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ABA and BAB type triblock copolymers of PEG and PLA: A comparative study of drug release properties and "stealth" particle characteristics

Guosen He, Lwin Lwin Ma, Jie Pan, Subbu Venkatraman *

Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue N4.1, Singapore 639798, Singapore

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Abstract

We synthesized two types of triblock copolymers containing PEG and PLA as controlled release carriers of hydrophobic drugs: these are the ABA type (PLA–PEG–PLA) and the BAB type (PEG–PLA–PEG). These polymers are amphiphilic and can form nanomicelles (40–200 nm) in aqueous medium. On the surface of PLA–PEG–PLA (ABA) type nanomicelles, the PEG content was enhanced somewhat over the bulk amount; whereas in the PEG–PLA–PEG (BAB type), surface segregation was much higher. The copolymers tested can entrap 35% of paclitaxel by weight on the average. In general, the diffusion-controlled release of paclitaxel is slower for the BAB polymers; furthermore, the actual release rates are influenced by the PLLA lengths in the BAB copolymers. Surface PEG contents influence the "stealth" characteristics of the nanomicelles. Compared with PLA particles, all nanomicellar particles tested, of both BAB and ABA types, showed a four-fold reduction in monocyte cell uptake, with the BAB type copolymer exhibiting a lesser uptake.

Keywords: Block; Copolymer; Drug carriers; Paclitaxel release; Immuno-cellular uptake; Opsonization

1. Introduction

Biodegradable materials have been attracting increasing interest for use in biomedical devices (Reis and Roman, 2004; Sachlos and Czernuszka, 2003; Laurencin et al., 1999; Vats et al., 2003). Although earlier applications focused on homopolymers of poly(L-lactide) (PLLA) or poly(glycolide) (PGA) and their random copolymers (PLGA), increasing attention is focused on the development of diblock copolymers such as PLA–PEG and PLGA–PEG, and their corresponding triblocks as drug carriers for controlled drug release (Kanjickal and Lopina, 2005; Bala et al., 2005; Pan et al., 2005). The amphiphilic character of these copolymers enables the formation of micelles in water through a self-assembly process at low concentration, with the hydrophobic PLA or PLGA serving as core reservoirs for drug and the hydrophilic PEG as the shell projecting into the aqueous environment. The use of PEG in the copolymers is also to reduce particle uptake by the mononuclear phagocytic system, sometime also referred to as the "stealth function", compared with the particles without PEG attachment (Gref et al., 1994, 2000). The opsonization-inhibiting property of PEG enables long circulation times of drug in vivo. In addition, proteins and plasmid DNA may also be incorporated into PEG-"coated" particles, indicating the potential application of the copolymers in immuno- and gene therapies (Vila et al., 2002; Jeong et al., 1997).

Besides possessing physical properties and functions similar to the PLA/PLGA–PEG–PLGA/PLA (ABA type) copolymers, mPEG–PLA/PLGA–mPEG (BAB type, also written as PEG–PLA/PLGA–PEG) copolymers were recently reported to undergo sol–gel transition from aqueous solutions at body temperature (Jeong et al., 1999; Li et al., 2003). The hydrogel of PEG–PLGA–PEG once formed in the body (in mice) can retain its integrity for more than 1 month, suggesting that it is ideal for slow-release systems to treat chronic diseases such as cancer and hypertension.

One of the most successful cancer drugs, paclitaxel, has shown its potency against a broad spectrum of cancers (Rowinsky et al., 1992; Rowinsky and Donehover, 1995; Lopes et al., 1993; Redhead et al., 2001). Paclitaxel is strongly hydrophobic and Taxol®, the dosage form of paclitaxel for administration, contains 50% Cremophor® EL to increase its

* Corresponding author. Tel.: +65 67904259; fax: +65 67909081.
E-mail address: Assubbu@ntu.edu.sg (S. Venkatraman).

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bioavailability. The Cremophor® El is believed to be responsible for side effects like dyspnea, flushing, rash and urticaria. Many attempts have been made to find alternative carriers for paclitaxel, particularly in nanoparticle form, made of PLGA–PEG and PLLA–PEG as the matrix materials (Matsumoto et al., 1999; Suh et al., 1999; Peracchia et al., 1997; Dong and Feng, 2004).

In an earlier paper (Venkatraman et al., 2005), we have reported on the ABA type copolymer and its characterization as a paclitaxel carrier. In this study, we compare this type copolymer with the BAB type for both surface properties (including an assessment of "stealth" properties) and drug release characteristics.

