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Backbone-collapsed intra- and inter-molecular self-assembly of cellulose-based dense graft copolymer

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ABSTRACT

The self-assembly of cellulose-based amphiphilic dense grafting copolymer ethyl cellulose graft poly(acrylic acid) (EC-g-PAA) in a selective solvent was measured by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Our aim is to realize the intra- or inter-molecular association by collapsing the backbone of dense grafting copolymers. The effects of grafting density, grafting length and solvent on the formation of the unimolecular or multimolecular micelles of EC-g-PAA were discussed. It was shown that the self-association of the grafting copolymers could occur in the mixed solvents of THF and water. The shorter graft chain could induce the multimolecular micelles, while the longer graft chain could lead to the unimolecular micelles. The possible micellization mechanism was proposed from the aspect of chain conformation.

1. Introduction

Recently, the self-assembly of block and graft copolymers has received much interest, due to their potential applications in many fields such as biomaterials and drug delivery (Liu et al., 2011; Qiu & Bae, 2007; Rosler, Vandermeulen, & Klok, 2001; Savic, Luo, Eisenberg, & Maysinger, 2003; Yu, Okano, Kataoka, & Kwon, 1998). Block or graft copolymers can associate to form micelles in selective solvents, which are thermodynamically good solvents for one of the components and comparatively poor solvents for the other (Gao, Wei, Li, & Han, 2008; Tang, Hu, & Pan, 2007). Intra-molecular association will lead to the unimolecular micelles, while inter-molecular association will induce the multi-molecular micelles (Hong, Mai, Zhou, Yan, & Cui, 2007; Hugouvieux, Axelos, & Kolb, 2011; Kikuchi & Nose, 1996b; Satoh et al., 2006). Linear block copolymers in selective solvents can form micelles with different morphologies (Bhargava et al., 2006; Ding & Liu, 1997; Jin, Liu, Liu, & Ji, 2010; Stewart & Liu, 1999; Zhang & Eisenberg, 1995), all of which are almost multi-molecular structures prepared by the general methods. In contrast to block copolymers, graft copolymers have special architectures containing a main chain or backbone and many side chains, which could cause the unimolecular association and result in the unimolecular micelles. In most cases, graft copolymers in selective solvents can form spherical micelles by the association of backbone or side chains to reach thermodynamic stability. The spherical shape of micelles is imposed by the excess free energy of the micelles-solvent interface arising due to unfavorable contacts of chains with the solvent. Furthermore, graft copolymers with complicated architectures can lead to various possible associated structures by adjusting the pertinent parameters, such as the side chain grafting density, the side chain length and the solvent quality with respect to the backbone and side chain (Wang, Li, & Guo, 2005; Zhang, Qiu, & Zhu, 2005). In previous work, graft copolymers with low graft density, named as sparse graft copolymers, were generally used to investigate the formation of unimolecular structures by the collapse of backbone or side chain, such as the flower type unimolecular and multimolecular micellar structures of poly(methyl methacrylate)-*graft*-polystyrene (PMMA-g-PS) formed in selective solvents (Kikuchi & Nose, 1996a, 1997), and the coil-to-globule

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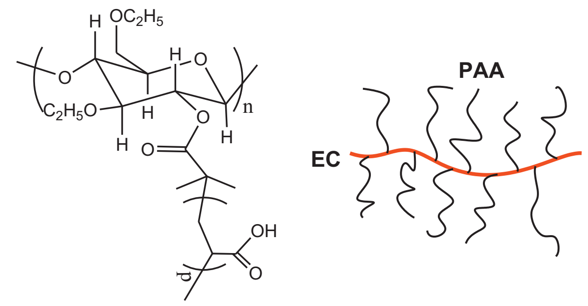
transition and the single chain core–shell nanostructure of poly(N-isopropylacrylamide)-*graft*-poly(ethylene oxide) (PNIPAM-*g*-PEO) (Chen et al., 2005; Qiu & Wu, 1997). On the other hand, the comb and brush polymers with a high graft density of side chains have been chiefly investigated on the focus of the unimolecular cylindrical structures (He, Merlitz, Sommer, & Wu, 2007; Lecommandoux et al., 2002; Polotsky et al., 2008; Rathgeber et al., 2006) and multimolecular micelles by the side chain collapse (Chang, Lin, Hob, Huang, & Lee, 2009). Moreover, the intra-molecular self-assembly of amphiphilic comb-like copolymers has been predicted by the scaling theory from the aspect of the chain conformations (Borisov & Zhulina, 2005). To the best of our knowledge, however, there are few reports about the self-assembly of dense graft copolymers by the intra-molecular collapse of a backbone. The possible reason is that the collapsing of a backbone is comparatively difficult because of the strong stretching of dense side chains, which prevents the backbone from collapsing in selective solvents. Therefore, there is a doubt as whether the backbone of dense graft copolymers can intra-molecularly collapse. How can the intra-molecular collapse of the backbone be realized in a comparatively dense graft copolymer?

