Additives induced morphological transition of molecular assemblies from PS-*b*-PGEA in aqueous solutions

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Polystyrene-*block*-poly[2-(β-D-glucosyloxy)ethyl acrylate] (PS-*b*-PGEA) can form a variety of molecular assemblies in aqueous media when initially dissolved in water-soluble organic solvents, such as *N*,*N*-dimethylformamide (DMF), THF, etc. The morphologies of these molecular assemblies are affected by the copolymer composition, the nature of the solvent and the initial copolymer concentration. Thus, morphological transformations can be realized by varying the above parameters. In this paper, the effects of additives, such as glucose, HCl, *Concanavalin A* (*Con A*), and $CaCl_2$, on the aggregates of PS-*b*-PGEA in dilute aqueous solutions have been investigated. PS_{77}-*b*-$PGEA_6$ yielded predominantly spheres in water when initially dissolved in DMF at a concentration of 0.1–0.2 wt %. A transformation of the morphology to bilayer structures upon addition of different amounts of glucose, HCl, *Con A* or $CaCl_2$ was observed. The morphological transitions can be ascribed to the decrease of the repulsive interactions among corona-forming blocks.

Much recent attention has been paid to the self-assembly *behavior* of amphiphilic block copolymers in water. These copolymers can self-*organize* into various ordered mesophases as the result of the association of insoluble blocks in water.[1–5] When the hydrophobic blocks of amphiphilic block copolymers are much longer than the hydrophilic blocks, the aggregates formed in water are of multiple morphologies, including spheres, rods, lamellae, vesicles, tubules, large compound micelles and large compound vesicles, *etc*.[6–7] The preparation method of these aggregates is different from that of amphiphilic diblock copolymers with long hydrophilic chains. Comparing with analog formed from small surfactant molecules, these molecular assemblies from amphiphilic block copolymers exhibit the following characteristics: (1) the range of molecular assemblies can be controlled by simply changing the copolymer composition; (2) the ordered structure can be easily changed from one type to another by slightly changing the preparation methods of a single polymer; and (3) these aggregates are much more stable in water. So far, several block copolymers have been investigated for their multiple morphologies of aggregates in aqueous media, including polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA), polystyrene-*block*-poly(ethylene oxide) (PS-*b*-PEO), polystyrene-*block*-poly(4-vinylpyridine) (PS-*b*-P4VP) and polyethylethylene-*block*-poly(ethylene oxide) (PEE-*b*-PEO) in water,[8,9] as well as polystyrene-*block*-poly(2-vinylpyridine) (PS-*b*-P2VP) and polystyrene-*block*-poly(2-cinnamoylethyl methacrylate) (PS-*b*-PCEMA) in organic solvents.[10,11]

We are interested in constructing molecular assemblies from amphiphilic block copolymers with glycopolymers as the hydrophilic segment. These assemblies should have potential to be used as models for cellular-specific target drug delivery systems or as building blocks for highly ordered supramolecular architectures. We have recently reported a new type of amphiphilic diblock copolymer, polystyrene-*b*-poly[2-(β-D-glucopyranosyloxy)ethyl acrylate] (PS-*b*-PGEA), which could form a variety of molecular assemblies in aqueous medium by first dissolving in an organic solvent, followed by addition of water.[12] The morphologies were affected by the composition of the block copolymer, the solvent nature and temperature.[13]

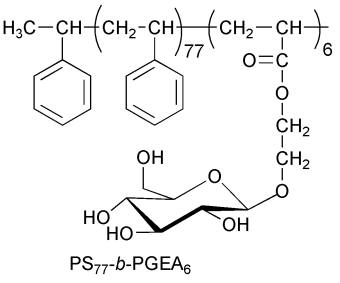
It has been reported by Eisenberg *et al.* that the addition of a small amount of ions into systems containing PS-*b*-PAA has a very important and complicated effect on the aggregation behavior through its influence on the repulsive interactions among the aggregates' corona chains.[8*b*,14] The morphology of PS-*b*-PAA aggregates can be controlled by the addition of HCl, NaOH, $CaCl_2$, $Ca(Ac)_2$, or NaCl to the initial copolymer solutions in DMF. The morphological change of PS-*b*-P4VP aggregates can be obtained by changing the apparent pH in DMF–H_2O mixtures, while the value of the apparent pH is related to the concentration of HCl or NaOH.[15] For PS-*b*-PEO, with nonionic hydrophilic blocks, the aggregate morphology can also be influenced by the addition of inorganic salts, such as KF, KI, KCl, NaCl, or LiCl.[9*b*] In addition, the pH-related aggregation behavior of P2VP-*b*-PEO was studied by Martin and co-workers.[16] With increasing pH, the degree of the protonation of P2VP decreases, and thus its solubility in water decreases. Micelles were obtained with P2VP block as the core and PEO block as the corona at pH > 5. Other examples of morphological transformations by changing pH or temperature for aggregates from diblock copolymers have also been reported recently by Armes *et al.*[4*c*]

