Colloid Polym Sci (2008) 286:327–333
DOI 10.1007/s00396-007-1782-x

SHORT COMMUNICATION

# Controllable synthesis and micelles preparation of tri-block copolymers from 2,2-dimethyl-trimethylene carbonate and ethylene glycol

**Ling Fan · Xu-Feng Ni · Zhi-Quan Shen**

Received: 10 June 2007 / Revised: 28 September 2007 / Accepted: 8 October 2007 / Published online: 25 November 2007


**Abstract** Amphiphilic triblock copolymer of poly(2, 2-dimethyl-trimethylene carbonate)–poly(ethylene glycol)–poly(2,2-dimethyl trimethylene carbonate) (PDTC–PEG–PDTC) was synthesized by dihydroxyl capped PEG with molecular weight of 1,000, 4,000, and 6,000 in the presence of rare earth tris(2,6-di-*tert*-butyl-4-methylphenolate)s. The rare earth phenolates/PEG system could prepare triblock copolymer with predictable molecular weights with narrow molecular weight distribution. The polymers were characterized by nuclear magnetic resonance spectroscopy, gel permeation chromotography, and differential scanning calorimetry to confirm the structure. The micelles formed from the amphiphilic triblock copolymer were determined by fluorescence spectrophotometer and dynamic light scattering. The critical micelle concentrations fell in the range of 1.67~5.25 mg/L. Transmission electron microscopy pictures showed that the micelles possess spherical morphology, and the diameters of micelles in number averaged scale ranged from 20–70 nm. The micelles formed from triblock amphiphilic copolymers were explored as carrier for indomethacin (IND), and they could enhance IND solubility in water dramatically.

**Keywords** Controllable synthesis · Micelles · Rare earth catalysts

L. Fan · X.-F. Ni · Z.-Q. Shen (✉)
Institute of Polymer Science, Key Laboratory of Macromolecular Synthesis and Functionalization of Ministry of Education, Zhejiang University,
Hangzhou 310027, People's Republic of China
e-mail: zhiquan_shen@163.com

## Introduction

Micelles formed by self-assembly from amphiphilic block copolymers have been explored in recent years for solubilization of poorly soluble drug [1–3]. The hydrophilic and hydrophobic blocks of copolymer can form core and corona of the micelles, respectively. These nano-sized micelles can avoid being quickly taken up by the reticuloendothelial system, prolong drug circulation time in blood, and target the loaded drug on specific site, which makes amphiphilic copolymers of much interest for drug delivery system research [4–6].

In most cases, the hydrophilic segment of copolymer refers to poly(ethylene glycol) (PEG) because PEG could increase the biocompatibility and enhance the colloidal stability of many types of delivery vehicles [1]. In contrast to the wide use of PEG as hydrophilic block, a much wider range of hydrophobic blocks have been explored such as polylactides, polycaprolactone, poly(aspartic acid), and others [7–9]. However, the poly(aliphatic carbonate)s, such as poly(trimethylene carbonate) (PTMC) and poly (2,2-dimethyltrimethylene carbonate) (PDTC), have called much attention owing to their biodegradability, biocompatibility, and good mechanical property [10, 11]. The copolymers with these polycarbonates as hydrophobic blocks might have much potential usage in biomedical field.

Many reports deal with the synthesis of various block or graft copolymers. However, a lot of copolymers containing PEG as hydrophilic block mainly originate from ring-opening polymerization of cyclic monomers, which are initiated by PEG with hydroxyl end group reacted with different metal catalysts, such as $Sn(oct)_2$, butyllithium, etc. [12, 13]. The former metal catalyst is commonly used in bulk polymerization at very high

