.9×10^{-4} (0.092)	5.1×10^{-4} (0.095)	5.6×10^{-4} (0.105)

[a] In the table, numbers in parentheses represent the molar ratio of added HCl to acrylic acid.

Tables 2 and 3, one can see that changes in the aggregate morphology can be achieved with much lower concentrations of $CaCl_2$ than of NaCl. At the onset of the morphological transition from spheres to vesicles for PS(410)-*b*-PAA(25), the total amount of $CaCl_2$ in the solution was equal to 2.3 calcium cations per 100 acrylic acid repeat units. In that case, even if all the added Ca^{2+} ions were to interact with the hydrophilic chains, on average two PAA blocks would be sharing one Ca^{2+}. When the aggregates are LCVs, the number of Ca^{2+} ions per PAA block is about 1.5. The morphogenic effect appears to be caused by strong Ca^{2+} binding and bridging to the carboxylate groups of the PAA segments. Because of this ion binding or bridging, a simultaneous HCl effect may also be involved. This speculation is based on the following consideration.

The addition of $CaCl_2$ to the solution is accompanied by binding of the Ca^{2+} ions to the carboxylate anions, which are present in the solution as a result of the slight dissociation of the poly(acrylic acid) segments. This binding thus decreases the carboxylate anion content. However, an acid dissociation equilibrium exists involving the carboxylate anions, protons, and carboxylic acid groups. Since the last two are present in great excess, relative to the Ca^{2+} added, the concentration of COOH group will not change appreciably. Thus, the product of $[H^+][COO^-]$ must remain constant. Since the concentration of COO^- was decreased by calcium binding, a small amount of the COOH groups must dissociate to re-establish the constant $[H^+][COO^-]$ product. In the process, the H^+ concentration increases slightly over what it was before $CaCl_2$ was added. Simultaneously, the COO^- content decreases, which can be regarded as a protonation effect. Thus, it is very possible that the strong morphogenic effect of added $CaCl_2$ on the aggregates is a combined result of calcium ion binding and protonation.

For $Ca(Ac)_2$, strong calcium ion binding and bridging are still operative. However, the protons produced as a result of the ion binding and bridging can combine with the acetate anions to yield acetic acid molecules, the morphogenic effect of which is very weak. From another point of view, the addition of $Ca(Ac)_2$ does not affect the ionization (or charge density) of the PAA segments appreciablly because acetic acid is a weak acid. Therefore, the effect of added $Ca(Ac)_2$ on the aggregate morphology is mainly a result of the Ca^{2+} binding and bridging to the PAA segments. As was shown in section 3.3., when the added $Ca(Ac)_2$ concentration was 650 μM ($R = 0.12$), the aggregates were still spherical and the average core diameter changed only very little. As the concentration was increased further, for example, to 810 μM ($R = 0.15$), the morphology changed directly from small primary spherical micelles to large compound spherical micelles (LCMs). No intermediate morphologies, i.e. rodlike or vesicular, were observed.

The formation of LCMs can be related to the phase separation behavior of the copolymer/DMF solution as the aqueous $Ca(Ac)_2$ is added. Light scattering studies[9] showed that when the added $Ca(Ac)_2$ concentration in a 1 wt % PS(410)-*b*-PAA(25) solution was below 650 μM ($R = 0.12$), the copolymers were still dispersed as single chains at a water content of 3 wt %. Subsequent addition of more water (at constant $Ca(Ac)_2$ concentration) induced PS block aggregation. However, when the added $Ca(Ac)_2$ concentration was increased to 810 μM ($R = 0.15$) (at a water content of 3 wt %), the PAA blocks of the copolymer started to self-assemble to form reverse micellelike aggregates; adding more water at that point induced PS block aggregation also, which produced the large compound micelles (LCMs). The degree of reverse micellelike aggregation increased further as the added $Ca(Ac)_2$ concentration was increased, which resulted in the formation of LCMs of increased size. The formation of reverse micellelike aggregates is thus most likely due to the interactions of the added $Ca(Ac)_2$ with acrylic acid units, i.e. binding and bridging, leading to the formation of calcium acrylate. As the fraction of calcium acrylate in the PAA segments increases, the PAA blocks become less soluble, and finally aggregate. Therefore, in view of the relationship between the aggregate morphology and the added $Ca(Ac)_2$ concentration, the formation of LCMs can be ascribed to the presence of reverse micellelike aggregation, not an increased degree of stretching of the PS blocks in the core. This possibility can also be deduced from changes of the average core diameter of the spherical micelles before the morphological transition occurs, which will be discussed in section 3.6.1. It is worth mentioning here that the phase separation behavior of copolymer/DMF solutions accompanying the addition of aqueous solutions of NaCl or NaOH or $CaCl_2$ was also examined.[9] It was found that in the additive concentration range employed in the present study, the addition of NaCl, $CaCl_2$, or NaOH does not induce reverse micellelike aggregation of the copolymers.

