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Incorporation and release behavior of hydrophobic drug in functionalized poly(D,L-lactide)-block–poly(ethylene oxide) micelles

Jaeyoung Lee, Eun Chul Cho, Kilwon Cho*

Department of Chemical Engineering, School of Environmental Engineering, Pohang University of Science and Technology, 790-784 Pohang, South Korea

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Abstract

The poly(ethylene oxide)–poly(lactide) (PEO–PLA) block copolymers containing a small quantity of carboxylic acid in the PLA block were synthesized. The microscopic characteristics of nanoparticles with carboxylic acid content in the copolymer were analyzed, and the effect of specific interactions between the copolymer and the model drug on the drug loading capacity and the release behavior were investigated systematically. The sizes of nanoparticles prepared by a dialysis method are within the range of 30–40 nm. The nanoparticles prepared from functionalized block copolymers have a very low critical micelle concentration (CMC) value as low as $\sim 10^{-3}$ mg/ml, which indicates a good stability of the nanoparticles in spite of the presence of carboxylic acid. The drug loading efficiency of nanoparticles dramatically increased when carboxylic acid content was increased in the block copolymer. This result may be attributed to the increase of interactions between the copolymer and the drug. The release rate of the drug was much slower from nanoparticles containing higher amounts of carboxylic acid in the copolymer, which might be associated with the enhanced interaction between the carboxylic group of copolymers and the drug. These experimental results suggest that the nanoparticles prepared from functionalized PEO–PLA block copolymers could be a good candidate for an injectable drug delivery carrier.

Keywords: Block copolymer; Micelle; Nanoparticle; Drug delivery; Careboxylic acid

1. Introduction

Over the past few decades, biodegradable nanoparticles have attracted considerable interest as an effective drug carrier device. Various polymers have been used in drug delivery systems. The amphiphilic block copolymer has been the focus of research especially because of the nano-character in self-assembling systems [1–5]. This type of diblock copolymer can form a spherical micelle structure in an aqueous media. The hydrophobic blocks of the copolymer form the core of the micelle, while the hydrophilic blocks form the corona or outer shell. The hydrophobic micelle core serves as a microenvironment for incorporating hydrophobic drugs such as anti-cancer drugs [6,7] while the outer shell serves as

* Corresponding author.
E-mail address: kwcho@postech.ac.kr (K. Cho).

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a stabilizing interface between the hydrophobic drug and the external medium. Since most drugs have a hydrophobic character, the drugs can be easily incorporated into the micelle by simple physical entrapment through dialysis or by an oil/water emulsion method. The solubility of hydrophobic drug in the aqueous media is greatly increased by the use of micelle. Thus, incorporating a drug in the micelle is an effective method of preparing an efficient drug delivery system. Biodegradable and biocompatible polymers such as poly(lactide) (PLA) [8], poly(ε-caprolactone) (PCL) [9–11], poly(β-benzyl L-aspartate) (PLBA) [12,13], and poly(γ-benzyl L-glutamate) (PLBG) [14] have been used mostly for the core material of micelle. Moreover, poly(ethylene oxide) (PEO) is a non-toxic, highly hydrated polymer that stabilizes the surfaces in aqueous systems, and it is effective in preventing the adsorption of proteins and adhesion of cells. Therefore, PEO has been used as the outer shell material of micelle for a long-circulating drug carrier.

Studies on the polymeric micelles composed by the PEO as hydrophilic block and PCL, PLA, PLBA, PLBG as hydrophobic block have been carried out by many groups [8–15]. The size, stability, drug loading capacity, release kinetics, circulation time and biodistribution of micelles are several key properties that have been studied. These properties are affected by the molecular weight, the composition, and the chemical structure of diblock copolymer. Therefore, these studies focused on correlating the structure of block copolymer and the properties of micelle. The block copolymers developed so far either contain no functional groups at all in the hydrophobic block like as PCL, PLA or contain excessive functional groups like as poly(aspartic acid) [16]. When the functional groups are not contained in the hydrophobic block of the copolymer, no special interaction such as hydrogen bonding can be expected between the copolymer and the drug. Consequently, drug is not very well loaded into the micelle. And this copolymer has a relatively low drug loading efficiency. In the case of copolymers containing excessive functional groups, the copolymers may be water-soluble and unable to form the micelle in an aqueous medium. Thus, attaining the amphiphilicity of the copolymer is encountered with a problem—that is, the chemical conjugation of a hydrophobic drug is needed with the functional group of core block.

To solve this problem and increase the drug loading capacity compared to unfunctionalized poly(ethylene oxide)–poly(lactide) (PEO–PLA) block copolymer, we synthesized the PEO–PLA diblock copolymers containing a small quantity of carboxylic acid in the PLA block and prepared functionalized nanoparticles. This study analyzed the microscopic characteristics of nanoparticles with carboxylic acid content in the copolymer and investigated the effect of specific interaction between the copolymer and the model drug on the drug loading capacity and the release behavior systematically.