Cellulose and its derivatives have the advantages of biodegradable and biocompatible properties and are of both extensive academic and industrial interest (Shi, Li, & Zhang, 2008; Song, Zhou, Zhang, & Wu, 2008; Tian et al., 2011). Moreover, cellulose-based graft copolymers have been used to self-assemble to form micelles for drug release (Dou & Jiang, 2007; Tan et al., 2010; Wang et al., 2011). Obviously, the micellar structures, which will be influenced by the structure of the graft copolymer, will play an important role in the process of drug release. In our previous work, we synthesized the dense graft copolymers ethyl cellulose graft polystyrene (EC-*g*-PS) and investigated the self-assembly behavior in a selective solvent of the backbone (Liu et al., 2009). It was found that the dense graft copolymers ethyl cellulose graft polystyrene (EC-*g*-PS) could self-assemble to form the uni- and multimolecular spherical micelles by the collapse of the side chains. However, the intra-molecular collapse of backbone was not realized in the dense EC-*g*-PS copolymers since it is difficult to find a suitable selective solvent for the side chains because of the similar hydrophobic properties of the backbone and the side chains. If the side chain is hydrophilic, the ethyl cellulose-based grafting copolymers will have amphiphilic properties and the self-assembly of such grafting copolymers may occur in the aqueous systems. Because of the backbone ethyl cellulose with excellent biocompatible properties, such self-assembly of the ethyl cellulose-based grafting copolymers will be applied in many biological fields.

Here we report the self-assembly of dense amphiphilic graft copolymers. The purpose of our research is to carry out the collapse of the backbone of dense graft copolymers by adjusting the property of solvent and the topological structure of graft copolymers, such as the grafting density and the grafting length. We used the relatively dense amphiphilic graft copolymers ethyl cellulose-grafting poly (acrylic acid) (EC-*g*-PAA), with two different side-chain lengths and grafting densities synthesized via ATRP to investigate the self-association behavior of the dense graft copolymers in solvents mixed with water. The intra- and inter-molecular backbone of the dense graft copolymers collapsed to form the micelles. The effects of the side chain length, the grafting density and selective solvents on the formation of the intra- or inter-molecular micelles of the EC-*g*-PAA copolymers were discussed. The possible process of the micellization of the dense graft copolymers was proposed from the aspects of chain conformation. In addition, it should be emphasized that the used backbone ethyl cellulose is biocompatible and usually applied in drug release, and the side chain PAA is a kind of functional polymers with pH-responsive properties and has many applications. Therefore, the graft copolymer EC-*g*-PAA would have many extensive potential applications with pH-responsive properties, especially in the filed of drug release. The basic research on the self-assembly of the graft copolymer may be interesting and important for potential applications.



EC-*g*-PAA

Scheme 1. Molecular structure and schematic illustration of the graft copolymers.

2. Experimental

2.1. Materials

The graft copolymers, ethyl cellulose grafting poly (acrylic acid) (EC-*g*-PAA) (Scheme 1), were synthesized via atom transfer radical polymerization (ATRP) by using ethyl cellulose 2-bromoisobutyryl ester (EC-Br) as the macro-initiator of ATRP. The details of the synthesis procedure are shown elsewhere (Kang et al., 2006). Two macro-initiators EC-Br with the average degree of substitution of bromide groups (DS$_{Br}$) of 0.30 and 0.50 were used for the preparation of the EC-*g*-PAA copolymers, respectively. This means that the graft density would be up to 30% and 50%, and the obtained graft copolymers belong to a kind of relatively dense graft copolymers. The molar mass and its distribution of the precursors ethyl cellulose grafting poly (*t*-butyl acrylate) (EC-*g*-P*t*BA) of EC-*g*-PAA were determined by gel permeation chromatography (GPC) (Waters 515 with a 2410 differential refractometer detector) with tetrahydrofuran (THF) as the eluting solvent (1 mL/min) and the monodisperse polystyrene was used as the standard. The composition of the copolymers was estimated by ^{1}H NMR spectra measured by a Bruker DMX 400 NMR spectrometer. The details of the precursor EC-*g*-P*t*BA (marked as EC$_{0.3}$-*g*-P*t*BA$_{21}$, EC$_{0.3}$-*g*-P*t*BA$_{43}$, EC$_{0.5}$-*g*-P*t*BA$_{28}$ and EC$_{0.5}$-*g*-P*t*BA$_{53}$) of EC-*g*-PAA copolymers used in this work are listed in Table 1. The corresponding amphiphilic EC-*g*-PAA graft copolymers, marked as EC$_{0.3}$-*g*-PAA$_{21}$, EC$_{0.3}$-*g*-PAA$_{43}$, EC$_{0.5}$-*g*-PAA$_{28}$, and EC$_{0.5}$-*g*-PAA$_{53}$, respectively, were then obtained by the hydrolysis of poly (*t*-butyl acrylate) grafting chain using trifluoroacetic acid. All the poly (*t*-butyl acrylate) side chains of the graft copolymers were almost hydrolyzed completely to become the PAA chain, which was confirmed by ^{1}H NMR spectra. The molecular structure and schematic illustration of the EC-*g*-PAA graft copolymers are shown in Scheme 1. Other chemicals were commercially available and used as received.

2.2. Sample preparation and characterization

The copolymers were first dissolved in methanol and tetrahydrofuran (THF) by stirring at room temperature for a few days to obtain the copolymer/methanol and the copolymer/THF stock solutions, respectively. The concentrations of all the stock solutions are 5 mg/mL. Under continuous stirring, deionized water was then added dropwise into the copolymer/methanol and copolymer/THF stock solutions, respectively. During the water-dropping procedure, the copolymer/THF solutions for all the graft copolymers turned into bluish THF/water solutions. However, the

Table 1
Molecular characteristics of the graft copolymers.