**Table 1** Triblock PDTC–PEG–PDTC prepared by PEG/Dy(ODTBMP)$_3$ system

| No. | $M_n$/PEG | [DTC], mol/L | [DTC]/[PEG] (molar ratio) | $t$ (h) | Yield[a] (%) | $M_{n,calc}$ $\times 10^{-4}$ | $M_{n,NMR}$ $\times 10^{-4}$ | $M_{n,GPC}$ $\times 10^{-4}$ | MWD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1,000 | 1.2 | 200 | 3.5 | 92.4 | 2.50 | 2.58 | – | – |
| 2 | 4,000 | 0.5 | 50 | 4 | 89.5 | 0.98 | 0.88 | – | – |
| 3 | 4,000 | 0.8 | 100 | 4 | 100 | 1.70 | 1.76 | 1.36 | 1.31 |
| 4 | 4,000 | 1.2 | 200 | 6 | 80.1 | 2.42 | 2.22 | 1.42 | 1.29 |
| 5 | 4,000 | 1.9 | 300 | 24 | 59.6 | 2.70 | 2.55 | – | – |
| 6 | 6,000 | 0.5 | 50 | 4 | 92.5 | 1.20 | 1.32 | – | – |
| 7 | 6,000 | 0.8 | 100 | 4 | 100 | 1.90 | 2.06 | 1.37 | 1.38 |
| 8 | 6,000 | 1.2 | 200 | 6 | 70.6 | 2.42 | 2.46 | 1.51 | 1.39 |
| 9 | 6,000 | 1.9 | 300 | 24 | 69.5 | 3.29 | 3.51 | – | – |

Conditions: Molar ratio of [PEG]/[Dy] = 3, 50 °C, THF
[a] Yield of DTC polymerization

temperature, and the latter metal catalyst often leads to the production of oligomers. According to our previous reports, lanthanide tris(2,6-di-*tert*-butyl-4-methylphenolate)s (Ln(ODTBM)$_3$)/aliphatic alcohol systems are efficient catalysts for preparation block copolymer of ethylene glycol and ring carbonates [14, 15]. In this paper, we report the controllable synthesis of tri-block copolymers of PDTC–PEG–PDTC by PEG with molecular weights from 1,000 to 6,000 in the presence of Ln(ODTBM)$_3$, the characterizations of the micelles formed from the resulting copolymer and the micelles loaded anti-inflammatory drug indomethacin (IND) evaluation.

## Experimental

### Materials

Dimethyltrimethylene carbonate (DTC) was synthesized according to the literature method [16]. Ln(ODTBM)$_3$ was prepared by the reaction of $LnCl_3$ and Na(ODTBM) according to the method described in [17]. Tetrahydrofuran (THF) was dried by refluxing over benzophenone–Na complex and distilled under nitrogen atmosphere. PEGs were commercial products and dried in vacuum before use. Pyrene obtained from Acros Organics was recrystallized twice from anhydrous ethanol before use. Indomethacin was kindly provided by the College of Pharmaceutical Sciences, Zhejiang University, China. Unless stated otherwise, all reagents and solvents were commercially available and used as received.

### Synthesis of triblock copolymers

All polymerizations were carried out in glass ampoules previously flamed and purged by dry nitrogen at 50 °C with Schlenk teckniques. PEG, THF, and Ln(OAr)$_3$ were added to the ampoule successively and reacted for a certain time, then DTC was added by syringe and polymerization began. The copolymer was precipitated by *n*-hexane, washed by dilute HCl aqueous solution, and then dried under vacuum to constant weight.

**Table 2** Triblock PDTC–PEG–PDTC prepared by PEG/Y(ODTBMP)$_3$ system

| No. | $M_n$/PEG | [DTC], mol/L | [DTC]/[PEG] (molar ratio) | $t$ (h) | Yield[a] (%) | $M_{n,calc}$ $\times 10^{-4}$ | $M_{n,NMR}$ $\times 10^{-4}$ | $M_{n,GPC}$ $\times 10^{-4}$ | MWD |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6,000 | 1.25 | 50 | 3 | 95.6 | 1.22 | 1.26 | 0.81 | 1.24 |
| 2 | 6,000 | 1.25 | 100 | 4 | 85.6 | 1.75 | 1.63 | 1.13 | 1.32 |
| 3 | 6,000 | 1.25 | 200 | 12 | 80.0 | 2.68 | 2.48 | 1.57 | 1.24 |
| 4 | 6,000 | 1.25 | 300 | 24 | 65.0 | 3.14 | 3.01 | 2.36 | 1.28 |
| 5 | 4,000 | 1.0 | 100 | 5 | 85.9 | 1.61 | 1.74 | 1.11 | 1.38 |
| 6 | 4,000 | 1.0 | 200 | 12 | 88.0 | 2.76 | 2.70 | 1.61 | 1.22 |

Conditions: Molar ratio of [PEG]/[Y] = 3, 50 °C, THF
[a] Yield of DTC polymerization