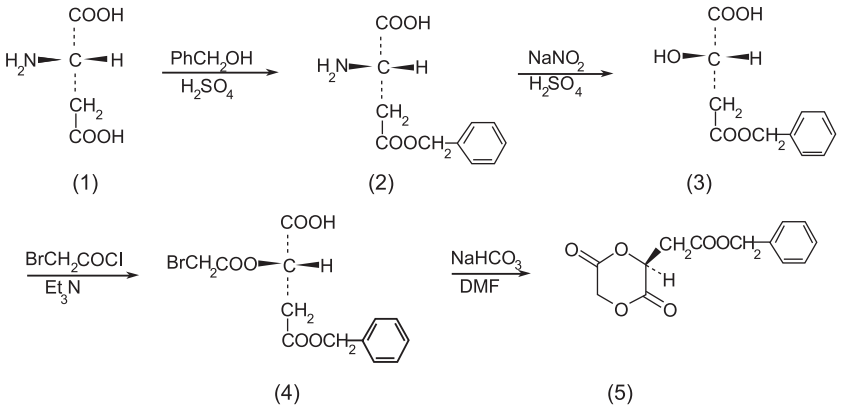
2. Experimental

2.1. Materials

Methoxy terminated poly(ethylene oxide) (MePEO) with molecular weight of 5000 was purchased from Fluka. LAC was supplied by Aldrich and recrystallized from ethyl acetate. Stannous 2-ethylhexanoate was provided by Sigma Chemical and purified by distillation. Papaverine(6,7-dimethoxy-1-veratrylisoquinoline) hydrochloride was obtained from Sigma Chemical, and a papaverine free base (PAP) was prepared by increasing the pH of solution above 10. L-Aspartic acid and bromoacetylchloride were purchased from Aldrich and Fluka, respectively. All other chemicals used were of reagent grade and used without further purification.

2.2. Snythesis of functionalized monomer and diblock copolymer

3-(*s*)-[(Benzyloxycarbonyl)methyl]-1,4-dioxane-2,5-dione (BMD) (5) was prepared from L-aspartic acid according to the following procedures (Scheme 1) [17]. The synthesis of PEO–PLA diblock copolymer containing carboxylic group (7) was also conducted according to the following procedures (Scheme 2). PEO–PLA diblock copolymers containing benzyl moiety (6) were synthesized by ring-opening copolymerization of LAC and BMD in the presence of MePEO homopolymer with stannous 2-ethylhexanoate as a catalyst. The weighed amounts of MePEO,



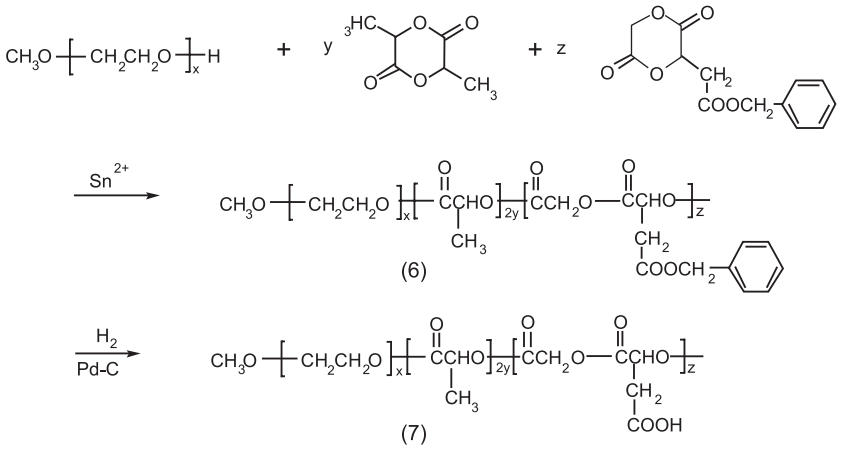
Scheme 1. Synthetic scheme of functionalized monomer (BMD).

LAC, BMD, and stannous 2-ethylhexanoate were placed in a 100 ml round flask equipped with a vacuum cock. The flask, filled with nitrogen gas and evacuated, was heated up to 160 °C, and the mixture was stirred for 2 h. While the mixture was being stirred under these conditions, the viscosity of the product increased gradually. The product was dissolved in 50 ml of chloroform and precipitated in an excess amount of diethyl ether, which was filtered and dried in a vacuum oven.

PEO–PLA diblock copolymer containing carboxylic group (7) was obtained by the catalytic debenzylation of its precursor (6) in the presence of hydrogen gas. A solution of 4 g of (6) in 125 ml of ethanol/dioxane (25:75) was poured into a flask of 500 ml. After 1 g of charcoal coated with palladium was dispersed to this solution, the flask was evacuated, connected to a hydrogen depot and filled with hydrogen gas. The mixture was then stirred vigorously with a magnetic stirrer and reacted with hydrogen for 15 h. After the theoretical amount of hydrogen had been absorbed, the mixture was filtered to remove the catalyst. The filtrate was condensed under reduced pressure and precipitated in a large excess of diethyl ether. The final product was filtered and dried in a vacuum.

2.3. Preparation of nanoparticles

The nanoparticles (micelles) containing a hydrophobic drug were prepared according to the dialysis method [12]. Diblock copolymer (200 mg) was dis-



Scheme 2. Synthetic scheme of PEO–PLA block copolymer containing carboxylic acid.

solved in 10 ml of DMF followed by adding 150 mg of drug (PAP) and stirred at room temperature. This solution was filtered to remove dust particles and dialyzed for 24 h against 2 l of ultra-pure water using cellulose dialysis membrane (Mw cut off: 6000–8000). The water was exchanged to fresh water once every 5 h. The nanoparticle solution was filtered to eliminate the unloaded drug and aggregated particles. The pore size of Teflon filter was 0.45 μm. A part of nanoparticle solution was frozen and lyophilized by a freeze dryer system to obtain dried nanoparticles and also to calculate the concentration of nanoparticles solution prepared in this process. The remaining part of the nanoparticles solution was frozen to prevent degradation of diblock copolymer. The nanoparticles that are not containing a drug were also prepared following the same method as above.

2.4. Characterizations

The chemical structure of synthesized monomer, and the composition and the molecular weight of the copolymers were determined by using a proton nuclear magnetic resonance (NMR) instrument (Bruker DRX500). The molecular weight and its distribution of diblock copolymer were characterized by using gel permeation chromatography (GPC) (Waters 600E) calibrated using the standard polystyrene at room temperature. Tetrahydrofuran was used as an eluent at a flow rate of 0.8 ml/min. The thermal properties of the PAP and drug containing nanoparticles were determined by a differential scanning calorimetry (DSC) instrument (Perkin–Elmer DSC 7) at a scanning rate of 10 °C/min. The X-ray photoelectron spectroscopy (XPS) instrument (VG instrument) was used to investigate the surface chemistry of nanoparticles. The source was a monochromated Al K-α X-rays.

