Communications

pH-Dependent Self-Assembly: Micellization and Micelle–Hollow-Sphere Transition of Cellulose-Based Copolymers**

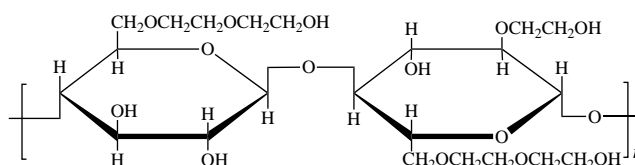*Hongjing Dou, Ming Jiang,* Huisheng Peng, Daoyong Chen, and Yan Hong*

pH-Sensitive micelles are usually made from double-hydrophilic block copolymers which can dissolve molecularly in water in a certain pH range and aggregate spontaneously upon an appropriate change in the pH value. Significant advances in this field have been achieved in recent years.[1]

Polymeric hollow spheres have great potential for the encapsulation of large quantities of guest molecules. Different approaches, such as by the use of block copolymers as precursors,[2] as well as colloidal particles[3a,b] or liposomes[3c,d] as templates, have been developed to obtain such nanocapsules. Our research group has produced micelles in which no chemical bonds connect the core and the shell,[4] thus enabling hollow spheres to be obtained by simple dissolution of the inner component of the micelles.[4a–c] However, these approaches are mostly irreversible, that is, once the hollow structure forms, the core–shell micelles cannot be reformed.
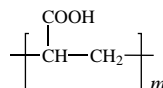
Hydroxyethylcellulose (HEC) has been extensively studied as a nonionic and water-soluble cellulose ether (Scheme 1).[5] However, studies on the self-assembly of HEC are limited, despite its biocompatibility and biodegradability. Here we report the self-assembly of HEC-*graft*-poly(acrylic acid) (HEC-*g*-PAA) in water, which was prepared by free-radical graft polymerization of acrylic acid from HEC backbones (Table 1). The results demonstrate its micellization and the transition between micelles and hollow spheres; both processes were found to be pH-dependent and reversible.

Figure 1 shows the relative scattering intensity I/I_0 of the solutions of two HEC-*g*-PAA copolymers, CAA-1 and CAA-2, as a function of the pH value (I_0 is the intensity of the starting solution at pH 13.5). The two solutions exhibit a

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HEC backbone

PAA graft

Scheme 1. Chemical structures of HEC and PAA.

Table 1: Characteristic data of HEC-*g*-PAA copolymers.

Sample no.	AA:AGU[a] (molar radio)	$M\eta \times 10^{-5}$ (HEC backbone)	$M\eta \times 10^{-5}$[b] (PAA grafts)	Average graft point	$Mw \times 10^{-5}$[c] (HEC-*g*-PAA)	$\langle D_h \rangle$ [nm] [d]
CAA-1	1.28:1	0.9	0.175	1.58	1.67	53.8
CAA-2	2.54:1	0.9	0.210	2.61	3.33	75.6

[a] The molar ratio of acrylic acid units to anhydroglucose units (AGU) was determined by enzymatic degradation. [b] Calculated from viscosity measurements. [c] Determined by SLS in 0.1 M NaOH, the Mw data were obtained using standard Zimm plot analysis. [d] Determined by DLS at the same condition as for SLS.
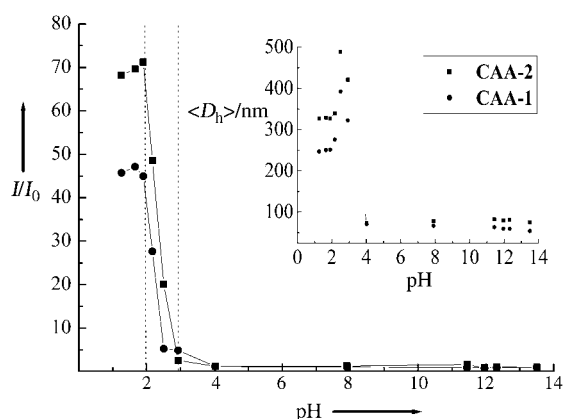

