

Electronic Supplementary Information

Novel “Star anise”-like Nano Aggregate by Self-assembling of Preformed Microcrystals from Branched Alternating Multi-block Copolymer

Si-Chong Chen, Jing Shi, Gang Wu, Yu-Zhong Wang*

Center for Degradable and Flame-Retardant Polymeric Materials, College of Chemistry, and State Key Laboratory of Polymer Materials Engineering, Sichuan University, Chengdu 610064, China

Experimental Section

Materials

p-Dioxanone (PDO) was provided by the Pilot Plant of the Center for Degradable and Flame-Retardant Polymeric Materials (Chengdu, China), and was dried over CaH₂ for 48 h, purified by vacuum distillation before use. (1K, 2K and 4K) poly(ethylene glycol) (PEG) with different molecular-weight were purchased from Alfa Aesar, which were performed by dissolving in dichloromethane followed by precipitation in diethyl ether and dried under vacuum at 70 °C overnight before use. Stannous octoate (SnOct₂) (≥95%) was purchased from Sigma-Aldrich (USA) and used without further purification. SnOct₂ solution was stored in glass ampoules under argon ambient after diluted with dry toluene. The solvent chloroform was distilled from calcium chloride before use. Trimethylolpropane (TMP), dicyclohexyl carbodiimide (DCC), 4-(dimethylamino) pyridine (DMAP), succinic anhydride, 1,4-dioxane are all AR grade and used as received.

* Corresponding author Tel./fax: +86 28 85410259 (Prof. Y. Z. Wang and Dr. S. C. Chen).

E-mail address: yzwang@scu.edu.cn (Y. Z. Wang); chensichong@scu.edu.cn (S. C. Chen)

Synthesis of the precursors

The hydroxyl-terminated 3-arm PPDO and carboxyl terminated PEG was synthesized according to the method we reported before ^{1,2}. Typically synthesis processes are described as follow:

The synthesis of hydroxyl-terminated 3-arm PPDO was performed in an argon-purged glass reactor with magnetic stirring, which was prepared by typical bulk ring-opening polymerization of PDO using TMP as an initiator, and SnOct₂ as a catalyst. After the polymerization was completed, the reactors were rapidly cooled to room temperature via ice cooling. Moreover the crude polymers were dissolved in chloroform and precipitated with methanol to eliminate the residue and the white trihydroxyl-terminated PPDO powder was obtained. At a fixed molar ratio of TMP to SnOct₂ (500:1), Different molar ratios of PDO to TMP ([P]/[T]) were performed and gave PPDO precursors with different molecular weight.

Dried PEG (2 mmol) was firstly dissolved in anhydrous 1,4-dioxane (8 ml). Succinic anhydride (5 mmol), 4-(dimethylamino) pyridine (DMAP) (5 mmol) and triethylanine (TEA) (5 mmol) were added to the solution at room temperature. The reaction was then conducted under mechanical stirring and nitrogen atmosphere for 24h. The dioxane was evaporated under vacuum, and then the residue was dissolved in chloroform; the insoluble solid was filtered off. The filtrate was precipitated via cold diethyl ether (4°C), filtered, and dried in vacuo overnight. The white carboxyl terminated PEG powder was obtained.

Synthesis of branched PPDOstar-b-PEG alternating multi-block copolymers

For the synthesis of alternating PPDOstar-b-PEG multi-block copolymers, trihydroxyl- terminated 3-arm star-like PPDO (2 mmol), dicarboxylated linear PEG (3 mmol), DMAP (1.2 mmol) and dicyclohexylcarbodiimide (DCC) (12 mmol) were dissolved in anhydrous chloroform, and reacted at room temperature for 24 h under dry nitrogen. To avoid the cross-linking, the concentration of the precursors were kept below the critical cross-linking concentration. Precipitated dicyclohexylurea (DCU) was removed by filtration and the filtrate was poured into an excess amount of diethyl ether. The precipitate was filtered, and dissolved in water; the unreacted PPDO was then removed by filtration. The solution was freeze-dried and then further dried in vacuo to give PPDOstar-b-PEG copolymer.

Nano aggregate preparation:

White powder of the multi-block PPDOstar-b-PEG copolymer was directly dispersed in water at 40°C, and the concentration of the copolymer is 1.0 mg/ml. The magnetic stirring was applied until clear homogeneous suspension of nano aggregate was obtained.

