Nanoparticles Prepared by Blending of Carboxylic Acid Terminated Poly(ε-caprolactone) and L-Phenylalanine Substituted Dextran

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ABSTRACT: Preparation and characterization of nanoparticles from L-phenylalanine substituted dextrans (Dex-Phes) and carboxyl-terminated poly(ε-caprolactone) (PCL-COOH) blends in water/dimethyl sulfoxide (DMSO) (10/1, v/v) solution are reported. Dex-Phe with degree of substitution (DS) = 0.67 has good solubility in both water and DMSO, which can form the nanoparticles with PCL-COOH in water/DMSO solution. The size and size distribution of the nanoparticles were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). The effect of weight ratio of PCL-COOH to Dex-Phes on particle size was investigated. A possible mechanism for the formation of nanoparticles was also proposed. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 830–836, 2011

Key words: nanoparticles; L-phenylalanine substituted dextran; carboxylic acid-terminated poly(ε-caprolactone)

INTRODUCTION

Biodegradable polymer-based nanoparticles with a core-shell structure exhibit many advantages in the control of drug administration, transport, and delivery at the desired site.[1,2] It was often obtained by micellization of amphiphilic block (or graft) copolymers in select solutions.[3–5] The core of a particle basically consists of compact insoluble blocks (or grafts) and is surrounded by a corona of solvated blocks (grafts). One block existing in core and the other in corona is connected by chemical bonds.

Nanoparticle-forming materials for biomedical applications need to be nontoxic, biocompatible, and should have high-disease site selectivity. Thus, they are commonly prepared from biodegradable polymer such as polysaccharides and biocompatible polyesters by self-assembling of polysaccharide–polyester copolymers.[6–8] Methods for preparing polysaccharide–polyester copolymer include a catalytic ring-opening polymerization of monomers (lactide or caprolactone) in the presence of polysaccharides[9–11] and bulk polymerization of monomers in the presence of partially protected hydroxyl groups of polysaccharides,[12–14] followed by deprotection. The first approach is the difficulty to obtain controlled structures because all the hydroxyl groups on the polysaccharides, except the ones sterically hindered, initiate the polymerization. The bulk polymerization leads to inhomogeneous substitution ratio and high polydispersity. The resulting copolymers with uncontrolled structures are difficult to form stable, homogenous nanoparticles. In addition, large number of grafted chains with variable length in the polysaccharidic backbone may modify its physicochemical properties. This is disadvantage for biomedical applications, where interactions of the biological environment with polymeric surfaces are importance for bioadhesion or targeting.

Recently, a new approach for building polymeric core-shell nanoparticles was introduced by assembling hydrophilic and hydrophobic polymer blends in mixed solvent, which is a precipitant for the former but solvent for the latter.[15–17] For example, Jiang and coworkers[17] blended the carboxyl group-terminated polystyrene and poly(4-vinyl pyridine) in chloroform/methanol mixed solvent to form micelle-like particles. In the particle, the core is composed of one polymer and shell is composed another one. Two components in the self-assembly structures connect by specific interactions existing between them other than chemical bond.

In this article, we reported the formation of core-shell nanoparticle by blending polysaccharide and polyester in mixed solvent. L-phenylalanine graft dextran (Dex-Phe) and carboxyl group-terminated poly(ε-caprolactone) (PCL-COOH) are selected as

