

A Facile One-Pot Construction of Supramolecular Polymer Micelles from α -Cyclodextrin and Poly(ϵ -caprolactone)**

Haiqing Dong, Yongyong Li, Shaojun Cai, Renxi Zhuo, Xianzheng Zhang,* and Lijian Liu*

Weak interactions such as hydrogen bonds, ionic bonds, hydrophobic interactions, and π - π interactions govern the structural conformation of all biological macromolecules,^[1] for example the double helix of DNA and cell membranes formed by lipids. In the past few decades, chemists have made significant progress in rationalizing the fundamental rules of these interactions and have developed various self-assembling polymer systems^[1b] including polymer micelles from amphiphilic block copolymers. Recently, block-copolymer-free strategies were developed to construct supramolecular polymer micelles (SMPMs) through the noncovalent interaction between a hydrophilic polymer host and a hydrophobic polymer guest.^[2] However, the fabrication of SMPMs still poses a tremendous challenge as it involves the multistep synthesis of carefully designed polymer hosts and guests; thus a more convenient method to construct SMPMs needs to be developed.

Cyclodextrins (CDs) are an ideal species for the development of new self-assembling systems.^[3] The cone-shaped cavities of CDs can act as hosts for a great variety of macromolecular guests containing multiple binding sites to form polyrotaxanes,^[4] with the inclusion driven by the geometric compatibility and hydrophobic interactions between the CDs and the polymers.^[5] Various cyclodextrin/poly(ϵ -caprolactone) (CD/PCL) based polyrotaxanes have been reported.^[4,6] These pioneering studies have provided a wealth of new insights into these CD-containing systems, but, from the standpoint of potential applications, a great challenge still exists as a result of the insolubility of polyrotaxanes in most solvents, especially water, because of the strong intermolecular hydrogen bonds that are formed between CDs. These bonds may be weakened by either physical or chemical methods, such as those already applied to cellulose.^[7]

We report here an entirely new approach for the construction of SMPMs in which α -CD and PCL are used as building blocks. These species initially self-assemble in THF/H₂O to form an amphiphilic complex of PCL, only part of

which is threaded through the α -CDs. Removal of the THF results in a second assembly process in which the supramolecular polymer amphiphiles form SMPMs (Figure 1). The second step occurs as the section of PCL threaded through the

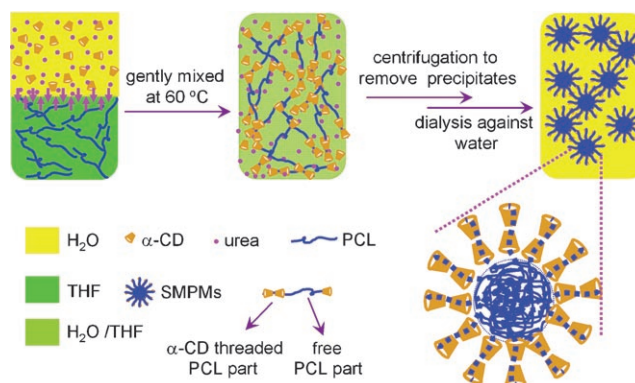


Figure 1. Schematic illustration of the formation of α -CD/PCL SMPMs.

α -CDs has a hydrophilic character and forms the micellar corona, whilst the segment of unthreaded PCL remains hydrophobic and forms the core. However, the key issue is to prevent the α -CD/PCL amphiphiles from precipitating which is induced by the strong hydrogen bonds between the PCL-threaded α -CDs in solution. To render the α -CD/PCL amphiphiles water-soluble, we used urea as well as a maleic anhydride modified α -CD (MAH- α -CD, see Figure S1 in the Supporting Information for the NMR spectrum) to weaken the strong intermolecular hydrogen bonds between the CDs and minimize the crystallization of the polyrotaxanes.^[7] High-molecular-weight PCL ($M_n = 37000$) was also employed to decrease the formation of crystallized α -CD-containing polyrotaxanes as it is known that low-molecular-weight PCL prefers to form crystallized polyrotaxanes with α -CDs.^[4c]

As shown in Table 1, assembly of the supramolecular α -CD/PCL micelles occurred, but only with a yield of 3.2%. This, as expected, was greatly improved to 35.1% by the

Table 1: Yields of SMPMs under different conditions.

