Synthesis and Characterization of Biodegradable Polymers Composed of 3,4-Dihydroxycinnamic Acid and Poly(ethylene glycol)

Weifu Dong, Yapeng Xu, Jingjiao Ren, Hong Chu, Jihang Li, Xiaoya Liu, Mingqing Chen

School of Chemical and Material Engineering, Jiangnan University, Wuxi 214122, People's Republic of China

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ABSTRACT: A novel series of biodegradable copolymers were synthesized by the thermal polycondensation of 3,4-dihydroxycinnamic acid (DHCA) and poly(ethylene glycol) (PEG). The copolymers were characterized by ^{1}H-NMR, Fourier transform infrared spectroscopy, and gel permeation chromatography. It was found that the incorporation of PEG reduced the glass-transition temperature (T_g) of the copolymers, and T_g decreased with increasing amount of PEG in the compositions. The fluorescence spectroscopy revealed that the homopolymer and copolymers of DHCA gave a higher fluorescence emission intensity than that of DHCA monomer, of which the strongest fluorescence emission peak occurred in the copolymers containing a small amount of PEG. X-ray diffraction spectra demonstrated that poly(3,4-dihydroxycinnamic acid) and copolymer were amorphous; this indicated the facile biodegradability of the copolymers. Furthermore, copolymer micelles formed by self-assembly were investigated. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 1657–1662, 2012

Key words: biodegradable; micelles; polycondensation

INTRODUCTION

Since 3 decades ago, biodegradable polymers have become more and more important and popular for pharmaceutical and biomedical applications.[1,2] Of these, poly(glycolic acid), poly(L-lactic acid), poly (ε-caprolactone), and poly(butane-1,4-diol succinate) have been widely used in surgical repair and drug-delivery carriers because of their biodegradability, biocompatibility, and proper mechanical properties.[3–8]

Cinnamoyl polymers are known as one biodegradable polymers, and they have recently been paid attention after being first reported by Akashi and coworkers[9–11] It has been reported that a series of biodegradable polymers with a biomonomer, a coumaric acid derivative, demonstrated many interesting properties, including liquid-crystalline behavior, high mechanical and thermal properties, photoreactivity, and cell compatibility. Known coumaric acid derivatives are 4-hydroxycinnamic acid, 3-hydroxycinnamic acid, 3-methoxy-4-hydroxycinnamic acid (MHCA), and 3,4-dihydroxycinnamic acid (DHCA; coffeic acid). The aforementioned monomers contain cinnamoyl groups, which is photoreactive and can undergo at least two photoreactions, a 2 + 2 cycloaddition and trans/cis isomerization.[12] Therefore, polymer nanoparticles containing cinnamate groups are expected to be useful for developing photoresponsive nanoscaled materials in biomedical applications. As an AB_2-type multifunctional monomer, DHCA can be used to create a branched architecture.[13] The homopolymer of DHCA [poly(3,4-dihydroxycinnamic acid) (PDHCA)] shows high cell adhesion. However, PDHCA is hydrophobic and hard to use to fabricate polymer micelles by self-assembly. Thereby, it is necessary to synthesize amphiphilic copolymers composed of DHCA and hydrophilic parts.

The copolymer poly(3,4-dihydroxycinnamic acid-*co*-4-hydroxycinnamic acid) exhibited various improved properties, such as in its mechanical and thermal properties, cell compatibility, and degradability, compared with PDHCA.[9] Another polyester composed of DHCA and L-lactide showed high thermal stabilities.[14,15] Poly (ethylene glycol) (PEG) is well-known as a water-soluble and nontoxic polymer; this makes it useful for obtaining the water dispersability and biocompatibility, especially the resistance against the nonspecific adsorption of serum proteins and hemocytes, of material and particle surfaces.[16,17]

To the best of our knowledge, copolymers from DHCA and PEG have not yet been reported. In this study, we attempted to obtain a novel kind of