Characterization

The ¹H-NMR spectra were obtained by Avance Bruker-II NMR spectrometer under 400MHz (Bruker, Germany) using CDCl₃ as solvent and tetramethyl silane (TMS) as internal reference. Number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PDI) of the PPDO precursor was obtained from gel permeation chromatography (GPC) (Waters 1515 isocratic HPLC pump, Waters 717 plus autosampler, Waters 2414 refractive index detector) at 30°C with a calibration curve constructed using polystyrene (PSt) standards. Chloroform was used as solvent and eluent with a flow rate of 1.0 ml/min.

The Critical Micelle Concentration (CMC) of the copolymers was measured by a HORIBA JOBINYVON Fluormax-4 spectrofluorometer and using pyrene as fluorescent probe, as a method widely reported in the literature.^[3]

The crystalline morphology of the PPDOstar-b-PEG copolymer was observed via a Nikon Fi1 polarizing optical microscopy (POM) (Japan) equipped with a hot-stage. Thin films were prepared between microscope cover slips. The sample film was firstly melted at 140°C for 3 min to erase the thermal history and then quickly cooled to the isothermal crystallization temperature on the hot-stage. Photographs were taken at different time by a digital camera.

DSC measurement was performed on TA Co. Q200DSC (USA) in aluminum pan under nitrogen atmosphere. The nano aggregates dispersed in water was freeze-dried at -50°C under vacuum. The frozen-dried nano aggregates as well as the bulk sample of PPDOstar-b-PEG were collected and then studied by DSC without erasing the thermal history. The X-ray diffraction (XRD) patterns were taken on

a Philips X'Pert X-ray diffractometer with Cu Ka radiation.

Results and discussion

Synthesis and characterization

The ¹H-NMR spectra of the star-like PPDO and carboxylized PEG precursors were in good accordance with those reported before by our group,^[1,2] which were therefore not listed and discussed here. The average molecular weight of PPDO precursors obtained from NMR, GPC, Intrinsic viscosity and the theoretical calculation (calculated from the feed ratio of monomer and initiator) were listed in Table S1.

Tab. S1 Average molecular weight of 3-arm PPDO precursors obtained from different methods.

Samples	PDO/TMP (mol/mol)	$M_{n,Theory}^a$ (g/mol)	$M_{n,NMR}^b$ (g/mol)	M_v^c (g/mol)	GPC		
					$M_{n,GPC}$	$M_{w,GPC}$	PDI
					(g/mol)	(g/mol)	
PPDO14	12/1	1360	1580	1840	3330	4160	1.25
PPDO32	30/1	3200	3370	3300	6940	8420	1.21

- a. $M_{n,Theory} = (PDO/TMP) \times 102 + 136$, where PDO/TMP is the feed ratio of PDO monomer to TMP initiator in mol; 102 and 136 is the molecular weight of PDO and TMP, respectively.
- b. $M_{n,NMR} = \frac{I_b/2}{I_f/3} \times 102 + 136$, where I_b and I_f are integrate of resonances of methylene group in repeating unit of PPDO segments and methyl group of esterified TMP core, respectively.
- c. The viscosity average molecular weight, M_v , was calculated using following Mark-Houwink equation: $[\eta] = KM_v^\alpha$, where $K = 7.9 \times 10^{-4} \text{ cm}^3/\text{g}$, $\alpha = 0.63$ ^[4]. The Intrinsic viscosity, $[\eta]$, was determined by Ubbelohde Viscometer Capillary using phenol/1,1,2,2 tetrachloroethane (1:4 mol/mol) as the solvent.

The number average molecular weight of PPDO precursors obtained from NMR ($M_{n,NMR}$) is in agreement with that of theoretical calculation from feed ratio ($M_{n,Theory}$) and the viscosity average

molecular weight (M_v). However, a large deviation was found when using GPC to determine the molecular weight of PPDO precursor, probably because the 3-arm star-like PPDO had a quite different nature both in repeating unit and the chain topology from the monodisperse linear PS, which was used as the standard for GPC measurement. Therefore, the PPDO precursors were expressed as PPDO $_x$, where x is degree of polymerization calculated from $M_{n,NMR}$.