Method	α -CD/ PCL ^[a]	(α - CD+urea)/ PCL ^[b]	MAH- α - CD/PCL ^[c] (DS ^[e] = 1)	MAH- α - CD/PCL (DS=5)	MAH- α - CD/PCL (DS=6)
Yield ^[d]	3.2 %	35.1 %	19.5 %	60.5 %	63.8 %

[a] SMPMs obtained from α -CD and PCL. [b] SMPMs obtained from α -CD, PCL, and urea. [c] SMPMs obtained from MAH- α -CD and PCL. [d] The yield was calculated based on the equation: weight of obtained SMPMs/weight of added PCL $\times$ 100%. [e] Degree of substitution.

[*] H. Dong,^[a] Y. Li,^[a] S. Cai, R. Zhuo, Prof. Dr. X. Zhang, Prof. Dr. L. Liu
Key Laboratory of Biomedical Polymers of Ministry of Education &
Department of Chemistry
Wuhan University
Wuhan 430072 (P.R. China)
Fax: (+86) 27-6875-4067
E-mail: liulj@whu.edu.cn
xz-zhang@whu.edu.cn

[†] Both authors contributed equally to this work.

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addition of urea and to 63.8% by using MAh- α -CD. The yield of the MAh- α -CD/PCL micelles increased from 19.5% to 63.8%, as the degree of substitution (DS) of the CD increased from one to six. SMPMs were also assembled from other hydrophilically modified α -CDs such as acrylated and methylated α -CDs (DS = 6, see the experimental procedure in the Supporting Information) with improved yields (43.9% and 67.5%, respectively). These results show that the assembly of α -CD and PCL to form SMPMs proceeded smoothly under our specific conditions, but was strongly suppressed under the conventional conditions that mainly give crystallized polyrotaxanes.^[4–6]

The complexation of PCL by α -CD could be monitored by ^1H NMR spectroscopy, as the proton environments of both the host and the guest change significantly upon binding. The internal H-3 and H-5 protons in α -CD are more sensitive to complexation than those outside the host cavity (H-1, H-2, and H-4), and an upfield shift of the H-3 and H-5 signals was observed in the ^1H NMR spectrum of the α -CD/PCL SMPMs (Figure 2). The 2D ^1H - ^1H gCOSY spectrum (see Figure S2 in

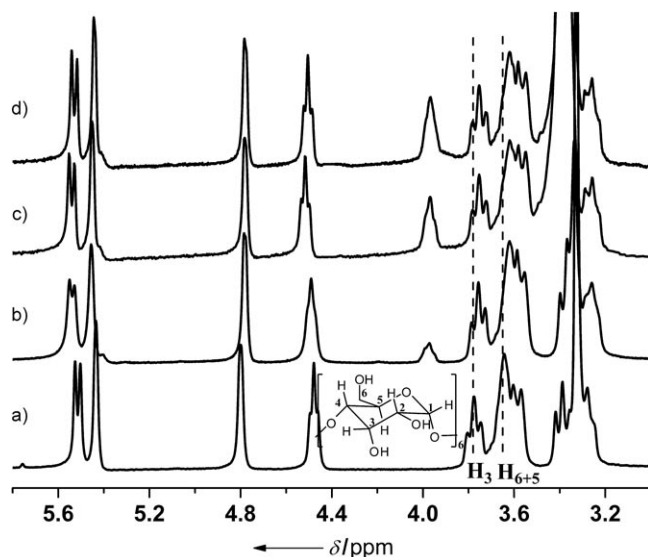


Figure 2. ^1H NMR spectra of α -CD (a), freeze-dried α -CD/PCL SMPMs (b), and the α -CD/PCL SMPMs with increasing amounts of phenol ((c) 1 mg and (d) 2 mg)