In this study, two series of copolymers of PLA–PEG–PLA ("ABA") and PEG–PLA–PEG ("BAB") have been synthesized as carriers for paclitaxel release, and also evaluated for their "stealth" characteristics using an in vitro assay. The synthesis of the copolymers, the formulation and characterization of the nanoparticles, drug release behaviour and immuno-uptake results (stealth function) are presented and compared here.

2. Materials and methods

2.1. Materials

Methyl-terminated poly(ethylene glycol) (mPEG, with only one hydroxyl group, $M_n = 5000$), poly(ethylene glycol) (PEG, with two hydroxyl groups, $M_n = 2000$, 4000, 8000) and stannous octoate were from Sigma–Aldrich (Singapore). mPEG and PEG were dried at 120 °C in vacuo for at least 6 h prior to use. L-Lactide was purchased from Purac Far East Pte. Ltd., Singapore, and recrystallized twice from dry ethyl acetate and dried in vacuo at 40 °C overnight. Hexamethylene diisocyanate (HMDI) was supplied by Tokyo Kasei Corp. (Tokyo, Japan) and used as received. Paclitaxel was purchased from Hande Biotechnology Ltd. (Yunnan, China), and coumarin 6 from Sigma–Aldrich (Singapore). All HPLC and analytical grade solvents include dichloromethane (DCM), tetrahydrofuran (THF) and acetonitrile (ACN) were from Merck (US) and used as received unless otherwise processed. Toluene was dried over sodium threads under reflux condition with benzophenone as indicator, and ethyl acetate was dried under reflux over diphosphorus pentaoxide. The pellets for pH 7.4 buffer preparations were supplied by Sigma–Aldrich (Singapore). The monocyte cell line (U937), for the immuno-uptake study of the copolymer particles was purchased from ATCC (US).

2.2. Triblock copolymers synthesis

2.2.1. PLA–PEG–PLA copolymers (ABA type)

The given amount of purified lactide was charged into a 250 mL three-neck round bottle flask containing pre-dried PEG, with the protection of purified nitrogen. Dry toluene was transferred into the flask followed by stannous octoate (0.05% (w/w), in toluene). The mixture solution was heated to 120 °C and allowed to reflux for 12 h. All syntheses were carried out under an oxygen and moisture free environment. The products were purified by repeated dissolution into dichloromethane and precipitated by cold ethyl ether or ethanol. The isolated polymer was dried at 40 °C under vacuum.

2.2.2. PEG–PLA–PEG copolymers (BAB type)

This type of copolymer synthesis starts with PEG–PLA diblock synthesis, followed by block coupling reaction with the chemical linker hexamethylene diisocyanate (HMDI) to produce the PEG–PLA–PEG triblock copolymers (Jeong et al., 1997).

PEG–PLA diblock copolymers were prepared by ring-opening polymerization (ROP) process. Briefly, mPEG was dried at 120 °C for 6 h in vacuo, and then dissolved with appropriate amount of lactide and 0.05%, w/w stannous octoate in toluene. The mixture was refluxed for 10 h under a dry nitrogen atmosphere. The solvent was removed under vacuum, and the product dissolved in DCM was precipitated by cold ethyl ether. The precipitation was done twice to get products with good quality. The diblock copolymers were then dried under vacuum at 40 °C for overnight.

To synthesize the BAB type copolymers, the mixture of PEG–PLA diblock and HMDI in toluene was thermostated at 60 °C for 10 h, followed by refluxing at 140 °C for overnight under nitrogen. Upon cooling to room temperature for a few hours, the solution gelled spontaneously. Upon warming, it became a solution again. The solvent was then removed in vacuo, and the resulting triblocks in DCM were purified by cold ethyl ether via fractional precipitation, followed by vacuum drying for 10 h. The diblocks, the ABA type triblocks and the fractions of BAB type triblocks were analyzed by GPC (Agilent series 1100, with the column of PLGel Mixed Bed C, 5 μm, 300 mm × 7.5 mm, Polymer Laboratories, USA), and ^{1}H NMR spectrum (Bruker, avance 400 MHz, Germany) to determine the molecular weights. $CDCl_3$ was used as solvent and TMS as internal reference.

2.3. Nanoparticle formation and characterization

2.3.1. Nanoparticle formation

In each experiment, 20 mg of triblock copolymer was dissolved in 1 mL DMF, and the solution was added slowly into 20 mL stirring deionized (DI) water. The water turned into cloudy during the polymer addition, and the aqueous emulsion was stirred for 4 h. Dialysis method was employed to remove DMF. Briefly, the micellar solution was transferred into a dialysis bag and dialyzed in a water bath. The bath water was changed at certain intervals. The micelles in the bag were harvested by freeze-drying (ALPHA 1-4 LSC, Christ GmbH, Germany) and kept in a desiccator, if not used immediately.