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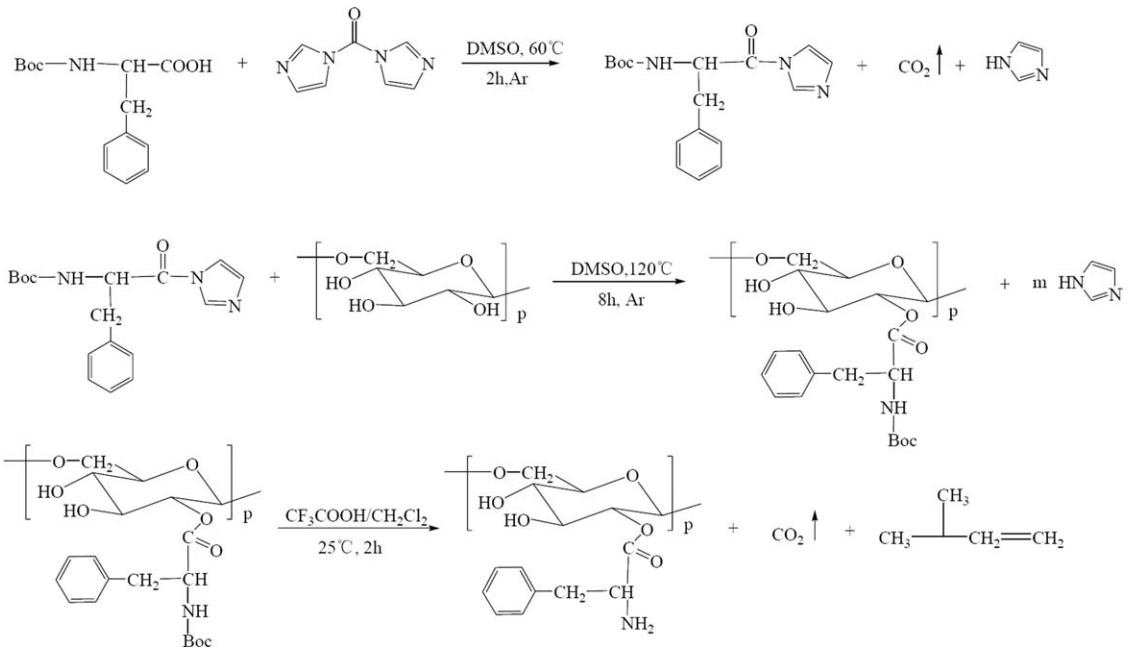


Scheme 1 Synthesis of Dex-Phe.

model polymers. The core-shell structure was fixed by coupling between a carboxylic function present on the polyester chains and an amino group on the polysaccharidic backbone. It was, therefore, necessary to first synthesize PCL-COOH of appropriate molecular weight and amino-graft dextran (Dex-Phe), and then PCL-COOH and Dex-Phe assemble into nanoparticle in dimethyl sulfoxide (DMSO)/water solution. The formation of the polymeric particles with core-shell structure in solvents by blending is the distinguishing feature of this investigation.

EXPERIMENTAL

Materials

ε-caprolactone (ε-CL) (Aldrich, 99%) was dried over CaH_2 for 24 h and distilled under reduced pressure before use. Dextran (Fluka) and Boc-L-phenylalanine (Alfa Aesar) were of analytical grade and used without purification. All other reagents were analytical grade and used as received.

Synthesis of PCL-COOH

PCL-COOH was synthesized by ring-opening polymerization of ε-CL using glycolic acid (GA) as the initiator (Scheme 1).[18,19] The typical procedure is given as following: 1.5 g of ε-CL with certain amount of GA and a mini magnetic stir bar in a vacuum-sealed ampoule (40 Pa) was polymerized in an oil bath at 120°C for 24 h. The ampoule was quenched in ice water to stop the polymerization. After cooling down, the product was dissolved in tetrahydrofuran (THF) and precipitated in methanol at room temperature. The precipitate was collected by filtration and dried in vacuum at 30°C for 24 h.

Synthesis of Dex-Phe

Two grams of *N*-(tert-butoxycarbonyl)-L-phenylalanine (Boc-Phe) was dissolved in 20 mL DMSO in a three-necked flask with mini magnetic stir bar. To this solution, 1.35 g of *N*,*N*-carbonyldiimidazole (CDI) (molar ratio of [CDI]/[Boc-Phe] = 1.1) was added. The reaction was carried out at 60°C for 2 h in an argon atmosphere, then calculated amount of dextran was added and the reaction was heated to 120°C in an argon atmosphere for 8 h. As the flask was cooled to room temperature, a mixture of methanol and distilled water (V/V = 2/1) was added drop wise into the reaction mixture to precipitate the product. The product was collected by filtration and washed thrice with distilled water before being vacuum-dried for 24 h. The obtained product was added to a mixture of trifluoroacetic acid and dichloromethane (V/V = 1/2) and stirred at 25°C for 2 h. Then the trifluoroacetic acid and dichloromethane was removed on a rotary evaporator. The obtained solid was dissolved in 10 mL 4% acetic acid, and precipitated in a mixture of methanol/triethylamine (V/V = 5/1). The precipitate was filtrated, washed with methanol thrice, and dried in vacuum at 30°C for 24 h.

