doi:10.1246/cl.2011.1074
Published on the web September 17, 2011

pH-Responsive Self-assembly of Partially Hydrolyzed Polyacrylamide in Aqueous Solution

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(Received June 8, 2011; CL-110478)

As a simulacrum of random copolymer poly(acrylamide-*co*-acrylic acid), partially hydrolyzed polyacrylamide (HPAM) self-assembled into polymeric micelles in pure aqueous solution in pH response. Spherical nanoparticles ($D = 150$ nm) were generated at pH 12, and giant semihexagonal nanoplates (450 nm × 250 nm × 50 nm) were obtained at pH 1.3, but no visible micelles were observed in pH 3–10. This is important for drug release considering the pH environment in human stomach and intestines. The giant semihexagonal micelles obtained at pH 1.3 are hierarchical multivesicular vesicles with the structure of (hydrophilic inner vesicle)@(hydrophobic continuous cyst wall)@(hydrophilic shell). These multivesicular vesicles were formed because the random copolymer has a random distribution of the hydrophilic units in the hydrophobic core. In addition, a new contrast enhancing strategy by in situ reduction gold doping that we established was efficient at illuminating the nanostructure of polymeric micelles with low contrast, and this strategy came with a way to obtain polymer nanoparticles smaller than 20 nm.

Unlike block or graft copolymers that have been reported intensively on their self-assembly behaviors, random copolymers, except polymeric surfactants,[1] are presented much less because of the uncontrollable driving force for self-assembly due to their random chain distribution. However, random copolymers are easy to synthesize and inexpensive. Continuous efforts have been made on their self-assembly in selective solvents.[1–8] For example, random copolymers of *N*-vinylformamide and acrylic acid showed complex phase behaviors,[9] assembling into round nanoparticles with a broad particle-size distribution under high acidity conditions, while poly(styrene-*co*-4-vinylpyridine) formed spherical nanoparticles of hydrodynamic diameters around 100 nm in DMF/H_2O in a pH range of 5–3 and multicore structures at lower pH.[10] Nevertheless, self-assembling in pure aqueous solution is much more beneficial to applications in life science and medicine than that in selective solvent, which urges research on the self-assembly of random copolymers in aqueous phase.

There are few reports on the self-assembly of random copolymers such as polymeric surfactants[1] in pure aqueous solution, although it is well-known that some block or graft polymers can self-assemble in aqueous solution.[11–16] We noticed that poly(acrylamide-*co*-acrylic acid), a random copolymer, is water soluble in neutral conditions, and its chain units of acrylamide or acrylic acid present different protonated forms under various pH. These pH-responsive species could arrange themselves into different hydrogen-bonding pairs under different pH as shown in Figure 1. This hydrogen bonding gives a possibility for hydrophobic self-assembly in aqueous solution

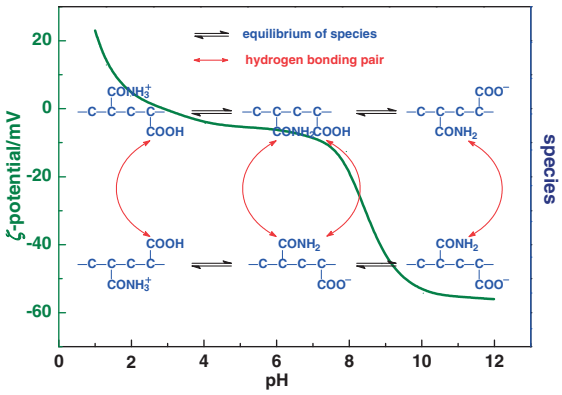

Figure 1. pH-responsive hydrogen-bonding pairs and ζ-potential of HPAM aqueous solution.

without any selective solvent assistance, in which the main driving force could be responsible for the hydrophobic self-assembly of the partially hydrolyzed polyacrylamide (HPAM). Subsequently, to provide a lower and controllable molecule weight for easier self-assembly, HPAM was synthesized and used as a mimic of poly(acrylamide-*co*-acrylic acid) in this experiment.[17,18]

As desired, the pH-responsive formation of HPAM micelles was observed as in Figure 2[19] (Figure 2A, pH 12, spherical nanoparticles, $D = 150$ nm; Figures 2B, 2C, and 2D, pH 1.3, semihexagonal nanoplates, 450 nm × 250 nm × 50 nm). No other obvious micelle morphologies were recorded in the pH range from 3 to 10, which is important for drug release considering the pH environment in human stomach and intestines. Yet this formation course fairly agrees with the ζ-potential trend as shown in Figure 1. The micellization was not possible in the pH range from 3 to 10 because HPAM micelles are unstable at zero ζ-potential or rapidly changing fields. Generally, for HPAM homologs, the higher the hydrolysis ratio, the higher the solubility of polymers in basic aqueous solution is, and the lower the hydrolysis ratio, the higher the solubility of the polymer in acidic aqueous solution is. Hence, the higher solubility results in the loose aggregates and even no aggregates formed when the solubility is high enough.