2.3.2. Nanoparticle characterization

2.3.2.1. Particle morphology. The surface morphologies of the nanoparticles were observed by a JEOL JSM-6340F field emission scanning electron microscope (FESEM) at 5.0 kV. The samples were mounted on a metal holder using double-sided

copper tape, and then coated with gold using a pulse plasma system before observation.

2.3.2.2. Particle size determination. The particle size determinations were carried out by BI-MAS (multi angle sizing option on the Zetaplus, Brookhaven Instruments Corporation). All measurements were performed at 25 °C in triplicate. To minimize the effect of stray light, a 90° angle was chosen for the measurement, with a wavelength of 632.8 nm Helium Neon Laser.

2.3.2.3. Critical micelle concentration (CMC) and micelle stability. Fluorescence spectroscopy method was employed for the CMC measurement (Dominguez et al., 1997). Firstly, 50 μL of 0.01 M pyrene in methanol was slowly added to 1 L stirring DI water to form the 5×10^{-6} M pyrene saturated stock solution. The DMF solution of the triblock was added into the pyrene stock solution with the volume ratio of DMF/water being 1/30, to reach a total copolymer concentration of 1 mg/mL. The micellar solution was then passed through a 0.8 μm syringe type filter, and diluted into different concentrations and stabilized in 60 °C water bath for 10 h. After cooling to room temperature, the solutions were tested by fluorescence spectrometer (Shimadzu RF-5301PC, Japan) to measure the pyrene fluorescence emission ratio of I_1/I_3, recorded at 371 and 383 nm, respectively, with excitation wavelength being 339 nm. The CMC values were determined from the best-fit curves of I_1/I_3 ratio against concentrations, at that concentration the I_1/I_3 value dramatically decreased.

Electrolytes promote the aggregation of micelles above a certain concentration. The micelle stability experiment followed the procedure reported by Riley et al. (1999). Briefly, each portion of 0.5 mL colloidal emulsion was added into 2.5 mL sodium sulfate solutions of varying concentration (0–0.8 M), and the turbidity was measured by UV spectrophotometer (Shimadzu UV-2501 PC, Japan), at a wavelength of 500 nm after 15 min. The critical flocculation point (CFPT) was taken as the electrolyte concentration at which a sharp increase of the turbidity was first detected.

2.3.2.4. Surface composition. XPS measurements were carried out on a VG Scientific ESCA LAB Mk II spectrometer equipped with a Mg Kα X-ray source (1235.6 eV photons) and a hemispherical energy analyzer. The copolymer samples were analyzed in film and particle forms. For film preparation, copolymers in DCM were spin-coated on the pre-cleaned glass slides. After vacuum drying, the films were tested by XPS. For particles, the freeze-dried nanoparticles were loaded on the glass slides by the double-sided adhesive tapes. The tapes should be fully covered by the nanoparticles. A series of survey spectra was recorded over a binding energy range from 0 to 1000 eV using passive energy 20 eV first. In all cases the survey spectra recorded the presence of only oxygen (O1s 533 eV) and carbon (C 1s 285 eV) on the surfaces. Detailed analysis of C 1s regions of each sample was carried out over a binding energy range from 280 to 300 eV. Charge referencing was performed using the C–H peak (285.0 eV) for PLA–PEG–PLA samples. The curve fitting was performed by the computer program Kratos, provided by the equipment manufacturer. A Gaussian–Lorentzian function was applied to all data. No asymmetry component was introduced.

2.3.2.5. Paclitaxel release from micelles. To load the drug in BAB type copolymers, 2.5 mg of paclitaxel and 50 mg copolymer were dissolved in 2 mL DMF, which was then added dropwise into 48 mL DI water, with stirring. After 4 h stirring, the micellar solution was filtered through a 0.8 μm syringe type filter, and centrifuged at 50,000 × *g* for 2 h (4 °C, Jounan® KR25i, France) to remove DMF and the un-trapped drug. The paclitaxel-loaded micelles were re-suspended in pH 7.4 buffer and incubated in 37 °C oven. For testing, the micelles were collected by centrifugation at 50,000 × *g* for 2 h, and re-suspended in the identical volume of fresh buffer. The supernatants were tested with HPLC (Agilent 1100 Series instrument equipped with a VW detector operating at 227 nm and a ZORBAX 300SB-C18 column, with the mobile phase being 50/50 acetonitrile/water in volume, and the flow rate of 1.0 mL/min, US) for the paclitaxel level. The drug entrapment is based on the amount found in particles, and the fed amount in formulation. The accumulated release calculation is based on the accumulated drug levels in buffer and the drug entrapment.