When compared to $CaCl_2$ and $Ca(Ac)_2$, the effect of NaCl is much weaker. The weaker effect on the morphology can be ascribed to a weak Na^+ binding to the PAA segments given that sodium acrylate is a strong electrolyte. The effect is possibly a screened electrostatic field of the partially charged PAA segments in the presence of NaCl.

3.6. Stability and Characteristics of the Various Aggregates. In this section, we discuss the regions of stability of the aggregates of different morphologies in DMF/water mixtures as well as some of their characteristics in water.

3.6.1. Spherical Micelles. In the absence of added ions, it was reported in previous papers[2,3] that the average core radii of spherical micelles vary with copolymer composition according to

$$R_{\mathrm{core}} \sim {N_A}^{0.4} {N_B}^{-0.15} \tag{1}$$

where N_A and N_B are the degrees of polymerization of

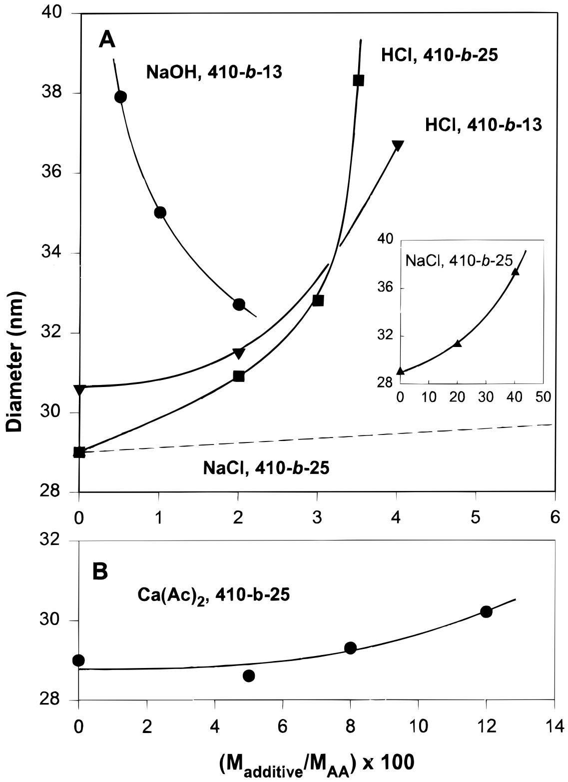

Figure 7. Plot of the average core diameter (D/nm) versus molar ratio of the different additives to acrylic acid repeat units ($M_{\mathrm{additive}}/M_{\mathrm{AA}}$) for spherical micelles from different block copolymers. The type of the additive and the copolymer are indicated near each curve. For the NaOH effect, the initial copolymer concentration in DMF was 2 wt %. For the others, the initial concentration was 1 wt %. The lines are shown as a guide for the eye.

the PS and PAA blocks, respectively. For copolymers with a PS block of constant length, eq 1 implies that the shorter the PAA block, the larger the spherical micelle core. In the present system, for a single block copolymer, the core sizes of the spherical micelles depend on the concentration of added ions. Thus, changing the added ion concentration has an effect on the aggregate morphology parallel to that when the copolymer composition is changed. Figure 7 shows a plot of the average core diameters of the spherical micelles as a function of the concentrations of the different additives.[23] Clearly, the changes of micelle core sizes are not a linear function of the additive concentration. For HCl or NaCl, as the additive concentration increases, the core size increases at first relatively slowly and then more rapidly and finally the morphology of the aggregates changes. For NaOH, the average diameter of the micelles decreases as the added NaOH concentration increases, at first rapidly and then more slowly. The changes in the PS core dimensions are a result of the fact that the addition of NaOH increases the repulsive interactions among the corona chains, while added HCl or NaCl decreases the interchain repulsion. The rapid change of the micelle core sizes just before or after the morphological transition suggests that the thermodynamic penalty from the PS block stretching is largely responsible for the morphological transition.