Correspondence to: M. Chen (mqchen@jiangnan.edu.cn).
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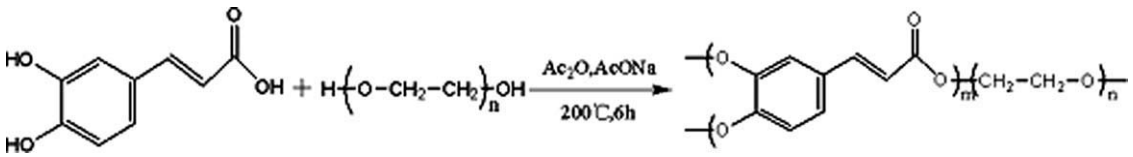

Scheme 1 Synthesis of the copolymers derived from 3,4-dihydroxycinnamic acid and PEG.

amphiphilic copolymer through the melt-polycondensation of DHCA and PEG and its micelles formed by self-assembly. Further investigation has shown that the copolymers exhibited a lower glass-transition temperature (T_g) and a higher fluorescence in comparison with PDHCA.

EXPERIMENTAL

Materials

DHCA, which was used as a monomer, was purchased from Huzhou Biological Material Co., Ltd., (Huzhou, China) and was recrystallized from *N*,*N*-dimethylformamide (DMF)/H_2O (1 : 50 v/v) before use. Anhydride acetic acid and sodium acetate (NaOAc), supplied by Shanghai Chemical Reagent Station, were used directly for polycondensation as a condensation agent and transesterification catalyst, respectively. Poly(ethylene glycol), with a molecular weight of 400 (PEG400), and other chemicals were also purchased from Shanghai Chemical Reagent Station and were used as received.

Synthesis of the copolymers

The copolymers were synthesized via the thermal polycondensation method, as previously reported.[8–11,18] The reaction process is described in Scheme 1. The DHCA monomer (10 mmol) and PEG400 (in a prescribed amount) were added to a round-bottom flask (100 mL) containing anhydride acetic acid (10 mL) and NaOAc (0.1 mmol; Table I). After being purged with dry nitrogen to remove moisture and oxygen in the flask, the reactor was placed in a silicon oil bath with a set temperature of 140°C and was mechanically stirred for about 1 h until the solution became homogeneous. The reaction system was heated further up to 200°C, and the polycondensation was performed for an additional 6 h with the protection of dry nitrogen. The viscosity of the melt increased gradually along with the reaction time. Then, the product was cooled to room temperature and was washed with water and ethanol two times. The yellow-colored powder of the copolymers was recovered after centrifugation and desiccation. All of the polycondensation reactions were carried out under shading conditions to avoid any light induction with consideration of the cinnamoyl group of the DHCA monomer. The yield was calculated with the actual and theoretical weights of the product (W_{actual} and $W_{theoretical}$, respectively):

$$\text{Yeild (wt\%)} = 100 \times (W_{actual}/W_{theoretical})$$

Preparation of the copolymer micelles

The copolymer, derived from 3,4-dihydroxycinnamic acid and PEG, was dissolved in DMF at a concentration of 2 mg/mL to form a transparent solution; this was followed by the dropwise addition of deionized water to induce the formation of micelles and create the suspension. The mixture was centrifuged to remove the supernatant and washed with distilled water three times. The micelles were obtained by lyophilization.

Measurements

Fourier transform infrared (FTIR) spectroscopy (FTLA2000, ABB Bomen, Québec, Ganada) and ^{1}H-NMR (Bruker Avance III, 400 MHz, Rheinstetten, Germany) were used to identify the chemical composition and structure of the synthesized PDHCA-*b*-PEG

TABLE I
Synthesis Conditions, Compositions, and Yields of the Copolymers

	DHCA/PEG monomer ratio (mol %)	DHCA (mmol)	PEG (mmol)	Compositions of the copolymers (DHCA/PEG; mol %)[a]	Yield (%)
PDHCA	—	10	—	—	80.6
PDP-1	100 : 2.4	10	0.24	100 : 2	68.8
PDP-2	100 : 5	10	0.50	100 : 7	68.2
PDP-3	100 : 8	10	0.80	100 : 15	68.0

NaOAc (0.1 mmol) was used as the catalyst (1 mol % DHCA). Thermal polycondensation was carried out at 140°C for 1 h and then at 200°C for 6 h.

