Q1 Construction and micellization of a noncovalent double hydrophilic block copolymer†

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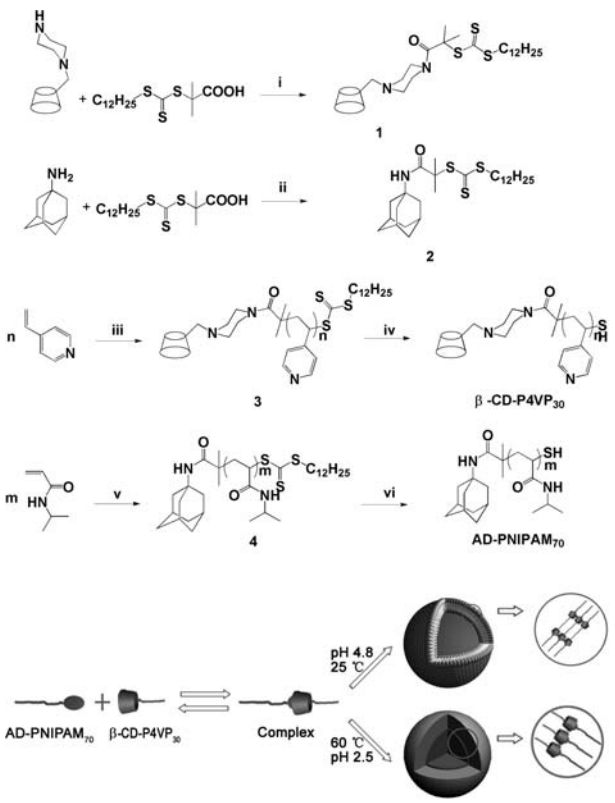
Q2 **The first noncovalent double hydrophilic block copolymer was constructed through inclusion complexation between β-cyclodextrin and the adamantyl group; it can further self-assemble into two distinctly different micelles in response to pH and temperature in dilute aqueous solution.**

Double hydrophilic block copolymers (DHBCs) consisting of two hydrophilic blocks can self-assemble into a variety of micellar structures, such as spheres, vesicles, rod-like micelles and lamellar structures in dilute aqueous solution. The micellar self-assembly of DHBCs has attracted much attention for their potential applications in drug delivery, nanotechnology and as biological vectors.[1–6] Recently, noncovalent interactions have been successfully employed in building supramolecular polymers[7–12] as well as supramolecular assemblies.[13–17] However, attempts to construct noncovalent DHBCs have not been reported yet. As a first step towards developing a noncovalent DHBC, herein we present a well-defined DHBC based on supramolecular interactions, and report on its micellization behavior in dilute aqueous solution.

As described in Scheme 1, the noncovalent DHBC was constructed through the host–guest inclusion between β-cyclodextrin (β-CD) and the adamantyl group (AD) with an association constant of 10^4–10^5 M^{-1}. In the first step, two novel chain transfer agents, β-CD containing trithiocarbonate **1** and AD containing trithiocarbonate **2**, were synthesized and applied in the reversible addition–fragmentation chain transfer (RAFT) polymerization of 4-vinylpyridine (4VP) and *N*-isopropylacrylamide (NIPAM), respectively. The resultant β-CD containing poly(4-vinylpyridine) (β-CD-P4VP) trithiocarbonate **3** has an M_n of 4800 and polydispersity (M_w/M_n) of 1.17; and the resultant AD containing poly(*N*-isopropylacrylamide) (AD-PNIPAM) trithiocarbonate **4** has an M_n of 5700 and polydispersity of 1.10. The degree of polymerization of **3** and **4** was calculated to be 30 and 70 from ^{1}H NMR spectra, thus they were denoted as β-CD-P4VP$_{30}$ trithiocarbonate and AD-PNIPAM$_{70}$ trithiocarbonate, respectively. After the thiocarbonylthio end group was removed, β-CD-P4VP$_{30}$ was mixed with equimolar amounts of AD-PNIPAM$_{70}$ in aqueous solution to generate a well-defined noncolvalent DHBC, β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex.

^{1}H NOESY measurement provided direct evidence for the construction of the noncovalent DHBC by the inclusion interaction. The NOESY spectrum of a mixed solution containing equimolar amounts of β-CD-P4VP$_{30}$ and AD-PNIPAM$_{70}$ in D_2O exhibits clear NOE cross-peaks between the signals at δ 3–4 ppm ascribed to the interior protons of β-CD and the signals at δ 1.5–2.2 ppm due to AD, which indicates that the AD groups are deeply included in the cavities of β-CD.