Aqueous dispersion of nanoparticles was examined by using a transmission electron microscopy (TEM) instrument (JEOL). Specimens were prepared by dropping the nanoparticles solutions onto carbon coated EM grids. The solution on the grid was refrigerated in liquid nitrogen and lyophilized by a freeze dryer. The nanoparticles on the grid were stained by 20 wt.% of phosphotungstic acid. Specimens were vacuum dried before examination. Average size of nanoparticles in the aqueous solution was determined by a dynamic light scattering (DLS) instrument (Brookhaven BI-9000AT) using an argon ion laser at 25 °C. The intensity of the scattered light was detected with a photomultiplier as a function of the scattering angle. The signal from the photomultiplier was digitized via an amplifier-discriminator and was fed into a correlator. Intensity autocorrelation function was fitted by using the second cumulant method. The samples for DLS measurement were prepared by diluting the nanoparticles solution. And then, the solution was filtered through a 0.45 μm Teflon filter. The concentration of solution was about 0.1 wt.%. This concentration is sufficiently diluted so that the multiple scattering due to high concentration of nanoparticles may not occur.

2.5. Fluorescence measurements

In order to determine the critical micelle concentration (CMC) of diblock copolymeric nanoparticles in distilled water, fluorescence measurements were carried out using pyrene as probe [18]. Pyrene predissolved in acetone was added to the test tube, and the solvent was evaporated. Different amounts of nanoparticles solution and distilled water were added to this tube and made a different concentration of diblock copolymer ranging from 10^{-6} to 10^{-1} mg/ml. The concentration of pyrene used was 6.0×10^{-7} M. The solution was incubated at room temperature with mild stirring for pyrene to equilibrate between the nanoparticle and the aqueous phase completely. Fluorescence excitation spectra were obtained as a function of the concentration of diblock copolymers using spectrofluorometer (Shimadzu RF-5301PC). Experiments were conducted with emission wavelengths of 390 nm. Excitation and emission bandwidths were 3.0 and 1.5 nm, respectively. Excitation spectra were obtained from the scanning excitation spectrum of each sample from 300 to 360 nm, fixing the emission wavelength at 390 nm. The CMC was determined by taking a mid-point of the copolymer concentration at which the relative excitation fluorescence intensity ratio measured at 335–333 nm was varied.

2.6. Drug loading amount

The drug containing nanoparticles were dissolved to dichloromethane. The amount of papaverine free base

(PAP) entrapped was determined by measuring the UV absorbance at 238 or 279 nm. A calibration curve was constructed using different concentrations of free PAP in dichloromethane. The weight % of the PAP content entrapped into the core of nanoparticles was calculated from the weight of the initial drug loaded nanoparticles and the amount of drug incorporated.

2.7. Drug release experiment

Nanoparticles solutions containing a known amount of drug were sealed in a dialysis bag (Mw cutoff: 6000–8000), and the drug was released into 250 ml of phosphate buffer solution (pH 7.4, 0.01 M) at 37 °C. The release medium (5 ml) was withdrawn at pre-determined time intervals and replaced with an equivalent volume of fresh buffer. The content of the drug released was directly determined from the absorbance at 238 nm.

3. Results and discussion

3.1. Synthesis of functionalized monomer

The synthesis of BMD (5) was carried out according to Scheme 1. The reactions of up to (4) progressed well, and the yields of products were higher than 95%. However, the yield of BMD was quite a low 20% because the reaction of the final step was progressed by the internal cyclization between bromoacetyl group and carboxylic group of (4) whereas BMD with high purity could be obtained by a sublimation method because BMD has the property of sublimation. The onset of melting peak of BMD synthesized is 150.8 °C (DSC thermogram of BMD is not shown here), which is quite consistent with the reference value (150 °C) [17]. The chemical structure of BMD is also analyzed by a proton NMR method. Each hydrogen atom of BMD is assigned to each peak of NMR spectrum. The integral area ratio of each peak corresponds accurately to the number of hydrogen of each group in the chemical structure of BMD. Especially, the observation of the characteristic quartet at $\delta = 5.0-5.1$ ppm in the spectrum was good proof for its cyclic structure [17]. Thus, BMD with high purity was successfully synthesized.

3.2. Synthesis of functionalized diblock copolymer

BMD (5) has a ring-opening polymerizability comparable to glycolide and lactide [17]. The copolymerization of MePEO, LAC, and BMD was carried out in bulk with stannous 2-ethylhexanoate. The MePEO/(BMD + LAC) weight ratio in the feed is fixed at 2/3, and only the BMD/LAC weight ratio is changed from 5/95 to 15/85. When the diblock copolymer has a large amount of carboxylic groups in the hydrophobic block, the diblock copolymers do not form the nanoparticles in the aqueous solution. Therefore, the weight ratio of BMD in the hydrophobic block is restricted up to 0.15. The NMR spectrum of PEO–PLA diblock copolymer containing BMD (6) shows the peak at $\delta = 7.28$ owing to the benzene group in the copolymer, which indicates that BMD should be introduced to the diblock copolymer. However, the NMR spectrum of PEO–PLA containing carboxylic acid (7) does not nearly show the peak at $\delta = 7.28$, which means that the pendant benzyl ester can readily be removed by catalytic hydrogenolysis after copolymerization to yield a new carboxyl-functionalized PEO–PLA diblock copolymer [19]. Also, GPC diagrams show only the single peak regardless of samples. These results confirm that PEO–PLA diblock copolymers containing carboxylic acid in the PLA block are successfully synthesized.