Figure 1. $\langle I/I_0 \rangle$ and $\langle D_h \rangle$ of HEC-*g*-PAA copolymers as a function of pH. The concentration was 1 mg mL^{-1}. I and I_0 are the scattering intensity at various pH values and at pH 13.5, respectively. The solution at pH 2–3 was unstable: the apparent $\langle D_h \rangle$ value increased with time. The $\langle D_h \rangle$ values measured 20 min after the solution preparation were used. However, the $\langle D_h \rangle$ values at pH < 2 and pH > 3 ranges did not change for several months.

similar behavior, namely, I/I_0 shows almost no change as the pH value decreases from about 13.5 to 4. However, as the pH value decreases further, the value of I/I_0 abruptly increases by about a factor of 50 over a small pH range (from pH 3 to 1.8), which indicates the formation of aggregates with high molar mass. Figure 1b shows the variation of the average hydrodynamic diameter $\langle D_h \rangle$ of the copolymers as the pH value decreases. The diameter of about 50–70 nm does not change between pH 13.5 and 4.[6] As the

pH value decreases to less than 4, the value of $\langle D_h \rangle$ jumps to 250 and 330 nm for CAA-1 and CAA-2, respectively. This abrupt increase in the $\langle D_h \rangle$ value between pH 2 and 4 reflects the transition from individual molecules to aggregates. In addition, the transition was found to be reversible: the aggregates dissociated into individual molecules when the pH value of the solution was increased to about 4.

It is well known that polyacrylic acid chains undergo dramatic conformational changes, from an extended chain to a hypercoil, as the pH value decreases.[8, 9a] Such a change occurs gradually over a large pH range. We also found that the viscosity of the PAA solutions continuously diminishes as the pH value decreases. However, as shown in Figure 1, the jump in the relative scattering intensity and the $\langle D_h \rangle$ value takes place over a narrow pH range. Therefore, it cannot be attributed to the conformational variation of the PAA chains.

Polyacrylic acid can be considered as a proton-donating polymer, and thus could form complexes with many proton-accepting polymers.[9b] Recently, Nikolaeva et al. reported the interpolymer complexation between HEC and PAA in water resulting from the formation of hydrogen bonds when the PAA chains are sufficiently protonated.[10] Therefore, the aggregation of CAA in the low pH region can be attributed, in our opinion, to interpolymer complexation between HEC and PAA. As no macroscopic precipitation took place in this process, we suggest that the HEC chains which have less complexed segments may still remain solvated and thus stabilize the complex aggregates.

Figure 2a shows the morphologies of CAA-1 micelles in an acidic medium. All the micelles have a perfect spherical shape with a distinct boundary between the core and the shell. The micrograph of CAA-1 at a high magnification clearly shows the details of the micellar structure (Figure 2b). The difference in rigidity between the HEC and PAA chains means that the core composed of the complex shows a much higher packing density than the shell composed mainly of semi-rigid HEC. Such a clear core–shell structure of the micelles is seldom seen in micelles composed of either coil–coil block copolymers[1d–f] or homopolymer pairs.[4a,b] The CAA-2 micelles show a much

larger proportion of the core and a less distinct core–shell boundary than CAA-1; this effect is a consequence of it containing a higher percentage of PAA (Figure 2c).

The PAA chains of the micelles were cross-linked simply by treating them with a desired amount of a cross-linker, 2,2′-(ethylenedioxy)bis(ethylamine), at room temperature[11] (see Experimental Section). Dialysis against water was then performed to remove all the impurities, which was accompanied by an increase in the pH value to 7. The bluish tinge characteristic of nanosized micelles was maintained in the resulting neutral medium, thus indicating that the cross-linking has successfully locked the integral structure.