Preparation of PCL-COOH/Dex-Phe nanoparticle solution

PCL-COOH (10 mg) and different weight ratio of Dex-Phe (100 mg, 20 mg, 10 mg, 5 mg, and 2.5 mg)

TABLE I
Preparation of PCL-COOH Initiated by GA

[ε-CL]/[GA]	Yield[a] (%)	M_n	M_w/M_n	Number of carboxyl group[b]
20	91.4	2300	1.66	0.91
30	94.2	3100	1.63	0.93
50	95.1	5700	1.65	0.87

[a] The yield was determined by gravimetric method.
[b] The number of carboxyl groups per polymer chain was determined by titration.

were dissolved in 1 mL DMSO, and then water was added drop by drop to the solution with mild stirring. The initial 2 mL of water was added at a rate of one drop per 10 s; after 2 mL, the rate was increased to one drop per 5 s. In the final solution, the volume ratio of water to DMSO is 10/1. The solution was then transferred into a dialysis tube (M_w cutoff 500 Da) and dialyzed against 2 L of distilled water for 24 h. Distilled water was refreshed at 2 h, 5 h, 8 h, and 24 h separately. The purified product was stored at 4°C for DLS and TEM tests.

Characterizations

Determination of chemical structure

^{1}H-NMR spectra were recorded on Mercury-Plus 300 (Varian, USA) spectrometer at 300 MHz, using tetramethysilane (TMS) as an internal standard and $CDCl_3$ or DMSO-d_6 as solvent. The concentration of the solution was 5 mg/mL.

Measurement of molecular weights

Number-average molecular weight (M_n) and polydispersity index (M_w/M_n) of PCL-COOH were measured on a Waters high-performance liquid chromatography system equipped with a model 2690D separation module, a model 2410 refractive-index detector, and Shodex K802.5 (pore size 60 Å) and Shodex k805 (pore size 500 Å) with Shodex K-G Guard column. The measurements were performed in chloroform at 35°C. It is difficult to get commercial PCL standard, so the column was calibrated using polystyrene standards, and the value of M_n was determined by universal calibration method using a viscosity detector. M_n and polydispersity index (M_w/M_n) of dextran and Dex-Phe were measured by using a Waters 515-410 gel permeation chromatograph equipped with Waters 410 detector and Ultrahydrogel column at 40°C. Water was used as the eluent at a flow rate of 0.6 mL/min.

Determination of content of carboxyl end group in PCL-COOH

The content of the carboxyl end group was determined by titration method. Briefly, purified PCL-COOH was dissolved in a mixture solvent of isopropyl alcohol and 1,4-dioxane (1/4, V/V) and titrated with 0.012 mol/L potassium hydroxide (KOH) in the same solvent, 1% phenolphthalein/pyridine was used as the pH indicator. The number of the carboxyl end groups in the PCL-COOH was calculated according to the following equation [eq. (1)]:

$$No = \frac{V_{KOH} \times 10^{-3} \times 0.012}{M/M_n} \quad (1)$$

where No is the number of carboxyl group in PCL-COOH, V_{KOH} is the volume (mL) of KOH consumed in the titration, 0.012 is the concentration of KOH (mol/L), M is the mass of PCL-COOH, and M_n is the number-average molecular weight of PCL-COOH as determined by GPC.