For the drug loading in ABA type copolymers, although three drug concentrations were tested, we report on one loading only, as it matches the 1:20 ratio of drug to polymer for the BAB type.

2.3.2.6. Immuno-uptake of copolymer particles. The copolymer nanoparticles were sterilized under UV light and the uptake study was performed under sterilized condition. About 10 mg of copolymer particulate and 5 μL of 0.01 mg/mL coumarin 6 in DMF were prepared similarly as described in this section. The suspension of copolymer and coumarin 6 was prepared by adding this stock solution dropwise in stirred DI water, followed by a dialysis process against autoclaved DI water. Positive control PLLA particles with the M_n of 10 K were prepared by the double emulsion technique (Huang and Venkatraman, 2006). In order to get pure nanoparticle, the copolymer and PLLA suspension were centrifuged (35,000 × *g*, 30 min) and re-suspended in DI water U937 suspension cells were cultured in 75 cm^2 culture flask in RPMI 1640 supplemented with 10% FCS at 37 °C, 5% CO_2 in humidified atmosphere. U937 cells were mixed (5×10^5 per well) in a six well plate (Nunc, Roskilde, Denmark) together with negative control U937 cells, the same amount (0.5 mg/well) of positive control PLA, coumarin 6 only and copolymers. For this experiment incubate the cells and coumarin 6 only to exclude the coumarin 6 absorbed by cells and intend to get the coumarin 6 loaded nanoparticle uptake by cells only After keeping in 37 °C incubator for 2 h, the cells were collected by centrifuge (10 min, 800 × *g*), and washed with 3 mL phosphate buffered saline (PBS, Sigma–Aldrich) and centrifuged again. Use the spectrofluorometer to study the fluorescence intensity of supernatant after centrifuge and the washing procedure was repeated until the fluorescence activity of the control coumarin 6 and cells were then re-suspended in 1 mL PBS and subjected to flow cytometry testing.

Table 1
Characterization of PLA–PEG–PLA triblock copolymers

Copolymer	^{1}H NMR			GPC	
	LA/EG in feed	LA/EG in product[a]	M_n	M_n[b]	M_w/M_n[c]
4	2.49	1.98	17940	17310	1.12

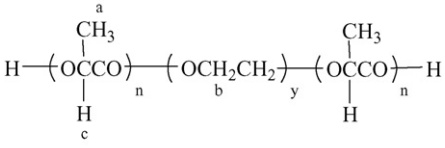
M_w: weight average molecular weight and M_n: number average molecular weight.
[a] LA/EG in product was determined from the integration ratio of resonances due to PEG blocks at 3.64 ppm (–O–CH_2–CH_2–) and to the PLA blocks at 5.17 ppm (Me–CH$^*<$) in the ^{1}H NMR.
[b] Based on polystyrene standards.
[c] Polydispersity of copolymers.

3. Results and discussion

3.1. Triblock copolymer syntheses

3.1.1. PLA–PEG–PLA copolymers

A series of ABA type copolymers was obtained successfully. From Table 1, it can be seen that, the molecular weight of the copolymers increased with the increase of the LA/EG feed ratio. The number average molecular weight of the ABA block copolymers fell into the range of 6400–18,000; and the molecular weight distributions were rather narrow, as seen in Table 1:



ABA type triblock copolymer

In Fig. 1, a typical ^{1}H NMR spectrum of the ABA type copolymer is shown. The presence of methine (CH) and methyl (CH_3) protons in PLA was observed at around 5.17 ppm (c) and 1.60 ppm (a), respectively. Tetralet and doublet split peaks were observed accordingly, which were in good accordance with the NMR split theory. The methene protons in CH_2 group of PEG were recorded at around 3.64 ppm.

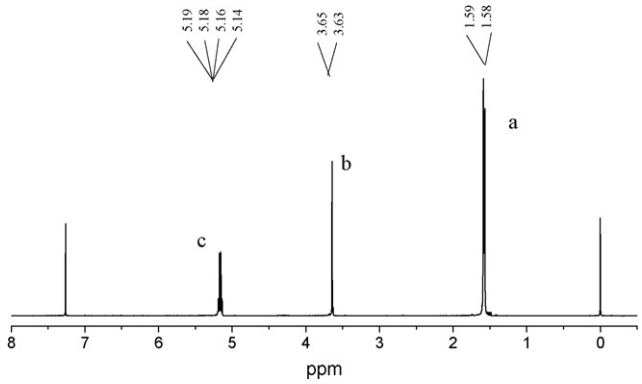


Fig. 1. ^{1}H NMR spectrum ($CDCl_3$) of typical ABA triblock copolymer.