Dynamic light scattering (DLS) studies showed the micelles had expanded after the cross-linking and dialysis. The size of the expansion depends on the composition of the parent copolymers and degree of cross-linking (Table 2). The most remarkable feature of the cross-linked micelles after dialysis, as found by TEM, is that the core–shell structure of the micelles is transformed into hollow spheres. A typical microimage of such hollow spheres of CAA-2 (Figure 2d) displays a clear contrast between the center and the shell that indicates the presence of a cavity. It is noteworthy that this cavitation was caused by neither degradation[2, 4d] nor dissolution of the core;[4a–c] a change in the pH value is exclusively responsible for the transition in morphology. DLS and static light scattering (SLS) measurements were made to estimate the parameter $\langle R_g \rangle / \langle R_h \rangle$, which is sensitive to particle morphologies, and so confirm the presence of a hollow structure. Both CAA-1 and CAA-2 have $\langle R_g \rangle / \langle R_h \rangle$ values in the range from 0.83 to 0.89 (Table 2), which are in accordance

Table 2: Light-scattering characterization data of the cross-linked CAA-1 and CAA-2 after dialysis against water.[a]

Sample no.	Cross-linker [%][b]	$\langle R_h \rangle$ [nm]	$\langle R_g \rangle$ [nm]	$\langle R_g \rangle / \langle R_h \rangle$	PDI
CAA-1	50	182	162	0.89	0.21
CAA-1	100	169	140	0.83	0.14
CAA-2	50	386	339	0.88	0.08
CAA-2	100	322	270	0.85	0.08

[a] The concentration of the measured solution here was very low (0.9×10^{-2} mg mL^{-1}) as required for the SLS apparatus. The original solution of $c = 0.9$ mg mL^{-1} was used for other DLS measurements and TEM observations. [b] This refers to the molar ratio of amine groups in the cross-linker to carboxylic acid groups of the PAA grafts. It reflects only the maximum possible degree of cross-linking.
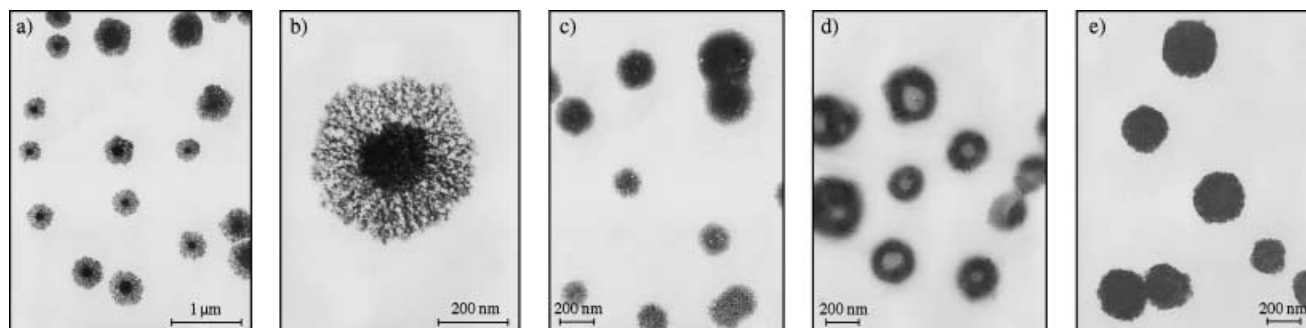

Figure 2. TEM image for a) the micelles of CAA-1 formed at pH 1.3 and b) that at a high magnification; c) the micelles of CAA-2 formed at pH 1.3; d) the hollow spheres of CAA-2 after cross-linking and dialysis, and e) the micelles of CAA-2 obtained by adjusting the acidity of the cross-linked and dialyzed CAA-2 solution to pH 1.3.

with the values derived from a model consisting of thick-layer hollow spheres.[12]

This transition between the micelle and hollow sphere can be rationalized as follows. Under the given conditions of cross-linking, the reaction proceeds mainly at the periphery of the micellar core. An increase in the pH value partially deprotonates the carboxylic acid in the PAA, which leads to de-complexation between the PAA and HEC, and hence a disintegration of the center part of the core.[14] As both the HEC and cross-linked PAA chains are highly hydrophilic, the cross-linked shell swells and forms hollow spheres. This swollen nanocage is expected to be very soft. Therefore, deformation and collapse of the network during the preparation of the TEM sample, led to a much smaller size (Figure 2d) than measured by DLS studies.