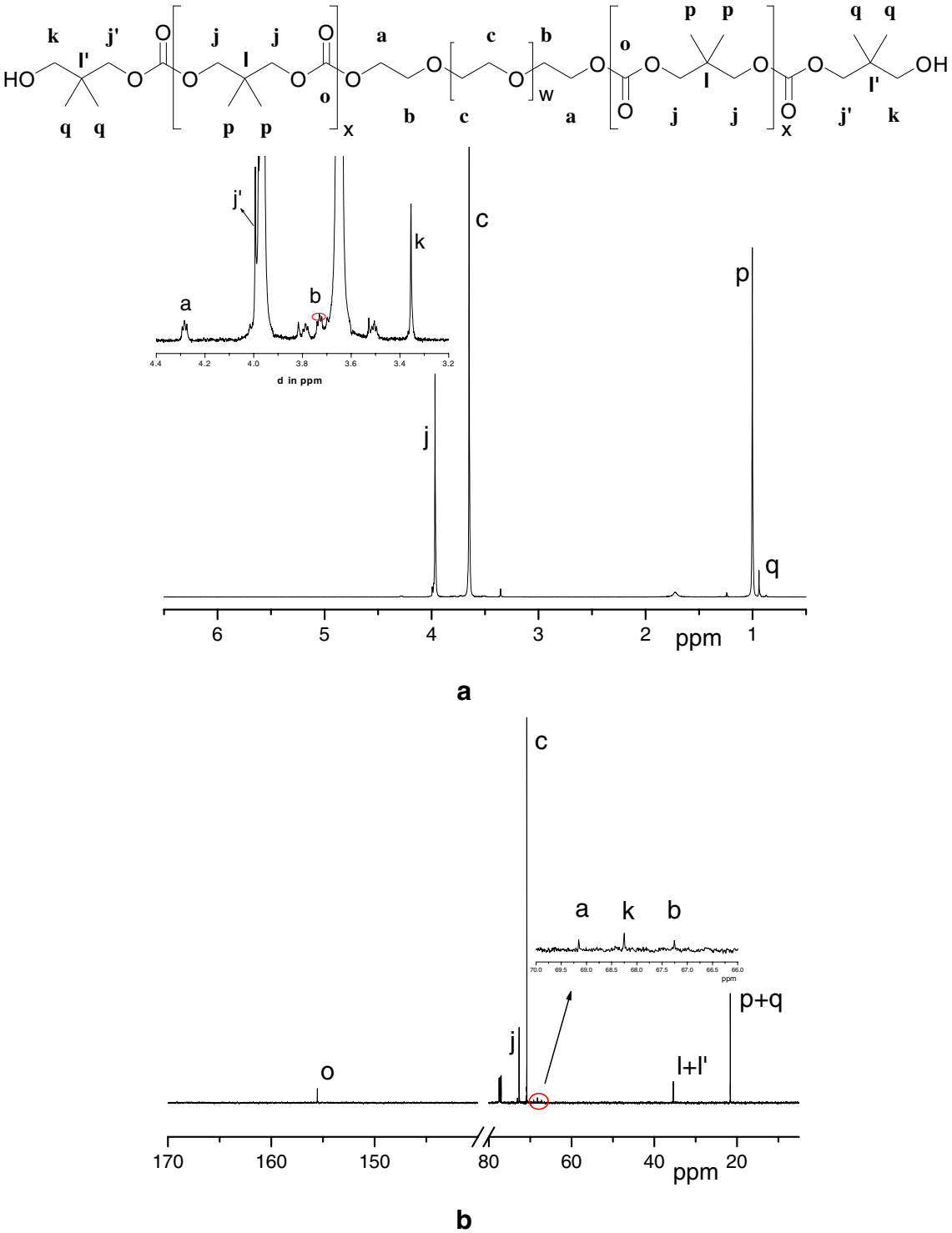


**Fig. 1** **a** $^{1}$H NMR of PDTC–PEG–PDTC. **b** $^{13}$C NMR of PDTC–PEG–PDTC

## Polymer characterization

Nuclear magnetic resonance (NMR) spectra were measured in $CDCl_3$ on a Bruker Avance DMX500 spectrometer at 25 °C with tetramethylsilane as internal standard. The molecular weight ($M_{n,GPC}$) was determined by a gel permeation chromatograph (GPC) at 30 °C (Waters 208 with Columns Styragel® HT3, HT4, and HT5). THF was used as solvent and narrow disperse polystyrene as calibration standards. Differential scanning calorimetry