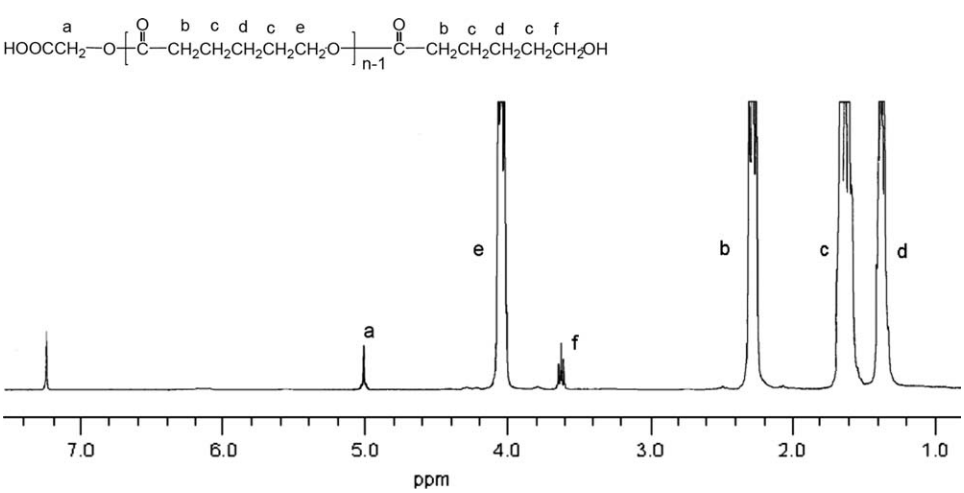


Figure 1 ^{1}H-NMR spectrum of PCL-COOH initiated by GA.

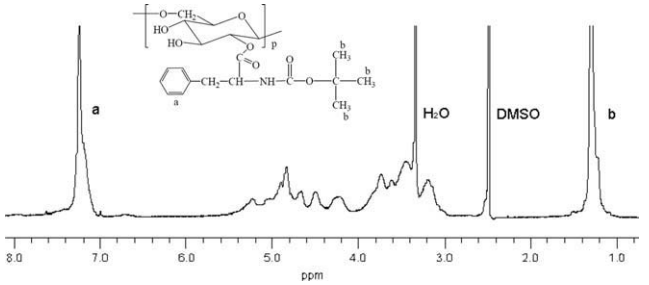


Figure 2 ^{1}H-NMR spectrum of dextran with Boc-Phe side group.

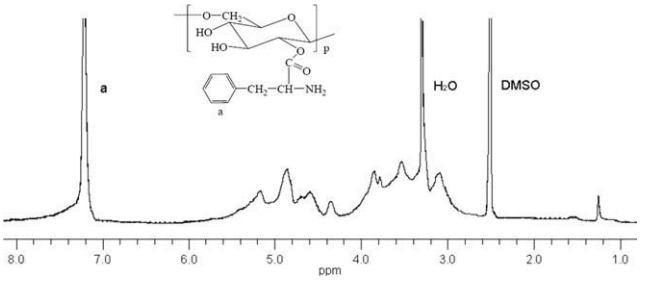


Figure 3 ^{1}H-NMR spectrum of Dex-Phe.

Determination of degree of substitution of L-phenylalanine on dextran

The degree of substitution (DS) of L-phenylalanine on dextran, which is defined as the molar fraction of L-phenylalanine per repeating unit of dextran, was derived from the percentage of nitrogen in Dex-Phe that was determined by elemental analysis [eq. (2)].

$$N\% = \frac{\mathrm{DS}14.01}{162.14 + \mathrm{DS}165.19 - \mathrm{DS}18.02} \quad (2)$$

where N% is the percentage of nitrogen in Dex-Phe as determined by element analysis, 14.01 is the atomic weight of nitrogen, 162.14 is the molecular weight of repeat unit of dextran ($C_6H_{10}O_5$), 165.19 is the molecular weight of L-phenylalanine, and 18.02 is the molecular weight of water that was produced in the reaction.

Determination of solubility of Dex-Phe in different solvents

To 1 g of Dex-Phe, 10 mL of solvent was added in three portions using a calibrated Pasteur pipette; after the addition of each portion, the mixture was stirred vigorously with a magnetic stir bar for 35–40 min at 25°C, and observations were made with care during this processing.

Dynamic light scattering measurements

The complex particle size and size distribution were determined by dynamic light scattering (DLS) using a BI-200SM Goniometer particle size analyzer (Brookhaven, USA). Each analysis lasted for 300 s and was performed at 25°C with angle detection of 90°. The concentration of the polymer solution was 2 mg/mL.

Transmission electron microscopy measurement

The TEM was performed using a JEM-2010HR high-resolution transmission electron microscope (TEM). A droplet of Dex-Phe/PCL-COOH nanoparticle solution containing 0.2 wt % phosphotungstic acid was deposited onto a 200-mesh copper grid coated with carbon. Excessive solution was removed with a Kimwipe delicate wipe. The shape and size of the nanoparticles were directly observed from each transmission electron micrograph.