Table 1 shows the results of the characterization for diblock copolymers. The compositions and Mw of copolymers were determined by proton NMR spectroscopy and GPC measurement. The molecular weight % of PLA block in the copolymers was obtained from the peak intensity of the methylene proton ($\delta = 3.65$) of the PEG chain and the methyl proton ($\delta = 1.58$) of the PLA chain. The Mw and the weight composition of each block copolymers are controlled similarly, but the content of carboxylic acid is controlled differently (Table 1). Thus, it was possible to examine only the effect of the carboxylic acid in the copolymer on the physicochemical properties of the nanoparticles. The probability of forming the homo sequence of BMD is negligibly small at the present relatively low BMD/LAC ratios in the feed composition. Consequently, the BMD unit sequences of PLA block are thought to be random [19]. The number of carboxylic acid introduced in the PLA

Table 1
Characteristics of functionalized PEO–PLA diblock copolymers

Sample	Feed weight % (PEO/LA/BMD)	Mn GPC[a]	Mn NMR	PDI[b]	Weight % (PEO/PLA)[c]	Weight % BMD[d]	Number of –COOH/chain
PEO		5350	6810	1.09			
P	40/60/0	10,500	12,300	1.13	55.3/44.7	0	0
F1	40/57/3	10,000	12,600	1.14	54.1/45.9	5.95	1.34
F2	40/54/6	9010	12,300	1.14	55.3/44.7	11.7	2.78
F3	40/51/9	8480	11,500	1.14	59.5/40.5	19.5	3.74

[a] Mn, number-average molecular weight.
[b] PDI, Mw GPC/Mn GPC.
[c] Estimated from NMR spectrum.
[d] Weight % of BMD introduced in the hydrophobic block (PLA).

block was about 1–4, which indicated a small quantity of carboxylic acid.

3.3. Size of nanoparticles

The size of functionalized diblock copolymeric nanoparticles was investigated by a DLS method. The correlation function obtained from scattered intensity was fitted by Eq. (1). The diffusion coefficient of nanoparticles (D) was obtained from the slope of decay rate (Γ_1) versus scattering vector (q^2) plot.

$$y = A\ \exp[-\Gamma_1 t + (\Gamma_2/2)t^2]^2 + b \quad (1)$$

Also, the hydrodynamic radius of nanoparticles was determined by using the Stokes–Einstein Eq. (2).

$$D = kT/6\pi\eta R_h \quad (2)$$

where, k, Boltzmann's constant; T, absolute temperature, and η, viscosity of the solvent. Generally, micelle formation occurs as a result of two forces. One is an attractive force that leads to the association of molecules while the other one is a repulsive force that prevents unlimited growth of the micelles. Therefore, the micelle size is mainly determined by the relative magnitude and balance of these two forces. The sizes of nanoparticles obtained from DLS experiment were about 30–40 nm (Table 2), which are suitable for an injectable drug carrier. However, within the experimental range of carboxylic acid content, there is no special relation between the size of nanoparticles and the carboxylic acid content in the copolymer. This means that a small quantity of carboxylic acid in the hydrophobic block does not give a large effect on the formation of nanoparticles. Also, the sizes of nanoparticles containing the drug were almost similar compared to the ones containing no drug. This result may be attributed to the low content of drug in the nanoparticles (Table 2).

The size and morphology of nanoparticles were further observed by a TEM technique. Fig. 1 shows the nanoparticles stained by the phosphotungstic acid. Because the phosphotungstic acid is a negative staining agent, the color of the stained region is shown as white brown on the TEM photograph [8]. The size and the size distribution of the nanoparticles are clearly investigated. TEM photographs show that the morphology of nanoparticles is of spherical type, that the diameter of nanoparticles is about 20 nm, and that its distribution is quite uniform. It is also observed that even in the case of containing BMD 19.5 wt.% in block copolymer (Fig. 1(b)), the nanoparticles are well formed. The diameter of nanoparticles observed by TEM is smaller than its

Table 2
Characteristics and drug solubility of PEO–PLA nanoparticles

Sample	Diameter of nanoparticles[a] (nm)		CMC (mg/ml)	Drug solubility[b] (mg/ml)
	Unloaded	Drug loaded		
P-N	41.2	42.8	1.19×10^{-3}	0.40
F1-N	33.6	34.0	1.22×10^{-3}	0.87
F2-N	31.0	32.0	1.98×10^{-3}	1.59
F3-N	36.6	36.8	2.26×10^{-3}	1.75

[a] Determined by a DLS experiment.
[b] The concentration of PEO–PLA nanoparticles used is 1 wt.%.

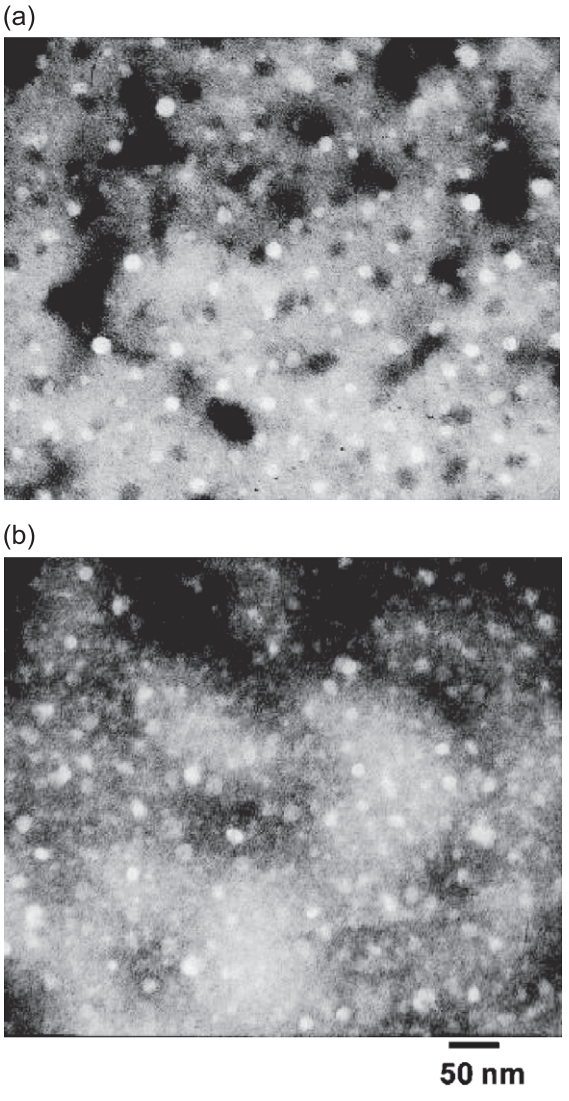


Fig. 1. TEM micrographs of PEO–PLA nanoparticles stained with phosphotungstic acid: (a) BMD 0 wt.%, (b) BMD 19.5 wt.%.