Secondly, the hydroxyl end groups of PEG were modified to carboxyl end groups by reacting with an excess amount of succinic anhydride. Thirdly, the three-arm star-like PPDO-PEG multi-block copolymers were obtained by polycondensation between the trihydroxyl-terminated PPDO and the dicarboxylated PEG. The products were denoted as PPDO $_x$ /PEG $_y$, where y is the degree of polymerization of PEG.

The $^1\text{H-NMR}$ spectra of PPDOstar-b-PEG multi-block copolymers were shown in Fig. S1. The resonances at 4.16ppm (a , $-\text{COCH}_2\text{OCH}_2\text{CH}_2\text{O}-$), 3.78ppm (b , $-\text{COCH}_2\text{OCH}_2\text{CH}_2\text{O}-$), and 4.33ppm (c , $-\text{COCH}_2\text{OCH}_2\text{CH}_2\text{O}-$) corresponded to the protons of three different methylene groups of the PPDO segments; the proton resonances visible at 3.65ppm (d , $-\text{OCH}_2\text{CH}_2\text{O}-$) and (e , $-\text{OCOCH}_2\text{CH}_2\text{OCO}-$) were attributed to carboxyl terminated PEG segments; the resonances at 0.90ppm (f , $\text{CH}_3\text{CH}_2\text{C}(\text{CH}_2\text{O})_3-$), 1.45ppm (g , $\text{CH}_3\text{CH}_2\text{C}(\text{CH}_2\text{O})_3-$), and 4.12ppm (h , $\text{CH}_3\text{CH}_2\text{C}(\text{CH}_2\text{O})_3-$) corresponded to the protons of esterified TMP residue of the star-like PPDO segments.

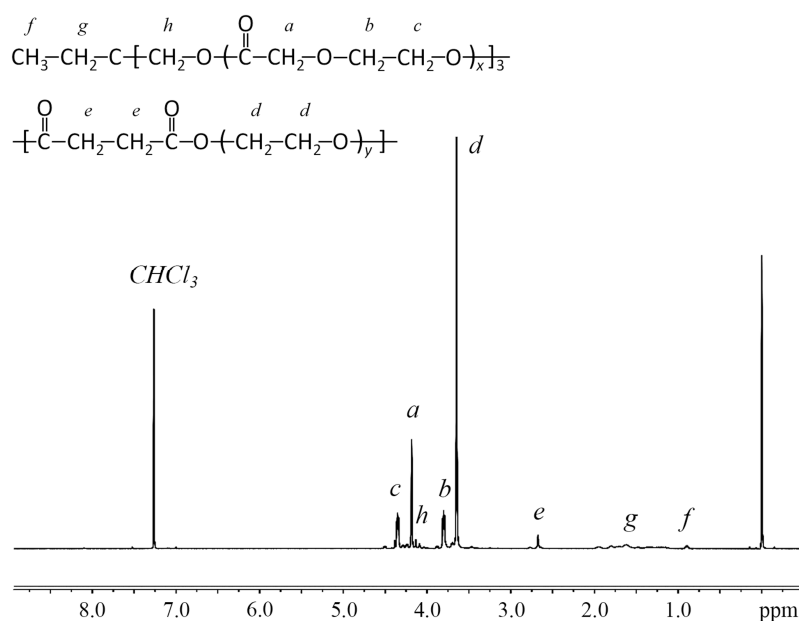


Fig. S1 $^1\text{H-NMR}$ spectrum of PPDOstar-b-PEG copolymer

The content of hydrophobic PPDO block, hydrophilic PEG block and the branch segments (the esterified TMP core of the star-like PPDO segments) in weight fraction (Tab. S2) could therefore be calculated from the ratio of resonance intensities as equation S1-S3, respectively.

$$\text{content of PPDO (wt\%)} = \frac{\frac{I_b}{2} \times 102}{\frac{I_b}{2} \times 102 + \frac{I_d}{4} \times 60 + \frac{I_f}{3} \times 136} \quad \text{eq. (S1)}$$

$$\text{content of PEG (wt\%)} = \frac{\frac{I_d}{4} \times 60}{\frac{I_b}{2} \times 102 + \frac{I_d}{4} \times 60 + \frac{I_f}{3} \times 136} \quad \text{eq. (S2)}$$

$$\text{content of branch segments (wt\%)} = \frac{\frac{I_f}{3} \times 136}{\frac{I_b}{2} \times 102 + \frac{I_d}{4} \times 60 + \frac{I_f}{3} \times 136} \quad \text{eq. (S3)}$$

where I_d is the integrate of resonances of methylene group in repeating unit of PEG segments, 60 is the molecular weight of PEG repeating unit.

