Front. Chem. China 2009, 4(1): 104–109
DOI 10.1007/s11458-009-0006-y

RESEARCH ARTICLE

Jieming GAO, Yingzhi GUO, Zhongwei GU, Xingdong ZHANG

Micellization and controlled release properties of methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide-*co*-trimethylene carbonate)

Abstract Amphiphilic block copolymers composed of D,L-lactide, trimethylene carbonate and the methoxy poly (ethylene glycol) (PETLA) were synthesized with ring-opening copolymerization. Studies on the micellization and drug-controlled release behavior of PETLA were performed. Both of the copolymers and the micelles were characterized with the methods of ^{1}H nuclear magnetic resonance (^{1}H-NMR), fluorescence spectroscopy, gel permeation chromatographic (GPC), dynamic light scattering (DLS), transmission electron microscopy (TEM) and ultraviolet-visible spectroscopy (UV). As a result, the critical micelle concentration of the copolymer was decreased with the increase of the hydrophobic chain length. DLS results indicated the diameters of the micelle were increased with increasing hydrophobic length. TEM photographs illustrated that micelles MT1 were regularly spherical with the diameter from 30 nm to 40 nm. Taking 9-nitro-20(S)-camptothecin (9-NC) for the model drug, the release profiles *in vitro* show that the release behavior from micelles was controllable and nearly in zero order after the initial burst release.

Keywords biodegradable, methoxy poly(ethylene glycol)-*b*-poly(D, L-lactide-*co*-trimethylene carbonate), micelle, controlled release

Amphiphilic copolymer micelles, with the diameters in mesoscopic scope, relatively narrow size distribution and unique physiological distribution characteristics, have attracted significant interest. They possess outstanding advantages in anticancer agent active/passive targeting delivery, controllable release and alleviation of toxic side-effect to healthy tissues [1,2]. Up to now, many studies have reported copolymer micelles self-assembled from methoxy poly(ethylene glycol)-b-poly(D,L-lactide) [3,4]. However, the acid degradation products degraded from poly(D,L-lactide) might lead to local inflammation because of the pH change of micro environment *in vivo* [5]. Polycarbonate is an important polyester with good biocompatibility and surface erosion properties. As reported by Mao [6], the degradation products of polycarbonate are carbon dioxide and dual alcohol (or phenol), which have less effect on pH change *in vivo* than that of poly(D, L-lactide) and will not lead to inflammation. However, the slow degradation rate is an important factor that restricts the wide usage of polycarbonates [7]. In addition, many reports show that it was hard to achieve zero-order release by drug encapsulation of poly (ethylene glycol)-polycarbonate copolymer micelles [8,9]. Thus, it is assumed that the copolymerization of D,L-lactide and carbonate as the hydrophobic part in the amphiphilic copolymers would not only effectively relieve the decreased pH value caused by degradation products from poly (D,L-lactide), but also regulate the drug release behavior through changing the copolymer compositions.

In this paper, amphiphilic block copolymers methoxy poly(ethylene glycol)-*b*-poly(D,L-lactide -*co*- trimethylene carbonate) (PETLA) were synthesized by ring-opening copolymerization. Studies on the copolymer's structure, composition, micellization behavior and release behavior *in vitro* with the model drug 9-nitro-20(S) camptothecin (9-NC) were also performed.

1 Materials

Trimethylene carbonate (TMC) was synthesized according to a literature [10]. The crude product was purified by reflux and recrystallization for three times in tetrahydrofuran/diethyl ether (volume ratio of 2:1). After vacuum-

Translated from *Acta Polymerica Sinica*, 2008, 2 (in Chinese)

Jieming GAO, Yingzhi GUO, Zhongwei GU (✉), Xingdong ZHANG
National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China
E-mail: zwgu@scu.edu.cn

drying, the pure TMC was obtained with the yield of 40 percent (melting point 46.5–47.5°C).

Stannous octoate (Aldrich) was purified by redistillation beforehand, and then dissolved in anhydrous toluene solution with the final concentration of 0.01 g/mL. Methoxy poly(ethylene glycol) (mPEG) (Aldrich, M_n = 2000) was dried under vacuum at 40°C for 72 h before use. D,L-lactide (LA) (Purac) was treated by sublimation after recrystallization twice in ethyl acetate. 9-NC (99.7%) was purchased from the Hetd Pharmaceutical Co., Ltd. in Hangzhou. THF (Merck, HPLC-grade). Pyrene (Acros Organics). Other chemical analytical reagents were obtained from the Beijing Chemical Reagent Company.