EC-*g*-P*t*BA copolymers[a]	DS_{Br} of EC-Br	M_n[b] (10^5 g/mol)	M_w/M_n[b]	Average repeated units of the side tBA chain[c]	EC-*g*-PAA copolymers[a]
$EC_{0.3}$-*g*-P*t*BA_{21}	0.3	0.67	1.55	21	$EC_{0.3}$-*g*-PAA_{21}
$EC_{0.3}$-*g*-P*t*BA_{43}	0.3	0.87	1.49	43	$EC_{0.3}$-*g*-PAA_{43}
$EC_{0.5}$-*g*-P*t*BA_{28}	0.5	1.26	1.46	28	$EC_{0.5}$-*g*-PAA_{28}
$EC_{0.5}$-*g*-P*t*BA_{53}	0.5	1.72	1.41	53	$EC_{0.5}$-*g*-PAA_{53}

[a] The graft copolymers were denoted by EC-*g*-P*t*BA, in which the footnotes were graft density and the graft length. For example, $EC_{0.3}$-*g*-P*t*BA_{21} was represented as the graft copolymer with graft density of 30% and graft length of 21 repeated units.
[b] Parameter determined by GPC is the result of corresponding EC-*g*-P*t*BA of EC-*g*-PAA.
[c] Average number of monomer repeated unit every graft chain was determined by ^{1}H NMR.

transparent methanol solutions for all the graft copolymers always remained transparent during the water-dropping procedure. The final concentrations of all the bluish THF/water solutions were 1 mg/mL. The obtained copolymer/THF/water solutions were then sealed and equilibrated at least overnight before preparing the samples for DLS measurements and TEM observations.

Dynamic light scattering (DLS) experiments were performed on a commercial spectrometer (ALV/SP-150), equipped with an ALV-5000 multi-τ digital time correlator and a solid-state laser (ADLAS DPY 425 II, the output power was 400 mW at λ = 632.8 nm) at a scattering angle of 90°. All light scattering experiments were performed at 25 °C. The apparent hydrodynamic radii (R_h) were obtained by fitting the correlation function with the CONTIN analysis and using the Einstein–Stokes equation. All the solutions for DLS experiment were filtered through filters of 0.45 μm pore size before DLS measurements.

Transmission electron microscopic (TEM) observations were carried out on a JEOL JEM-2200FS (Japan) high-resolution transmission electron microscope at an accelerating voltage of 200 kV. The sample for TEM observations was prepared by dropping the copolymer micellar solution on copper grids coated with carbon film. After a few minutes, the excess solution was blotted away with filter paper. The grids were then dried at room temperature and atmospheric pressure at least for 12 h, and were kept in a vacuum desiccator for 2 days before the TEM observation.

3. Results and discussion

As shown in Fig. 1, the bluish solutions would appear with the addition of deionized water into the copolymers/THF solutions, while the solutions were always transparent and colorless with the addition of water into the copolymers/methanol solutions. The experimental phenomena indicated that the copolymers were self-assembled to form micelles in such bluish copolymer/THF/water systems, whereas the micelles were not formed in the colorless copolymer/methanol/water solutions. As we know, water is a poor solvent for the backbone EC and is a good solvent for the side chain PAA. With the addition of water into the copolymers solution, the backbone would collapse because of the hydrophobic interaction of the backbone. The self-assembled micelles would be formed through the collapse of the backbone in the selective solvent of the side chains. Therefore, it could be concluded by the experimental phenomena that the backbone collapsed to form the micelles in the bluish THF/water solutions, while the backbone did not collapse and the micelles were not formed in the colorless methanol/water solutions.

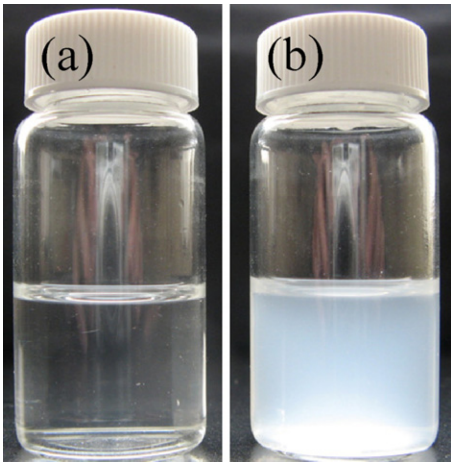


Fig. 1. The typical photos of the copolymer solutions in the mixed solvents of methanol/water (a) and THF/water (b) indicating colorless methanol/water solution (a) and bluish THF/water solution (b).

The micelles in the bluish THF/water solutions were further confirmed by DLS and the result of the hydrodynamic radius R_h distribution is shown in Fig. 2(a). It is clear that the average hydrodynamic radius $\langle R_h \rangle$ of $EC_{0.3}$-*g*-PAA_{21} in the THF/water solvents was up to 60 nm, which is much larger than the $\langle R_h \rangle$ of $EC_{0.3}$-*g*-PAA_{21} in a common solvent of methanol (~17 nm). The large $\langle R_h \rangle$ indicated that the large micelles or aggregates were indeed formed in the THF/water solvents by the self-assembly of the graft copolymer and the obtained micelles were of multimolecular structures. The self-assembly morphologies of the copolymer were examined by transmission electron microscopy (TEM). As shown in Fig. 2(b), the spherical aggregates with diameters in the range of 20–80 nm were observed. It is noted that the $\langle R_h \rangle$ of the micelles in the solution measured by DLS (~60 nm) is apparently larger than those observed by TEM, which is due to that the micelles are swollen in the solution, while TEM observations are of the dried state.