In this paper, we present the results of a transmission electron microscopy (TEM) study of the effects of additives (glucose, HCl, *concanavalin A* (*Con A*), NaOH, KF, and $CaCl_2$) on the aggregates of PS_{77}-*b*-$PGEA_6$ (Scheme 1) in aqueous medium.

Experimental

Materials

Glucose and inorganic materials were of analytical reagent grade from China; they were used as aqueous stock solutions: [glucose] = 5 M, [HCl] = 1 M, [$CaCl_2$] = 0.2 M. *Concavalian*



PS_{77}-*b*-$PGEA_6$

Scheme 1

A was from Sigma and used as a 20 mg mL^{-1} aqueous solution. PS-*b*-PGEA was prepared by atom transfer radical polymerization (ATRP) and the detailed procedure can be found in a previous paper.[17] Briefly, styrene was first polymerized with 1-phenylethyl bromide (1-PEBr), $Cu^{I}Br$ and 2,2'-bipyridine (bpy) in chlorobenzene at 110 °C for about 20 h (conversion was about 90%) to obtain bromo-end-capped polystyrene, which was used as a macroinitiator for the polymerization of the second monomer, [2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-*D*-glucopyranosyloxy)ethyl acrylate] (AcGEA), at 90 °C in chlorobenzene. The purified polystyrene-*block*-poly[2-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucosyloxy)ethyl acrylate] (PS-*b*-PAcGEA) was obtained by reprecipitating from THF into a large amount of methanol and drying in vacuum. Deacetylation of PS-*b*-PAcGEA was carried out with freshly prepared sodium methanoxide in a mixture of chloroform and anhydrous methanol (9/1, V/V) at room temperature. Polystyrene-*block*-poly[2-(β-D-glucosyloxy)ethyl acrylate] (PS-*b*-PGEA) was precipitated gradually as the organic solvent was removed. PS-*b*-PGEA may contain some homopolystyrene. The elimination of homopolystyrene from block copolymer samples was achieved by cyclohexane extraction. The copolymers were characterized by gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR). The diblock copolymer used in this study was PS_{77}-*b*-$PGEA_6$ with a polydispersity of 1.27 (polydispersity of PS block is 1.10), while the subscripts, 77 and 6, represent the number of repeat units of the PS block and PGEA block, respectively.

Preparation and observation of molecular assemblies

To prepare the aggregates in aqueous media, the copolymers were first dissolved in DMF to give a copolymer stock solution. The stock solution was divided into several 1 mL portions. Different amounts of additives were added (as concentrated aqueous solutions) to each of the portions. While vigorously stirring the solution, deionized water was added at a rate of 0.2–0.3 wt % per 10 s. When *ca.* 6 wt % of water had been added to the DMF solutions, the clear solutions became turbid, indicating that aggregation had taken place. The addition of water was continued until 30 wt % of water had been added to make sure that the structure of the formed assemblies was kinetically frozen. Then the sample was put into a dialysis bag (molecular weight cut: 10 000) and dialyzed against deionized water for 4 days to remove the organic solvents.

After being diluted by a factor of 10, the aggregate solution free of the organic solvent was used to make TEM samples. A drop of the aqueous solution of aggregates was mixed with a drop of saturated aqueous solution of uranyl acetate (concentration *ca.* 2.0 wt %) and the mixed solution was placed onto a copper EM grid that had been precoated with a thin film of Formvar and coated with carbon. The aqueous solution was blotted away with a strip of filter paper after 15 min. After drying in air for an hour, the grids were observed on a TEM (JEM-100CXII) operated at an acceleration voltage of 100 kV.