RESULTS AND DISCUSSION

Preparation and characterization of PCL-COOH

GA was used to initiate the ring-opening polymerization of the ε-CL to obtain low molecular weight PCL-COOH.[19] The GPC results in Table I showed that precipitates with M_n of 2300, 3100, and 5700 g/mol were obtained by adjusting the feed ratio of ε-CL to GA. Chemical structure of obtained precipitate was characterized by ^{1}H-NMR spectroscopy (Fig. 1). ^{1}H-NMR ($CDCl_3$): δ = 1.35 (—$CH_2CH_2CH_2COO$—), 1.61 (—$OOCCH_2CH_2CH_2CH_2OCO$—), 2.25 (—CH_2COO—), 3.62 (—CH_2OH), 4.05 (—$OOCCH_2$—), 5.02 (HOOC CH_2—).

The content of carboxyl end groups in the polymer was determined quantitatively by titration. The number of the carboxyl group per PCL-COOH chain with M_n = 2300, 3100, and 5700 g/mol were 0.91, 0.93, and 0.87, respectively. The results of titration and ^{1}H-NMR indicated that the carboxyl group (—COOH) was introduced to one end of the polymer.

Characterization of Dex-Phe

The dextran grafted with Boc-L-phenylalanine was characterized with ^{1}H-NMR (Fig. 2). Peaks at 1.27 and 7.28 ppm can be attributed to the methyl (CH_3—) and phenyl (C_6H_5—) protons in the Boc-L-phenylalanine side group; the 2.80–5.50 ppm signals

TABLE II
Molecular Weight of Dex and Dex-Phe by GPC

Material	[L-phe]/[Dextran]	Yield[a] (%)	M_n (g/mol)	M_w/M_n
Dextran	–	–	23,100	1.64
Dex-Phe	0.8	81.2	17,300	1.79
Dex-Phe	1.6	76.5	18,700	1.76

[a] Based on dextran feed.

TABLE III
Element Analysis and Solubility of Dex-Phes

[Boc-L-Phe]/[Dextran repeating unit] in feed	N%	SD	Solubility				
			H_2O	DMSO	DMF	Methanol	Acetone
0.8	3.6	0.67	s	s	p	n	p
1.6	4.62	1.04	p	s	s	n	s

DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; s, dissolve; p, partly dissolve; n, cannot dissolved.

were due to the protons in dextran backbone, the peaks of protons in —CH_2—, —CH— of Boc-Phe side groups overlapped with those of dextran backbone.

Figure 3 showed the ^{1}H-NMR spectrum of Dex-Phe. When compared with Figure 2, it was found that the peak at 1.27 ppm, which belongs to the CH_3— in Boc-group, decreased significantly, suggesting most of the Boc-protected amino groups were deprotected.

The molecular weights of the Dex-Phes and their molecular weight distribution were listed in Table II, the M_n values varied between 17,300 and 18,700 g/mol. When compared with that of the Dextran, the Dex-Phe has lower M_n values and slightly wider M_w/M_n. This was probably caused by the degradation of the dextran during the reaction process.

DS of L-phenlalanine in Dex-Phe was calculated from the nitrogen content in Dex-Phe determined by element analysis. The solubility of two Dex-Phes were investigated (Table III) in different solvents. Dex-Phe with DS of 1.04 has good solubility in DMSO, *N*,*N*-dimethylformamide (DMF), and acetone but partially dissolved in water. Dex-Phe with DS of 0.67 was found to dissolve in both water and DMSO. The Dex-Phe with DS of 0.67 was selected for preparation of nanoparticles in the following experiment.

Formation and characterization of the nanoparticles

PCL-COOHs (10 mg) with different M_n and 20 mg Dex-Phe or dextran were dissolved in 1 mL DMSO, and then water was added drop by drop to the DMSO solution with mild stirring. A slightly blue solution formed by PCL-COOH with M_n of 2300 and 3100 g/mol, indicating the presence of nanoparticles. As for the PCL-COOH with M_n of 5400 g/mol, a slightly blue to milky suspension was obtained, which suggested the aggregation of the nanoparticles. For comparison purpose, dextran was used for instead of Dex-Phe, no nanoparticle suspension but precipitate was obtained in the same conditions.