In the case of the copolymer $EC_{0.3}$-*g*-PAA_{43} with the longer side chains, the DLS result is shown in Fig. 3. The curve of the R_h distribution showed the bimodal distribution. One peak is corresponding to the small R_h of about 10 nm, the other is corresponding to the large R_h in the range of 50–400 nm. The small R_h (~10 nm) is smaller than that of the single chain dissolved in the good solvent of methanol, which implied that the intra-molecular association occurred and resulted in the formation of single molecular micelles. The large R_h is much larger than that of the single chain in the good solvent, which suggested that the inter-molecular association arose and the multimolecular micelles or aggregates were formed. The typical TEM results are shown in Fig. 4. The apparent network-like structures constituted by nanowire-like structures were observed in a large size range (Fig. 4(a)), which are different from the spherical particles from $EC_{0.3}$-*g*-PAA_{21}. The network-like structures seemed to correspond to the large R_h measured by DLS. At a higher magnification, a few isolated small spherical particles were observed in the light areas (Fig. 4(b)). Interestingly, even in all wire-like network structures, many small sphere-like particles were clearly observed in a further higher magnification, which constituted the network-like structures (Fig. 4(c)). Moreover, the size of the small spherical particles in the network structures was almost equal to that of the isolated small

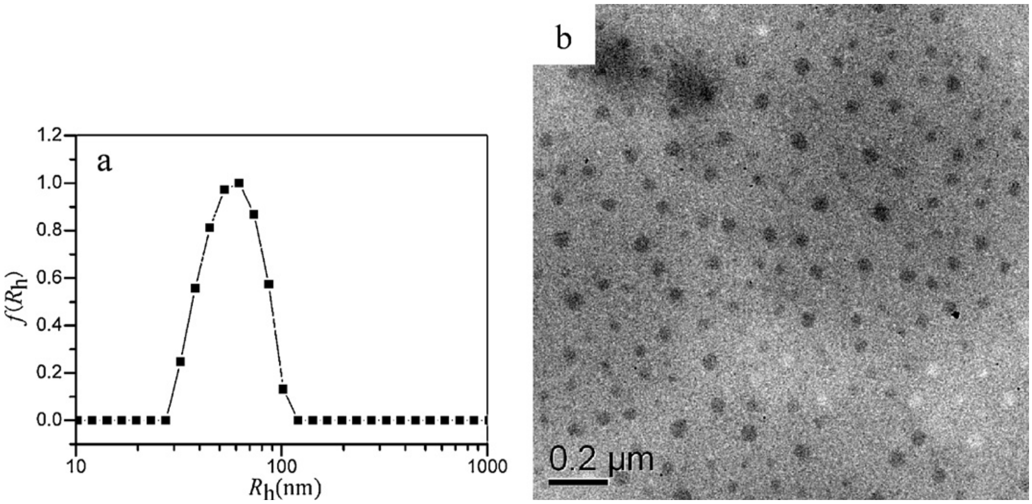


Fig. 2. The hydrodynamic radius distribution function (a) of the copolymer $EC_{0.3}$-*g*-PAA_{21} in the THF/water solvent and a typical TEM image (b) from the copolymer $EC_{0.3}$-*g*-PAA_{21}.

particles, which indicated that all of the small particles had the same kinds of micelles. Fig. 4(d) displays the size distribution histogram of the small spherical particles based on a statistical analysis of 100 particles. The average diameter of the small particles was only 3.2 ± 0.5 nm. Herein, the mole molecular mass of the small particles as the spheres could be theoretically calculated by the formula of $M_n = \rho \cdot (4/3)\pi r^3 \cdot N_A$ (ρ is the mass density, π is the circular constant, r is the radius, N_A is Avogadro's constant). Given the whole backbone collapse and the mass density of the particles is equal to that of the bulk EC ($\rho = 1.07 \times 10^6$ g/m^3), the mole molecular mass of the small particles is estimated to be 1.04×10^4 g/mol. The order of the estimated M_n value is just the same as that of the obtained M_n value of EC by GPC. From these estimated results, it is reasonable to believe that the small spherical particles are of single chain structures. Therefore, the small particles should correspond to the small R_h determined by DLS, which are of single molecular micelles. Obviously, because the solvents are poor for the backbone and good to the side chains, the single micelles are formed in such solvent systems by the intra-molecular backbone collapsing with a core–shell structure, in which the core was made up of the EC backbone and the shell was constituted by the PAA side chains.

However, it is noted that the size of the small particles observed by TEM is much smaller than that measured by DLS. This is due to that the observed particles are only part of the backbone EC, while the PAA side chains are invisible in the TEM micrograph. The backbone in the poor solvents compactly collapsed to form the core, resulting in the larger electron density and could be visible by the darker appearance in the TEM micrographs. If the size of the core part of the small particles in the solutions is approximately equal to that observed by TEM, the shell radius of the PAA side chains is equal to the value of the subtraction of the core radius from the small R_h ($10 - 3.2/2 = 8.4$ nm). The estimated value of the shell radius is much larger than that of the core radius. Therefore, the obtained single molecular micelles are of the so-called star micelle structure. According as the shell radius of 8.4 nm, the length of each monomer unit of the side chains would be up to 0.20 nm (=8.4/43), which is close to the contour length (~0.25 nm) of the side chain unit. The deduced result suggested that the side chain-constituted shell of the single molecular micelles was of an extended state. This is due to the very strong repulsive interaction between the side chains, originated from the collapsed backbone-induced high density of the PAA side chain on the core surface.

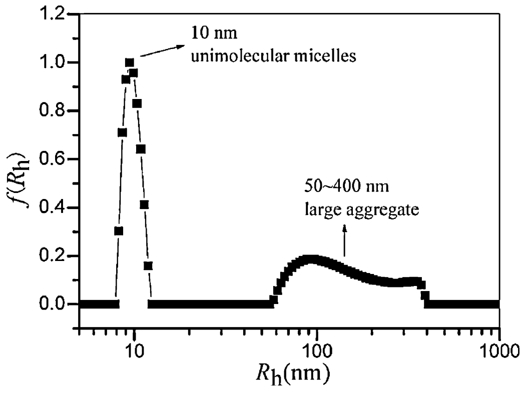


Fig. 3. The hydrodynamic radius distribution function of the copolymer $EC_{0.3}$-*g*-PAA_{43} in the THF/water solvent.