[a] The molar ratios were estimated by ^{1}H-NMR spectroscopy.

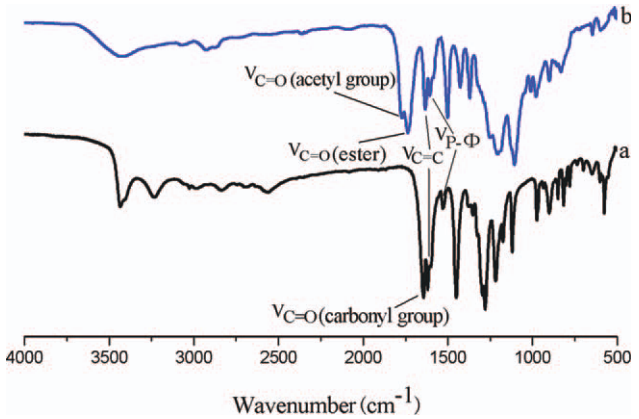

Figure 1 FTIR spectra of (a) DHCA and (b) PDP-3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

copolymers. Deuterated trifluoroacetic acid (TFA) was used as a solvent for ^{1}H-NMR measurement.

The average molecular weight and molecular weight distribution of the PDHCA-*b*-PEG copolymers were determined by gel permeation chromatography (Aligent 1100), Palo Alto with a Hewlett-Packard PLGel Mixed-C column with DMF as an eluent and polystyrene as a standard with a flow rate of 1 mL/min at 35°C.

Differential scanning calorimeter (DSC 822e, Mettler-Toledo), Zurich, Switchland was applied to investigate the thermal properties of the obtained copolymers in a nitrogen atmosphere with flow rate of 200 mL/min. The copolymer samples were scanned at a heating rate of 20°C/min from 20 to 200°C. X-ray diffraction (D8 Advance) was used to investigate the crystallinity of the copolymers.

Fluorescence spectra (Shimadzu RF-5301PC), Kyoto, Japan were collected at 25°C. The samples were dissolved in ethanol (1 mg/mL), and the bandwidth of the emission monochromators was set 5 nm.

Polymer micelles dispersed in aqueous solution were examined in a JEOL JEM-2100 (Tokyo, Japan) transmission electron microscope operating at 200 kV. Samples for transmission electron microscopy (TEM) were prepared by the casting of one drop of the micellar solution on a carbon-coated copper grid; this was followed by drying.

Dynamic light scattering (ALV-5000/E), ALV/Laser Vertriebsgesellschaft m.b.H, Hamburg, Germany was used to measure the diameter and the distribution of the copolymer micelles in water at a concentration of 0.04 mg/mL three times at room temperature.

RESULTS AND DISCUSSION

Chemical composition of the copolymers

Figure 1 shows the FTIR spectra of the DHCA monomer and its copolymers. The absorbance bands at 1728–1736 and 1768–1780 cm^{-1} in [Fig. 1(b)] were assigned to the carbonyl (C=O) stretching vibrations of the ester and acetyl groups of the copolymers, respectively. The C=O stretching peak of carboxylic acid at 1648 cm^{-1} in Figure 1(a) from the DHCA monomer disappeared in the spectra of the copolymers; this indicated that carboxylic acids were successfully converted to esters. The peak at 1632–1634 cm^{-1}, assigned to the characteristic C=C stretching vibration, remained; this meant that the C=C of cinnamoyl groups was unchanged during the high-temperature polycondensation.[19] The stretching peak at 1580–1605 cm^{-1} in the spectra of the copolymer corresponded to C=C of the aromatic group.