the Supporting Information) of the SMPMs shows significant correlation peaks between H-3 of α -CD and the central proton of the ϵ -caprolactone (CL) units. The X-ray diffraction pattern (Figure 3) of the freeze-dried SMPMs is substantially different from those of pure PCL and α -CD. The most intense diffraction peak at $2\theta = 19.8^\circ$ in the α -CD/PCL inclusion complex (IC) spectra of freeze-dried SMPMs and crystallized polyrotaxane is a fingerprint for the channel structure of α -CD ICs, which indicates that the α -CDs are stacked on top of each other.^[6a] The X-ray diffraction pattern of PCL (Figure 3) shows diffraction peaks at 21.3° and 23.6° , which are also present in the spectrum of the freeze-dried SMPMs, thus indicating the existence of the unthreaded PCL segment within the SMPMs. However, the polyrotaxane formed from

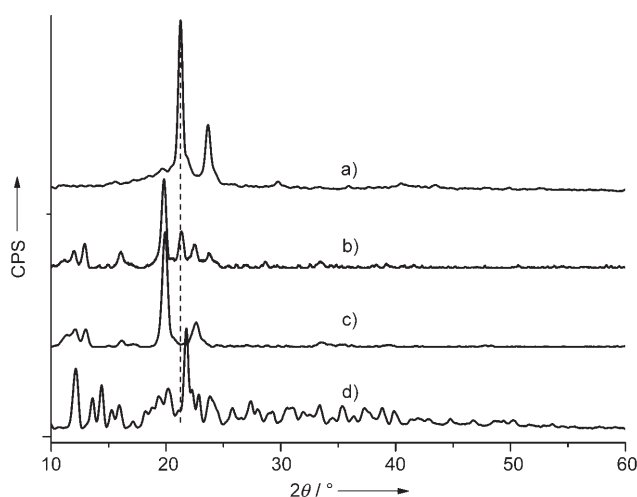


Figure 3. X-ray diffraction patterns of pure PCL (a), freeze-dried α -CD/PCL SMPMs (b), α -CD/PCL polyrotaxane (c), and α -CD (d). CPS = counts per second.

PCL with $M_n = 1000$ and α -CD does not show any PCL diffraction peaks as the chains are completely threaded inside the α -CD cavities in a 1:1 molar ratio (CL/ α -CD). This finding confirms the coexistence of the threaded IC and the free PCL segments in each PCL strand, which provides the basis of SMPM formation. In contrast, the diffraction patterns of MAh- α -CD and its micelles show no diffraction peaks (see Figure S3 in the Supporting Information) which indicates they have an amorphous nature. The FTIR spectra of freeze-dried SMPMs (see Figure S4 in the Supporting Information) show a strong band at 1735 cm^{-1} corresponding to the stretching vibration of the carbonyl group in PCL. This is a higher frequency than that found for pure PCL (1726 cm^{-1}) because in the former case the carbonyl groups of the PCL chains are included in the α -CD cavities.^[6b]

To verify the unique structure of the α -CD/PCL micelles, phenol was used as a competitive guest to expel the threaded PCL segments from the α -CD. This process was followed by ^1H NMR spectroscopy, in which the enhanced proton signal of the hydrophobic part of the micelles was monitored. The intensity of the signal of the $-\text{CH}_2\text{O}-$ unit in PCL at $\delta = 3.98\text{ ppm}$ significantly increased upon addition of phenol (Figure 2), which indicates dethreading of the PCL segments from the cavities of α -CDs and exposure of the disengaged PCL segments to the solvent.