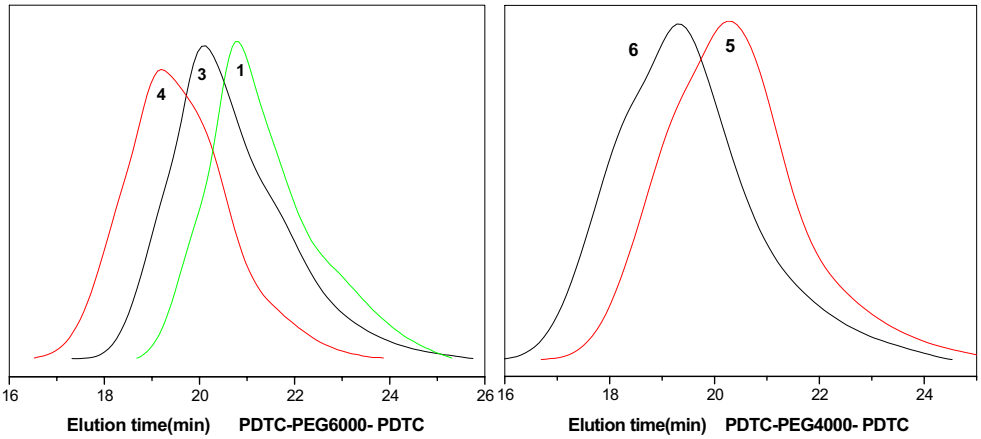


**Fig. 2** GPC curves of PDTC–PEG–PDTC (corresponding to samples in Table 2)

(DSC) curves were taken on a Universal V3.8B TA Instrument. The samples were cooled to −100 °C, heated to 150 °C for the first scan, then cooled to −100 °C, and heated to 150 °C again for the second scan. The rate was 10 °C/min for both heating and cooling.

## Polymer micelles preparation

PDTC–PEG–PDTC micelles loaded with indomethacin were prepared by the dialysis method [18]. Briefly, the copolymer and indomethacin were dissolved in THF and the solution was transferred to a dialysis bag (molecular weight cutoff 8,000) and dialyzed against 1 L of ultrapure water for 24 h. The dialysate water was changed after 0.5, 1, 2, 5, 8, 16, and 24 h from the beginning of the dialysis. The micelle solutions obtained were filtered (0.8 μm) to remove the aggregates. Blank micelles were produced by the same procedure.

## Micelle characterization

The critical micelle concentration (CMC) of micelles was determined by fluorescence method [19], and pyrene was used as the probe. Typically, a known amount of pyrene in acetone was added to a 10-ml vial and then the acetone was evaporated. The micelle solution was added into the vial and then kept at 50 °C atmosphere overnight. The final concentration of the probe and micelle were $6.0\times10^{-7}$ and $10^{-7}$–2.0 g/L, respectively. Steady-state fluorescence spectra were recorded on an F-4500 fluorescence spectrophotometer (Hitachi High Technologies Corp., Tokyo, Japan) with a slit width of 2.5 nm at 25 °C. For fluorescence emission spectra, the excitation wavelength was set at 339 nm, and for excitation spectra the emission wavelength was 390 nm. The ratio of intensities of the excitation at 338 ($I_{338}$) and 333 nm ($I_{333}$) can be plotted as a function of concentration; the crossover value represents the CMC.

The micelle size was determined by dynamic light scattering (DLS; 90 Plus Particles Size Analyzer, Brookhaven Instruments Corp.) The scattering angle was kept at 90° and the wavelength in vacuum was set at 658 nm.

Transmission electron microscope (TEM) images were obtained using a JEM 1230 operating at an acceleration voltage of 80 kV. All of the TEM samples were prepared at 15 °C by dipping a TEM grid into a copolymer solution and the extra solution was blotted out with filter paper; then the samples were negative stained with phosphotungstic acid and air dried before test.

The loading amount of IND detection was performed by absorption at a wavelength of 320 nm using UV spectrometer. The entrapment efficiency was expressed as the ratio of the actual amount of IND in micelles to the IND feed. For this analysis, a calibration curve of standard solutions containing various IND concentrations in DMF was obtained. The standard equation for IND in DMF at 320 nm was $Y = 0.00632 + 18.925X$; $Y$ represented