Tab. S2 The compositions of PPDOstar-b-PEG

Entry ^[a]	Content of PPDO block (wt%)	Content of PEG block (wt%)	Content of esterified TMP residue (wt%) ^[b]
PPDO32/PEG45	52.7	45.3	2.0
PPDO14/PEG90	19.6	78.5	1.9
PPDO14/PEG45	32.4	64.8	2.8

^[a] calculated from intensity ratio of proton signals of each segments in ¹H-NMR spectrum

^[b] TMP is the trimethylolpropane which was used as initiator for preparing 3-arm PPDO precursor.

Pyrene was used as fluorescence probe to determine the critical micellization concentration (CMC) of the copolymers. The excitation spectrum of pyrene with altered concentration from 0.001 to 1mg/ml of copolymer was measured (Fig. S2A). A red shift of the fluorescence excitation spectra of pyrene from 333 nm to 336 nm was observed with the increase of concentration, suggested that the pyrene molecules transferred to the hydrophobic core of micelles. Therefore, the formation of micelles could be determined according to the red shift. The variation of the intensity ratio of the absorbencies at 336 nm and 333 nm (I_{336}/I_{333}) could be divided into two stages (Fig. S2B), when the concentration of the copolymer lower than CMC, I_{336}/I_{333} value almost remained constant, and it commonly increased when the concentrations risen above the CMC. Thus, by finding the intersection between the horizontal line with relatively constant I_{336}/I_{333} values and the diagonal line with rapid increased I_{336}/I_{333} values, the CMC values were determined. The results indicated that the CMC were increased with the increase of the PEG segments content. In other word, the increase of hydrophobicity is favorable for micellization of the amphiphilic PPDOstar-b-PPDO copolymer under low concentration.

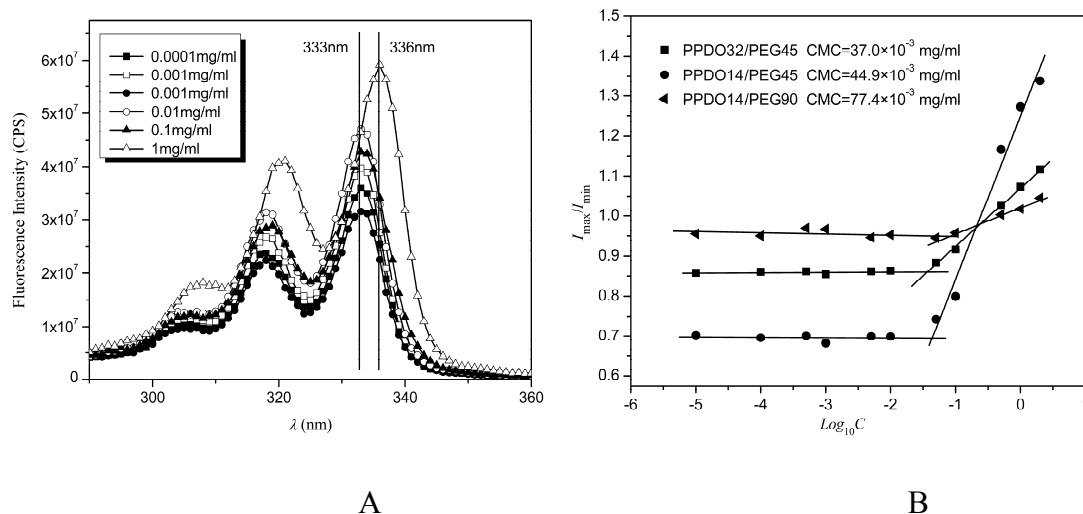


Fig. S2 Einstein shift of micelles measured by Fluorescent Probe in aqueous solution of PPDO14/PEG90 at different concentration (A); CMC measured by Fluorescent Probe (B)

Since the samples prepared for TEM characterization had experienced a drying process under vacuum at 40°C, the collapse in PEG shell may inevitably occur. The average diameters of samples obtained from