Moreover, the chemical composition of the copolymers was further identified by ^{1}H-NMR. The ^{1}H-NMR spectrum of PDP-3 (PDHCA-b-PEG copolymers with a molar ratio of 100:15 for DHCA/PEG in the compositions) is shown in Figure 2. The TFA proton peak (δ = 11.7) was set as an internal standard. The multiple peaks in the region of the chemical shifts (δ_b = 7.52 ppm, δ_d = 7.76 ppm, and δ_e = 7.26 ppm and δ_a = 8.21 ppm and δ_c = 6.93 ppm) were assigned to the aromatic ring and vinylene protons, respectively. The chemical shifts (δ_f = 4.60 ppm and δ_g = 4.09 ppm) were attributed to the different H protons of PEG400. For PEG400, the number-average molecular weight (M_n) was 400, and the repeat unit was 9. In theory, there was one unit of —CH_2CH_2O— connected with DHCA directly and eight adjacent units in the copolymers. The NMR spectra showed that the integrated area ratio of peak f to peak g was 1 : 7.87; this was very close to the theoretical value (1 : 8). This result further implies that the reaction between PEG and DHCA was successfully performed. In addition, the spectra of the monomers showed sharp peaks, whereas the spectra of copolymers exhibited broad peaks; this also indicated that the copolymers were successfully prepared. For the DHCA monomer, the 3,4-hydroxyl

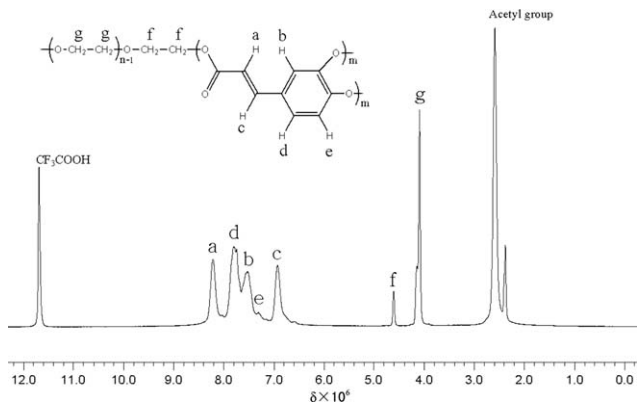

Figure 2 ^{1}H-NMR spectrum of PDP-3 (deuterated TFA was used as the solvent).

TABLE II
T_g Values and Molecular Weights of PDHCA and the Copolymers

Sample	T_g (°C)	$M_n \times 10^{-4}$	M_w/M_n	Color
PDHCA	124.3	2.05	1.81	Yellow
PDP-1	112.1	2.22	1.75	Yellow
PDP-2	98.3	2.61	1.69	Yellow
PDP-3	73.4	2.98	1.61	Primrose yellow

groups on the end of the benzene ring were not acetylated; the chemical shift was observed at $\delta = 5$. In our spectrum, the peak of $\delta = 5$ was not found; instead, $\delta = 2.58$ appeared. This was attributed to the acetyl proton. This demonstrated that 3,4-hydroxyls were acetylated. The peak at $\delta = 2.37$ was assigned to the hydroxyl proton of the PEG chain end; this indicated the presence of hydroxyl groups in the copolymer. The compositions of the PDHCA-*b*-PEG copolymers were investigated by ^{1}H-NMR, and the results are shown in Table I. The higher the feed ratio of PEG was, the higher the content of PEG in the compositions was. This convinced us that the PDHCA-*b*-PEG copolymers were successfully synthesized according to the characterization results of FTIR and ^{1}H-NMR spectroscopy.

Molecular weights and T_gs of the copolymers

The average molecular weight and its distribution of copolymers were obtained by gel permeation chromatography measurement and are listed in Table II. M_n and the molecular weight distribution (M_w/M_n where M_w is the weight-average molecular weight) of the copolymers were in the range 2×10^4 to 3×10^4 and 1.61–1.81, respectively. It was found that M_n of the copolymers increased initially and then decreased with increasing PEG amount.