TEM images of the particles formed by Dex-Phe (DS = 0.67, M_n = 18,200 g/mol) with PCL-COOH (M_n= 2300 g/mol) and PCL-COOH/Dex-Phe (1/2 w/w) in aqueous were shown in Figure 4. The isolated particles took an approximately spherical appearance with diameter of 60–120 nm and core–shell structure can be observed. The hydrophobic PCL-COOH was difficult to be stained by phosphotungstic acid and formed the white core. The black shell presented the stained Dex-L-phe chains.

From the structure of the particles, we deduce the forming process of the particles as illustrated in Scheme 2. DMSO is a good solvent for both Dex-Phe and PCL-COOH, blending of Dex-Phe and PCL-COOH in DMSO, and thus could form graft-like complexes by the interaction between terminal carboxyl group of PCL-COOH and amino group of Dex-Phe. Water is the poor solvent for PCL-COOH but good solvent for Dex-Phe, when adding water to the solution of Dex-Phe and PCL-COOH in DMSO,

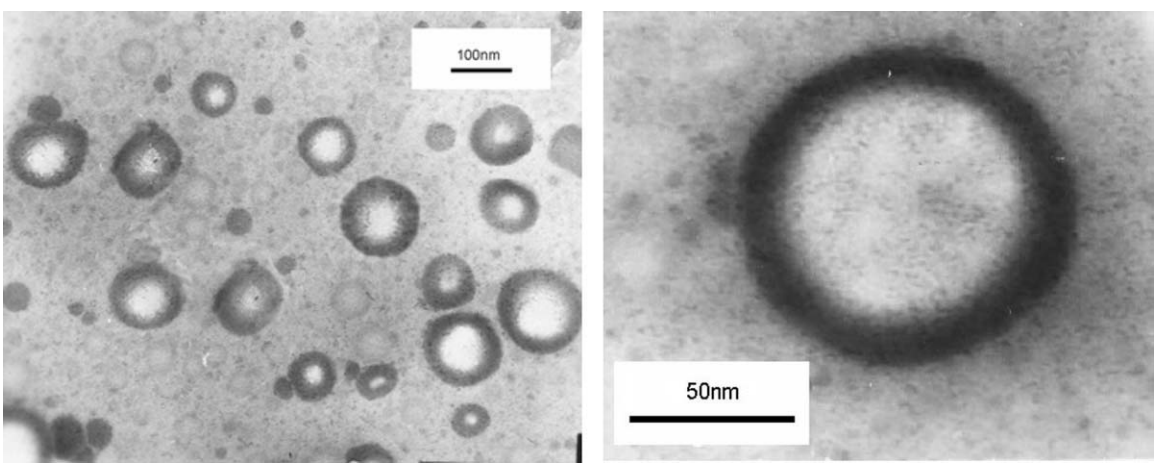
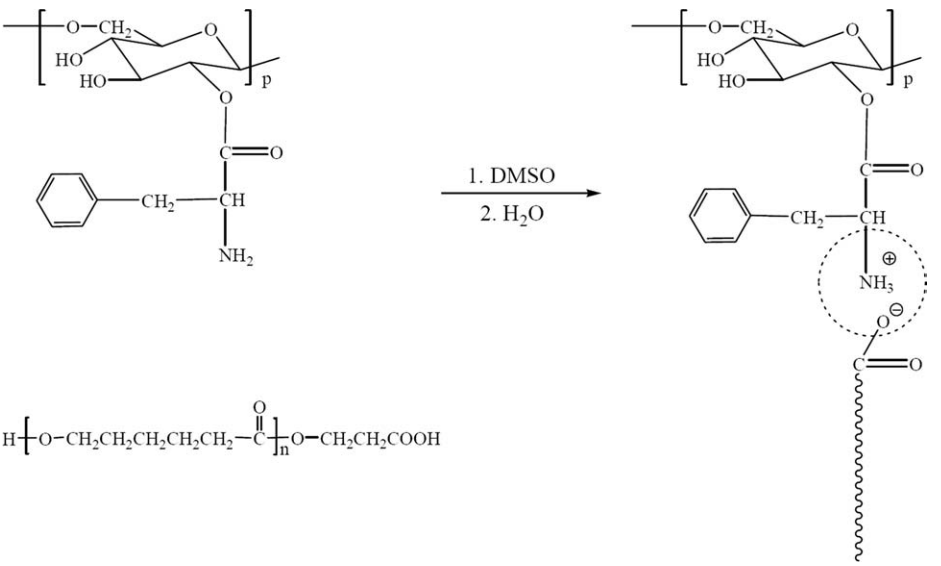


Figure 4 TEM images of the particles with core-shell structure.