Results and discussion

We have previously reported that PS-*b*-PGEA can form a variety of molecular assemblies depending on the composition of the block copolymer, the nature of the solvent and the initial copolymer concentration.[12,13] PS_{77}-*b*-$PGEA_6$ can be molecularly soluble in DMF up to a concentration of 5 wt %. When water is added to the homogeneous PS_{77}-*b*-$PGEA_6$ solution in DMF, the solvent becomes progressively worse for the PS block; after a critical amount of water has been added, the copolymer solution undergoes microphase separation and PS segments start to aggregates and, as a result, micelles are formed. At this stage, the micelle cores are highly swollen and the mobility of the polymer chains is high; further addition of a small amount of water may induce the formation of other types of thermodynamically favored morphologies. If the water content in the mixture is beyond the effective window in solution, the rate of morphological transition can be slower than that of the experimental factors, such as temperature or added water content; the morphology of the aggregates can thus be kinetically stabilized by either dropping the temperature or by quenching the sample into excess water. In our case, the morphologies were trapped by the second method, quenching the sample by addition of excess water upto a water content of 30 wt %, and then dialysis to finally remove DMF. The isolated structures can be visualized by electron microscopy. It should be pointed out that these structures are equilibrium ones under the present conditions, since their sizes depend on the water content and can be changed reversibly. Nevertheless, they can be compared because they are prepared under the same conditions except for the addition of additives. The copolymer concentrations we used were 0.1 or 0.2 wt %, because our former studies revealed that at these concentrations, spheres or rods were the main structures when the copolymer was initially dissolved in DMF; above 0.2 wt %, other types of structures with a bilayer nature appeared. In this paper, we will concentrate on the TEM manifestation of additive effects on the morphological change of molecular assemblies from micelles or rods to bilayer structured morphologies.

Glucose effect

Figure 1 presents a set of TEM pictures that show various morphologies of molecular assemblies prepared from PS_{77}-*b*-$PGEA_6$ with different amounts of added glucose. The initial concentration of PS_{77}-*b*-$PGEA_6$ was 0.2 wt % in DMF. When there was no glucose, this polymer yielded mixtures of spheres and rods. Small vesicles were also occasionally observed [Fig. 1(A)]. The average diameters of the spheres and rods were around 50 nm. When the added glucose concentration was 100 mM, the aggregates were still spheres and small vesicles [Fig. 1(B)], but the content of rods decreased and the content of small vesicles increased. When the glucose concentration was increased to 200 mM, this copolymer still yielded partial vesicles and spheres [Fig. 1(C)], but the outer diameter of the vesicles increased, ranging from 80 to 200 nm. The wall thickness of vesicles was uniform, at *ca.* 20 nm. As the glucose concentration was increased to 240 mM, the aggregates were mainly vesicles, the content of other aggregates like spheres and rods having dramatically decreased. The shape of the vesicles became irregular and their sizes became larger, ranging from 100 to 350 nm, but the wall thickness did not change obviously [Fig. 1(D)]. As the glucose concentration was further increased to 300 mM, this copolymer started to form large compound vesicles (LCVs) [Fig. 1(E)] that coexisted with a few single-room vesicles. The outer diameter of the LCVs was highly polydispersive, ranging from 400 to 800 nm. When the glucose concentration reached 400 mM, the dominant morphologies were LCVs [Fig. 1(F)]. The sizes of the LCVs (800–1500 nm) increased appreciably compared to those in Fig. 1(E).

