Short-Life Core−Shell Structured Nanoaggregates Formed by the Self-Assembly of PEO-*b*-PAA/ETC (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Methiodide) and Their Stabilization

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Core−shell structured polymeric nanoaggregates of various morphologies have raised great interest in the scientific literature.[1] Among them, the water-soluble ones formed by double-hydrophilic block copolymers (DHBCs) in water are particularly attractive due to their potential applications in the fields of biological and medical sciences.[2] Usually, the polymeric nanoaggregates are the results of the self-assembly of block copolymers in selective solvents.[3]

Although water is the common solvent for DHBCs under normal condition (room temperature and pH 7), it may become the selective solvent when the pH[4] or the temperature is changed.[5] So, in some cases, altering the pH or the temperature can lead to the self-assembly of DHBCs in water, resulting in the polymeric nanoaggregates being sensitive to the environmental changes. In addition, it is reported that the complexation of DHBCs in water with either the oppositely charged polymer chains[6] or surfactants[7] can also induce the self-assembly, producing water-soluble polymeric nanoaggregates as well. The complexation of DHBCs with surfactants has been especially focused on, since there are a wide variety of available surfactants for one's choices and the composition of the resultant complexes is readily adjustable.[8] Through introducing surfactants to the aqueous solutions of DHBCs, Kabanov and Eisenberg and Jerome et al. prepared water-dispersible spherical micelles, vesicles, and their mixtures.[7] Considering that the complexes formed by single-tail surfactants are not stable enough to keep their integrity upon dilution in biological fluids, Kabanov and Eisenberg further stabilized them by chemically linking the surfactant components with each other.[9]

This paper deals with self-association of complexes of ETC (1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide) and PEO-*b*-PAA. ETC is a small molecular mass organic compound. It was selected for the following reasons: it can be molecularly solubilized in water and react with AA units promptly; it is the necessary catalyst to cross-link PAA with a water-soluble diamine at room temperature.[10] This catalyzed cross-linking reaction was used to stabilize formed aggregates for the preparation of "SCK" particles.[10e] A more remarkable work,[10a] concerning the use of ETC and the cross-linking reaction, demonstrates a rod-to-sphere morphological transition, induced by the reaction between ETC and the PAA block in the shell of rodlike aggregates, and shows clearly the trapped intermediate morphology of the transition by subsequent cross-linking reaction with diamine. In our study, the reaction of ETC with the PAA block of molecularly dispersed PEO-*b*-PAA in water leads to the self-association, resulting in the aggregates with the morphologies of short rods, vesicles, encapsulated spheres, and long fibers. It is interesting to find that the aggregates have the unique feature of "autodissociation" and exist in the aqueous solutions only for 1−3 weeks. On the other hand, these aggregates can be readily stabilized by the ETC-catalyzed cross-linking reaction.

The block copolymer used for this study is PEO_{113}-*b*-PAA_{70} (the subscripts indicate the average number of repeat units for each block; the polydispersity index of the precursor PEO_{113}-*b*-P*t*BA_{70} measured by SEC is 1.20). Either the block copolymer or ETC can be molecularly dispersed in their respective aqueous solutions, while PEO_{113}-*b*-PAA_{70} was mixed with ETC in water at a fixed polymer concentration of 0.5 mg/mL but with varying [ETC]/[carboxyl group] ratios (denoted as MR) of 0.5, 1.0 and 1.5, a bluish tint appeared simultaneously. The resultant solutions were measured by dynamic light scattering (DLS) 3−4 h after the mixing (it is proven by DLS measurements that after the duration, no remarkable changes were detected for the solutions in the subsequent 3−4 days). The characterization data are presented in Table 1 (line 1).