The pH-dependent cavitation of the micelles was also found to be reversible. Figure 3 shows the variation in the
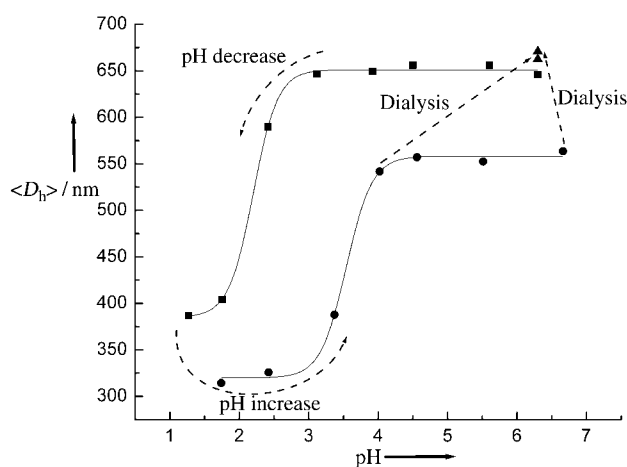
Figure 3. Average hydrodynamic diameter $\langle D_h \rangle$ of CAA-2 aggregates as a function of pH during the transition from micelle to hollow sphere (one reversible cycle). The concentration was 0.9 mg mL^{-1}.

$\langle D_h \rangle$ value of CAA-2 during pH cycling. The starting point corresponds to the cross-linked micelles in neutral solution. The $\langle D_h \rangle$ value does not change until the pH decreases to around 3. A sudden decrease in the $\langle D_h \rangle$ value occurs between pH 3 and 1.5, which is the same range where complexation and micellization occurs. Therefore, it is clear that complexation between the HEC and PAA is resumed as the pH value decreases to this range and, consequently, the hollow spheres revert to core–shell micelles. As shown in

Figure 2e for the micelles at pH 1.3, the cavity has disappeared and the morphology clearly resembles the original micelles prior to cross-linking (Figure 2c).

The second half of the $\langle D_h \rangle$ versus pH cycle in Figure 3 shows the reverse process, that is, the micelles revert to hollow spheres as indicated by a jump in the size at a higher pH range (pH 3–4). The $\langle D_h \rangle$ value of 550 nm at the plateau of the second half is somewhat smaller than its value in the first half, 644 nm. This discrepancy could be caused by a difference in the ionic strength between the solutions. This argument is confirmed by the following fact: The micelle solutions at pH 4.0 and pH 6.7 in the second half of the cycle were dialyzed against water. After dialysis, the $\langle D_h \rangle$ values in both cases jumped to approximately 650 nm, a value very close to the plateau value of $\langle D_h \rangle$ in the first half of the cycle (Figure 3).

Our findings can be summarized as follows (Figure 4): The PAA chains are sufficiently protonated at pH 3 to be able to form a complex with HEC and form small aggregates which are stablized by the free HEC; thus micellization takes place. Cross-linking of the PAA chains in the micelles can efficiently lock the integrality of the micelles. Decomplexation occurs in neutral medium, which causes disintegration of the micellar core; thus pH-induced cavitation takes place. This morphological transition from core–shell micelles to hollow spheres was found to be reversible by DLS studies and TEM. The resultant spheres are characterized by large hydrophilic cavities and have an on-off character: open at pH > 3 but closed at pH < 3. Such a unique micelle is expected to be useful in broad application fields.