Under basic conditions (pH >10), –COOH groups of acrylic acid units were deprotonated to $–COO^-$, where the negative-charged acrylic acid rich domains became a hydrophilic micelle shell, while the acrylamide–acrylamide hydrogen-bonding pairs made up micelle cores. Then the ζ-potential at the micelle's surface declined corresponding with the increase of pH and finally stayed at −55 mV in pH 10–12, and the electrostatic repulsion kept the polymeric micelles from aggregation. Therefore, the hydration of $–COO^-$ was the accessorial driving force

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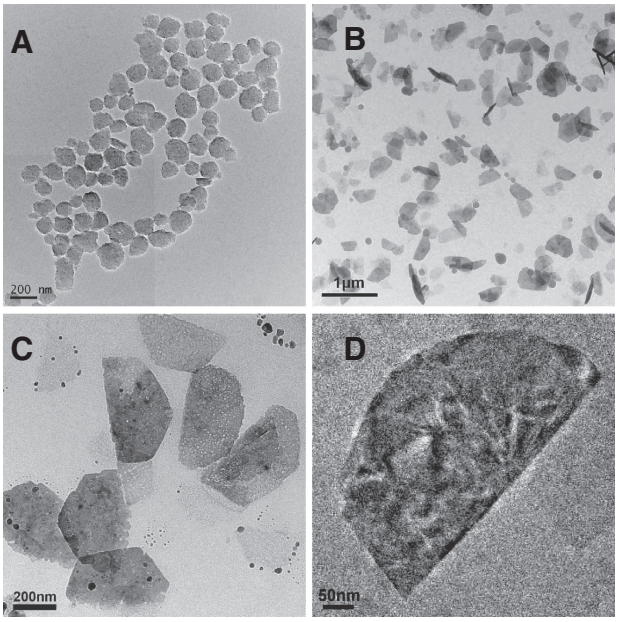

Figure 2. TEM images of the HPAM micelles at different pH: (A) 12; (B), (C), and (D) 1.3.

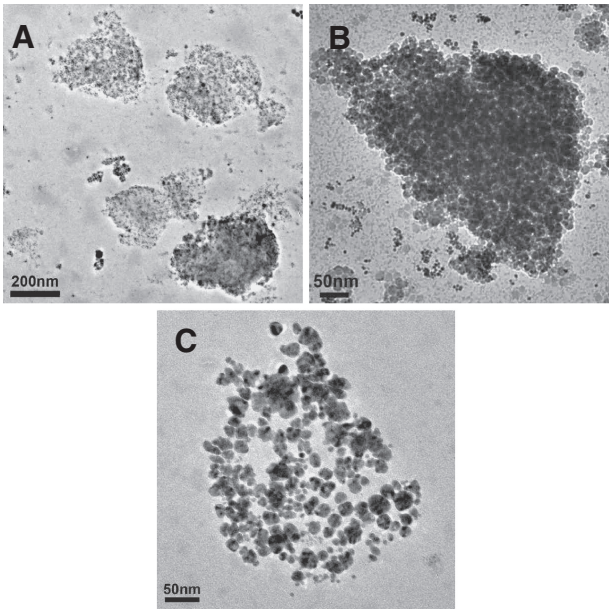

Figure 3. TEM images of semihexagonal plate HPAM micelles at pH 1.3 after in situ reduction in (A) 25, (B) 25, and (C) 60 min.

for the self-assembly under basic conditions. In comparison, under acidic conditions (pH <3), $–CONH_2$ groups of acrylamide units were protonated to $–CONH_3^+$. The acrylic acid–acrylic acid hydrogen-bonding pairs constructed the micelle core, and the weak cationic acrylamide-rich domains became the positively charged hydrophilic micelle shell. The hydration of $–CONH_3^+$ is the accessorial driving force for the self-assembly under acidic conditions. Meanwhile, ζ-potential at the surface climbed to 15 mV with the pH declining, which made it possible that the HPAM micelles could further aggregated to bigger microplates. It is well-known that block or graft polymers in aqueous environment place their hydrophobic segments in micelle cores and make micelle shells only or mainly with their hydrophilic segments. This is not the case for random copolymers. Herein, comparing to what happened at pH 12, the HPAM micelle core at pH 1.3 was much less hydrophobic since the –COOH groups in acrylic acid rich domains are somewhat hydrophilic; and the $–CONH_3^+$ random distributed into acrylic acid-rich domains repulsed one another, which potentially induced a loosened core structure. Actually, the bright spots and the nonuniformity in Figures 2C and 2D imply where the micelle structure might be loosened. Unfortunately, the weak cationic micelle has a lower electron density that induces a weaker contrast and makes it difficult to identify clearly the nanoscale details of the micelle morphology.