2 Copolymer synthesis and micelles preparation

Certain amounts of TMC, LA and mPEG were added into a dried ampoule. The ampoule was purged with nitrogen for six times and then, the prescribed stannous octoate was added with determined molar ratio of monomer to catalyst ([M]/[C] = 2000). After evacuation of solvent, the ampoule was sealed and polymerization was carried out at 160°C for 12 h. The product was purified three times by dissolving in dichloromethane and precipitating in excess anhydrous diethyl ether, after that it was vacuum-dried at 30°C to constant weight.

PETLA copolymer (20 mg) and 9-NC (2 mg) were dissolved in 5 ml dichloromethane in a branched tube. The mixture was stirred under the protection of nitrogen in a water bath at 60°C until the solvent was completely evaporated. Then, the vials were left in a vacuum oven at 30°C for 24 h and a transparent polymer film was received. After that, 5 ml of tri-distilled water was added into the tube for rehydration. The solution was allowed to be mixed and stirred in water bath for 10 min and then filtrated (0.22 μm), freeze-dried, and the drug encapsulated micelles were obtained. Micelles from MT1 to MT4 were prepared from PETLA1 to PETLA4 (as shown in Table 1), respectively.

3 Characterization methods

The copolymer composition was determined by ^{1}H nuclear magnetic resonance (^{1}H-NMR) (Bruker ARX-400 instrument) with $CDCl_3$ as solvent and tetramethyl silane as internal standard at room temperature. The molecular weight of copolymers was measured by Waters 2695 gel permeation chromatographic (GPC) (Waters, USA) equipped with Waters 2414 refractive index detector and Waters Styragel HT3 & Styragel HT4 & Styragel HT5 Columns. THF was used as the eluent at a flow rate of 1 mL/min at 30°C. Micellar morphology evaluation was characterized by transmission electron microscopy (TEM) (J EOL JEM 1010, Japan). A drop of sample solution (0.5 mg/mL) was placed on the Formvar support film and then stained with phosphotungstic acid solution (1%). The sample was observed until it was dried at room temperature. The critical micelle concentration (CMC) was measured by fluorescence spectroscopy (Hitachi, Japan) with wavelength ranging from 350 nm to 500 nm and the excitation wavelength was at 335 nm. In the pyrene emission spectra (λ_{ex} = 335 nm), the intensity ratio of the third (385 nm) peak to the first (373 nm) peak (I_{385}/I_{373}) of pyrene solution (6×10^{-7} mol/L) was calculated, and the mutation value corresponded to CMC. The aggregation performance of micelles was characterized by GPC (Waters, USA) with standard samples glucan for data correction, in which $NaNO_3$ solution (0.01 M) was used as the eluent at a flow rate of 1 mL/min at 30°C. The size of micelles was measured by a Zetasizer Nano Z particle size analyzer (Malvern Instruments, UK). Micelles were diluted with tri-distilled water to 1 mg/mL, and then filtered through 0.22 μm filters. The reported diameter was the mean value from three independent samples. The method for release profiles test was as follows: certain amounts of 9-NC loaded micelles were dissolved in 3 mL phosphate buffer (PBS, pH 7.4) and put into a dialysis tube (MWCO 7000, USA). The dialysis tube was then immersed into 50 mL PBS (pH 7.4) and the media was constantly stirred with 120 rpm at 37 ± 0.5°C. At a special time point, the 3 mL medium was taken out and the same volume of fresh PBS was added. The concentration of

Table 1 ^{1}H-NMR and GPC characterization of PETLA copolymers

Copolymer	Feed [a]	$\overline{M_n}$ [b]	Product [a,c]	$\overline{M_n}$ [c]	$\overline{M_n}$ [d]	$\overline{M_w}/\overline{M_n}$ [d]	Yield/%
	mPEG:LA:TMC		mPEG:LA:TMC				
PETLA1	11.5:30:70	3000	18.5:35.1:64.9	2600	2900	1.05	75.5
PETLA2	5.4:30:70	4000	6.7:33.2:66.8	3900	4100	1.25	89.7
PETLA3	3.8:30:70	5000	3.7:28.8:71.2	5300	5300	1.43	94.1
PETLA4	2.9:30:70	6000	2.5:29.1:70.9	6800	6700	1.53	89.7