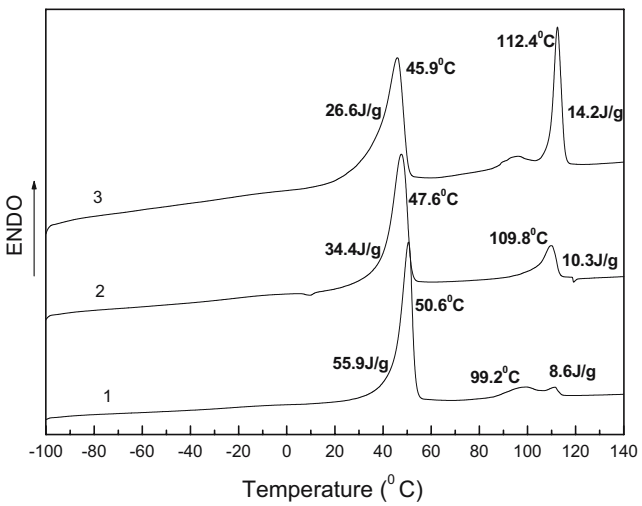


**Fig. 3** DSC curves of PDTC–PEG6000–PDTC (2nd scan)

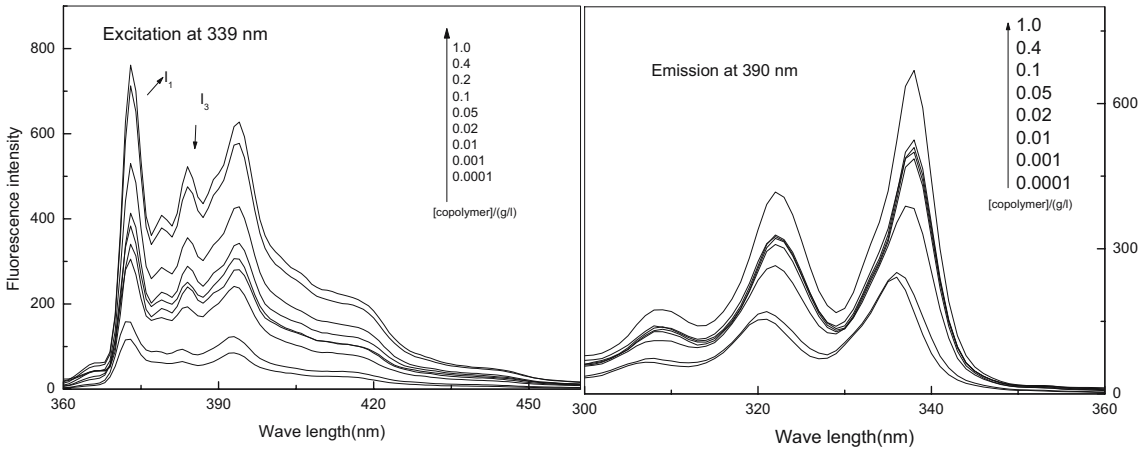


**Fig. 4** Emission (*left*) and excitation (*right*) fluorescence spectra of PDTC–PEG–PDTC copolymer

absorbance, and $X$ took the entrapment efficiency. Each drug loading experiment was assayed twice.

## Results and discussion

### Triblock copolymer synthesis and characterization

Triblock copolymer (PDTC–PEG–PDTC) were successfully synthesized by dihydroxyl-capped PEG with different molecular weights (PEG1000, PEG4000 and PEG6000) with rare earth catalysts. These PEG serve as macroinitiators in the presence of dysprosium tris(2,6-di-*tert*-butyl-4-methylphenolate)s ($Dy(ODTBMP)_3$) (Table 1) or yttrium tris(2,6-di-*tert*-butyl-4-methylphenolate)s ($Y(ODTBMP)_3$) (Table 2). PEG and $Ln(ODTBM)_3$ reacted in THF for 30 min to form an in situ initiator with the Ln–OR bond before the initiation of DTC polymerization.

$$M_{n,calc} = \frac{[DTC] \times 130 \times \text{conversion}}{[PEG]} + M_{n,PEG} \quad (1)$$

$$M_{n,HNMR} = \frac{[I(CH_{2,DTC})] \times 130}{[I(CH_{2,PEG})] \times 44} \times M_{n,PEG} + M_{n,PEG} \quad (2)$$

$M_{n,calc}$, the theoretical molecular weight, was calculated by consumed amounts of DTC and PEG as shown by Eq. (1); $M_{n,NMR}$, the number average molecular weight from $^1H$ NMR spectrum, was calculated by Eq. (2). $M_{n,calc}$ and $M_{n,NMR}$ matched each other quite well (Tables 1 and 2), which indicated that triblock copolymer with predictable molecular