Scan-TEM images were compared with those obtained from Dynamic Laser Light Scattering (DLS) with a same concentration in water solution. All samples show only a little decrease in diameter after drying, suggested that the PEG shell of the micelle is relatively compact. For example, the average diameters of PPDO14/PEG45 aggregates obtained from TEM and DLS are approximate 200nm and 240nm, respectively (Fig. S3). Owing to the alternating multi-block sequence and branched structure of the copolymer, many corona-forming PEG blocks must loop back to insert the PPDO blocks connected with it into the microcrystalline core and therefore could not fully extend in water, resulting in some interweaving and interpenetrating of the PEG segments in the corona of the aggregates. The TEM image exhibits the 2D projection of the 3D nano aggregate with different-tilting angle. The tilting angles of some aggregates in the image are almost 90° while some of others are nearly 0° . Therefore, the diameter and the thickness of the star anise-like aggregates can be measured by scaling these projections, respectively.

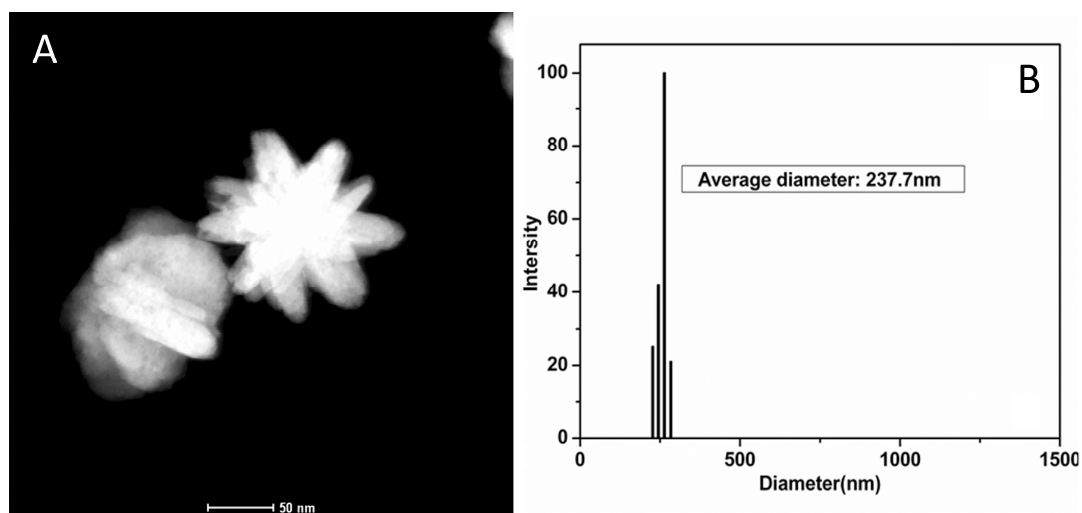


Fig. S3 Scan-TEM image (A), Effective diameter of nano aggregates measured by DLS in aqueous solution (B), of the PPDO14/PEG45 aggregates

The PPDO block of the multi-block copolymer can experience crystallization when cooling from the melt at room temperature. Owing to the multi-block and branched molecular structure, the immiscibility between PPDO and PEG block may result in an obvious microphase-separation. As shown in the POM photos of Fig. S4, the copolymer could only form some microcrystalline with relative small size at 40°C .

Moreover, the crystallization rate of the copolymer was very slow, and the complete crystallization was achieved after 24 hours.

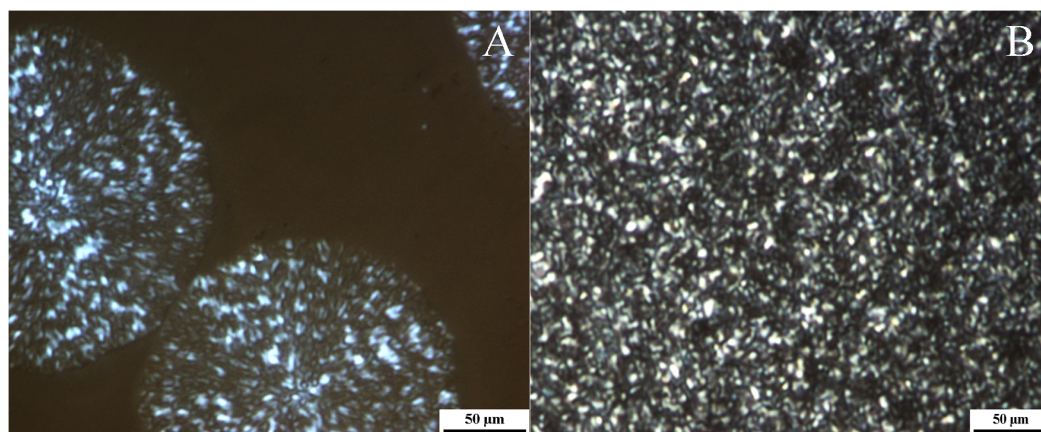


Fig. S4 Polarized optical microscopy micrographs for isothermally crystallized PPDO14/PEG45 samples under 40°C after 40min (A); and complete crystallization (B).