The strategy we developed to ease the micelle morphology observation is enhancing the contrast by doping gold on the micelle surface, based on the fact that $HAuCl_4$ could be in situ reduced to gold atoms by polymers with weaker reducing ability,[20] which was then deposited onto the micelle surface. Hence, if the micelle was loosened or porous, the in situ deposited gold atoms could magnify the contrast of where it was doped. The time course of the in situ reduction of $HAuCl_4$ is illustrated in Figure 3. The reducing in 25 min indeed enhanced the contrast (Figure 3A), made a porous morphology visible and simultaneously kept the semihexagonal profile, as shown in the magnified single plate (Figure 3B). The extended reduction (60 min) further loosened the micelle, and even partially departed the giant semihexagonal profile into subunit micelles, which surprisingly pointed out a way for making polymer nanoparticles smaller than 20 nm.[21]

Based on the discussion aforementioned, we made the hypothesis that the semihexagonal nanoplate was composed of a hierarchical structure of (hydrophilic inner vesicle)@(hydrophobic continuous cyst wall)@(hydrophilic shell), and the reflection on TEM images represents the hierarchical multivesicular vesicles with lower contrast. Because of the random distribution of acrylamide units, the cationization was enhanced with the pH decreasing in both the micelle shell and the core. This cationization increment not only induced richer cationic hydrophilic layer of the shell, but also increased the electrostatic repulsion in the rich acrylic acid segments, resulting in the microphase-separation in the kinetically stable hydrophobic core, as well as the cavitation, which caused loosened or porous structures. The thin semihexagonal shape, we think, is a kind of mutant half-moon morphology but the reason resulting in them still remains unknown.

In conclusion, the partially hydrolyzed polyacrylamide can self-assemble in pure aqueous solution with pH response. The hydrogen-bonding complex is the primary driving force for the self-assembly of the random copolymers or its mimics which induced the formation of the hydrophobic polymer core; and the hydration of electronic charged units is the secondary driving force, which induced the formation of the hydrophilic polymer shell. In addition, the contrast enhancing strategy by in situ reduction gold doping is efficient at illuminating the nanostructure of micelles with low contrast, and this strategy also comes with a way to obtain polymer nanoparticles smaller than 20 nm. Moreover, the semihexagonal micelles obtained at

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pH 1.3 might be hierarchical multivesicular vesicles with the structure of (hydrophilic inner vesicle)@(hydrophobic continuous cyst wall)@(hydrophilic shell). These multivesicular vesicles formed because HPAM has a similar random distribution of the hydrophilic units in the hydrophobic core.

We gratefully thank the support of National Natural Science Foundation of China (No. 20871059) and Professor Jiang Ming for his helpful comments.

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16 K. Matoishi, S. Nakatsuka, K. Nakai, M. Isokawa, N. Nagai, T. Fujita, *Chem. Lett.* **2010**, *39*, 1028.
17 Preparation of polyacrylamide (PAM): 15-mL acrylamide solution (20 g acrylamide dissolved in 40-mL H_2O) and 7-mL ammonium persulfate solution (15 mmol L^{-1}) was added dropwise into a 250-mL three-neck flask containing 20-mL 2-propanol, 5-mL aforementioned acrylamide solution, and 5-mL ammonium persulfate solution at 65 °C in 1.5 h. The polymerization was performed at 65 °C for another 3 h under stirring. The resulting polymer was vaporized and then dried under vacuum at 40 °C for 24 h.
18 Preparation of partially hydrolyzed polyacrylamide (HPAM): 2 g PAM was dissolved in 40-mL NaOH solution (1.25 mol L^{-1}), stirred at 50 °C for 0.5 h, the reaction mixture was then precipitated in 300-mL methanol. The precipitates were washed three times with methanol and then dried under vacuum at 40 °C for 24 h, a white powder HPAM was obtained ($M_w = 2.84 \times 10^4$, $M_n = 1.13 \times 10^4$). The hydrolysis ratio of the resulting HPAM was 36%.
19 Preparation of HPAM micelle solutions: 0.1 mol L^{-1} of HCl or NaOH solution was added (40 μL min^{-1}) into 25-mL aqueous HPAM solution at 25 °C to the desired pH. The final HPAM concentration was about 1 mg mL^{-1}. The ζ-potential was recorded (Zetasizer 2000) at 25 °C, all data was measured 5 times. For TEM image, a drop of the resulting micelle solution was sprayed onto a copper TEM grid covered with a Formvar supporting film precoated with a thin carbon film, and the excess solution was blotted away using a strip of filter paper immediately. All samples were left to dry at room temperature before staining. Samples were viewed by using a JEOL-JEM2100 TEM operated with an accelerating voltage of 200 kV.
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21 In situ reduction gold doping: 0.1 mol L^{-1} of HCl was added into a 10 mL tube containing 2-mL HPAM (5 mg mL^{-1}) and 6-mL water under room temperature, adjusting the solution to pH 1.3 and made the final volume of the solution to 9-mL with water. The solution was stirred at 40 °C for another 30 min before 1-mL $HAuCl_4 \cdot 4H_2O$ (1 mmol mL^{-1}) was added. The reduction was finished in 25 or 60 min.

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