[a] Mol ratio of mPEG, LA, TMC;

[b] Calculated molecular weight of PETLA copolymer based on the feed ratio of mPEG, LA, and TMC;

[c] Determined from the integrations from ^{1}H-NMR spectra;

[d] Obtained by GPC with respect to polystyrene standards

released drug was detected by Ultraviolet spectrophotometer (UV) (Perkin Elmer, USA), and the relationship between accumulated release amount and release time could be established.

4 Structure and composition for PETLA

As the synthesis scheme shows in Fig. 1, amphiphilic block copolymers with different hydrophobic chain lengths were synthesized by modulating the input amounts between LA/TMC (3∶7, molar ratio) and mPEG on the following conditions: [M]/[C] = 2000, T = 160°C and t = 12 h. The ^{1}H-NMR spectrum of block copolymer PETLA2 is shown in Fig. 2. The proton signals from methyls and methylenes of mPEG appear at δ 3.38 (H^a) and δ 3.65 (H^b), respectively. Similarly, the proton chemical shifts of methines and methyls in PLA are shown at δ 5.17 (H^c) and δ 1.53 (H^d), and the proton peaks of methylenes in PTMC are shown at δ 4.25 (H^e) and δ 2.06 (H^f), respectively. The copolymer composition could be calculated according to the hydrogen integral strength in different positions of ^{1}H-NMR spectrum. As shown in Table 1, the results from GPC and ^{1}H-NMR illustrated that the obtained block copolymers had the consistent molecular weights and compositions compared to our design.

5 Determination of critical micelle concentration

CMC is the lowest concentration for micelle formation from amphiphilic copolymers, through which the micelle's thermodynamic stability could be reflected. Fluorescent probe has been proven to be an effective tool for micelle study [11]. In this paper, pyrene was chosen as the fluorescent probe because it was highly sensitive to the polarity change in the surrounding environment, and it would preferentially transfer into hydrophobic cores of micelles during the micelles formation in water [12–14].

In a series of solutions with different copolymer concentrations, the pyrene emission spectra ranging from 350 nm to 500 nm were measured. The intensity ratio I_{385}/I_{373} of pyrene was calculated as the Y-axis, and the copolymer concentrations were acted as the X-axis. From the relationship of I_{385}/I_{373} and copolymer concentrations