Further evidence for the structure of α -CD/PCL SMPMs came from a fluorescence experiment using pyrene as a hydrophobic probe, which can preferentially partition into hydrophobic microdomains with a concurrent change in photophysical properties. From the plots of fluorescence intensity versus the concentration of α -CD/PCL SMPMs shown in Figure 4, an abrupt increase in the total fluorescence intensity of pyrene is observed as the micelle concentration increases, which indicates transfer from the aqueous environment to the hydrophobic micellar cores. The transfer is also confirmed by a red-shift of the (0,0) band from about 373 to 378 nm in the pyrene emission spectra.^[8] The same result is obtained from the MAh- α -CD/PCL SMPMs (see Figure S5 in

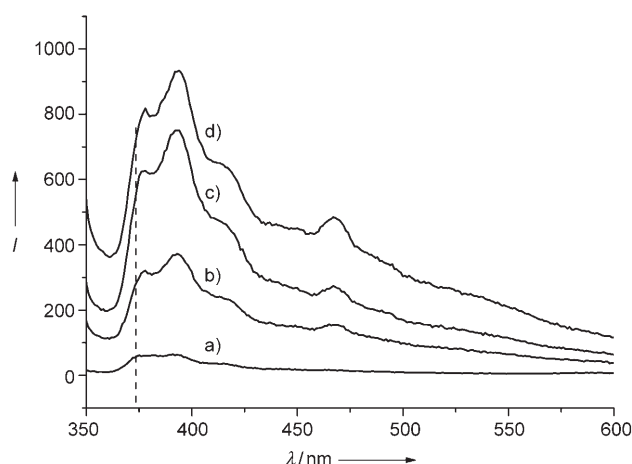


Figure 4. Emission spectra of pyrene with different concentrations of freeze-dried α -CD/PCL SMPMs (a: 0 mg L⁻¹, b: 100 mg L⁻¹, c: 200 mg L⁻¹, d: 400 mg L⁻¹).

the Supporting Information). The fluorescence intensity of pyrene in the presence of α -CD/PCL micelles weakens slowly over the first eight days, but an attenuation of 50 % takes place over the next eight days (see Figure S6 in the Supporting Information). However, in the case of MAh- α -CD/PCL micelles, the pyrene emission shows almost negligible changes over this time interval, thus indicating that the MAh- α -CD/PCL micelles are more stable than those of α -CD/PCL.

We also investigated the influence of urea on the stability of α -CD/PCL micelles by comparing the fluorescence spectra of the pyrene/micelle system obtained after dialyzing until it was free of urea and after dialysis at a constant urea concentration. Almost the same spectra were recorded for the two dialysis processes, which indicates that urea has little effect on protecting the α -CD/PCL micelles from dethreading or precipitation. It seems that the movement of α -CD-threaded PCL in the micellar corona is limited by the host-guest interaction and the hydrophobic micellar core, and thus the hydrogen bonds between PCL-threaded α -CDs are restricted to some extent by their directional nature.

The micellar morphology was also investigated by TEM. As shown in Figure 5a, the SMPMs were individually

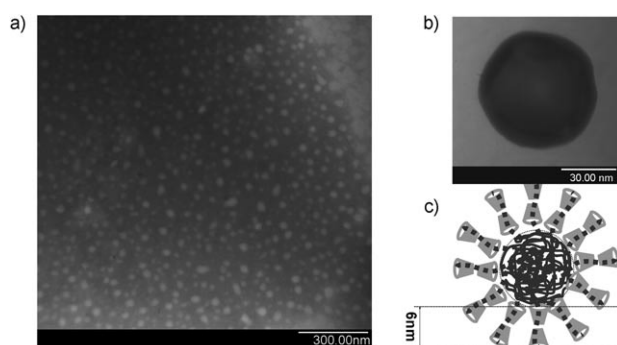


Figure 5. TEM image of α -CD/PCL SMPMs stained by 0.1 % phosphotungstic acid (a), MAh- α -CD/PCL SMPMs in which the shell of the micelles was stained with CsOH (b), and a schematic structure of the SMPMs (c).

dispersed with a regular spherical shape and a diameter of (30 ± 5) nm. The images remained practically unchanged two months after freeze-drying and depositing at room temperature (see Figure S7 in the Supporting Information).