Table 2
Characterization of PEG–PLA–PEG triblock copolymers

Copolymer	^{1}H NMR		GPC	
	LA/EG in product	M_n	M_n	M_w/M_n[a]
5	0.18	13000	17100	1.21
6	0.95	25600	27300	1.19
7	2.24	39300	46800	2.49

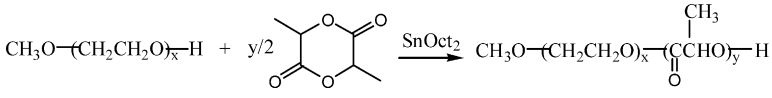
[a] Determined by GPC.

3.1.2. PEG–PLA–PEG copolymers

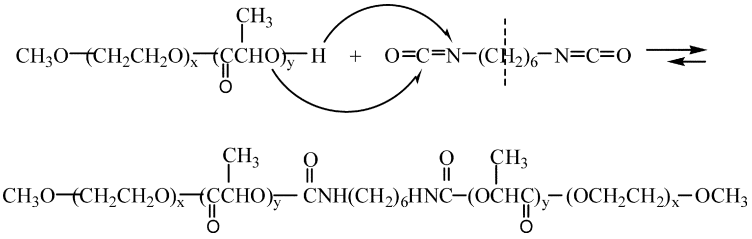
The ROP of lactide yielded PEG–PLA diblock copolymers in moderate yields and good quality. Each diblock contained only one hydroxyl group, which undertaken nucleophilic addition with the isocyanate group to form the corresponding triblock.

Due to the extreme similarity in physical properties between the diblock and triblock, the purification of triblock is rather difficult. In this study, we used fractional precipitation to isolate the triblocks, and GPC to verify their purity. Three BAB type copolymers were obtained. The compositions and molecular weights of the trio were summarized in Table 2 (Schemes 1 and 2).

Fig. 2 shows the representative NMR spectrum of PEG_{114}–PLA_{512}–PEG_{114}. The protons of CH_2NCO group from the HMDI are clearly present at 3.15 ppm, and both PEG and PLA protons are recorded at 3.65, 1.60 and 5.15 ppm, respectively, which is in accordance with the report (Jeong et al., 1997).



Scheme 1. Diblock copolymer synthesis.



Scheme 2. BAB type triblock copolymer synthesis.

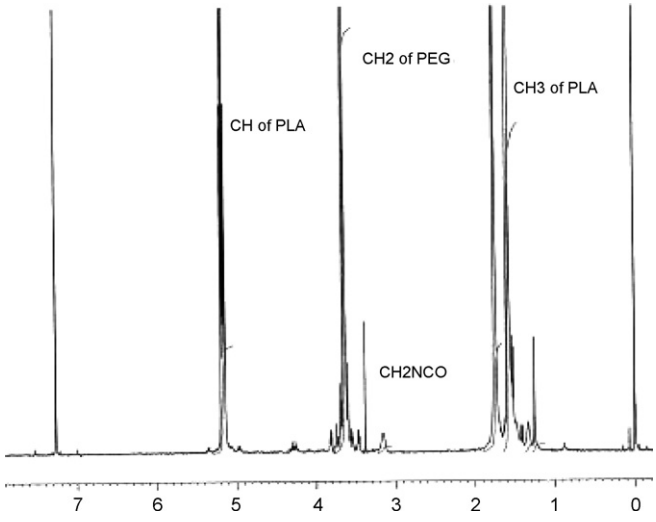


Fig. 2. ^{1}H NMR spectrum ($CDCl_3$) of copolymer PEG_{114}–PLA_{512}–PEG_{114}.

Notably, due to the low proton content of HMDI in the triblock, it is necessary to amplify the NMR spectrum vertically to make the HMDI protons visible, thus the trace amount of impurities have also been amplified.

3.2. Nanoparticle characterization

3.2.1. Particle morphology

The morphology of the ABA type copolymer particles is well documented (Ruan and Feng, 2003; Lim and Park, 2000; Liu et al., 2001). In contrast to the commonly used solvent evaporation method, we employed DMF to dissolve the polymers for the particle formation and eventually removed it by dialysis. The freeze-dried nanoparticles were observed with FESEM. This image was typical of those obtained for all the samples, confirming that the ABA and BAB type copolymers formed spherical, discrete particles in aqueous media (Fig. 3).