diameter obtained from the DLS experiment. The diameter of nanoparticles obtained from the DLS experiment reflects the hydrodynamic diameter of nanoparticles that are swelled by water molecules, whereas the diameter of nanoparticles observed by TEM shows that of dried nanoparticles. Therefore, an increase in the nanoparticles size obtained from DLS compared to that of TEM is assumed to be caused by the hydration of the shell portion of nanoparticles.

3.4. Critical micelle concentration

The CMCs of functionalized diblock copolymers were determined by a fluorescence spectroscopy measurement. Pyrene was chosen as a fluorescent probe because of its photochemical properties suitable for an effective probe [18,20]. Pyrene molecule had a strong hydrophobic character and a very low solubility in water. Because pyrene preferentially solubilized itself into the hydrophobic region of nanoparticles, the fluorescence intensity was greatly affected by the environmental change around pyrene. We can observe the shift of the peak at the excitation spectra with the increasing concentration of block copolymer (Fig. 2(a)). Specifically, the maximum peak for pyrene, which is at 333 nm in water, was shown to shift to 335 nm upon addition of block copolymer. The CMC was determined by taking a mid-point of the copolymer concentration at which the relative excitation fluorescence intensity ratio of I_{335}/I_{333} was varied (Fig. 2(b)). The CMC values of block copolymers are slightly increased with the content of carboxylic acid in the hydrophobic block (Table 2). This result may be attributed to the increase in the hydrophilicity of hydrophobic block with the increasing content of carboxylic acid. Because the absolute value of CMC is very low, however, the stability of nanoparticles is not affected greatly by the content of carboxylic acid within this range.

The physical state of the inner core region of nanoparticles was further characterized by the NMR study. The freeze-dried nanoparticles were dispersed into the D_2O, which were filtered to remove the aggregates, and then measured by using 500 MHz proton NMR instrument. Fig. 3(a) shows the NMR spectrum of nanoparticles (BMD 19.5 wt.%) measured in the D_2O solution. Due to the limited mobility of PLA chains in the core of the nanoparticles, the intensity of proton peak ($\delta = 1.55$) originated from PLA was dramatically reduced compared to the one in the $CDCl_3$ solution, where the formation of nanoparticles was not expected (Fig. 3(b)). The small, broad signals in the NMR spectrum indicate restricted motions of these protons within the core of nanoparticles. This suggests that the core of the polymeric nanoparticles is a very rigid structure. This result also offers one of the evidences that functionalized PEO–PLA diblock copolymers may associate to form

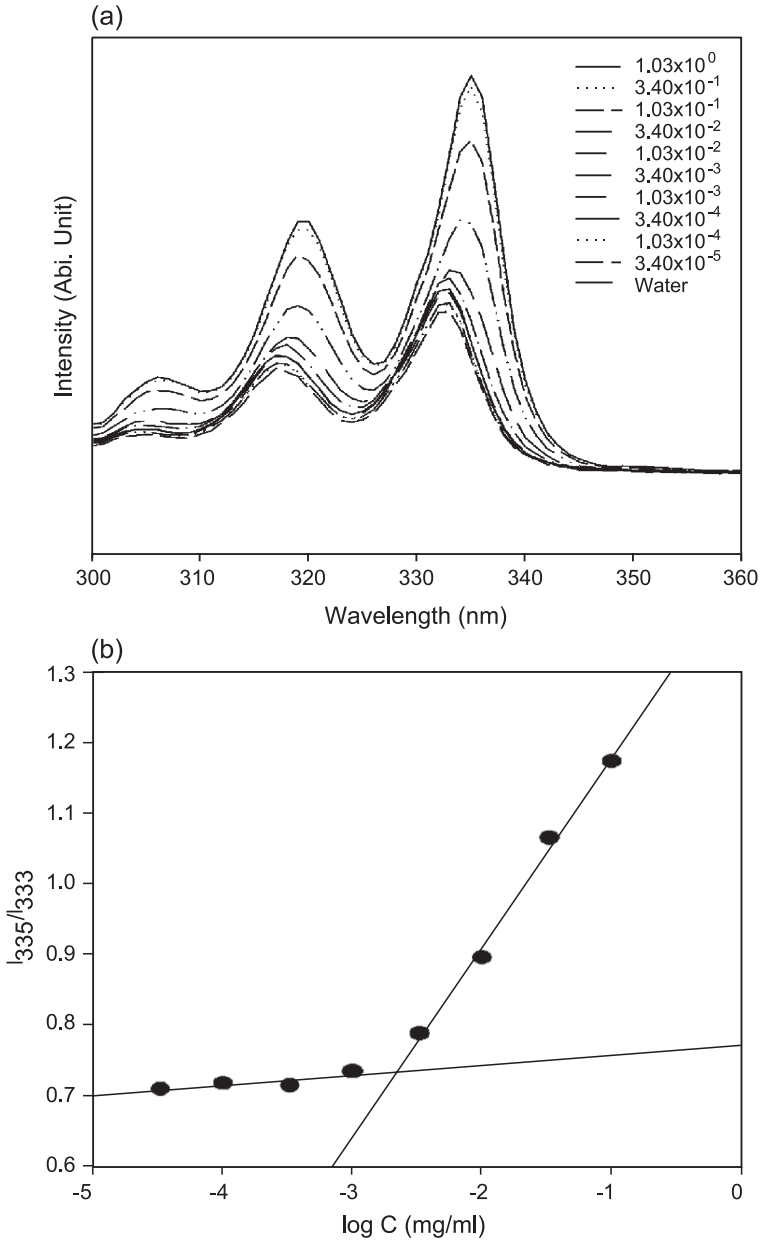


Fig. 2. Fluorescence excitation spectra (a) of pyrene as a function of PEO–PLA (BMD 19.5 wt.%) concentration (mg/ml) in water. (b) Plot of the intensity ratio from pyrene excitation spectra vs. log C. $[Py] = 6.0 \times 10^{-7}$.