Scheme 2 Schematic illustration for the formation of nanoparticles.

the hydrophobic PCL-COOH chains and the L-phenylalanine side groups in Dex-Phe aggregate to form the compact core and the hydrophilic dextran backbone form the shell surrounding the core to prevent the aggregation of the particles.

The size distribution and hydrodynamic radius of the nanoparticles were also measured by the dynamic light scattering (DLS) technique. The hydrodynamic radius (R_h) of the nanoparticle was found to be 169.5 nm and the size distribution of the particle in aqueous was shown in Figure 5.

Figure 6 depicted the R_h of the nanoparticle with different weight ratios of PCL-COOH to Dex-Phe with M_n of 2300 g/mol. Generally, the R_h of the nanoparticles increased with the weight ratio of PCL-COOH to Dex-Phe. R_h of the nanoparticles varied within 150–200 nm at PCL-COOH/Dex-Phe $\leq$ 1, then the R_h reached abruptly over 250 nm at PCL-COOH/Dex-Phe $\geq$ 2.0, this phenomenon may be explained that the decreasing of the Dex-Phe content reduces the stability of the particles and causes them aggregation.

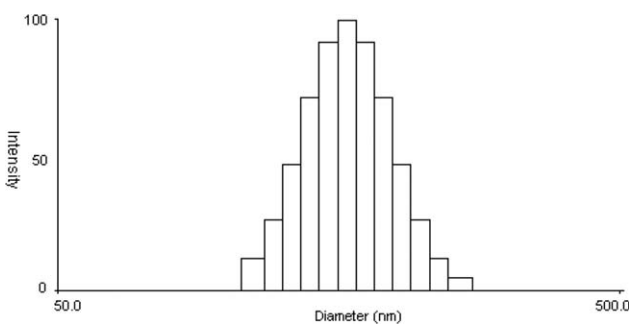


Figure 5 Size distribution of the particles in aqueous.

CONCLUSIONS

L-phenylalanine substituted dextrans (Dex-Phes) and PCL-COOH were synthesized for the purpose of making core–shell type nanoparticles by blending in water/DMSO mixed solution. The formation of the nanoparticles relates to DS of the Dex-Phe and molecular weight of the PCL-COOH; R_h of the nanoparticle was affected by the weight ratio of Dextran-phe to PCL-COOH. TEM study showed the nanoparticles had core–shell structure. Core of PCL-COOH and shell of Dex-Phe was connected by physical interaction instead of chemical bonding. This method is different from those that utilized polysaccharide/polyester amphiphilic block copolymer or graft copolymer, in which the hydrophilic and hydrophobic components co-exist in one polymer and are connected by chemical bonding.

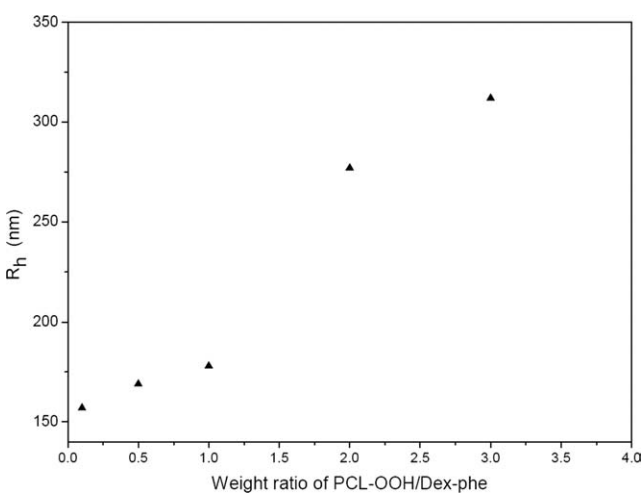


Figure 6 The relationship between the R_h of the particles and the weight ratio of PCL-COOH to Dex-Phe.

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