In the case of added Ca(Ac)$_2$, the core sizes of the small spherical micelles change only slightly as the concentration of added Ca(Ac)$_2$ increases (Figure 7B). Before the morphological transition from small spherical micelles to LCMs, the average core diameter is only around 30 nm, while for the other additives, i.e. HCl, NaOH, or NaCl, the values are around 38 nm. This implies that the penalty from the PS block stretching in the cores is not crucial at this core dimension. Therefore, the morphological transition from spherical micelles directly to LCMs by addition of Ca(Ac)$_2$, as discussed in section 3.5.3, appears to be due to the formation of reverse micellelike aggregates, rather than an increased degree of PS block stretching.

3.6.2. Aggregates of Other Morphologies. As the concentration of added acid or salt increases, the core diameter of the spherical micelles increases. At some point, the aggregates change their morphology progressively from spheres to rods, to vesicles, and eventually to LCVs, in order to reduce the entropic penalty due to the stretching of the PS chains in the micelle core. Therefore, along with the morphological changes, the dimensions of PS core change also. The addition of NaCl to PS(410)-*b*-PAA(25) copolymer solution (*cf.* Figure 3) provides a typical example. When added NaCl concentration is 2.1 mM ($R = 0.4$), the core diameter of the spherical micelles increases to 37 nm. As the added NaCl concentration is increased to 3.2 mM ($R = 0.6$), the PS core diameter of the rodlike micelles decreases to 34 nm. When vesicles form, the thickness of the PS wall decreases again to 25 nm. The decrease of PS core dimension accompanying the morphological transitions is also related to an effect of different geometries of the aggregates on the force balance during micellization. A more detailed discussion can be found elsewhere.[3,24]

Changing the additive concentration has an effect on the aggregate morphology similar to that upon changing the copolymer composition; however, it is more easily controlled if one wishes to obtain a desired morphology. It has been shown that the range of stability of various morphological aggregates is a function of the copolymer composition and molecular weight.[3] However, when the PS block of the copolymers gets longer, the preparation of rodlike micelles and vesicles becomes more difficult because the composition range for these aggregates becomes very narrow. Specifically, when the PS block length of the copolymers exceeds 600 repeat units, it has not been possible to prepare rodlike micelles or vesicles by changing copolymer composition alone without added ions. However, aggregates of various morphologies from PS(660)-*b*-PAA(24) copolymer are obtained easily by adding NaCl to different final concentrations.

The addition of NaCl to PS(410)-*b*-PAA(25) changes the morphology gradually from spheres to rodlike micelles, to vesicles, and to large compound vesicles (LCVs); however, rodlike micelles are missing among the aggregates made from PS(410)-*b*-PAA(13). Even for PS(410)-*b*-PAA(25), when CaCl$_2$ is used as the additive, rodlike micelles have not been observed. A possible reason for the absence of rodlike micelles may be the delicacy of the force balance involved in the formation of different morphologies. The control of the repulsive interactions among the corona chains by the addition of NaCl is relatively easy because of the relatively weak morphogenic effect of NaCl on the aggregates. Therefore, relatively large quantities have to be added to achieve changes in morphology, and the rodlike micelle region can be located even if it is narrow. By contrast, when CaCl$_2$ is used, much smaller quantities of CaCl$_2$ are needed to achieve changes in morphology because of its strong morphogenic effect on the aggregates. Therefore, the region of rodlike micelles is more difficult to locate, in view of the fact that the change of added

$CaCl_2$ concentration is only about 20 μM for the morphological transition from spheres to vesicles for PS(410)-*b*-PAA(25) aggregates, and only 5 μM for PS(410)-*b*-PAA(13) aggregates (*cf*. Table 2). Another possibility is that the region of stability of the rodlike micelles is narrow and depends not only on the PS block length but also on the PAA block length. As the PAA block length decreases, the originally narrow region for the rodlike micelles becomes even more narrow, and more difficult to locate, as it is for PS(410)-*b*-PAA(13).