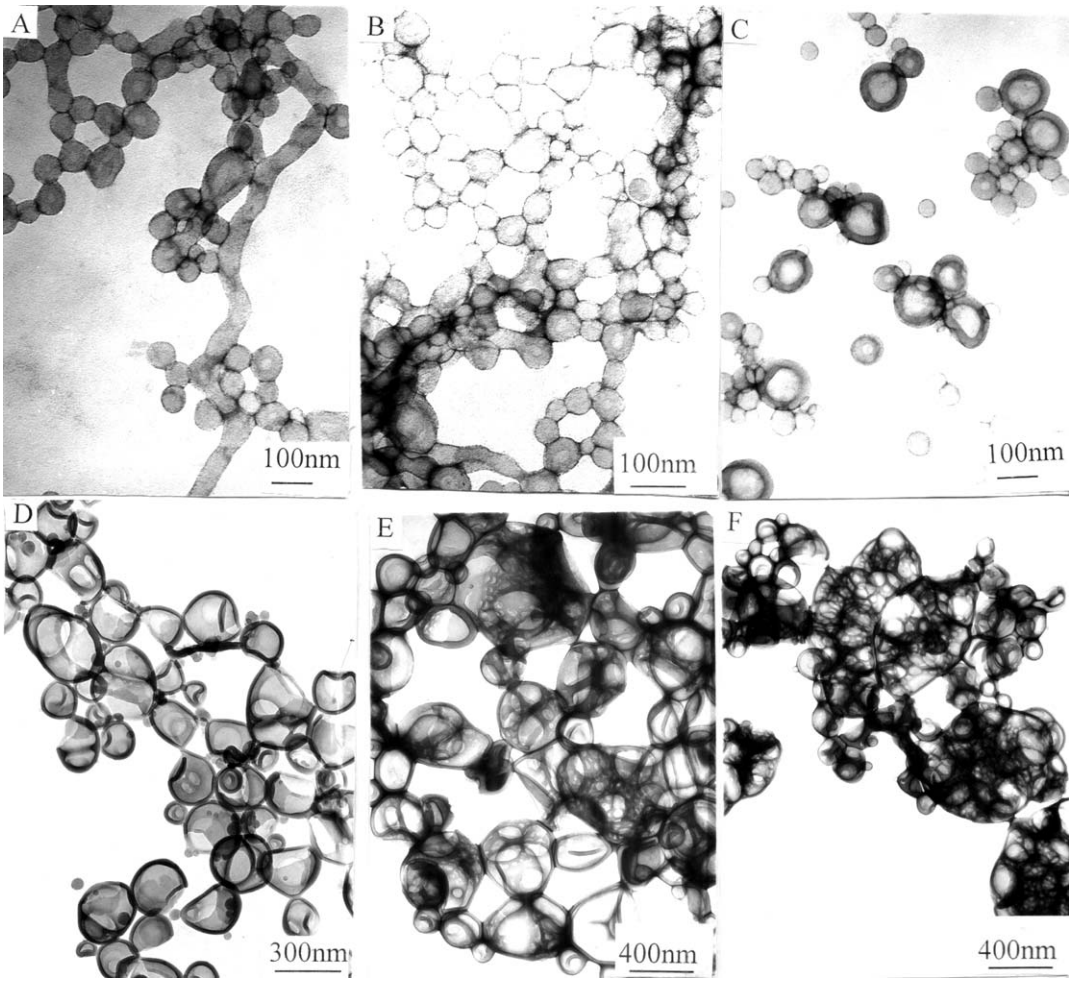


Fig. 1 Effect of glucose on the aggregate morphology of PS_{77}-*b*-$PGEA_6$: [glucose] = (A) 0; (B) 100; (C) 200; (D) 240; (E) 300; (F) 400, mM. (The initial copolymer concentration in DMF is 0.2 wt %.)

HCl effect

Addition of HCl can also induce a morphological change of the aggregates. The initial copolymer concentration in DMF was 0.1 wt %. Shown in Fig. 2 is a set of TEM pictures of various morphologies observed with different amounts of added HCl. When the HCl concentration in the initial copolymer solution of DMF was 0.5 mM, the morphologies, shown in Fig. 2(B), were the intermediate morphologies of the transition from rods to tubules. Spheres could only be observed occasionally. As the HCl concentration was increased to 1 mM, this copolymer yielded predominant short and bent tubules with a few single vesicles [Fig. 2(C)]. The tubular nature was evidenced from their higher transmission in the central parts compared to their peripheries. The ends of the irregular tubules were capped by hemisphere. Further increase in the HCl concentration to 2 mM resulted in the formation of aggregates, shown in Fig. 2(D). It can be seen that the length and outer diameter of the irregular tubules increased. Moreover, the number of tubules with large oscillatory perturbations at the outer diameter increased. As the HCl concentration was further increased to 5 mM, the aggregates were mainly transformed into vesicles as shown in Fig. 2(E). Further increase in the concentration of HCl to 10 mM yielded large compound vesicles (LCVs) [Fig. 2(F)]. The size of some LCVs was so large that they may precipitate to the bottom of the container in the preparation.

