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Degradation and drug release properties of poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide]-*g*-poly(2,2-dimethyltrimethylene carbonate)

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Abstract A series of biodegradable amphiphilic graft copolymers with poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA) as the backbone and poly(2,2-dimethyltrimethylene carbonate) (PDTC) segments with different lengths as the grafted branches were synthesized and characterized. The in vitro degradation of the obtained PHEA-*g*-PDTC copolymers was studied. With particular branch lengths, PHEA-*g*-PDTC can form self-assembling micelles in an aqueous solution. Transmission electron microscopy (TEM) images demonstrated that the micelles were regularly spherical in shape. The particle size and distribution of the micelles were measured. Nanoparticle drug delivery systems were prepared by the direct dissolution method. The in vitro release behaviors of two drugs, prednisone acetate and tegafur, with different water solubilities were investigated.

Introduction

Amphiphilic biodegradable polymers can be used for a wide variety of applications in biomedical fields, such as drug carriers and matrices in tissue engineering. Compared with amphiphilic block copolymers, amphiphilic graft copolymers most commonly can easily form micelles and form smaller micelles because of the possibility of forming micelles within one or several polymer chains. Moreover the graft copolymers have multi-grafted hydrophobic/hydrophilic branches along a hydrophilic/hydrophobic polymer backbone, so the micelle properties and the delicate hydrophilic/hydrophobic balance can be easily varied by simply adjusting the graft frequency and length of the branches [1]. Thus the adjustment of the physicochemical properties such as degradation rate and drug controlled release property can be realized. Another advantage of graft copolymers is that many reactive functional groups exist along each backbone, which allows further incorporation of various biorecognizable ligands for fabricating the surface engineered nanoparticles, microspheres, or porous scaffolds to realize the positive targeted drug delivery or to achieve the enhanced cell adhesion and recognition. Because of these favorable properties, amphiphilic graft copolymers are of special interest as biomaterials.

For amphiphilic polymers, when their amphiphilicity is in a certain range, generally they can form self-assembled colloids, and are able to encapsulate drugs with different properties such as different hydrophilicities and polarities. As drug carriers, amphiphilic polymers enjoy several advantages [2]. They can significantly enhance the water solubility of hydrophobic drugs to improve bioavailability [3–8], and have lower tendency to aggregate due to the hydrophilic parts which improve the stability in aqueous environments thermodynamically. In addition, as we know, the hydrophobic nanoparticles are usually eliminated by the reticuloendothelial system after injection. While nanoparticles formed by the self-assembling of amphiphilic polymers exhibit increased blood circulation time. So it is possible to delivery the encapsulated drugs to specific regions via an active and/or a passive mechanism.

Most reported backbones for these graft amphiphilic polymers are natural biodegradable polymers such as polysaccharides including dextran [9, 10], starch [10],

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pullulan [11], chitin [12], chitosan [13] and cellulose derivatives [14, 15], and nondegradable synthetic polymers like poly(vinyl alcohol) [16]. In our previous work, we synthesized a series of amphiphilic copolymers with poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA) as the backbone and poly(2,2-dimethyltrimethylene carbonate) (PDTC) segments with different lengths as the grafted chains [17]. PHEA is a water-soluble, nontoxic, nonantigenic biodegradable polymer, with a well-defined chemical structure. PDTC is a hydrophobic biodegradable polycarbonate. In our investigation, by initiating the ring-opening polymerization of 2,2-dimethyltrimethylene carbonate by the hydroxyl groups in PHEA, amphiphilic graft copolymers were obtained by one-step reaction conveniently. Moreover, the graft polymers were obtained by the ring-opening polymerization without adding any catalyst, this synthesis route is very useful in biomaterial fields since it permits the synthesis of biodegradable polymers with good biocompatibility without toxic impurities. By varying the feed ratio of the macroinitiator PHEA to the monomer, graft copolymers with different hydrophobic branch lengths can be obtained. Thus the adjustment of the physicochemical properties such as amphiphilicity, degradation rate, and drug controlled release property can be realized. In the present work, we further investigated the formation of nanoparticles by self-assembling of the amphiphilic copolymer. The nanoparticle drug delivery systems were prepared by the direct dissolution method. Prednisone acetate was chosen as a hydrophobic drug and tegafur was selected as a hydrophilic drug to be entrapped within the nanoparticles. The in vitro release behaviors of drugs were studied.