The crystallization of PPDO14/PEG45 before (in bulk) and after dispersed in water (in nano aggregate) at 40°C were studied using DSC. The nano aggregates of this sample dispersed in water was prepared according to aforementioned method. After stable dispersion obtained, the solution was then quenched in liquid nitrogen. The frozen sample was freeze-dried at -50°C under vacuum. The dried nano aggregates were collected and then studied by DSC without erasing the thermal history. The glass transition temperature (T_g) of the PPDO precursor is -26.6 °C, therefore, the microcrystalline of PPDO block in nano aggregates could be preserved under the condition of freeze-drying. The melting temperature (T_m) and the melting enthalpy (ΔH_m) of the PPDO block of the nano aggregate showed almost same values as those of from the bulk (Fig. S5 and Tab. S3), suggested the microcrystalline of PPDO block was stable and preserved during the dissolving of PEG block in water.

The melt of crystallization of PEG block was also observed during DSC heating scan. Because of the multiblock and branched molecular structure, it is very difficult to obtain crystalline with high regularity and large scale for the multiblock copolymers comparing to homopolymer. Therefore, the melting points (T_m) both of PPDO and PEG segments are much lower than their homopolymer, respectively (Tab. S3). Since the melting point of the PEG block is lower than 30°C, therefore it can melt and dissolve in the

water quickly when preparing nano aggregate at 40°C. The freeze-drying temperature of sample is -50°C, while the T_g of PEG block is lower than -60°C (the T_g of PEG is too low to be detected by our DSC instrument, it had not been recorded in this work), the PEG block may also experience crystallization during the freeze-drying. Because the PEG block in nano aggregate experienced a different drying process comparing to that of in bulk, the samples of nano aggregate whose PEG block crystallized under lower temperature showed a higher melting point and melting enthalpy.

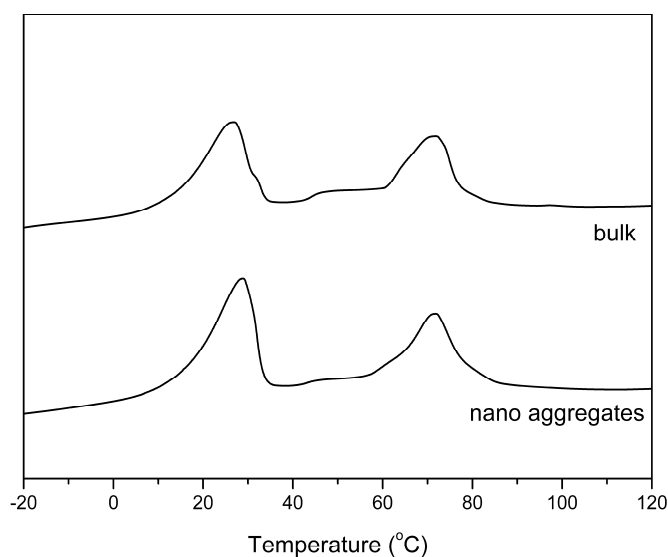


Fig. S5 DSC heating scan curves of PPDO14/PEG45 samples without erasing the thermal history

Tab. S3 Thermal characteristic of the PPDO14/PEG45 and the precursors from DSC heating scan

	PPDO block			PEG block	
	T_m (°C)	ΔH_m (J/g)	χ_c (%) ^[a]	T_m (°C)	ΔH_m (J/g)
Bulk	72.0	22.5	49.2	26.5	32.9
Nano aggregate	72.0	23.0	50.3	28.7	35.3
PEG precursor	—	—	—	53.1	143.5
PPDO precursor	94.4	77.3	54.8	—	—

^[a] the degree of crystallization (χ_c) of the PPDO was calculated by using following equation:

$$\chi_c = \frac{\Delta H_m}{\Delta H_m^0 \times PPDO(\text{wt}\%)}$$

where ΔH_m^0 is the melting enthalpy per gram of PPDO in its completely crystalline state (14.4 kJ/mol) ^[5], PPDO(wt%) is the content of PPDO block in weight fraction.