Con A effect

The effect of *Con A* on the aggregate morphology of PS_{77}-*b*-$PGEA_6$ is shown in Fig. 3. The initial copolymer concentration in DMF was 0.1 wt %. When *Con A* was added to the initial copolymer solution in DMF, the aggregate morphology started to change. When the *Con A* concentration was 0.1 mg mL^{-1}, PS_{77}-*b*-$PGEA_6$ yielded a mixture of spheres, vesicles and a few short tubules [Fig. 3(B)]. The outer diameter of the vesicles ranged from 60 to 200 nm. When the *Con A* concentration was increased to 0.3 mg mL^{-1}, the morphology changed to dominant vesicles [Fig. 3(C)], which transformed into long irregular tubules at a concentration of 0.5 mg mL^{-1} for *Con A* [Fig. 3(D)]. Further increasing the *Con A* concentration to 1.0 mg mL^{-1} resulted in the formation of large compound vesicles and tubules [Fig. 3(E)]. When the *Con A* concentration reached 2 mg mL^{-1}, the morphologies were still large compound tubules and vesicles [Fig. 3(F)], but their sizes were increased compared with those in Fig. 3(E).

$CaCl_2$ effect

Figure 4 shows the effect of added $CaCl_2$ on the aggregate morphologies of PS_{77}-*b*-$PGEA_6$. For a $CaCl_2$ concentration of 10 mM, the aggregate morphologies are shown in Fig. 4(A). The content of rod-like aggregates increased compared to that without any additive. When the $CaCl_2$ concentration was increased to 20 mM, the aggregates were mixtures of spheres, vesicles and a few short tubules as shown in Fig. 4(B). As the $CaCl_2$ concentration was further increased to 30 mM, this copolymer yielded short tubules with oscillatory perturbations in the outer diameter. When the $CaCl_2$ concentration reached 50 mM, the predominant morphologies were large compound vesicles [Fig. 4(D)].