stabilized nanoparticles in the water. This behavior of PEO–PLA nanoparticles was in contrast with low molecular weight amphiphiles, which typically exhibited liquidlike cores and a relatively higher mobility. On the other hand, the proton signal ($\delta = 3.65$) originated from the outer corona region (PEO) of nanoparticles was very high like that of free molecules. This result indicates that there is no difference on the mobility of the PEO chain between nanoparticles and free molecules. Based on fluores-

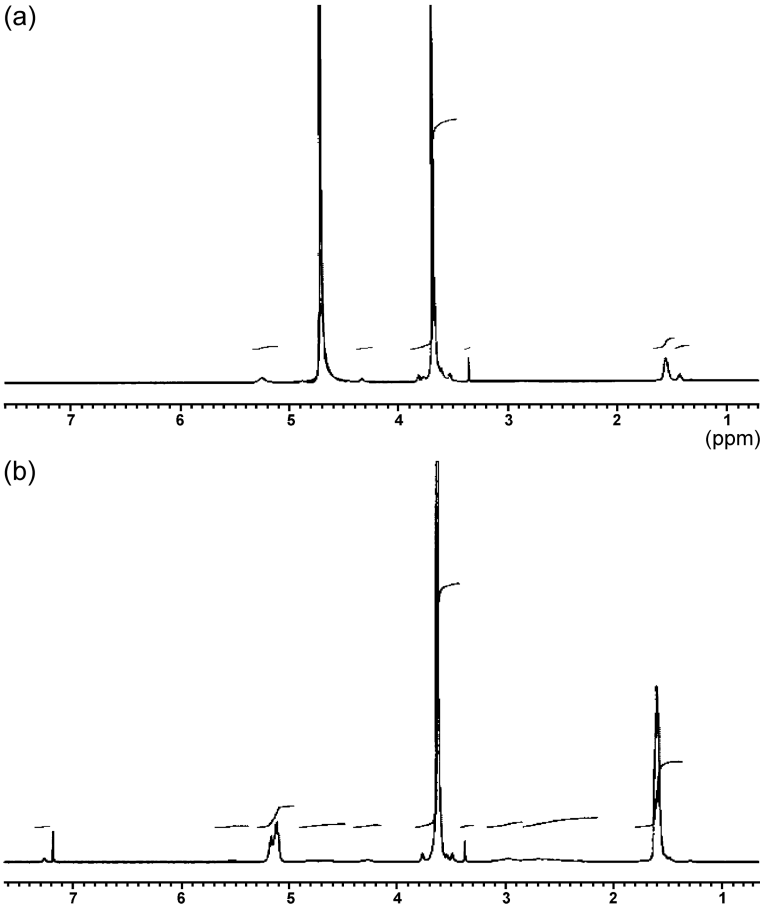


Fig. 3. NMR spectra of PEO–PLA nanoparticles (BMD 19.5 wt.%) with different solvent: (a) D_2O, (b) $CDCl_3$.

cence spectroscopy and NMR measurement, it is speculated that a small quantity of carboxylic acid in the PLA block does not have much effect on the formation and stability of nanoparticles.

3.5. Drug loading capacity

The amount of drug incorporated into PEO–PLA nanoparticles was measured by UV spectrometer. The freeze-dried nanoparticles were dissolved into $CHCl_3$ solvent, and then UV spectra were obtained. The content of drug loaded within the nanoparticles was calculated from the absorbance of PAP at 279 nm. This wavelength was not interfered by the presence of block copolymer. When the nanoparticles solution was prepared, the copolymer and a large excess of drug were dissolved into the DMF solvent together, and dialyzed against water, then the precipitate was filtered out, which was unloaded PAP. It is estimated that the aqueous solution was saturated with PAP, and the PAP was loaded as a maximum content into the nanoparticles. Therefore, the content of the drug determined by this method indicates the drug loading capacity of nanoparticles. The drug loading capacity of nanoparticles was greatly increased with the content of the carboxylic acid in the block copolymers (Fig. 4). In the case of functionalized nanoparticles (BMD 19.5 wt.%), the weight % of the loaded drug into the nanoparticles was 14.9. This value is more than four times the unfunctionalized nanoparticles, which do not contain carboxylic acid. In order to explain this result, we had to consider the physical and

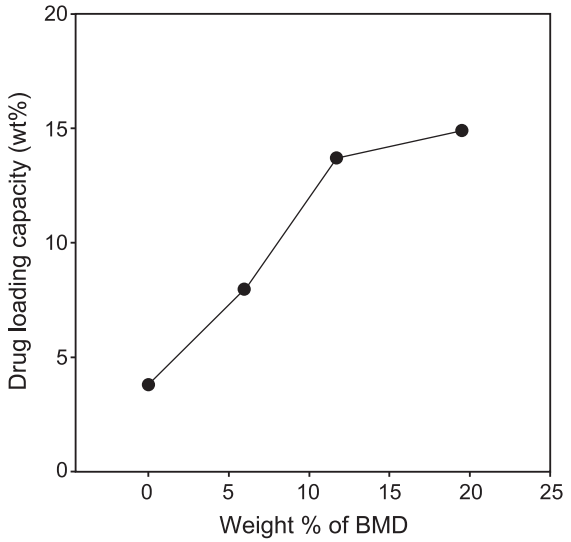


Fig. 4. Drug loading capacity of PEO–PLA nanoparticles as a function of weight % of BMD in hydrophobic block.