As mentioned above, the wire-like structures were clearly made up of the single molecular small particles and formed by the junction of the small particles. This implied that there was a certain force to induce the junction or aggregation between the single molecular particles. On the basis of the molecular characteristic of the graft copolymer, it is reasonable to believe that the strong hydrogen bond interaction of the side chains between the small particles resulted in the large network-like structures, since the side chains are of units of acrylic acid and the hydrogen bonds are easily formed between the AA units. The hydrogen bond interaction should exist in the solution, leading to large aggregations, which corresponded to the large R_h confirmed by DLS.

In the case of the denser graft copolymer $EC_{0.5}$-*g*-PAA_{28}, the hydrodynamic radius R_h distribution is shown in Fig. 5(a). It is clear that the average hydrodynamic radius $\langle R_h \rangle$ of $EC_{0.5}$-*g*-PAA_{28} in the THF/water solvents was also up to 65 nm. The large $\langle R_h \rangle$ indicated that the large micelles or aggregates were indeed formed in the THF/water solvents by the self-assembly of the denser graft copolymer and the obtained micelles were also of multimolecular structures. The self-assembly morphologies of the copolymer were examined by transmission electron microscopy (TEM). As shown in Fig. 5(b), the similar spherical particles with large size (20–100 nm) as the copolymer $EC_{0.3}$-*g*-PAA_{21} were observed. Obviously, the large particles from the copolymer $EC_{0.5}$-*g*-PAA_{28} were also of multimolecular micelles as those from the copolymer $EC_{0.3}$-*g*-PAA_{21}. In

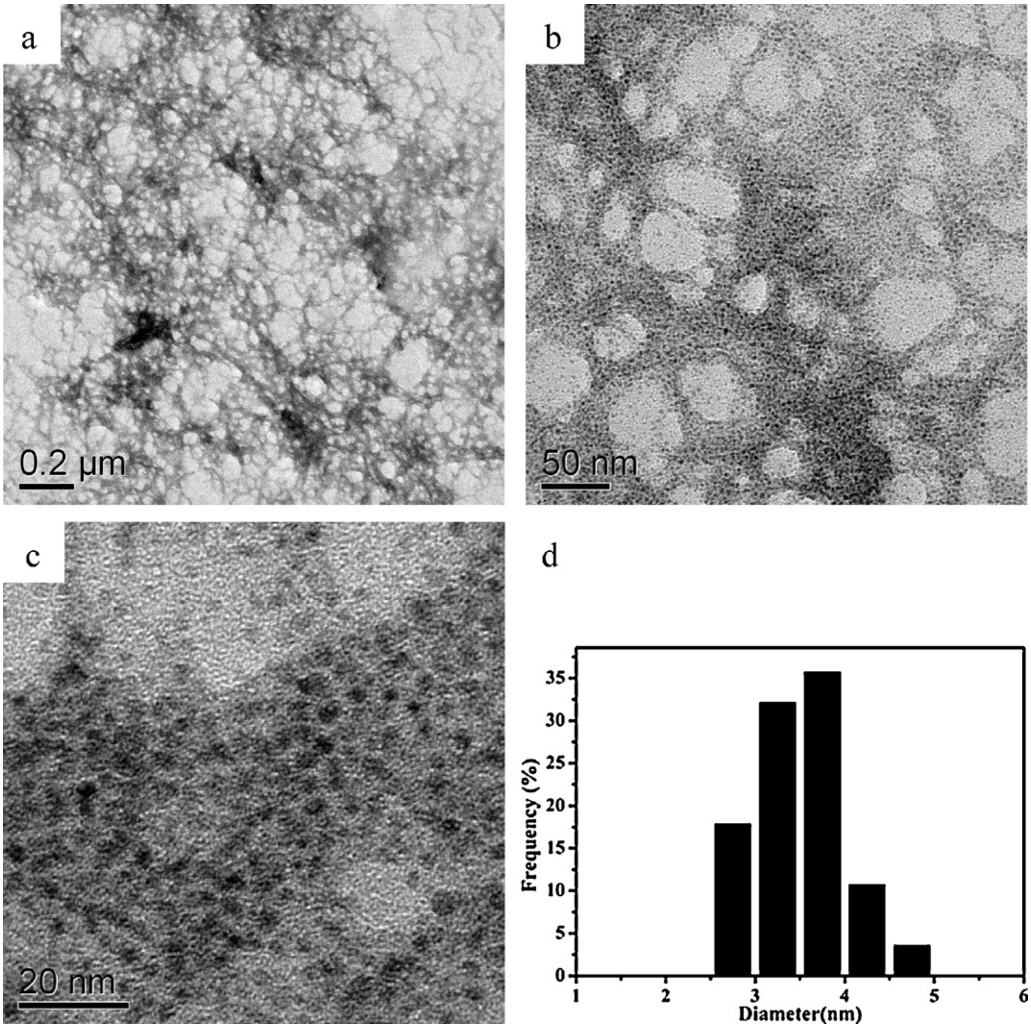


Fig. 4. The typical TEM images (a)–(c), and the size histogram of small spherical particles (d) from the copolymer $EC_{0.3}$-*g*-PAA_{43}.

a word, the results illuminated that the multimolecular micelles were formed in such systems.