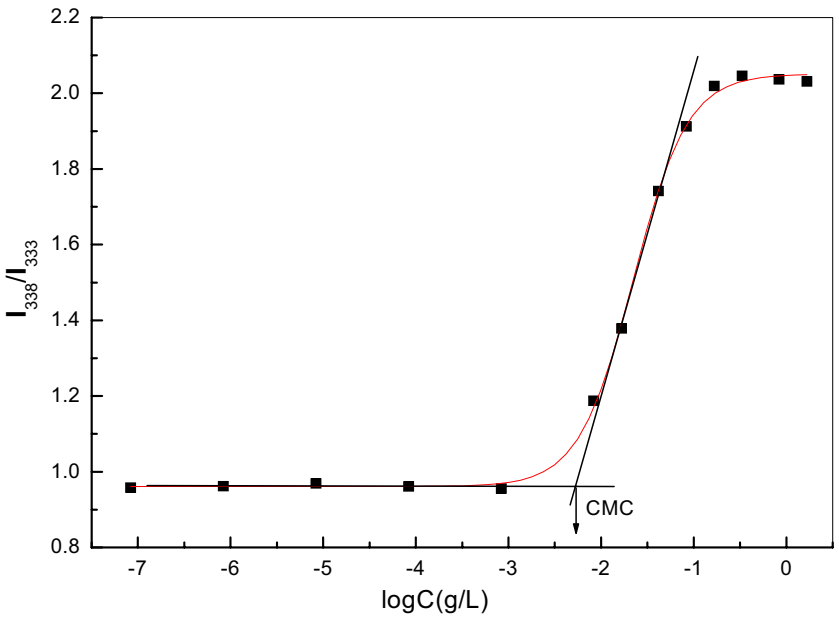


**Fig. 5** Plots of the intensity ratio $I_{338}/I_{333}$ vs. log $C$ (excitation spectra, no.1 of Table 3)

**Table 3** The characterization results of triblock polymer micelle [no. 1 to 6 from Dy(ODTBM)3 system, others from Y(ODTBM)3 system]

| No. | PEG | $\frac{[DTC]}{[PEG]}$ | CMC (mg/L) | PEG wt% | Particle size[a] (nm) | Poly-dispersity |
|---|---|---|---|---|---|---|
| 1 | 4,000 | 100 | 5.25 | 41.2 | 47.1 | 0.123 |
| 2 | 4,000 | 200 | 2.48 | 49.5 | 59.0 | 0.122 |
| 3 | 6,000 | 50 | 5.25 | 83.7 | 27.7 | 0.211 |
| 4 | 6,000 | 100 | 5.01 | 51.0 | 42.4 | 0.170 |
| 5 | 6,000 | 200 | 3.62 | 48.8 | 51.2 | 0.138 |
| 6 | 6,000 | 300 | 1.67 | 37.8 | 72.4 | 0.127 |
| 7 | 6,000 | 50 | 3.45 | 72.8 | 22.6 | 0.209 |
| 8 | 6,000 | 100 | 2.77 | 63.3 | 25.6 | 0.182 |
| 9 | 6,000 | 200 | 2.29 | 48.6 | 33.6 | 0.169 |
| 10 | 6,000 | 300 | 1.76 | 39.6 | 46.5 | 0.131 |

[a] Micelle concentration, 0.2wt%

weights can be prepared by varying the ratio of [DTC] to [PEG]. Triblock copolymers with almost near length of PDTC blocks (nos. 3 and 7, 4 and 8 in Table 1) have close $M_{n,GPC}$ values coinciding with the previous report [12]. This phenomenon was caused by the complete collapse of PEG segment in THF, so that the hydrodynamic volume of the copolymer is governed by the PDTC blocks of the triblock copolymer.

In conclusion, dihydroxyl PEG/Ln(ODTBM)$_3$ systems are active to proceed DTC polymerization and can prepare triblock copolymers with predictable molecular weights under mild conditions.

The microscopic structures of obtained triblock copolymers were characterized by NMR analyses. Figure 1 illustrated $^1$H NMR and $^{13}$C NMR spectra of PDTC-PEG-PDTC. Signals at 4.30 and 3.73 ppm in $^1$H NMR were assigned to H$^a$ and H$^b$, respectively, the direct linkage of the PDTC and PEG unit. No PEG–$CH_2CH_2OH$ end group signals were found between 3.60 and 3.65 ppm; the single peak of H$^k$ and the corresponding $^{13}$C NMR spectrum indicated that pure triblock PDTC–PEG–PDTC was formed.