The data in Table 1 (line 1) indicate that the self-association took place after the mixing, leading to the formation of the aggregates with the sizes around 110 nm. The morphologies of the resultant aggregates were observed by transmission electron microscopy (TEM, the preparation of the specimens for TEM observations is described in the Experimental Section). The TEM images for different MR are shown in Figure 1. As is shown in Figure 1, parts a, b, and c, different morphologies of resultant aggregates were obtained corresponding to different values of MR. When MR is 0.5/1, the resultant aggregates are mainly of a short rodlike morphology with the aspect ratios between 2 and 5 (Figure 1a), leading to a relatively large value of PDI measured by DLS (0.25, Table 1). At MR of 1/1, vesicles with a wall of uniform thickness and with a relatively low PDI (0.13) are obtained (Figure 1b). In principle,[10] when MR is 1.5/1, there should be a remarkable amount of ETC molecules free from reacting with the block copolymer. Nevertheless, it is found that these unreacted ETC molecules have a great effect on the behavior of the self-association, compared with that at MR 1/1. The self-association at MR of 1.5/1 results in nanoaggregates with the morphology of a solid sphere surrounded by a peripheral cycle (Figure 1c), and the solid sphere appears to be separated with the cycle. This morphology is different from that of core−shell micelles but similar to that of the encapsulated ones.[12] It is noted that the average diameters of the vesicles (Figure 1b) and the aggregates shown in Figure 1c based on the TEM observations are 60 and 40 nm, respectively, smaller than that observed by DLS. A similar phenomenon was reported by Jerome et al. and was accounted for by the fact that the contrast of the PEO shell is too low to be seen by TEM.[13] In addition, Chi Wu et al.[14] proved that a core of a copolymer aggregate contained more than

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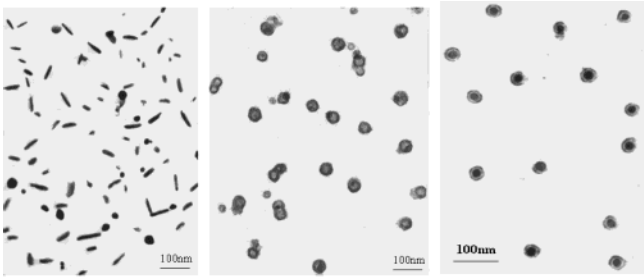
Table 1. Characterization Data of the Resultant Nanoaggregates Measured by Dynamic Light Scattering with an LLS Spectrometer (Malvern Autosizer 4700) with a Laser Source of Wavelength 514.5 nm and a CONTIN Analysis Model[a]

	MR	0.5/1	1/1	1.5/1
line 1	$\langle D_h \rangle$ (nm); $\mu_2/\langle\Gamma\rangle^2$ before cross-linking	106; 0.25	107; 0.13	119; 0.12
line 2	$\langle D_h \rangle$ (nm); $\mu_2/\langle\Gamma\rangle^2$ after cross-linking	102; 0.15	95; 0.18	104; 0.21
line 3	$\langle D_h \rangle$ (nm); $\mu_2/\langle\Gamma\rangle^2$ cross-linked aggregates, after dialysis	98; 0.15	91; 0.09	98; 0.14

[a] $\langle D_h \rangle$: the average hydrodynamic diameters of the resultant nanoparticles, measured at a scattering angle of 90° and 25 °C. $\mu_2/\langle\Gamma\rangle^2$: polydispersity index (PDI) of the size distribution; see ref 11.



a. MR: 0.5/1 c: 0.5mg/mL b. MR=1/1 c: MR=1.5/1

Figure 1. TEM images of aggregates obtained at different MR.

50% of solvent even in its highly packed state, so the shrinking of the core (or the wall of the vesicles) upon drying should make another contribution to the difference.

DLS measurements (conducted at the scattering angle of 90° and 25 °C) have proven that the resultant aggregates have a short life. Thus, 4−6 days after the preparation, the sizes and the polydispersity indexes of the aggregates begin to increase whereas the light scattering intensities decrease, indicating that the aggregates start to dissociate. Then 1−3 weeks after the preparation, the aggregates disappear. It is reported[10a] that the reaction of ETC with the acrylic acid functionalities will produce acylisourea and anhydride active intermediates and finally return to the acrylic acid functionalities again. As is indicated in Scheme 1, the production of acylisourea makes a part of PAA block be positively charged due to the attachment with the quaternary ammonium groups. It is reasonable to suppose that the complexation should take place between the quaternary ammonium groups and remained carboxylic anions. The complexation taking place among different PAA chains and the anhydride produced by the reaction of the initially formed *O*-acylisourea with excess carboxylic acid groups may drive the self-association of the block copolymer, forming the above-mentioned aggregates. (Note: In addition, our experiment demonstrates that mixing PAA homopolymer (M_w = 110 000) at different molar ratios (MR) of ETC to the AA units leads to the simultaneous formation of aggregates or precipitates. We analyzed the particles and precipitates by FT-IR. It is found that the carboxyl groups are detectable with a specific intensity at any stage after the mixing even when excess ETC was used, which makes the complexation between quaternary ammonium groups and the carboxylic anions possible. According to ref 10a (supporting Figure 9), it seems that the production of a considerable amount of anhydride may take a specific time. Therefore, we are inclined to think that the complexation is the initial driven force for the association.) Some time later, a considerable part of the acylisourea groups should change to anhydride groups, and the latter change back to carboxyl groups, so the aggregates start to dissociate. Because of this reaction cycle, the polymeric nanoaggregates appear as an intermediate and exist in the aqueous solutions for 1−3 weeks (Scheme 1). This unique "self-association and self-dissociation" feature makes the resultant aggregates very promising in the uses as carriers for controllable drug release. In this sense, the duration of 1−3 weeks for the existence of the aggregates is also desirable.