In the copolymer $EC_{0.5}$-*g*-PAA_{53}, the DLS measurement also showed the bimodal distribution. As shown in Fig. 6, one peak is corresponding to the small R_h of about 14 nm, the other is corresponding to the large R_h in the range of 50–400 nm. The small R_h, which was similar to that from the copolymer $EC_{0.3}$-*g*-PAA_{43}, also indicated that the intra-molecular association occurred and

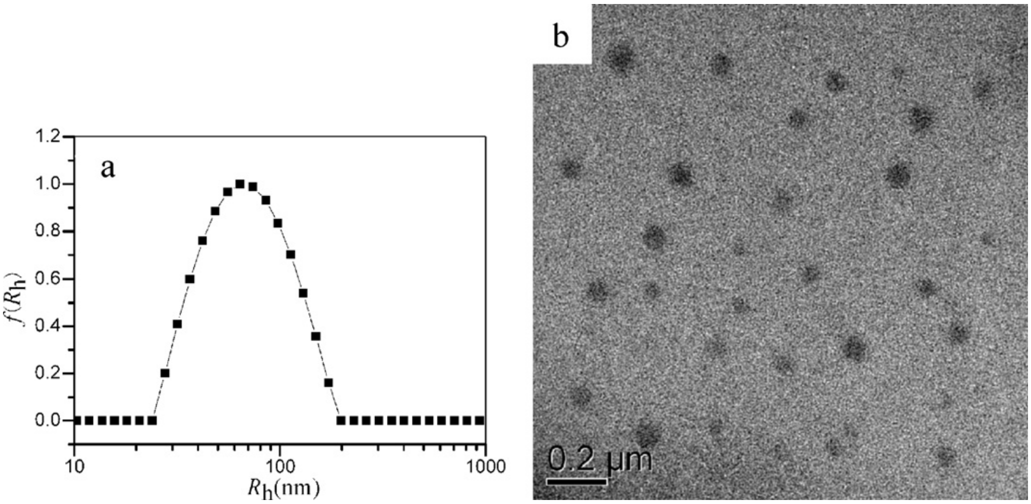


Fig. 5. The hydrodynamic radius distribution function (a) of the copolymer $EC_{0.5}$-*g*-PAA_{28} in the THF/water solvent and a typical TEM image (b) from the copolymer $EC_{0.5}$-*g*-PAA_{28}.

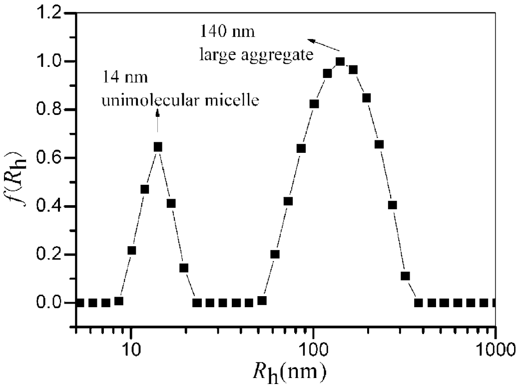


Fig. 6. The hydrodynamic radius distribution function of the copolymer EC$_{0.5}$-*g*-PAA$_{53}$ in the THF/water solvent.

resulted in the formation of single molecular micelles. The large R_h also suggested that the inter-molecular association occurs and the multimolecular micelles or aggregates were formed, which is also similar as that from the copolymer EC$_{0.3}$-*g*-PAA$_{43}$. The typical TEM results are shown in Fig. 7. Similar network-like structures to the copolymer EC$_{0.3}$-*g*-PAA$_{43}$ were also found (Fig. 7(a)). At a higher magnification, the isolated small particles and the network-like structures made up of many small spherical particles obviously coexisted (Fig. 7(b) and (c)). As shown in Fig. 7(d), the average size of the small particles from EC$_{0.5}$-*g*-PAA$_{53}$ was 3.5 ± 0.5 nm, which was almost the same as that from EC$_{0.3}$-*g*-PAA$_{43}$. As discussed above, the small spherical particles were also of unimolecular structures formed by the intra-molecular backbone collapse. The same size of the small particles from EC$_{0.5}$-*g*-PAA$_{53}$ as that from EC$_{0.3}$-*g*-PAA$_{43}$ indicated the same collapse degree of the backbone from copolymer EC$_{0.5}$-*g*-PAA$_{53}$ as that from EC$_{0.3}$-*g*-PAA$_{43}$.

From above results, it could be concluded that the similar large size and spherical morphology of the micelles or aggregates were obtained from the two copolymers with shorter side chains, and the similar small size and spherical morphology of the single molecular micelles were obtained from the two copolymers with longer side chains. Here, the two different graft densities did not distinctly influence the self-assembled structures. However, under the condition of the same graft density, the different side chain lengths resulted in different micellar sizes, which suggested that the graft length played an important role in the self-assembly of the dense graft copolymers in such systems. The large spherical particles (about 50 nm observed by TEM) from the copolymer EC$_{0.3}$-*g*-PAA$_{21}$ and EC$_{0.5}$-*g*-PAA$_{28}$ indicated the onset of multimolecular self-assembly to form large aggregates by the inter-molecular backbone association. On the contrary, the isolated small spherical particles and the small spherical particles in the network structures from the copolymer EC$_{0.3}$-*g*-PAA$_{43}$ and EC$_{0.5}$-*g*-PAA$_{53}$ implied the presence of a single molecular self-assembly in