chemical state of the core of nanoparticles. Due to the rigid and glassy state of the core, which was estimated from NMR study (Fig. 3), the carboxylic acid could be non-ionized, and the water content could be very low in the core of nanoparticles. The hydration of carboxylic acid was not sufficient to effectively block the hydrogen bonding between polymer and drug. This led us to speculate that the increase of drug loading in the functionalized nanoparticles was mainly attributed to the hydrogen bonding between the hydrogen of carboxylic acid in the copolymer and the unpaired electron of nitrogen or oxygen atom in the drug (Fig. 5). Because the number of carboxylic acid in block copolymer was very low, direct evidence on the existence of hydrogen bond between polymer and drug could not be found by spectroscopic technique. However, the drug loading results clearly show that a small quantity of carboxylic acid in the block copolymer has a profound effect on the drug loading capacity of nanoparticles.

The compatibility between the loaded drug and the core-forming block determines the efficiency of drug incorporation [1]. Polymer drug interaction suggests that the largest amount of drug loaded per micelle will be reached when the core-forming block is most suitably matched with the drug to be loaded. Therefore, in order to enhance the encapsulation of the drug, the compatibility between polymer and drug should be increased. For example, the compatibility increase can be achieved by attaching a compatible moiety such as fatty acid to the core-forming block [21,22]. In our study, we utilize the hydrogen bonding between polymer and drug as another method to improve the drug loading capacity of nanoparticles. So, we could anticipate that any kind of drug containing oxygen or nitrogen in their structure could be efficiently encapsulated in the nanoparticles containing carboxylic acid.

Meanwhile, when only the drug without the copolymer was dialyzed in the same condition, the saturated concentration of drug was a very low 0.0287 mg/ml. However, when the block copolymer was used for incorporation of drug, the solubility of drug in water was greatly increased, especially in the case of functionalized diblock copolymer (Table 2).

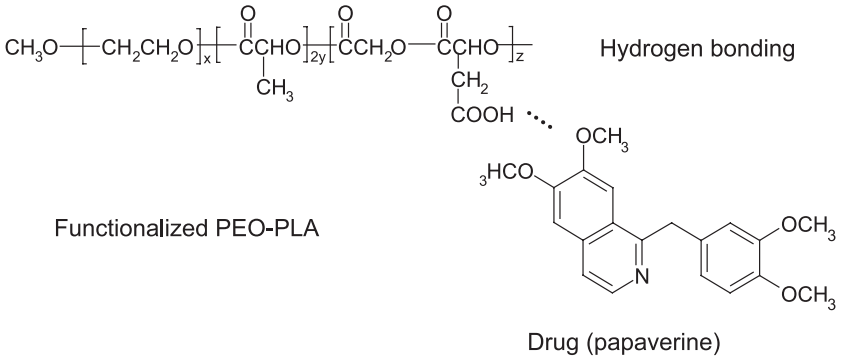


Fig. 5. Schematic diagram showing the interaction between PEO–PLA copolymer and drug (PAP).

Therefore, in order to increase the water solubility of hydrophobic drug, the use of PEO–PLA diblock copolymer containing a small quantity of carboxylic acid would be a very useful method.

We further characterized the drug loaded nanoparticles by DSC and XPS. Fig. 6(a) shows the DSC thermograms of drug (PAP) and drug loaded nanoparticles (BMD 19.5 wt.%). Although the weight % of drug in the nanoparticles was 14.9, the characteristic melting peak of drug (160 °C) was not present at all. Moreover, the XPS spectrum of nanoparticles does not show the peak of nitrogen from the included drug (Fig. 6(b)). These results indicate that the drug is dispersed in the core of nanoparticles as an amorphous form and does not nearly exist at the surface of nanoparticles.

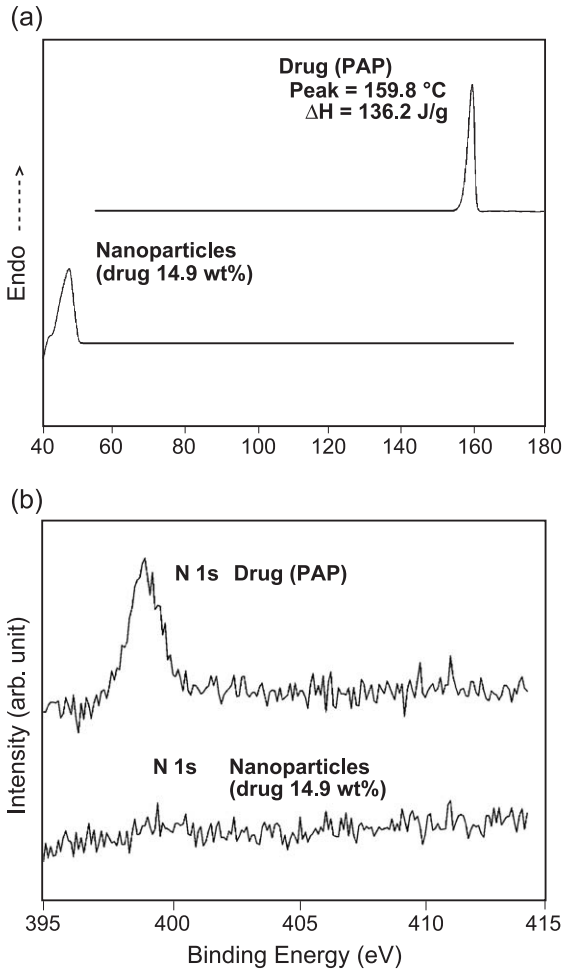


Fig. 6. DSC thermograms (a) and XPS spectra (b) of drug (PAP) and PEO–PLA nanoparticles (BMD 19.5 wt.%) containing drug (14.9 wt.%).