The XRD patterns of the PPDO14/PEG45 and the precursors were showed in the Fig. S6. Obvious reflection peaks attributed to the crystalline of PPDO and PEG segments were observed in the XRD profiles of the copolymer indicated that the unit-cell dimensions of PPDO and PEG segments did not disturb by each other and both segments crystallized separately.

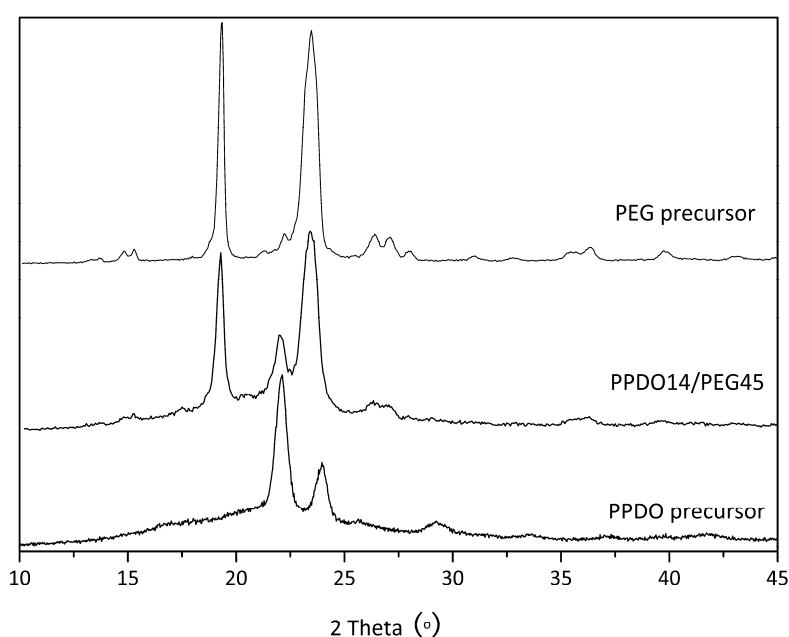


Fig. S6 WAXD patterns of PPDO14/PEG45 and the precursors.

Acknowledgement: This work was financially supported by the Research Fund for the Doctoral Program of Ministry of Education of China (No. 200806100012) and the National Natural Science Foundation of China (No. 20874065). The Analytical and Testing Center of Sichuan University provided TEM analysis.

Reference

- (1) a) Huang, H. X.; Yang, K. K.; Wang, Y. Z.; Wang, X. L.; Li, J. J. *Polym. Sci. A Polym. Chem.* **2006**, *44*, 1245-1251;
b) Chen, F.; Chen, S. C.; Yang, K. K.; Wang, X. L.; Wang, Y. Z. *Eur. Polym. J.* **2010**, *46*, 24-33.
- (2) Wang, X. L.; Mou, Y. R.; Chen, S. C.; Shi, J.; Wang, Y. Z. *Eur. Polym. J.* **2009**, *45*, 1190-1197.

- (3) a) Wilhelm, M.; Zhao, C. L.; Wang, Y. C.; Xu, R. L.; Winnik, M. A.; Mura, J. L. Riess, G.; Croucher, M. D. *Macromolecules* **1991**, *24*, 1033-1040; b) Kabanov, A. V.; Nazarova, I. R.; Astafieva, I. V.; Batrakova, E. V.; Alakhov, V. Y.; Yaroslavov, A. A.; Kabanov, V. A. *Macromolecules* **1995**, *28*, 2303-2314; c) Lee, S. C.; Chang, Y.; Yoon, J. S.; Kim, C.; Kwon, I. C.; Kim, Y. H. Jeong, S. Y. *Macromolecules* **1999**, *32*, 1847-1852
- (4) Sabino, M. A.; Feijoo, J. L.; Müller, A. J. *Macromol. Chem. Phys.* **2000**, *201*, 2687-2698.
- (5) Ishikiriya, K.; Pyda, M.; Zhang, G.; Forschner, T.; Grebowicz, J.; Wunderlich, B. *J. Macromol. Sci. Phys.* **1998**, *B37*, 27-44.