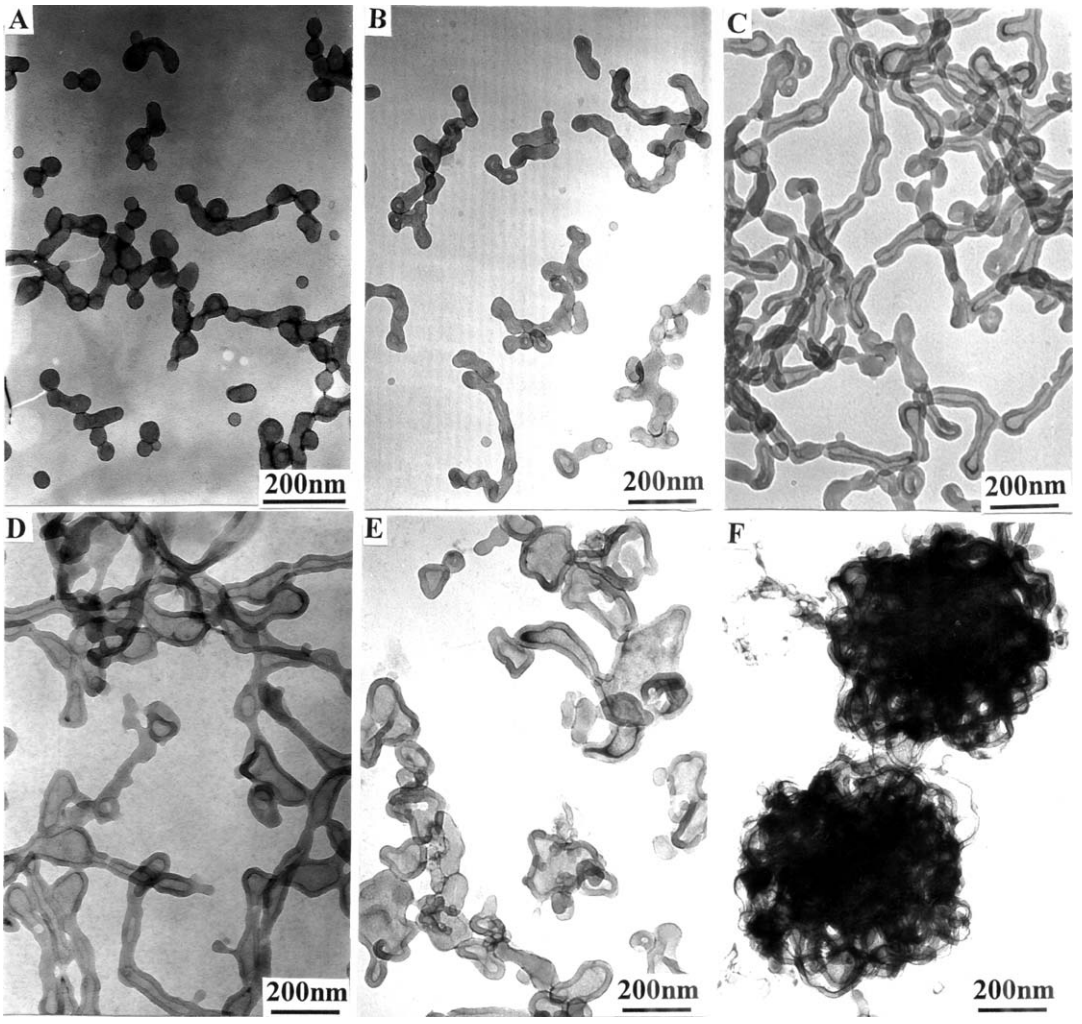


Fig. 2 Effect of HCl on the aggregate morphology of PS_{77}-*b*-$PGEA_6$: [HCl] = (A) 0; (B) 0.5; (C) 1.0; (D) 2.0; (E) 5.0; (F) 10 mM. (The initial copolymer concentration in DMF is 0.1 wt %.)

Cause of the additive effect on the aggregate morphology

Zhang and Eisenberg[7] have summarized that the "crew-cut" aggregate morphology is mainly controlled by three factors, that is the stretching of the hydrophobic blocks (PS) in the cores, surface tension between the core and the solvent, and the repulsive interactions among the corona-forming chains. In all cases, spheres are the dominant morphologies that form at the critical water content. But they are not stable in most systems, and may transform into rods and vesicles when a small amount of water is added subsequently. All these morphologies, as well as the transitions between them, are basically under thermodynamic control. Addition of more water may finally freeze the morphologies, which will no longer change in the following dialysis process, and can be observed under TEM. For example, increasing the PS_{77}-*b*-$PGEA_6$ concentration in DMF will induce the morphological transition from spheres at lower copolymer concentration to bilayer structures at higher copolymer concentrations upon water addition. The reason is that increase of the copolymer concentration results in a lower water content when PS begins to aggregate, thus the stretching of the PS block increases. As the amount of water added increases, the aggregation number and the core dimension also increase, but the stretching of the PS block is entropically unfavorable, so at some point when the mobility of the PS blocks in the core is still high, the aggregates start to transform.

The effects of different added ions on the morphological transformations of the PS-*b*-PAA or PS-*b*-P4VP crew-cut aggregates have been ascribed to the changes of repulsive interactions among the hydrophilic PAA segments or P4VP segments, due to neutralization by NaOH, protonation by HCl, ion-binding or bridging by Ca^{2+}, and electrostatic screening by NaCl.[8b] For nonionic amphiphilic block copolymer PS-*b*-PEO, the effect of the addition of electrolytes on aggregates is also due to the decrease of the repulsive interactions among PEO blocks induced by electrolytes.[9b]

For the present system, we have observed a similar trend of morphological transitions from spheres or rods to bilayer structures when different additives were added in the initial copolymer solutions in DMF. When the copolymer concentration was lower than 0.2 wt %, similar phenomenon can be observed for each system. However, the efficiency of the different additives is quite different, judged by the concentration, *Con A* may be the most effective one and glucose the least effective one. The present additive effects on aggregate morphology transformation can also be explained by the influence of additives on the interactions among corona-forming blocks,[7] that is the glycopolymer segment. However, the detailed causes for the effect of the additives on aggregates probably differ for different additives.

Glycopolymer tends to form intermolecular hydrogen bonds in water, but this tendency may decrease in DMF solution due to the stronger interaction of DMF with the glycopolymer. When a high concentration of glucose (100–400 mM) was added to the solution of PS_{77}-*b*-$PGEA_6$ in DMF, the interactions between glycopolymers and DMF or water decreased, and at the same time, the interactions between glucose and