3.6. Degradation of nanoparticles

The degradation of nanoparticles in phosphate buffered solution (pH 7.4, 0.01 M, 37 °C) was monitored in a DLS experiment [23]. The concentration of nanoparticles solution was 0.1 wt.%, which is suitable for DLS measurement. As the degradation of nanoparticles progressed, the correlation function of scattered intensity deviated from a single exponential decay. Because of the broad distribution of particle size, the correlation function could not be fitted by Eq. (1). Thus, this point of time was taken as the time required for degradation of nanoparticles. Although the degree of the degradation of nanoparticles could not be quantified by using this method, it could be compared with the carboxylic acid content in the block copolymer. The time required for degradation of nanoparticles was decreased with the content of carboxylic acid in the block copolymer. In the case of functionalized nanoparticles (BMD 19.5 wt.%), the time required for degradation of nanoparticles is about 6 days. Whereas in the case of unfunctionalized nanoparticles (BMD 0 wt.%) it is about 20 days. It is attributed to the autocatalytic effect of carboxylic acid in the copolymer on the degradation of nanoparticles [19]. That is, the rate of the degradation of core-forming block was increased by the acidic catalyst in the hydrophobic block, which leads to a rapid collapse of nanoparticles. This result implies that the degradation rate of nanoparticles can be controlled by the content of carboxylic acid in the copolymer.

3.7. Drug release behavior

The drug release behavior of nanoparticles was investigated using a dialysis membrane in phosphate buffered (pH 7.4, 0.01 M, 37 °C). The concentration of copolymer containing the drug was fixed 0.5 wt.%. As shown in Fig. 7, nanoparticles without carboxylic acid exhibited a rapid release of 90% of drug within 10 h, whereas the nanoparticles containing carboxylic acid showed controlled release of 50–90% for 7 days. In addition, nanoparticles containing higher amounts of carboxylic acid in the copolymer showed a much

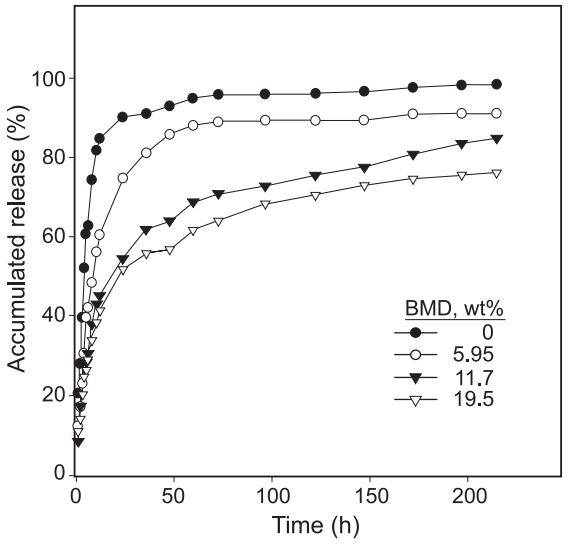


Fig. 7. Release profiles of drug (PAP) from functionalized PEO–PLA nanoparticles in phosphate buffer solutions (pH 7.4, 0.01 M, 37 °C). Solution concentration: 0.5 wt.%.

slower release rate. This implied that the carboxylic group of copolymer enhances the interaction between nanoparticles and drug, leading to a decrease in the drug release rate and in the amount.

Several mechanisms of drug release from biodegradable carriers have been proposed: [24] Fickian diffusion through the polymer matrix, diffusion through pores in the matrix, and drug liberation by polymer erosion. However, it is difficult to predict a drug release profile because this profile is governed by various factors such as solubility of drug, degradation of polymer, and polymer–drug interaction. In this study, although the degradation rate of nanoparticles is increased with the content of carboxylic acid, the time required for degradation is longer than 6 days even for nanoparticles containing BMD (19.5 wt.%). Therefore, it is speculated that the drug release from nanoparticles is carried out mainly through diffusion within this experimental period.

Diffusion of a material in a polymer matrix is governed by the excluded volume, hydrodynamic interaction, and specific interaction such as coulombic interaction and hydrogen bonding. When such a specific interaction takes place, the probe diffusion coefficient is affected significantly. For example, it was reported that the diffusion of methyl red was substantially more retarded in a toluene solution of poly(vinyl acetate) (PVAc) than in that of plystyrene (PS) [25]. The slower diffusion of MR in the presence of a PVAc matrix was ascribed to the hydrogen bonding between the probe and polymer. In our case, the release of drug from nanoparticles was achieved by the diffusion of drug from the core of nanoparticles to the aqueous medium because the nanoparticles are not degraded within the experimental period. Thus, the hydrogen bonding between the core-forming block and the drug retards the diffusion of drug, which plays an important role in the release of drug in nanoparticles.

4. Conclusions

The PEO–PLA block copolymers containing a small quantity of carboxylic acid in the PLA block were successfully synthesized. The sizes of nanoparticles prepared by a dialysis method are within the range of 30–40 nm, and they are suitable for an injectable drug carrier. The nanoparticles prepared from the functionalized block copolymer have a very low CMC value, which suggests good stability of the nanoparticles in spite of the presence of carboxylic acid. The drug loading efficiency of nanoparticles was dramatically increased with the content of carboxylic acid in the block copolymers. This result may be attributed to the hydrogen bonding between copolymer and drug. The release rate of drug was much slower from nanoparticles containing higher amounts of carboxylic acid in the copolymer, which might be associated with the enhanced interaction between the carboxylic group of copolymers and the drug. These experimental results suggest that the nanoparticles prepared from functionalized PEO–PLA block copolymers should be a good candidate for an injectable drug delivery carrier.

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