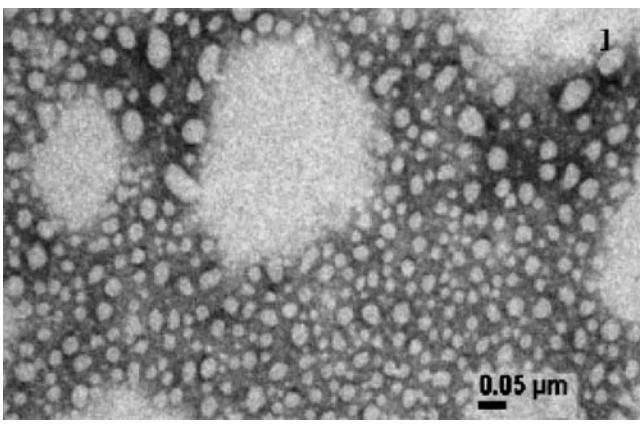


**Fig. 6** TEM micrograph of PDTC–PEG–PDTC micelle

Figure 2 illustrated GPC curves of triblock copolymers prepared by PEG6000 and PEG4000 in the presence of Y(ODTBMP)$_3$ (Table 2). All the samples showed unimodal curves with narrower distributions. Copolymer with higher molecular weight could be prepared as the ratio of [DTC]/[PEG] was increased.

Copolymers with same length of PEG block but different lengths of PDTC block show different thermal behaviors in DSC analysis (samples 1, 2, and 3 in Table 2 and in Fig. 3). On the second heating, all the samples exhibit two endothermic peaks which belong to PEG and PDTC segments, respectively. The melting enthalpy of the PDTC block increased from 8.6 to 14.2 J/g and that of the PEG block decreased from 55.9 to 26.6 J/g as the length of PDTC blocks was increased; this effect was attributed to the fact that the mobility of PEG segment was hindered by the crystallized PDTC.

## Micelle preparation and characterization

Information about the onset of micellization of the PDTC–PEG–PDTC tri-copolymer was derived from steady-state fluorescent probe method. Pyrene was used as probe due to its photophysical and strong hydrophobic properties. In Fig. 4, the emission and excitation spectra of pyrene ($6\times10^{-7}$ M) were shown in the presence of varying concentrations of PDTC–PEG–PDTC copolymer, respectively. An increase in the total fluorescent intensity and red shift occurring were observed with the rise of PDTC–PEG–PDTC copolymer concentration in both spectra. The ratio of intensities of the excitation spectrum at 338 ($I_{338}$) and 333 nm ($I_{333}$) was plotted as a function of concentration; the crossover value represents the CMC (Fig. 5). The plot indicated that pyrene was in water at low concentrations and transferred into more hydrophobic environments at higher concentrations. The CMC of these triblock copolymers with different PEG and PDTC length and micelle size in aqueous were determined by fluorescence and DLS

**Table 4** Results of IND loading into triblock polymer micelle

| No. | [DTC]/[PEG] (molar ratio) | Drug feed (%) | Drug loading (%) | Entrapment efficiency (%) | Particle size (nm)[a] |
|---|---|---|---|---|---|
| 1 | 50 | 5 | 1.28 | 25.5 | 29.6 |
| 2 | 50 | 10 | 4.0 | 39.9 | 32.6 |
| 3 | 50 | 20 | 6.4 | 31.6 | 39.6 |
| 4 | 50 | 30 | 8.7 | 28.9 | 38.7 |
| 5 | 100 | 10 | 5.5 | 51.2 | 35.8 |
| 6 | 200 | 10 | 9.0 | 90.1 | 48.9 |

[a] Size of drug loaded micelles by DLS

measurements, respectively, and the results were summarized in Table 3.

According to Table 3, the CMC values decreased as the length of hydrophobic PDTC segment increased, which suggested that PDTC–PEG–PDTC forms micelle more easily with the longer hydrophobic segments. The size of micelles is controlled by several factors, among which are the length of the core-forming block and the length of corona-forming block. So the opposite phenomenon was observed for the polymer micelle diameters as the length of core-forming block increased, which was consistent with the previous report [20].

Micelle morphology was investigated by TEM. Figure 6 shows the image observed from sample 1 (no. 1, Table 3) after negative staining with phosphotungstic acid. Well-defined nanoparticles with approximate sphere morphology are observed. The average diameters of nanoparticles from Fig. 6 are less than 50 nm.

UV spectroscopy was performed to quantify the weight content of IND in PDTC–PEG–PDTC micelles [21]. Table 4 shows the IND loading efficiency of micelles based on the drug-added contents and different length of PDTC block. Maximum entrapment efficiency could be achieved when drug feed reaches 10% for fixed lengths of hydrophobic and hydrophilic blocks (nos. 1 to 4). So this drug-feed value was further used for micelles with different length of PDTC block.