The fluorescence emission spectra of pyrene were used to determine the micellization behavior of the aqueous solution of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex. The intensity ratio of the first vibration band to the third one (I_1/I_3)



Scheme 1 Synthetic route for β-CD-P4VP$_{30}$ and AD-PNIPAM$_{70}$, and schematic illustration of the construction and micellization of noncovalent DHBC, β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex. (i) DMF, DCC–DMAP, 0 °C; (ii) CH_2Cl_2, DCC–DMAP, r.t.; (iii) DMF, AIBN, **1**, 70 °C; (iv) DMF, hexylamine, 0.5 M $Na_2S_2O_4$, r.t.; (v) DMF, AIBN, **2**, 70 °C; (vi) DMF, hexylamine, 0.5 M $Na_2S_2O_4$, r.t.

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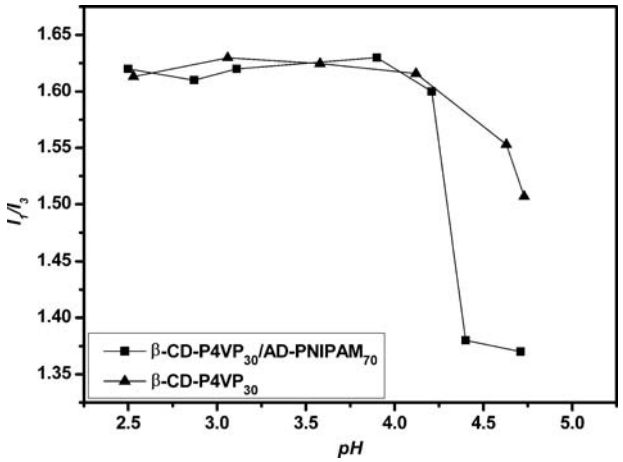

Fig. 1 Dependence of the intensity ratio I_1/I_3 on pH value for 0.2 mg mL^{-1} aqueous solution of β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at 25 °C (β-CD-P4VP$_{30}$ at a concentration of 0.2 mg mL^{-1} used as a control).

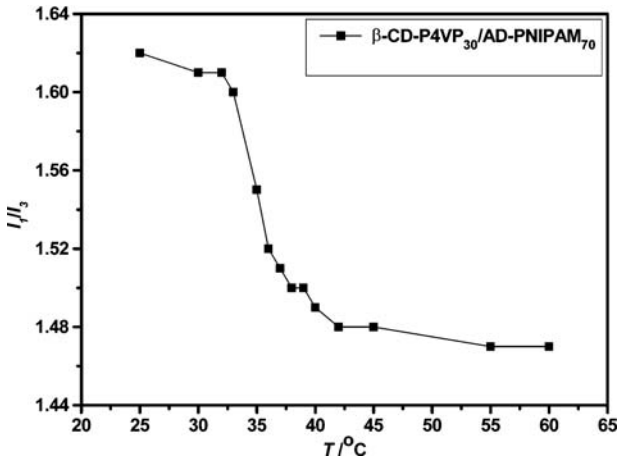

Fig. 2 Dependence of the intensity ratio I_1/I_3 on temperature for 0.2 mg mL^{-1} aqueous solution of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at pH 2.5.

in the emission spectra of pyrene is rather sensitive to the polarity of the external medium. When micelles are formed in aqueous solution, the pyrene molecules transfer from a hydrophilic to a hydrophobic microenvironment that will lead to a significant decrease of I_1/I_3.[18–20]

Fig. 1 shows the change of I_1/I_3 in an aqueous solution of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex associated with variation of the pH value at 25 °C. The attached PNIPAM chain is fully soluble at 25 °C. The inclusion complex is well dissolved at low pH values due to the protonation of the P4VP chain. I_1/I_3 only exhibits a slight variation with increased pH value in the solution. However, a pronounced fall of I_1/I_3 occurs when the pH value is higher than 4.2. The increased pH value of the medium could weaken the protonation of the P4VP chain, and decrease the water-solubility of P4VP. The reduced solubility of P4VP will cause the collapse of P4VP chains and the formation of micelles. In contrast, the decrease in I_1/I_3 in the aqueous solution of β-CD-P4VP$_{30}$ is much less than that in the solution of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at pH $>$ 4.2 since only loose aggregates could be formed in the absence of the hydrophilic PNIPAM chains.