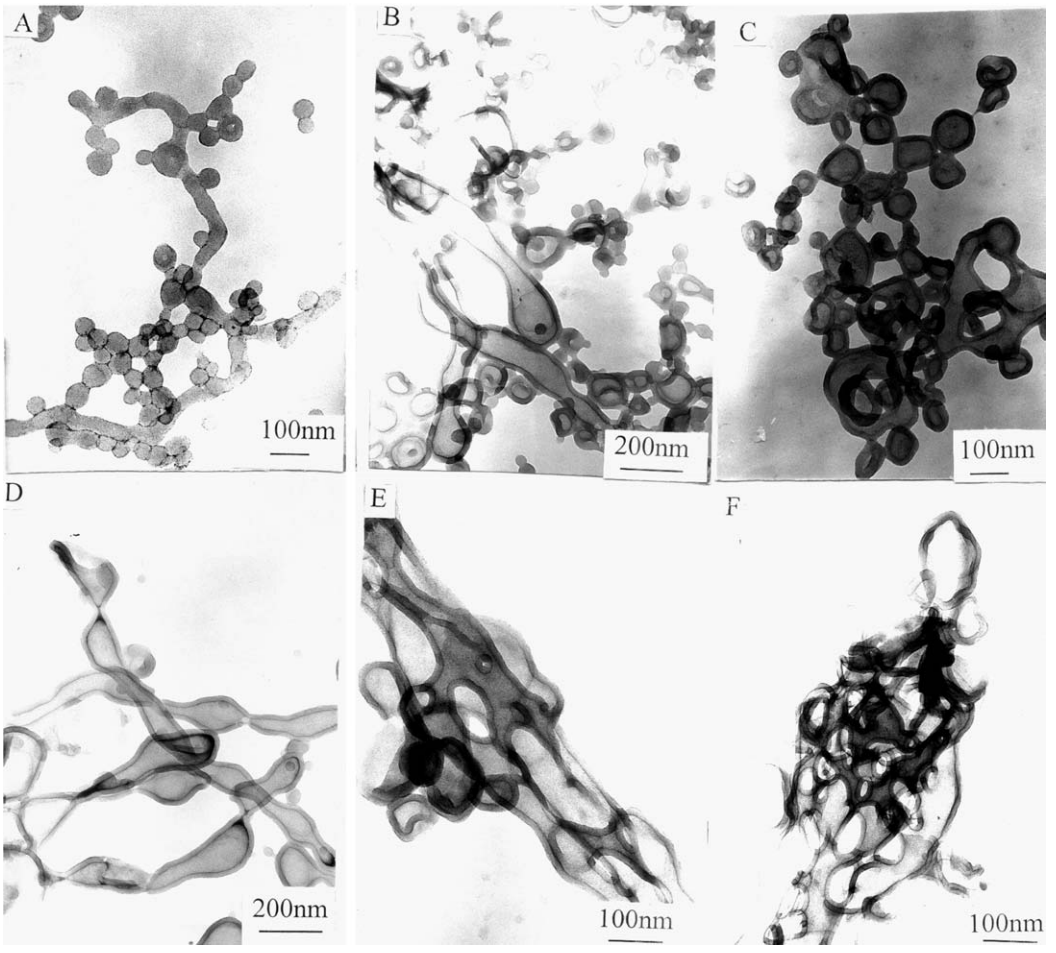


Fig. 3 Effect of *Con A* on the aggregate morphology of PS_{77}-*b*-$PGEA_6$: [*Con A*] = (A) 0; (B) 0.1; (C) 0.3; (D) 0.5; (E) 1.0; (F) 2.0 mg mL^{-1}. (The initial copolymer concentration in DMF is 0.1 wt %.)