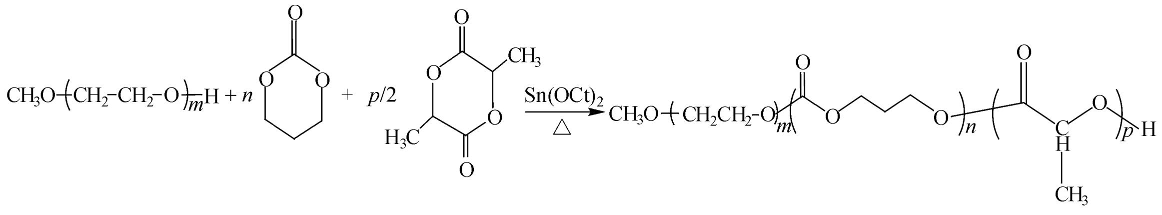

Fig. 1 Synthesis of copolymer PETLA

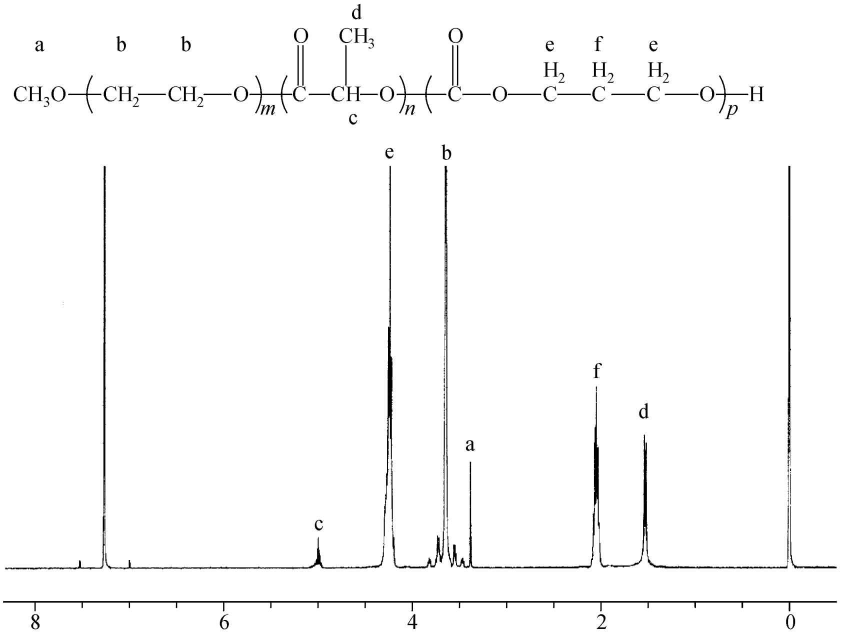

Fig. 2 ^{1}H-NMR spectra of block copolymer PETLA2

illustrated in Fig. 3, it was obvious that the mutations, which corresponded to CMC, took place in the copolymer concentration ranging from 10^{-4} g/L to 10^{-2} g/L. Comparing the CMC values of different hydrophobic length copolymers, the values were decreased with increasing hydrophobic part length. For instance, the hydrophobic part lengths of PETLA1 and PETLA4 detected by ^{1}H-NMR were 600 and 4800, respectively. The corresponding CMC values were 5×10^{-3} g/L and 2.5×10^{-4} g/L. The above results revealed that the CMC was much lower for those long hydrophobic length copolymers than the short counterparts when they self-assembled to form micelles in water.

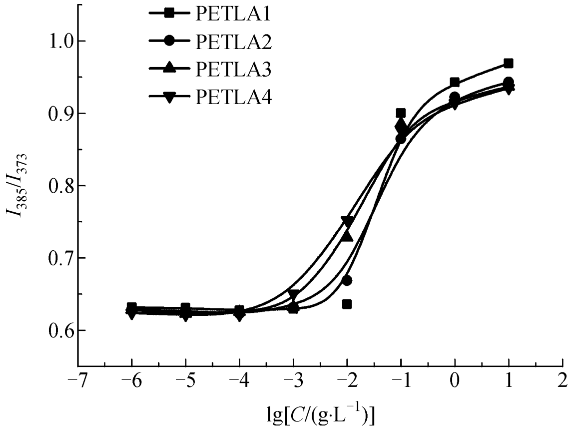

Fig. 3 Plots of the intensity ratio I_{385}/I_{373} from pyrene emission spectra versus concentrations (g/L) of copolymers from PETLA1 to PETLA4 in tri-distilled water

6 Structure, size and morphology of micelles

GPC was used to characterize micellar formation because the micelle was the aggregation of multiple copolymer molecules, and the molecular weight of micelle was much larger than that of the copolymer [15,16]. The GPC curve of micelles MT1 in water is shown in Fig. 4. Two peaks appear at 13.9 min and 18.2 min, respectively and the corresponding weight-average molecular weights are 277 500 and 2700 based on the standard sample of glucan. Since the coexistence of micelles and copolymer molecules in micellar solutions, the peak with larger molecular weight is attributed to micelles, while the other one corresponds to block copolymer. The GPC results of blank copolymer micelles MT2 and MT3 are listed in Table 2. Since micelles MT1, MT2 and MT3 have the same hydrophilic part of mPEG2000 and different hydrophobic lengths according to ^{1}H-NMR detection (600, 1900 and 3300, respectively), it could be concluded from Table 2 that the molecular weight of the micelle was increased with the increasing hydrophobic length of the copolymer.

Table 2 Micelles molecular weight and size analysis through GPC and DLS

Micelles	M_w of micelle ^{a}	M_w of copolymer ^{a}	Diameter /nm^b
MT1	277500	2700	32.7 ± 5.3
MT2	462200	4000	48.3 ± 6.4
MT3	644800	5100	61.3 ± 10.2

^{a} GPC result; ^{b} DLS result

The dynamic light scattering (DLS) results of block copolymer micelles, as shown in Table 2, illustrated that the micellar size was increased with the increase of hydrophobic chain length. For example, the diameter from MT1 to MT3 was increased from 32.7 nm to 61.3 nm. This kind of increase could be attributed to two reasons: one is related to the increase of hydrophobic part length as well as the hydrophobic part volume in the micellar cores. The other reason might be related to the increased hydrophobic interaction between copolymers, and this led to larger volume since more copolymers aggregated in one micelle. These results agreed well with the changing trends of micellar molecular weights detected by GPC.