Experimental

Materials

2,2-Dimethyl-1,3-propanediol (Shanghai Chemical Co., China) was dried in a vacuum oven at 60 °C for 2 h. Triethylamine was distilled over CaH_2. Ether chloroformate, L-aspartic acid and phosphoric acid (Shanghai Chemical Co., China) were of analytical grade and used as supplied. Ethanolamine (Shanghai Chemical Co., China) was distilled before use. *N,N*-Dimethylformamide (DMF) was purified by distillation over P_2O_5 and CaH_2. Prednisone acetate was purified from prednisone acetate tablets (Zhejiang Xianju Pharmaceutical Co. Ltd., China). Tegafur was purified from the tegafur tablets (Jinan Pharmaceutical Factory, China). The detailed purification procedure is as follows. The drug tablets were ground and then dissolved in a solvent (chloroform for prednisone acetate tablets, and ethanol for tegafur tablets). After the removal of the starch in the drug tablets by filtration, purified drugs were obtained after recrystallization and vacuum drying.

Polymer synthesis

PHEA-*g*-PDTC graft copolymers were synthesized by the ring-opening polymerization of DTC using PHEA with pendant hydroxyl groups as a macroinitiator without adding any catalyst as previously reported [17]. Graft polymers coded PHEA-*g*-PDTC-1, PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3 with different compositions were prepared. The feed ratios of hydroxyl group in PHEA to the DTC monomer were 50:50, 20:80 and 10:90 for PHEA-*g*-PDTC-1, PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3 respectively. Using PHEA-g-PDTC-1 as an example, the details of graft polymerization are as follows. 0.451 g DTC and 0.549 g PHEA were well mixed and were placed in a dried silanized glass flask with a magnetic stirring bar. The flask was evacuated, purged with argon three times and sealed, then immersed in an oil bath at 200 °C for 5 min. Then the reaction was allowed to proceed for 10 h at 120 °C. After the graft polymerizations, the product was dissolved in water and dialyzed for 48 h. Then the solution was concentrated under vacuum and the polymer was dried in a vacuum oven. To obtain PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3, the products of graft polymerizations were dissolved in dichloromethane and poured into ether to precipitate the polymers, which were further dried in a vacuum oven at a temperature of 60 °C.

For comparison, homopolymer PDTC was synthesized by the ring-opening polymerization under the same conditions without adding PHEA and other initiators.

In vitro degradation study

To study the in vitro hydrolytic degradation of copolymers, polymer films with a thickness of 0.2 mm were prepared by solvent casting method. Then the hydrolytic degradation study was carried out in 10 mL of phosphate buffer solution (PBS) with pH 7.4 at 37 °C in a shaking water bath. The medium was refreshed every week. At preset time intervals, the samples were taken out, rinsed with distilled water and subsequently vacuum dried at 40 °C for 24 h before being subjected to measurements. The weight loss of samples was measured gravimetrically.

Preparation and characterization of micelles

The micelle suspension was prepared by dissolving 30 mg of PHEA-*g*-PDTC-1 in 10 mL of distilled water while stirring at room temperature for 12 h. Nanoparticles of PHEA-*g*-PDTC-1 were then obtained by freeze drying the micelle suspension in a LABCONCO (FreeZone 4.5 L)

freeze dry system. The morphology of nanoparticles was visualized by a JEOL JEM-100CXII transmission electron microscope (TEM). Before visualization, a drop of nanoparticles suspension was placed on copper grid with Formvar film and dried. The particle size and distribution of the nanoparticles were measured using a Zetasizer 3000 (Malvern Instruments).

Preparation of nanoparticle drug delivery systems and in vitro drug release study

Nanoparticle drug delivery systems were prepared by the direct dissolution method [2]. To the micelle suspension of PHEA-*g*-PDTC-1 (30 mg) in distilled water (10 mL), a dichloromethane solution (2 mL) containing 4 mg of drug (prednisone acetate or tegafur) was added dropwise with vigorous stirring at room temperature overnight to remove the organic solvent completely. To remove the free drug, the solution was dialyzed against distilled water for 24 h using the dialysis membrane (MWCO 8000–12000). The dialyzed suspension was freeze dried to obtain drug-loaded nanoparticles.