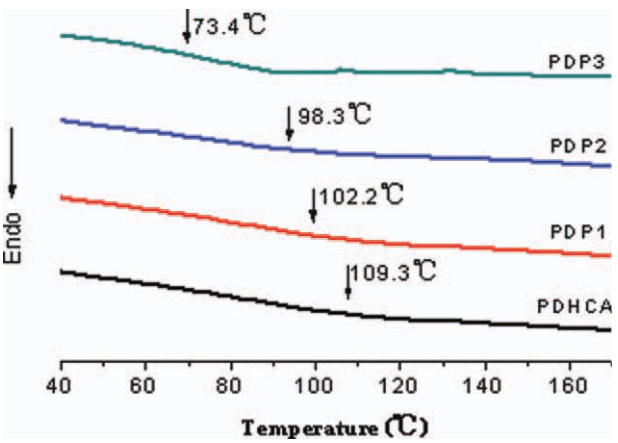

Figure 3 Differential scanning calorimetry curves of PDHCA and the copolymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

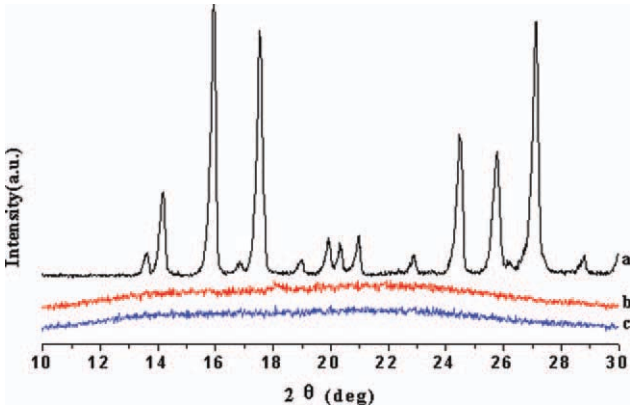

Figure 4 WAXD patterns of (a) DHCA, (b) PDHCA, and (c) PDHCA-*b*-PEG at 25°C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The effects of the PEG amount in the compositions on T_g of the copolymers are shown in Figure 3, and the data are given in Table II. It was found that the copolymers had a lower T_g in comparison with the homopolymer, and T_g decreased from 124.3 to 73.4°C with increasing amount of PEG in the copolymers; this resulted from the flexibility of PEG segment chains in the copolymer.

Crystallization of the copolymers

To investigate the effect of PEG on the crystallization, the copolymers were characterized with wide-angle X-ray diffraction (WAXD). Figure 4 shows the WAXD patterns of DHCA [Fig. 4(a)], PDHCA [Fig. 4(b)], and PDP-3 [Fig. 4(c)]. PDHCA and PDP-3 did not show any sharp peaks; this indicated that the PDHCA homopolymer and copolymers were amorphous. Although the PEG segment was easy to

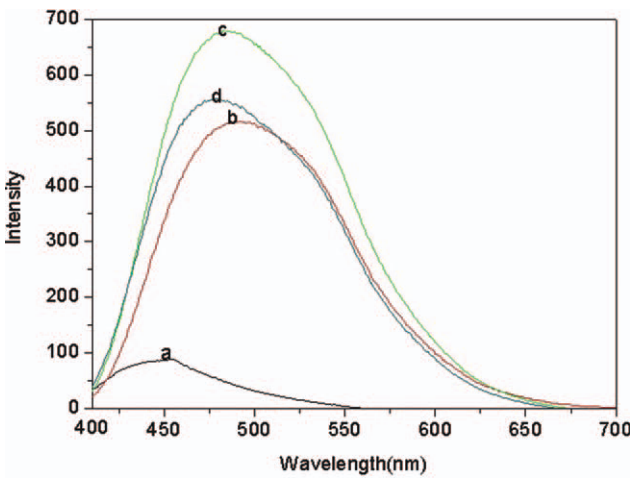

Figure 5 Fluorescence emission spectra of (a) DHCA, (b) PDHCA, (c) PDP-1, and (d) PDP-2 solutions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III
Solubility of PEG400, DHCA, and the Copolymers