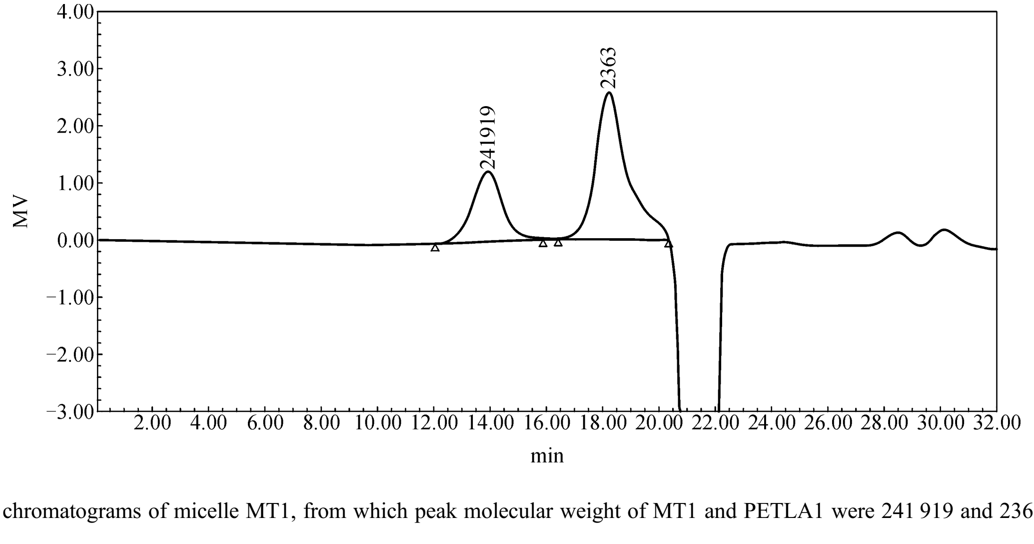

Fig. 4 GPC chromatograms of micelle MT1, from which peak molecular weight of MT1 and PETLA1 were 241 919 and 2363 respectively

Figure 5 is the TEM photograph of micelles MT1 after freeze drying, resolving in water and negative staining by phosphotungstic acid. As shown in this photo, micelles were close to spherical in shape and well dispersed. The diameters of these micelles were about 30–40 nm, which was basically coincident with the DLS results reported in Table 2.

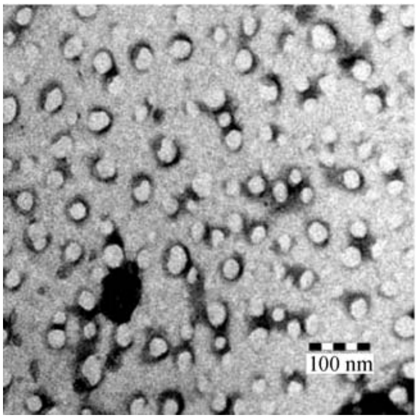

Fig. 5 Transmission electron micrographs of micelle MT1 composed of copolymer PETLA1

7 Release profiles *in vitro*

Figure 6 shows the micelles (MT1 to MT4) release profiles in the PBS (pH 7.4, 37°C), in which the micelles have constant drug loading content of 3 percent. From the release profiles, the initial burst release happened in the first two hours. It was assumed that this portion of drug was deposited in the region near or within the PEG shell and could get access to the aqueous medium without longtime diffusion. After burst release, the release rate of 9-NC slowed down nearly to zero order. As a result, only 40 to 55 percent of drug was released after 40 h, this might be related to the stronger interaction between drug molecules and the hydrophobic part in micelles. According to the results illustrated in Fig. 6, the drug release rate decreased from MT1 to MT4 with the increase of hydrophobic part length in copolymers that self-assembled into micelles. Since micelles composed of high percentage of hydrophobic composition copolymers could form larger micelles by a stronger hydrophobic interaction, it would cost more time for drug molecule diffusion from the core to the outside medium. As stated above, the controllable and sustained release system for 9-NC would be realized by adjusting the hydrophobic length of copolymer micelles.