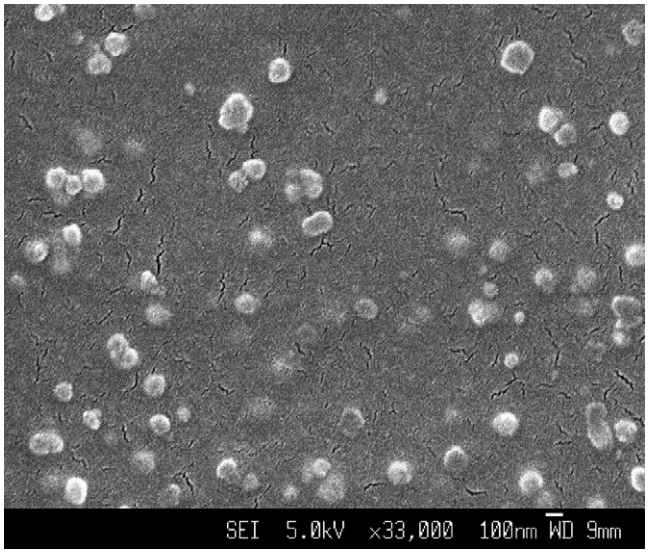


Fig. 3. SEM photo of the nanoparticles of typical ABA triblock copolymer.

Table 3
Particle size, CMC (critical micelle concentration) and CFPT (critical flocculation point) of ABA and BAB type micelles

Copolymers[a]	Particle diameter mean (nm)	CMC (mg/mL)	CFPT (mol/mL)
PLA_{90}–PEG_{91}–PLA_{90} (4)	210.7	0.005	0.50
PEG_{114}–PLA_{217}–PEG_{114} (6)	63	0.006	0.65
PEG_{114}–PLA_{512}–PEG_{114} (7)	80	0.003	0.60

[a] The low molecular weight copolymers 1 and 5 were omitted.

3.2.2. Particle size, CMC and stability of ABA and BAB type copolymers

ABA type copolymers particle size: the hydrodynamic diameter of particles was determined by DLS (dynamic light scattering). The effective diameter and polydispersity of particulate dispersions are presented in Table 3. It can be observed from Table 3 that for a fixed length of PEG, increasing the PLA block length increased particle size. We believe the ABA copolymers may form double layers with the PEG units forming the polar heads, and the PLA tails may form layers with neighbouring molecules.

BAB type copolymers particle size: in aqueous media, the BAB type copolymers may form simple micelles, with the molecule forming a U-shape to concentrate the PLA units in the core and the PEG tails projecting out into the water.

The BAB micelles are smaller than their ABA counterparts, at comparable PLA chain lengths. The particle sizes are in the 60–80 nm diameter range. The particle sizes observed showed a similar dependence on PLA chain length, in copolymers PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114}. This observation is in good accordance with one report (Liu et al., 2001).

3.2.2.1. CMC of the ABA and BAB type copolymers. Upon formation of micelles, pyrene would move into the micelle cores (hydrophobic) from the aqueous phase, which results in an alteration in the intensity ratio (I_1/I_3) of pyrene fluorescence bands I (371 nm) and PEG_{114}–PLA_{512}–PEG_{114} III (383 nm). Because of the amphiphilic property of these copolymers, all of them can form micelles. Table 3 lists the CMC values of the five triblocks. The ABA and BAB polymers show distinctly different trends. For ABA micelles, increasing LA length at fixed EG length increases CMC; for the BAB polymers, behaviour is more along expected lines, i.e., the CMC decreases with increasing LA length. However, it must be stated that the CMCs are all low, and do not show much variation in the range studied here.

We believe this could be due partly to the vesicle structure observed in the ABA copolymers; it appears that these copolymers form an elongated vesicle, with many (aggregated) molecules forming the micelle.

3.2.3. Surface chemistry

XPS analysis provides quantifiable data on biomaterial surface composition. Gref et al. found that most of the PEG is concentrated within 5 nm of the outer layer of the PLGA–mPEG diblock nanospheres (Gref et al., 1994). However, Ruan et al.

observed the distribution of PEG segments was homogenous in the PLA–PEG–PLA microspheres (Ruan and Feng, 2003), different from the diblock copolymer PLA–mPEG nanoparticles (Kim et al., 2001), in which PEG migrated to the surface. We found that surface composition of the ABA type copolymers were dependent on the overall composition (Table 4A). Copolymers with longer PLA chain, such as PLA_{90}–PEG_{91}–PLA_{90}, showed homogenous PEG distribution.

As for the BAB type copolymers tested, PEG chain length is fixed and it is attached to the ends of PLA of different molecular weights. In contrast to the ABA type copolymers, the composition percentages of PEG in PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114} are relatively higher. The XPS data (Tables 4B and 4C) show that the two films recorded much higher PEG concentration than the calculated bulk values, suggesting spontaneous PEG migration to the surface. The trend is accentuated even more with the nanoparticles. The higher surface segregation of PEG is attributed to the structural features of BAB type copolymer and its conformation in solution. Similar results have been reported (Mukose et al., 2004).