3.6.3. Large Compound Vesicles. The LCVs appear to be a new morphology for block copolymer aggregates in solution.[4] Their structure bears some resemblance to that of aggregated soap bubbles, and their outer and inner surfaces must be hydrophilic because of the presence of the short PAA chains. The LCVs are irregular in shape when they are made from the copolymers with relatively long PAA segments, for example, PS(410)-*b*-PAA(25) (Figure 3I) or PS(660)-*b*-PAA(21). However, those made from the copolymers with relatively short PAA segments are generally spherical, for example PS(410)-*b*-PAA(13) (Figure 4F). Furthermore, as the added ion concentration increases, the LCVs become more spherical and their sizes shrink because of smaller primary cell dimensions (Figure 1G and 1H). The differences in the geometric shapes of the LCVs may be related to differences in the PAA block length, the dimension of the primary vesicles, the softness of the wall, and the surface energy and the shear conditions under which the LCVs are formed. In the present study, we do not see a further morphological transition from the LCVs to large compound micelles (LCMs), probably because the PAA blocks are not short enough. However, in view of the characteristics of LCVs made from the copolymers with different PAA block lengths and different added salt or acid concentrations, the structure of LCMs has a superficial resemblance to that of LCVs when the inner diameter of the vesicular cell of the LCVs becomes very small so that it looks like a core of reverse micellelike aggregates. Finally, it should be pointed out that LCVs are subject to settling due to gravity. However, they are very stable and do not coalesce at room temperature since they can be resuspended after settling.

While the spherical micelles, rodlike micelles, and individual vesicles resemble morphologies formed mainly under thermodynamic control, the LCVs probably result from kinetic control. Because the thicknesses of both inside and outside walls of these aggregates are uniform and equal, their formation may involve a secondary aggregation of individual vesicles and a subsequent fusion process. For small molecule surfactants, vesicle fusion is believed to occur very rapidly and is usually over within 0.1−1 ms.[25] However, the fusion process of the vesicles made from the copolymers may be much slower because of the lower mobility of these high molecular weight molecules. Therefore, as the added ion concentration increases, the rate of effective collisions of the colloid particles increases. The captured vesicles fuse with each other to form large, less curved, vesicles, as shown in Figures 1, 3, and 4. When the rate of effective collisions of vesicular aggregates becomes faster than the subsequent fusion process as the added ion concentration increases further, the LCVs may result. In Figure 4F, a range of aggregates, the structures of which are intermediate between univesicular and LCV, can be seen. This may be taken as evidence of kinetic control.

The self-assembly of vesicles has become a topic of current interest. In one case,[26] chemical species such as bis(phospholipids) were incorporated into the vesicle walls, to aid their aggregation into higher order (multivesicular) structures. As is shown here, the LCVs can be formed spontaneously under a range of conditions. They are thus a “normal” aggregate morphology. It has been suggested[26] that these higher order structures might be of interest in leading to new methods for processing artificial tissuelike composites and soft biomaterials. In addition, controlled drug delivery appears a distinct possibility since multiple concentric layers might provide a convenient timing mechanism.

4. Conclusions

The morphology of the block copolymer aggregates formed from a single block copolymer can be controlled via the addition of micromolar $CaCl_2$, $Ca(Ac)_2$, NaOH, or HCl or millimolar NaCl. Ion-induced morphological changes in dilute solutions of block copolymer aggregates at such low ion contents are novel and significant. The effect of added ions on the aggregate morphology is parallel to a change in the soluble block length, but it is more easy to control. Although the morphogenic effect of added ions may differ for different additives, it can be ascribed to a changed repulsion among the coronal chains, due to protonation by HCl, ionization by NaOH, ion-binding and bridging by Ca^{2+}, and a screened electrostatic field of charged PAA segments by NaCl. In addition to the various aggregates, i.e. spherical micelles, rodlike micelles, vesicles, and large compound micelles, which had been described before,[3] large compound vesicles, a new morphology, are obtained. The formation of the LCVs may be a result of kinetic control involving collisions of vesicular aggregates and a subsequent fusion process. A number of novel features of the LCVs may make them especially useful as drug delivery vehicles. Furthermore, the spontaneous formation of the LCVs might provide a practical method for making stable artificial tissuelike composites and soft biomaterials with tunable properties. Thus, the versatility of crew-cut micelles is much greater than was originally suspected. From a fundamental point of view, the discovery of the strong effect of added ions on the morphology of polymeric amphiphilies in dilute solution may lead to an improved understanding of the nature of interactions in these systems, and their consequences for the structure of macromolecular assemblies.

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