Solvent	PEG400	DHCA	PDHCA	PDP-1	PDP-2	PDP-3
Water	+	+[a]	−	−	−	−
Methanol	+	+	+[a]	+[a]	+[a]	+[a]
Ethanol	+	+	+[a]	+[a]	+[a]	+[a]
Acetone	+	+	−	−	−	−
DMF	+	+	+	+	+	+
DMSO	+	+	−	−	−	−
Toluene	+	−	−	−	−	−
Hexane	+	−	−	−	−	−
THF	+	+	−	−	−	−
Chloroform	+	−	−	−	−	−
TFA	+	+	+	+	+	+

When the sample was completely dissolved in the solvent to form a transparent solution at a concentration of 10 mg/mL, it was considered to be soluble in the solvent. Otherwise, it was considered insoluble in the solvent. +, soluble; −, insoluble.

[a] These samples were dissolved at 60°C; others were dissolved at room temperature.

crystallize, in this case, it did not change the crystallization of the copolymers. It was assumed that the molecular arrangement of the copolymers was hindered by their branched structure.[13] As we know, polymer hydrolysis always starts from the amorphous phases. The proportion of amorphous phase in the polymer greatly influences its hydrolysis ability. With regard to the amorphous attributes of the copolymers, they may have had good degradability and could be used as one kind of biodegradable material.

Fluorescence properties of the copolymers

The fluorescence emission was characterized under excitation at 402 nm. It can be observed from Figure 5 that DHCA [Fig. 5(a)] had the characteristic fluorescence emission, which showed broad and weak emission peaks around 410–550 nm. PDHCA [Fig. 5(b)], PDP-1 [Fig. 5(c)], and PDP-2 (PDP-2 and PDP-1 are PDHCA-b-PEG copolymers with a molar ratio of 100:7 and 100:2 for DHCA/PEG in the compositions separately) [Fig. 5(d)] had characteristic fluorescence emission peaks that were broad and strong around 410–680 nm. The maximum emission peak intensity of PDP-1 at 484 nm [Fig. 5(c)] was almost 7.7 times that of DHCA at 450 nm [Fig. 5(a)], and the redshift in the maximum emission peak was about 34 nm. The wide range of the emission peak around 410–680 nm corresponded to the benzene ring and double-bond conjugated system of the copolymers. This change was mainly attributed to the reaction between the major part carboxyl group of coffeic acid and the hydroxyl group of PEG; this also indicated that the reaction between DHCA and PEG was accomplished successfully.

From these results, it was obvious that the fluorescent emission intensity of the copolymers was much higher than that of the DHCA monomer, and the strongest fluorescence emission peak was observed for PDP-1. This could be explained from the π-conjugated molecule structures. The PDHCA-*b*-PEG copolymers contained more benzene rings and double bonds than the DHCA monomer; this meant that more conjugated structures were present in the copolymers. As a result, the fluorescence emission intensity of all of the copolymers was enhanced and had a redshift compared to DHCA. For PDP-1, a small amount of PEG alleviated the steric hindrance of the copolymer, which could have contributed to the lower conjugation energy. Consequently, the fluorescence intensity of PDP-1 was higher than that of PDHCA. On the other hand, an increasing PEG amount in the copolymers resulted in a decreasing amount of cinnamoyl groups; this led to the reduction of an overall conjugated molecular structure. As a result, the fluorescence emission intensity of the copolymers decreased when the amount of PEG in the successive copolymer increased (Fig. 5).[8,18] If copolymers micelles are prepared by self-assembly, these micelles can be used as probes in biological fields because of their good fluorescence properties.[19–21]