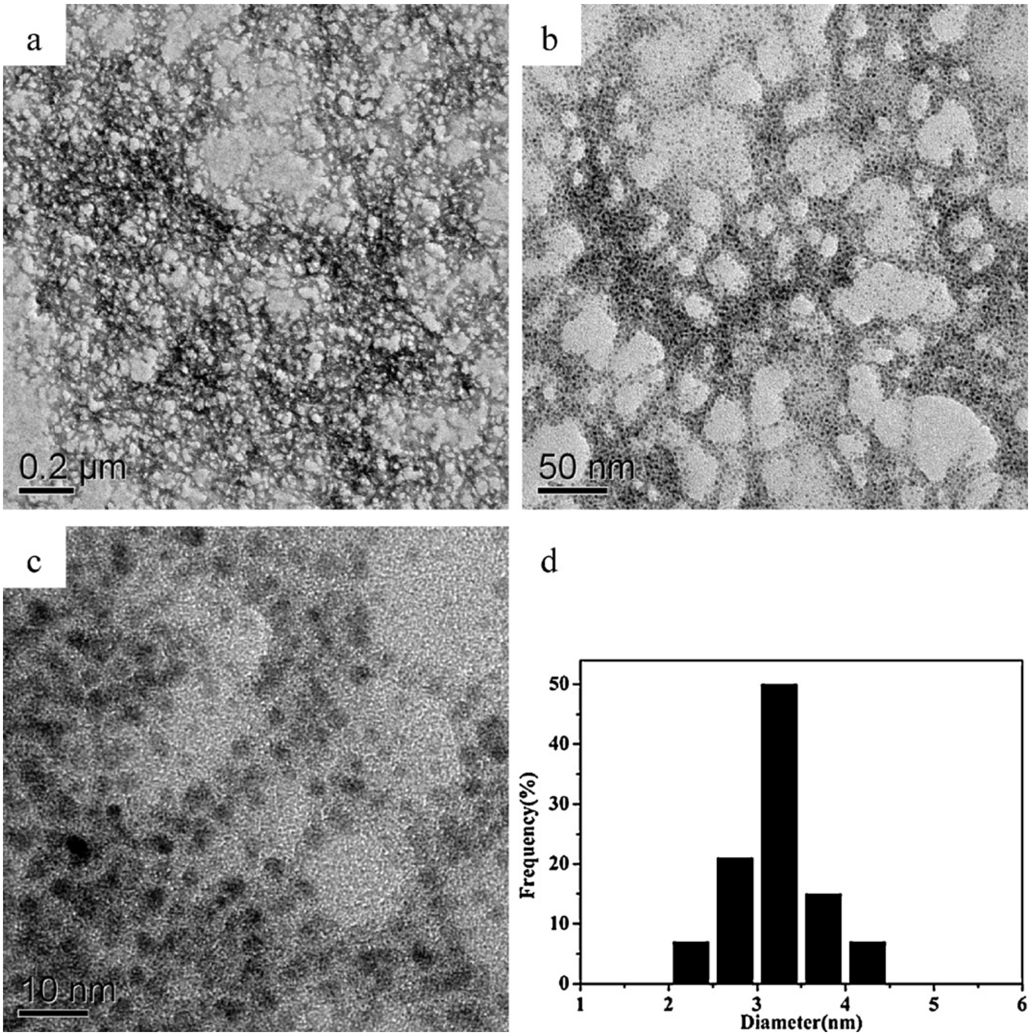
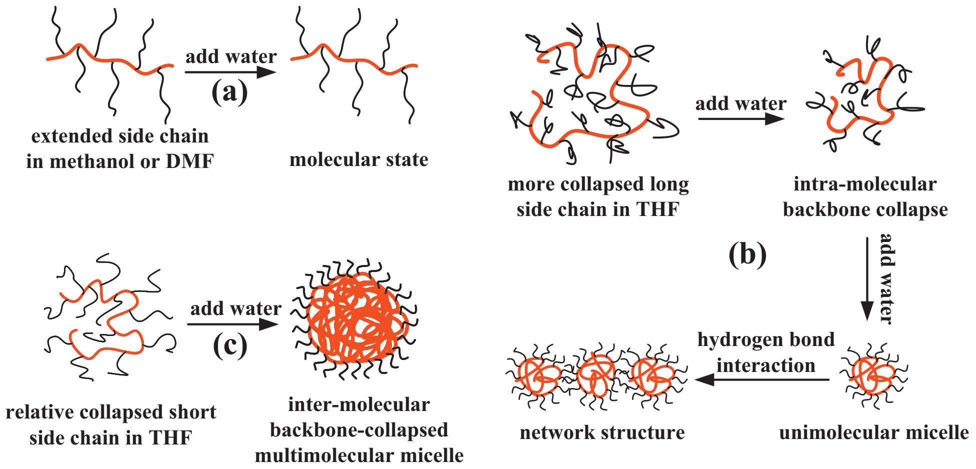


Fig. 7. The typical TEM images (a)–(c), and the size histogram of small spherical particles (d) from the copolymer EC$_{0.5}$-*g*-PAA$_{53}$.



Scheme 2. A proposed mechanism leading to molecular dissolved state in methanol or DMF/water system (a), unimolecular micelles constituting network-like structure (b) and large multimolecular micelles (c) in THF/water system from the dense EC-*g*-PAA graft copolymers.

solution by the intra-molecular backbone association. Therefore, the copolymers with longer side chain from THF/water mixed solvent were intra-molecularly self-assembled to form unimolecular spherical structure and those with short side chain did multimolecular large spherical aggregates by inter-molecular association. It is suggested that the length of side chain primarily determined the self-assembly morphologies from these graft copolymers.