It should be noted that pH-responsive micellization would not happen if pH $>$ 5 because of the equilibrium between free β-CD-P4VP$_{30}$, AD-PNIPAM$_{70}$ and the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex in aqueous solution. When pH $>$ 5, the P4VP chain will be highly deprotonated and free β-CD-P4VP$_{30}$ will deposit which would lead to the dissociation of this inclusion complex.

We also determined the thermo-responsive micellization of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at pH 2.5. At this pH value, the P4VP chain is completely soluble in aqueous solution because of the protonation effect. However, the conformation and water-solubility of the thermo-responsive PNIPAM chain can be changed by a high temperature,[21,22] and the PNIPAM chain will be hydrophobic when the temperature is higher than its lower critical solution temperature (LCST) which leads to the formation of micelles.

Fig. 2 shows the temperature-dependence of the intensity ratio I_1/I_3 of an aqueous solution of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at pH 2.5. The ratio of I_1/I_3 is relatively constant when the temperature is lower than 33 °C, but the value is dramatically decreased at $T > 33$ °C. The decrease is attributed to the formation of micelles. In this experiment, we did not use AD-PNIPAM$_{70}$ as a control since it could not present as a unimer form when its AD end group was not included in the cavity of β-CD.

Both of the pH- and thermo-responsive micelles were characterized by dynamic light scattering (DLS), static light scattering (SLS), transmission electron microscopy (TEM) and laser scanning confocal microscopy (LSCM).

The ratio of a radius of gyration $\langle R_g \rangle$ to the intensity-average hydrodynamic radius $\langle R_h \rangle$ is an important parameter and this ratio is dependent on the shape of the scattering objects in the solution or dispersion.[23] At pH 4.8 and 25 °C, $\langle R_h \rangle$ and $\langle R_g \rangle$ are 82.4 nm and 87.8 nm, respectively, thus $\langle R_g \rangle / \langle R_h \rangle$ is 1.07 and very close to the theoretical value of typical vesicles ($\sim$1.0).[23] The vesicular morphology of pH-responsive micelles was also determined by TEM. The vesicles with a radius around 90 nm can be clearly observed in Fig. 3a.

The study with LSCM indicates that the interior cavity of the micelles is hydrophilic, which demonstrates further that the pH-responsive micelles are vesicles. A plausible structure of the shell of the vesicle should be a bilayer structure with the

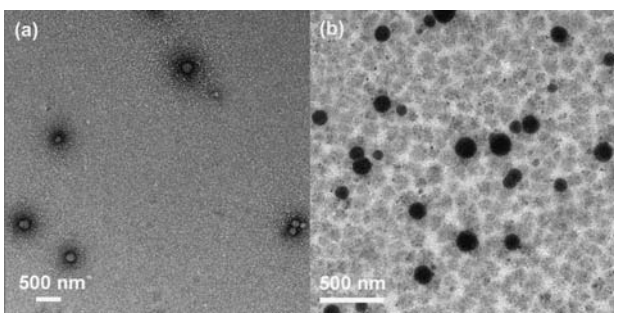

Fig. 3 TEM micrographs of the micelles of the β-CD-P4VP$_{30}$–AD-PNIPAM$_{70}$ inclusion complex at (a) pH 4.8, 25 °C; (b) pH 2.5, 60 °C, respectively (0.2 mg mL^{-1}, stained with OsO_4).

hydrophobic P4VP chains towards the interior while the hydrophilic PNIPAM chains are exposed to the exterior.

At 60 °C and pH 2.5, $\langle R_g \rangle$ and $\langle R_h \rangle$ of the thermo-responsive micelles are 63.1 nm and 78.4 nm, respectively, resulting in a ratio $\langle R_g \rangle / \langle R_h \rangle$ of 0.81. It is in reasonable agreement with the value of typical spherical micelles in theory ($\sim$0.775).[24] The actual spherical micelles with a radius of 40–90 nm are also observed by TEM (Fig. 3b). The thermo-responsive micelles consist of PNIPAM core and P4VP corona.

In summary, the first noncovalent DHBC, the β-CD-$P4VP_{30}$–AD-$PNIPAM_{70}$ inclusion complex, was successfully constructed based on host–guest inclusion interactions. Our study provides a new approach to prepare well-defined DHBCs. In dilute aqueous solution, the β-CD-$P4VP_{30}$–AD-$PNIPAM_{70}$ inclusion complex can further self-assemble into two distinctly different micelles in response to pH and temperature. The pH-responsive vesicles are particularly attractive for applications in the fields of drug delivery and gene transport.

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