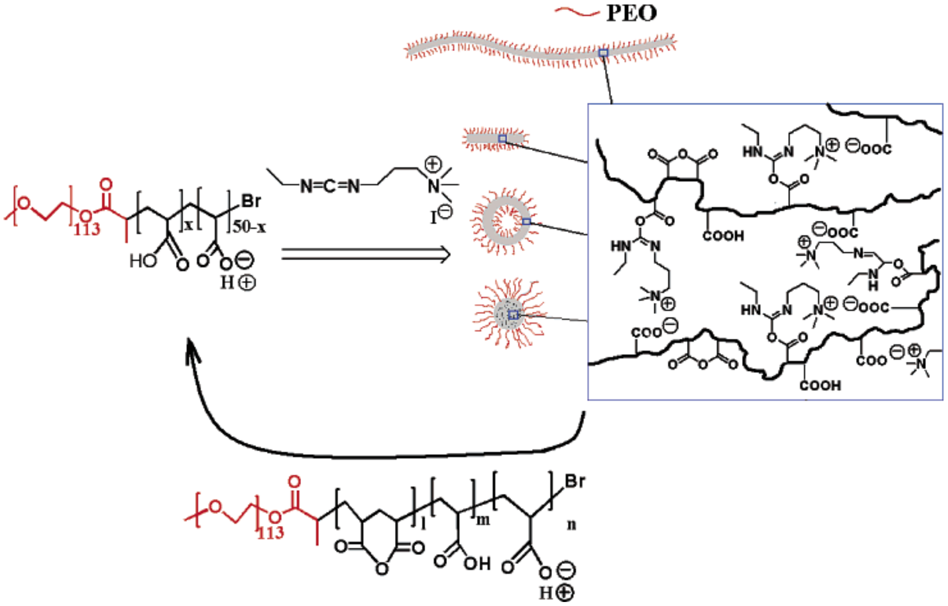
As mentioned above, ETC is also the catalyst for the cross-linking of PAA block. All the resultant aggregates can be readily stabilized by introducing a cross-linker 2,2′-(ethylenedioxy)bis(ethylamine) into the solutions 3−4 h after the mixing of ETC with the copolymer. In our study, a half of stoichiometric amount of the cross-linker was used for each of resultant solutions. Table 1 (line 2) presents the values of $\langle D_h \rangle$ and polydispersity indexes of the cross-linked aggregates. After the cross-linking, the $\langle D_h \rangle$ values decrease a little, indicating that no remarkable interparticle cross-linking reaction has taken place. According to the results we reported previously,[15] due to the shielding effect of the PEO shell, the cross-linking reaction should be localized within each individual aggregate. The cross-linked aggregates are very stable since no dissociation of the aggregates was found by DLS measurements taken months after the preparation.

The solutions of cross-linked aggregates were further dialyzed against water. The $\langle D_h \rangle$ and PDI values of cross-linked aggregates after the dialysis are presented in Table 1, line 3. It is exhibited that little change due to the dialysis is found in the $\langle D_h \rangle$ values at MR of 0.5/1, 1/1, and 1.5/1 and the PDI value at MR of 0.5/1. Remarkable changes due to the dialysis are detected in the PDI values at MR of 1/1 and MR of 1.5/1. These values drop from 0.18 and 0.21 to 0.09 and 0.14, respectively. These changes maybe related to the release of ETC from the aggregates due to the dialysis. The release of the ETC also leads to the decrease in the contrast of the aggregates in TEM image (Figure 2 a).