Experimental Section

HEC-*g*-PAA was synthesized by cerium(IV)-initiated free-radical graft polymerization of acrylic acid from HEC.[15] The HEC backbones were degraded in the presence of cellulase for 3 days to enable characterization of the grafts.[16] The molecular weight of the PAA grafts was calculated from viscosity measurements: $[\eta] = 42.2 \times 10^{-3} M^{0.64}$ (in 2 M NaOH at 25 °C).[17]

Micellization: A 5 mg mL^{-1} stock solution of HEC-*g*-PAA in 0.1 M NaOH was further diluted to 1 mg mL^{-1} and the required pH value. The core cross-linking reaction of the micelles prepared at pH 1.26 was performed by adding the desired amount of 2,2′-(ethylenedioxy)-bis(ethylamine) as cross-linker in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide at room temperature for 10 h.[11] The cross-linked product was dialyzed against distilled water for 3 days to remove the by-products.

Malvern Autosizer 4700 and ALV/SP-125 laser light scattering (LLS) spectrometers were used. DLS measurements were performed at a fixed scattering angle (θ) of 15°. Static LLS studies were
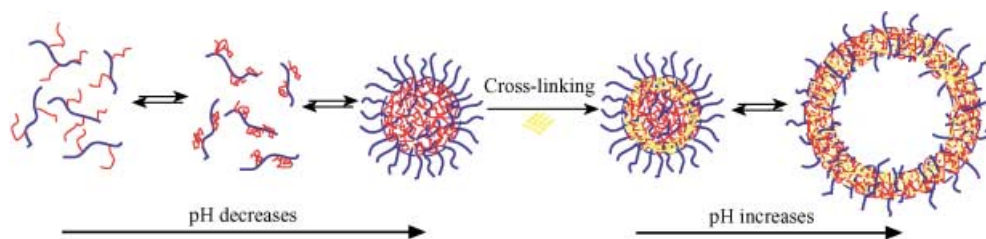
Figure 4. A proposed scheme of the pH-dependent micellization and transition of HEC-*g*-PAA from micelle to hollow sphere.

conducted at a low scattering angle range of 15 to 40°. The $\langle D_h \rangle$ and polydispersity index (PDI, that is, $(\langle \mu_2 \rangle / \Gamma^2)$) were obtained by a cumulant analysis. The respective dn/dc data of CAA-1 and CAA-2 were determined to be 0.124 and 0.150 in 0.1 M NaOH aqueous solution by using an Optilab DSP differential refractometer. TEM observations were performed on a Philips CM 120 electron microscope at an acceleration voltage of 80 KV. For all TEM observations, carbon-coated copper grids were dipped in the solutions of micelles followed by drying in a desiccator for at least 72 h.

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[12] The $\langle R_g \rangle / \langle R_h \rangle$ values for polymer coils, uniform spheres, and thin-layer hollow spheres are 1.50, 0.77, and 1.0, respectively, see reference [13]. For a hollow sphere with an outer radius R and wall thickness $R/3$, the $\langle R_g \rangle / R$ value is 0.86 according to a simple calculation. The $\langle R_g \rangle / \langle R_h \rangle$ values of CAA-1 and CAA-2 molecules in water at pH 13.5 are 1.48 and 1.58, respectively. The $\langle R_g \rangle / \langle R_h \rangle$ results support the hollow-sphere structure of CAA-2 shown in Figure 2d. However, as a major change in the particles may take place during the TEM sample preparation, for example, drying and subsequent shrinking of the swollen micelles, the image in Figure 2d can not be attributed to the hollow spheres exclusively.

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[14] Prolonging the cross-linking time to 24 h and increasing the amount of cross-linker to 200 % led to different results, namely, homogeneous microspheres without a central cavity were observed by TEM. The $\langle R_g \rangle / \langle R_h \rangle$ values of the heavily cross-linked CAA-1 and CAA-2 particles after dialysis are 0.74 and 0.76, respectively. In our opinion, strengthening the cross-linking reaction enabled the reaction to occur in the center as well as the periphery of the core. Thus, swollen microgel instead of hollow spheres was obtained when the pH value of the solutions increases.

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