On the other hand, as discussed by the experimental phenomena, all the copolymers from methanol/water mixed solvent were not self-assembled to form micelles. This indicated that the solvent also had a distinct effect on the self-assembly behavior. The solvent effect was further confirmed by another parallel experiment, in which deionized water were added into the copolymers/N,N-dimethylformamide (DMF) solutions. However, all the copolymers/DMF/water solutions were always transparent and no bluish tint appeared in solution. This indicated that the self-assembly did not occur in such DMF/water systems, which is similar to that in the methanol/water solutions. On the contrary, the graft copolymers were self-assembled to form the micelles in the THF/water systems. In other words, with the addition of water, the intra- or inter-molecular backbones of the graft copolymers in THF solutions could be associated, while those in methanol and DMF solutions could not be done. From this point, it could be concluded that the solvent critically determined the self-assembly behavior of the graft copolymers. As reported in block copolymers, different common solvents could lead to different micellar morphologies (Yu, Zhang, & Eisenberg, 1998) in spite of the same selective solvents. However, when the common solvent is methanol or DMF in this work, the micelles could not be formed, which is different from block copolymers. This may originate from the different architectures of dense graft copolymers from block copolymers and the different conformation of side chain and backbone induced by different solvents. The possible procedure of the micellization could be explained by the chain conformation, which is shown in the schematic illustration (Scheme 2).

For the graft copolymers EC-*g*-PAA, the backbone is hydrophobic and the PAA side chains are hydrophilic. Both methanol and DMF are common solvents for the graft copolymers (a good solvent for both backbone and side chain). In good solvents for both the backbone and side chain such as methanol and DMF, both the backbone and PAA side chain are in a relatively extended coil conformation. In contrast to methanol or DMF, THF is only a relative good solvent for backbone and a relative poor solvent for PAA side chain. Thus, the side chain may be in a collapsed conformation in THF. On the other hand, water is a good solvent for PAA side chain and a poor solvent for backbone, in which the backbone would be collapsed and the side chain in a coil state. When water was added, the mixed solvents became poorer for the backbone and induced the collapse and aggregation of the backbone. Meanwhile, the mixed solvents became better for the PAA side chain and resulted in the extension of the side chains. In methanol or DMF, the good solvent for both the backbone and side chains, the backbone and the side chains were in a relatively extended state induced by the steric repulsion because of the relatively dense side chains (Scheme 2(a)). The prior extension of side chain leads to the stronger stretching force to the backbone, which may be stronger than the backbone-hydrophobic interaction and prevented the backbone from collapsing with the addition of water. Therefore, the copolymers were still in a dissolved molecular state (Scheme 2(a)) and no micelles could be formed in such methanol/water and DMF/water systems. In the THF/water systems, however, the side chain was in a relatively collapsed state because of the solvophobic interaction of the side chains with THF (Scheme 2(b) and (c)). The steric repulsion force between the relatively collapsed side chains may be weaker than the hydrophobic interaction of the backbone. Thus, the backbone could collapse with the addition of water. Under the same condition, the longer side chains in the copolymers $\mathrm{EC}_{0.3}$-*g*-PAA_{43} and $\mathrm{EC}_{0.5}$-*g*-PAA_{53} should acquire a more collapsed conformation because of the relatively stronger solvophobic interaction of the longer side chains, which possibly favors the intra-molecular backbone-collapsing to form single molecular micelles (Scheme 2(b)). On the other hand, the shorter side chains in the copolymers $\mathrm{EC}_{0.3}$-*g*-PAA_{21} and $\mathrm{EC}_{0.5}$-*g*-PAA_{28} should adopt a relatively slightly collapsed conformation because of the relatively weak solvophobic interaction of the shorter side chains, which makes the intra-molecular collapsing very difficult and favors the inter-molecular collapse of the backbone for stabilizing the copolymer/THF/water system. Therefore, with the addition of water, inter-molecular collapse tended to self-assemble to form multimolecular micelles and large spherical aggregates (Scheme 2(c)). In a word, the prior extension of side chain in dense graft copolymer would prevent the backbone from collapsing, while the prior collapse of side chain would favor the collapse of a backbone with the addition of a selective good solvent for side chain. Therefore, the self-assembly of dense graft copolymer could be controlled by selecting suitable solvent systems.

It is obvious that the self-assembly of the grafting copolymers is based on the interaction between polymer and solvent, and the selected solvent is of important. In general, the self-assembly of block copolymers or grafting copolymers is realized by dissolving firstly the polymers in common solvents and adding selective solvents into the polymers solutions (Jiang, Eisenberg, Liu, & Zhang, 2006). However, when the common solvent of methanol or DMF was used in this work, the self-assembly of the dense grafting copolymers failed. In other words, the general method for preparing micelles of block copolymers or grafting copolymers did not fit in these systems. Fortunately, when the relatively poor solvent THF for the side chains was used, the self-assembly of the grafting copolymers was successfully realized. According to these results, it could be concluded that the backbone-collapsed self-assembly of relatively dense grafting copolymers could be achieved by choosing relatively poor solvents for side chains. Moreover, the single macromolecular micelles could be obtained by adjusting the side chain length. This method is clearly different from the common method of preparing micelles because of the special chain structure of dense grafting copolymers. The strategy adopted in this work is based on the selected solvent, but it is different from the common method. The strategy extends the method of preparing solvent-induced micelles and may help us further understand the mechanism of micellar formation. This may provide a novel method to manipulate the backbone-collapsed self-assembly of dense graft copolymers.

4. Conclusions

In summary, we have studied the self-assembly behavior of the dense EC-*g*-PAA graft copolymers with two different graft densities and side chain lengths in THF/water system by DLS and TEM. The intra-or inter-molecular association of the backbone in the dense graft copolymers, leading to the formation of micelles, were successfully realized by selecting a suitable solvent. The solvent and graft length influence the self-assembly behavior. In THF/water systems, the unimolecular spherical micelles were obtained from the graft copolymers with longer side chain, and the multimolecular spherical micelles were obtained from those with shorter side chain, while no micelles were formed in methanol/water and DMF/water systems. The effects of solvent and graft length could be illuminated by the chain conformation in solvents. This provides a strategy to control the micellization of graft copolymer by adjusting the chain conformation.

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