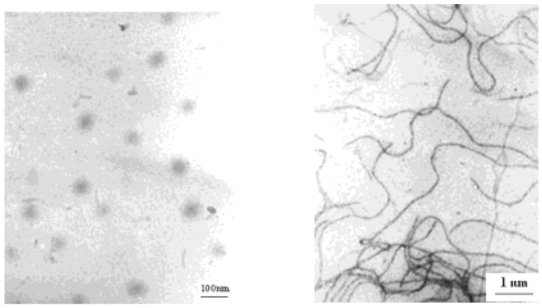
In addition, the self-assembly of ETC/PEO-*b*-PAA at the concentration of the block copolymer of 0.1 mg/mL and MR of 1/1 leads to the formation of long nanofibers of ∼30 nm wide and tens micrometers long or even longer (Figure 2b). This fiberlike morphology was viewed by TEM and AFM. The fiberlike aggregates can be readily stabilized by the ETC-catalyzed cross-linking reaction as well.

It is well-known that the self-assembly of block copolymers in selective solvents can result in nanoaggregates of different morphologies such as spheres, vesicles and wormlike micelles.[1] According to Eisenberg,[16] the formation of different morphologies can be explained by considering a balance between three major forces acting on the system: the stretching of the core-forming blocks, the interfacial tension between the core and the solvent, and the intercorona repulsion. In the present study, the change in MR will result in variation in the composition of the core of the aggregates, leading to alteration in the Coulombic interactions within and

Scheme 1. Schematic Description of the Complexation and the Resultant Self-Association and the Dissociation[a]



[a] An additional explanation is given as a note. The details of the reaction of ETC molecules with carboxylic anions can be found in ref 10.



a b

Figure 2. (a) TEM image of cross-linked micelles after dialysis (MR = 1.5/1). (b) TEM image of the nanofibers resulting from the self-association at the concentration of block copolymer of 0.1 mg /mL and MR of 1/1.

the hydrophilicity of the core. This alteration should be responsible for the morphological transition. The self-association and morphological transition of a diblock copolymer driven by a similar mechanism was reported by Jerome et al.[6d] In addition, when MR increases to 1.5/1, each of the resultant aggregates in the TEM image (Figure 1c) contains a solid sphere in the center. It was also reported for the complexes of block copolymers and surfactants that the excess surfactants may be encapsulated.[17] So, we are inclined to think that in this case the excess ETC molecules are encapsulated as well. The encapsulated ETC molecules and the PAA connected ones maybe separated during evaporation, as is indicated by an empty gap between them.

While the concentration of PEO-*b*-PAA is 0.1 mg/mL, at MR of 1/1, long nanofibers are produced. This effect of dilution may be related to the concentration sensitive equilibriums between the reactants and the products of the mentioned reactions. Further study on the mechanism is needed.

In conclusion, the self-association takes place in the aqueous solution of PEO-*b*-PAA/ETC at the early stage of the reaction between PAA and ETC. At the concentration of PEO-*b*-PAA of 0.5 mg/mL, when MR is 0.5/1 and 1/1, rodlike aggregates and vesicles were obtained respectively; while MR is 1.5/1, the excess ETC molecules maybe encapsulated and the resultant aggregates are with the morphology of a solid sphere encapsulated by a peripheral circle in the TEM image. The self-association at MR of 1/1 and the concentration of the block copolymer of 0.1 mg/mL leads to the formation of long nanofibers. Because of the reaction cycle of ETC with PAA, the aggregates have a limited life in water. After staying unchanged for several days in the aqueous solutions, the aggregates dissociate and finally disappear 1−3 weeks after their formation. While through the ETC-catalyzed cross-linking reaction, the aggregates can be stabilized. Except for the vesicles, the morphologies of short rods, encapsulated spheres, nanofibers are seldom reported for the systems of DHBCs/small molecules. Besides, the biocompatible and water-soluble properties along with the autodissociation feature of the un-cross-linked aggregates will make this system very promising in addressing various theoretical and practical problems.

Experimental Section

The PEO$_{113}$-*b*-PAA$_{70}$ was prepared from hydrolysis of a PEO$_{113}$-*b*-P*t*BA$_{70}$ copolymer through treating this precursor with trimethylsilyl iodide and subsequently with aqueous acid. The precursor was synthesized by bulk ATRP using PEO−Br as the macroinitiator.[18] The polydispersity index of the precursor is 1.20, measured by SEC.

The specimens for TEM observations were prepared 3 to 4 h after the mixing of ETC with the PEO-*b*-PAA by immersing a copper grid, which was coated with thin films of Formvar and carbon successively, into a micelle solution. Then the copper grid was freeze-dried in vacuo for 2 days.

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