Solubility of the copolymers

The solubility of the monomers and copolymers in different solvents were evaluated, and the results are shown in Table III. The monomers could dissolve in

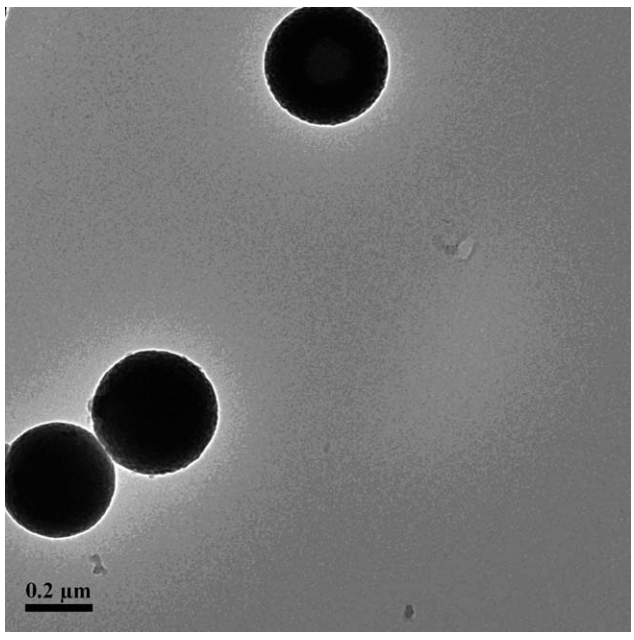

Figure 6 TEM image of the copolymer micelles (PDP-3). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

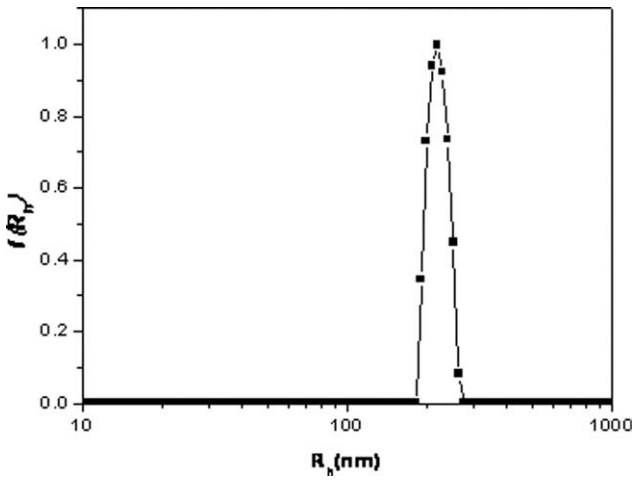

Figure 7 Diameter distribution of the copolymer micelles (PDP-3).

various organic solvents, but the solubilities of PDHCA and the copolymers were poorer. Different solubilities between the polymers and monomers suggested that the copolymers were synthesized by this thermal polycondensation method. The copolymers were soluble in DMF and insoluble in water; this indicated that the copolymers could be used to form micelles or nanoparticles by self-assembly.

Copolymer micelles

Figure 6 shows the TEM image of copolymer micelles. It was found that the sizes of the micelles were around 400 nm; this agreed well with the results from dynamic light scattering (Fig. 7). The formation of micelles containing cinnamoyl groups will enlarge the application of the copolymers in medical and biological fields, which will be investigated in our future work.

CONCLUSIONS

A novel biodegradable copolymer was synthesized successfully by the thermal polycondensation of DHCA and PEG. The introduction of PEG into the copolymers led to a decrease in T_g. The homopolymer and copolymers of DHCA showed a higher fluorescence emission intensity than the DHCA monomer, and the strongest fluorescence emission peak occurred in the copolymer (PDP-1) containing a small amount of PEG. The X-ray diffraction patterns indicated that the copolymers were amorphous. Moreover, copolymer micelles with a size of 400 nm were obtained by self-assembly. Further investigations of the micelles are in the progress.

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