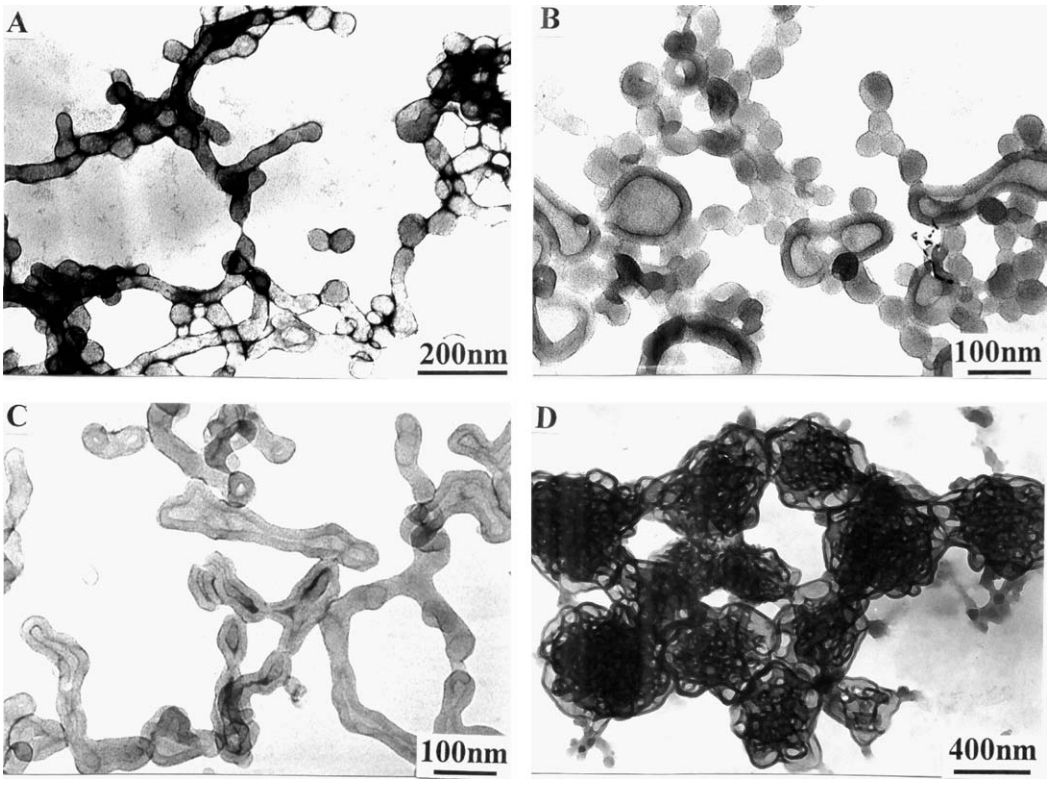


Fig. 4 Effect of $CaCl_2$ on the aggregate morphology of PS_{77}-*b*-$PGEA_6$: [$CaCl_2$] = (A) 10; (B) 20; (C) 50; (D) 100 mM. (The initial copolymer concentration in DMF is 0.2 wt %.)

DMF or water increased. As a result, the repulsive interactions among the glycopolymer blocks, the corona-forming blocks, decreased, which allowed the morphological change of aggregates from spheres to vesicles, and to large compound vesicles. This explanation is quite speculative; we do not have any direct evidence to support it. But one thing is true, the effect of glucose is not so strong, because a very high concentration of glucose is needed for the transition.

Con A is one of the most widely utilized lectins in studying the interaction of specific saccharides with proteins, particularly for non-reducing D-mannosyl and D-glucosyl residues.[18] The binding of *Con A* with glycopolymer will result in the precipitation of *Con A* cross-linked aggregates.[19] Recently, it has been found that there also exists a specific interaction between *Con A* and the aggregates formed from PS-*b*-PGEA or other molecular assemblies whose surfaces are covered with glucose groups.[20] When adding *Con A* to the PS_{77}-*b*-$PGEA_6$ copolymer solution, *Con A* will interact with the PGEA segments and complexation between different polymer chains may occur, thus the repulsive interactions among glycopolymer segments decrease. The aggregate morphology changes from spheres to bilayer as *Con A* concentration increases.

Though the exact effects of HCl and $CaCl_2$ are not so clear at present, it is assumed that addition of HCl and $CaCl_2$ to the PS-*b*-PGEA solution may also increase the interaction of the glycopolymer segment through hydrogen bonding or complexation, and lead to the morphological transitions from spheres to bilayer.

The above-discussed morphologies are the final structures that have been observed under TEM. One may argue that they are not the true morphologies in the solution. However, recent studies of Eisenberg *et al.*[21] on PS-*b*-PAA copolymers in dioxane–water mixtures revealed that the aggregates were formed over a narrow range of water contents; morphological transitions may occur by a small jump of water content. Intermediates encountered during morphological transitions can be trapped if the kinetics is slow. Once more water is added beyond this range, these structures become frozen and they retain their structural integrity throughout further water addition and subsequent dialysis, though vesicles may deform from their spherical state by partly indenting, collapsing or even breaking during the preparation of TEM samples. The transformed vesicles, tubules or large compound vesicles discussed above cannot be due to the TEM method; they can reveal the true nature in solution. So for each system, the trend of transitions can at least be compared, as caused by the addition of different types or amounts of additives.

Conclusion

The aggregate morphology of PS_{77}-*b*-$PGEA_6$ in aqueous medium can be controlled by the addition of glucose, HCl, *Con A*, or $CaCl_2$ to the initial copolymer solution in DMF. The morphology of the aggregates prepared from PS_{77}-*b*-$PGEA_6$ transform from spheres to vesicles, and to large compound vesicles with an increase of glucose concentration. The effects of HCl, *Con A* and $CaCl_2$ on the morphologies of the molecular assemblies show similar trends.

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