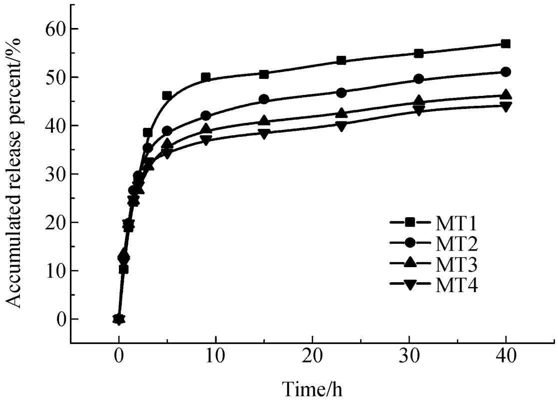

Fig. 6 9-NC release profile from MT1 to MT4

Acknowledgements The authors are indebted for the financial support by the National Basic Research Program of China (973 Program, Grant No. 2005 CB623903).

References

1. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev, 2001, 47: 113–131
2. Allen C, Maysinger D, Eisenberg A. Nano-engineering block copolymer aggregates for drug delivery. Colloids Surf B, 1999, 16: 3–27
3. Dong Y C, Feng S S. Methoxy poly(ethylene glycol)-poly (lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials, 2004, 25: 2843–2849
4. Riley T, Govender T, Stolnik S, Xiong C D, Garnett M C, Illum L, Davis S S. Colloidal stability and drug incorporation aspects of micellar-like PLA-PEG nanoparticles. Colloids Surf B, 1999, 16: 147–159
5. Younes H, Nataf P R, Cohn D, Appelbaum Y J, Pizov G, Uretzky G. Biodegradable PELA block copolymers: In vitro degradation and tissue reaction. Biomater Artif Cells Artif Organs, 1988, 16: 705–719
6. Mao J, Guo Y Z, Xia Y Z, Gu Z W. Synthesis and characterization of poly(D,L-lactide-co-cyclic carbonate)s with hydroxyl pendant functional groups. Acta Polymerica Sinica, 2006 , 9 : 1121–1124 (in Chinese)
7. Cai J, Zhu K J. Preparation, characterization and biodegradable characteristics of poly(D,L-lactide-co-trimethylene carbonate). Polymer International, 1997, 42: 373–379
8. Zhang Z, Grijpma D W, Feijen J. Thermo-sensitive transition of monomethoxy poly(ethylene glycol)-block-poly (trimethylene carbonate) films to micellar-like nanoparticles. J Control Release, 2006, 112: 57–63
9. Zhang Y, Zhuo R X. Synthesis and drug release behavior of poly (trimethylene carbonate)-poly(ethylene glycol)-poly(trimethylene carbonate) nanoparticles. Biomaterials, 2005, 26: 2089–2094
10. Zhu K J, Hendren R W, Jensen K, Pitt C G. Synthesis, properties, and biodegradation of poly(1,3-trimethylene carbonate). Macromolecules, 1991, 24: 1736–1740
11. Prochazka K, Limpouchova Z, Webber S E. Polymerica Materials Encyclopaedia. Baca Raton, New York, London, and Tokyo: CRC Press, 1996, 743–754
12. Kwon G, Naito M, Yokoyama M, Okano T, Sakurai Y, Kataoka K.

Micelles based on AB block copolymers of poly (ethylene oxide) and poly(β-benzyl L-aspartate). Langmuir, 1993, 9: 945–949

13. Yoo H S, Park T G. Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. J Control Release, 2001, 70: 63–70
14. Lee J, Cho E C, Cho K. Incorporation and release behavior of hydrophobic drug in functionalized poly(D, L-lactide)-block-poly (ethylene oxide) micelles. J Control Release, 2004, 94: 323–335
15. Inoue T, Chen G, Nakame K, Hoffman A S. An AB block copolymer of oligo (methyl methacrylate) and poly(acrylic acid) for micellar delivery of hydrophobic drugs. J Control Release, 1998, 51: 221–229
16. La S B, Okano T, Kataoka K. Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly (ethylene oxide)-poly (beta-benzyl L-aspartate) block copolymer micelles. J Pharm Sci, 1996, 85: 85–90