The increase of PEG surface concentration in the particles is quite dramatic. To form micelles in aqueous media, BAB molecule has to fold like a U letter, with PLA segment constrained in the micelle core and PEG chains projecting to the aqueous environment. After drying, the micelles keep their integrity so by XPS, higher PEG concentrations were detected on the surface. In the copolymers studied here, a longer PLA segment leads to a larger particle size, and also a higher surface PEG concentration. Compared with the ABA copolymers, BAB polymers simply find it easier to form smaller particles (not aggregated), and project higher PEG concentration on the surface, which make them ideal candidates for "stealth" behaviour.

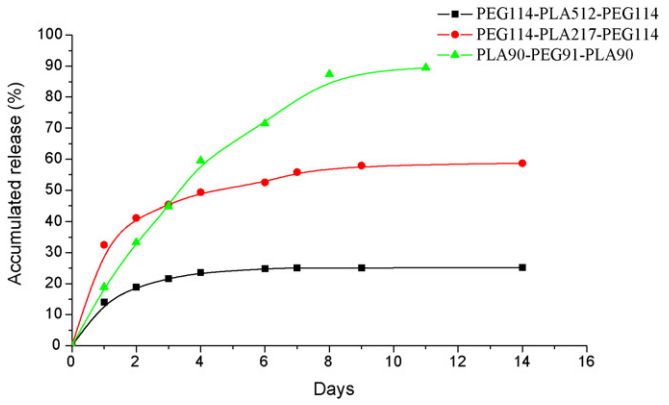


Fig. 4. Paclitaxel release from the micelles of copolymer PEG_{114}–PLA_{217}–PEG_{114}, PEG_{114}–PLA_{512}–PEG_{114} and PLA_{90}–PEG_{91}–PLA_{90} (at same drug: polymer ratio of 1:20).

3.2.4. *Paclitaxel release from micelles*

We chose the BAB type copolymers PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114} to test the paclitaxel release profiles in pH 7.4 buffer. It can be found in Fig. 4

Table 4A
XPS data (C 1s regions) for particles and film of PLA_{90}–PEG_{91}–PLA_{90}

Peak	Pure copolymer: film			Nanoparticles	
	Binding energy (eV)	Theoretical (%)	Experimental (%)	Binding energy (eV)	Experimental (%)
a	285.0	22.68	22.22	285.0	21.16
c	287.3	22.68	22.22	287.1	23.01
d	289.2	22.69	22.98	289.3	23.44
b	286.2	31.95	32.57	286.1	32.39

Table 4B
XPS data (C 1s regions) for particles and film of PEG_{114}–PLA_{217}–PEG_{114}

Peak	Pure copolymer: film			Nanoparticles	
	Binding energy (eV)	Theoretical (%)	Experimental (%)	Binding energy (eV)	Experimental (%)
a	285.0	16.23	14.53	285.0	12.25
c	287.1	16.22	15.71	287.4	12.44
d	289.5	16.36	8.44	289.6	7.05
b	286.4	51.31	61.47	285.9	68.31

Table 4C
XPS data (C 1s regions) for particle and film of PEG_{114}–PLA_{512}–PEG_{114}

Peak	Pure copolymer: film			Nanoparticles	
	Binding energy (eV)	Theoretical (%)	Experimental (%)	Binding energy (eV)	Experimental (%)
a	285.0	23.05	27.60	285.0	22.84
c	287.6	23.05	24.80	287.5	13.85
d	289.6	23.10	13.90	289.5	13.52
b	286.2	30.94	33.70	286.4	49.93

Table 5
Immuno-uptake of particles by U937 cell line

	Copolymer			Control
	PLA_{90}–PEG_{91}–PLA_{90}	PEG_{114}–PLA_{217}–PEG_{114}	PEG_{114}–PLA_{512}–PEG_{114}	PLA
Uptake (%)	24.7	16.1	15.6	110.1
Relative to PLA particles	22.4	14.6	14.2	100

that the accumulated release of paclitaxel from copolymer PEG_{114}–PLA_{217}–PEG_{114} is higher than that from copolymer PEG_{114}–PLA_{512}–PEG_{114}, and the drug release is sustained over several days.

The length of PLA in the two copolymers may contribute to the observed difference in release rates. The PLA length ratio of copolymer PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114} is 1:2.4, and the amount of drug entrapment is similar (33.9% and 38.8%, respectively). The diffusion path length through copolymer PEG_{114}–PLA_{512}–PEG_{114} could be higher than in copolymer PEG_{114}–PLA_{217}–PEG_{114}, due to the longer PLA segment.