To confirm the core-shell structure of the SMPMs, the shell of the MAh- α -CD/PCL micelles was stained with cesium hydroxide to increase the contrast between the free PCLs and the α -CD-threaded PCL. The excess CsOH was removed by dialysis. Figure 5b shows that the stained micelles remain spherical (a more characteristic image is shown in Figure S7 in the Supporting Information) and a clear interface between the core and corona can be observed. The thickness of the dried shell is calculated to be around 6 nm.

Prednisone acetate, an anti-inflammatory drug with a very low solubility in water, was employed as a model drug to evaluate the effect of drug loading and controlled release characteristics of the SMPMs. The release data (shown in Figure S8 in the Supporting Information) indicate that the drug was released from the micelles gradually and that the release continued for around 700 h. The drug was released a little faster at the initial stage and at a gentle rate thereafter. Compared with other traditional micelles, the SMPMs show higher drug loading (39.5 %) and much more sustained drug release. Furthermore, α -CD and PCL, which are materials approved by the Food and Drug Administration (FDA) as for use in the human body, for drug delivery devices, for example, are good candidates for biomedical materials, and the SMPMs are expected to have great potential in biomedical applications. The exploration of further applications of the SMPMs, such as the templated synthesis of inorganic nanoparticles or as a nanoreactor, is under way.

In summary, a novel method to construct SMPMs based on the inclusion complex between α -CD and PCL has been demonstrated. ¹H NMR spectroscopy, XRD, fluorescence methods and TEM show that the micelles possess a hydrophobic PCL core surrounded by a hydrophilic shell, with the PCL chains threaded through the α -CDs. The controlled drug release behavior of the SMPMs was evaluated using prednisone acetate as a model drug.

Experimental Section

Formation of α -CD/PCL SMPMs: A solution of α -CD (400 mg) and urea (400 mg) or of MAh- α -CD (400 mg) in deionized water (10 mL) was added dropwise to a stirred solution of PCL (M_n 37 000, 100 mg) in THF (10 mL) at 60 °C, and the resulting mixture was stirred continuously for 24 h. THF, urea, and the excess α -CD in the mixture were removed by dialysis against water; small amounts of precipitate were removed by centrifugation after cooling the mixture to room temperature. The resulting solution was freeze-dried before characterization.

Drug loading of α -CD/PCL SMPMs: The procedure is the same as for the formation of SMPMs, but using a solution of PCL and a predetermined weight of prednisone acetate in THF. The lyophilized drug-loaded SMPMs were dissolved in DMF and the loading calculated from the UV absorbance at 270 nm by using a standard calibration curve obtained experimentally with solutions of prednisone in DMF solutions. It was found that approximately 39.5 wt % of the feed drug was loaded into the micelles.

In vitro drug release: The dialyzate (2 mL) was transferred to a new dialysis bag and directly immersed into distilled water (200 mL).

Aliquots (2 mL) were withdrawn periodically from the solution. The volume of solution was kept constant by adding distilled water (2 mL) after each sample was removed. The amount of prednisone acetate released from the micelles was calculated from the UV absorbance of the solution at 242 nm. The concentration of prednisone acetate in distilled water (c) was obtained from the calibration curve: c [gm L^{-1}] = $A/0.04762$, where A is the UV absorbance at 242 nm. The cumulative drug release was calculated from the following relationship: cumulative drug release [%] = $(M_t/M_0) \times 100$, where M_t is the amount of drug released from micelles at time t , and M_0 is the amount of drug loaded into the micelles.

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