Several factors which influence drug loading content and entrapment efficiency are the copolymer composition, the compatibility between drug and core forming block, and the nature of the drug. Drug load and entrapment efficiency rise as the length of hydrophobic PDTC segment increased, which could be explained by the fact that the increase in the length of core-forming block causes an increase in the core size per micelle which, in turn, results in an increased loading capacity per micelle [22]. According to the previous reports on interaction carboxyl group of IND to polar group of core-forming block [23], high entrapment efficiency (summit of 90.1%) might originate from the interaction effect between carboxyl group of IND to carbonyl dioxy group of PDTC core and the very poor solubility of IND in water (0.016 mg/ml at 37 °C). The micelle size does not change much after drug loading, which might be due to the low drug loading content.

## Conclusion

The amphiphilic triblock copolymers of PDTC-PEG-PDTC have been synthesized by dihydroxyl-capped PEG with molecular weight of 1,000, 4,000, and 6,000 in the presence of rare earth tris(2,6-di-*tert*-butyl-4-methylphenolate)s. The catalytic system could prepare triblock copolymer with predictable molecular weights with narrower distribution. The micelles formed from the amphiphilic triblock copolymer were determined by fluorescence spectrophotometer and DLS. TEM pictures showed that micelles possess approximate spherical morphology and DLS showed that the diameters of micelles fall in the range of 20–70 nm depending on the length of hydrophobic and hydrophilic blocks. The micelles were used as the carrier of IND which greatly enhanced the solubility of IND in water, and the entrapment efficiency could reach to 90%, which suggested that the PDTC–PEG–PDTC copolymer might have potential as carriers of some hydrophobic drugs.

**Acknowledgements** The authors are grateful to the financial supports of National Natural Science Foundation of China (Key Program No: 20434020), Special Fund for Major State Basic Research Projects (No. 2005CB623802), and the Committee of Science and Technology of Zhejiang Province.

## References

1. Allen C, Maysinger D, Eisenberg A (1999) Colloids Surf B 16:3
2. Jones MC, Leroux FC (1999) Eur J Pharm Biopharm 48:101
3. Jeong YI, Cheon JB, Kim SH, Nah JW, Lee YM, Sung YK, Akake T (1998) J Control Release 51:169
4. Kwon G, Suwa S, Yokoyama M, Okano T, Kataoka K (1994) J Controlled Release 29:17
5. Bae Y, Fukushima S, Harada A, Kataoka K (2003) Angew Chem Int Ed 42:4640
6. Nardin C, Widmer M, Winterhalter M, Meier M (2001) Eur Phys J E 4:403
7. Ramaswany M, Zhantg X, Burt H, Wasan KMJ (1997) Pharm Sci 86:460
8. Allen C, Yu Y, Maysinger D, Eisenberg A (1998) Bioconjugate Chem 9:564
9. Harada A, Kataoka K (1998) Macromolecules 31:169
10. Inoue S, Tsuruta T (1975) Appl Polym Symp 26:257
11. Pego AP, Poot AA, Grijpma DW, Feijin J (2003) Polymer 44:6495
12. Muller AJ, Keul J, Jocker H (1991) Eur Polym J 27:1323
13. Wang H, Dong JH, Qiu JY (1998) J Polym Sci A Polym Chem 36:695
14. Chen W, Ling J, Shen ZQ (2005) Science in China Ser B Chemistry 48:334
15. Chen W, Ling J, Shen ZQ (2005) J Polym Sci Part A Polym Chem 43:1787
16. Hu B, Zhuo RX, Fan CL (1998) Chemical Reagents (in Chinese) 20:366
17. Hitchcock PB, Lappert MF, Singh AJ (1983) Chem Soc Chem Commun 24:1499
18. Shin IG, Kim SY, Lee YM, Cho CS, Sung YK (1998) J Control Release 51:1
19. Wilhelm M, Zhao CL, Wang Y, Xu R, Winnik MA, Mura JL, Riess G, Croucher MD (1991) Macromolecules 24:1033
20. Forster S, Zisenis M, Wenz EJ (1996) J Chem Phys 104:9956
21. Zhu KJ, Zhang JX, Wang C, Yasuda H, Ichimaru A, Yamamoto K (2003) J Microencapsulation 20:731
22. Gadelle F, Koros WJ, Schechter RS (1995) Macromolecules 28:4883
23. Zhang JX, Li XJ, Qiu LY, Li XH (2006) J Control Release 116:322