The paclitaxel entrapment in ABA type copolymers is slightly lower than their BAB counterparts. However, both types appear to have good capacity for loading paclitaxel.

Copolymer PLA_{90}–PEG_{91}–PLA_{90} was tested to release paclitaxel at the same 1:20 ratio, although other concentrations have been studied (Venkatraman et al., 2005). In this comparison (Fig. 5) the BAB type copolymer exhibits a slower diffusion-controlled release over the first 10 days, with no apparent contribution from degradation. This difference is most likely due to the longer PLA segments in the two BAB copolymers, as clearly increasing the PLA length decreases the diffusion rate for the BAB copolymer. Clearly, the effect of PLA length is seen here at fixed EPG segment length; it is possible that PEG segment lengths also make a difference.

In summary, we may conclude that this kind of copolymer is suitable for use as a paclitaxel carrier, can carry a reasonable amount of drug and sustain its release over several days (without the hypersensitivity reactions associated with Cremophor®). The copolymer composition may be manipulated to yield desired release characteristics.

3.2.5. *Immuno-uptake of copolymer particles*

To investigate the stealth function of the PEGylated copolymers, PLLA served as an ideal standard with its molecular weight close to 10 K, and the PLA nanoparticles prepared by double emulsion technique were around 62 nm in diameter, making both the polymer and the nanoparticle size comparable to the PEG-based copolymers tested. It was believed that the flexibility and hydrophilicity of the PEG chains in outer-shell of particles play important roles to prevent opsonization (Blume and Cevc, 1993; Torchilin and Papisov, 1994). PLLA does not have these features, and its uptake by monocytes was ascertained to be much higher than the amphiphilic diblock and triblock copolymers (Jaeghere et al., 2000).

As seen from Table 5 that the uptake rates of the copolymers tested are 25% lower than that of PLA, which is in good accordance with the report (Jaeghere et al., 2000). Furthermore, there are differences between the ABA and BAB type copolymers, which can be explained by their structural features. The PEG chain in ABA type copolymer is shorter than the one in BAB counterparts and conformationally, it is much more constrained than the PEG segments in the BAB type polymer. Thus the PEG chain in BAB copolymers can create a big "flexible cloud" to surround the particle and prevent opsonization (Woodle and Lasic, 1992). From the XPS data of the copolymers, it is clear that the nanoparticles of copolymer PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114} possess higher PEG density on the particle surface than copolymer PLA_{90}–PEG_{91}–PLA_{90}, and accordingly resulted in lower immuno-uptake (Photos et al., 2003). Interestingly, although the XPS data of copolymer PEG_{114}–PLA_{217}–PEG_{114} and PEG_{114}–PLA_{512}–PEG_{114} are significantly different, they produced similar results for monocyte uptake, which may suggests that once a certain amount of PEG coverage is available, it is sufficient to prevent cellular uptake; increases beyond this amount are inconsequential.

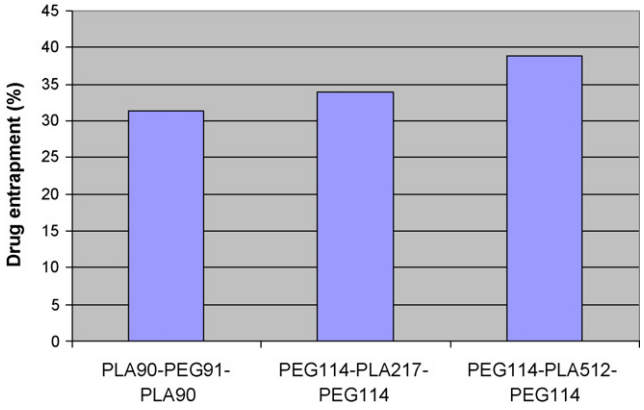


Fig. 5. Paclitaxel incorporation efficiencies for different copolymers.

4. Conclusions

Both ABA and BAB type copolymers of PEG and PLA were studied systematically for the various properties that are required of a passive-targeting paclitaxel carrier. These copolymers easily form nanomicelles in water, at low concentrations, and show reasonable stability. Paclitaxel incorporation is efficient at 30–40%. The drug is released over several days, and the release is influenced by diffusion and degradation. Due to the unique structural feature of BAB type copolymers, migration of PEG to the micellar particle surface was observed, to be higher than previous reports, and higher than that observed for ABA type polymers. In vitro assessment of the stealth function for these polymers

using monocyte uptake rates showed BAB type copolymers to be more adept at avoiding uptake, although whether this difference will be significant in vivo remains to be studied (Fig. 5).

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