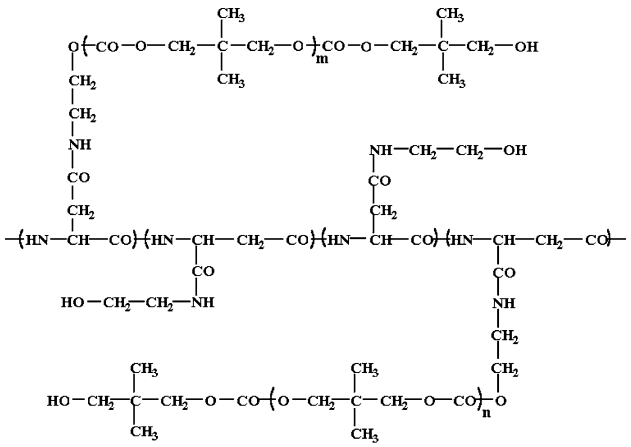
Drug-loaded nanoparticles (28.4 mg of nanoparticles containing prednisone acetate or 27.0 mg of nanoparticles containing tegafur) were suspended in 5 mL of PBS (pH 7.4, 0.1 M) in a dialysis bag. The dialysis bag was sealed and then shaken in 25 mL of PBS at 37 °C in a shaking water bath. At predetermined intervals, the drug concentrations were determined by UV spectroscopy (Perkin-Elmer Lambda Bio 40 UV/VIS spectrometer) at 242 nm for prednisone acetate and 271 nm for tegafur, respectively.

Results and discussion

Structure and properties of graft copolymers

The chemical structure of graft copolymers is shown in Scheme 1. The amphiphilic graft polymers with different branch lengths were synthesized by the ring-opening polymerization of 2,2-dimethyltrimethylene carbonate (DTC) initiated by the macroinitiator poly-α,β-[*N*-(2-hydroxyethyl)-L-aspartamide] (PHEA) bearing hydroxyl groups without adding any catalyst. In our previous study, the chemical structure of the copolymers was verified by FTIR, ^{1}H-NMR, and combined size-exclusion chromatography (SEC) and multiangle laser light scattering (MALLS) analysis [17]. By controlling the feed ratio of the macroinitiator to the monomer, copolymers with different PDTC branch lengths can be obtained. Their structure and molecular weight are summarized inTable 1.

Among the amphiphilic copolymers we synthesized, PHEA-*g*-PDTC-1 is the most hydrophilic because of its high content of PHEA backbone. PHEA-*g*-PDTC-1 can form micelles in an aqueous solution. Copolymers PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3 cannot be dissolved in water. Therefore, we are able to measure the weight loss of PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3 during hydrolytic degradation. Compared with homopolymer PDTC, higher weight loss values can be observed for graft copolymers, which are mainly due to the increased hydrophilicity of the copolymers. As shown in Fig. 1, among the three polymers we studied, PHEA-*g*-PDTC-2 has the highest weight loss rate. This is due to its relatively high content of hydrophilic PHEA backbone, which could facilitate the water absorption and diffusion, and the cleavage of carbonate bonds thereof. With an increase in the hydrophobic branch content, the degradation rate becomes slow.



Scheme 1 Chemical structure of PHEA-*g*-PDTC copolymers

In our study, the copolymer PHEA-*g*-PDTC-1 forms micelles in an aqueous solution. Therefore, nanoparticles of PHEA-*g*-PDTC-1 were prepared by the direct dissolution method. Morphology of polymeric nanoparticles was visualized by TEM. The TEM image (Fig. 2) demonstrates that the nanoparticles are individually separated and regularly spherical in shape, with the size less than 100 nm. The particle size and distribution were measured using a Zetasizer. The particle size determined by the Zetasizer is 64.8 nm with a polydispersity index (PI) of 0.325, which is in agreement with the TEM observation.

Drug release behaviors of amphiphilic graft copolymer

In our study, PHEA-*g*-PDTC-1 nanoparticle drug delivery systems were prepared by the direct dissolution method. Two drugs with different hydrophilicities were encapsulated in the PHEA-*g*-PDTC-1 nanoparticles. The drug loading content and entrapment efficiency of the two kinds of nanoparticles we prepared are summarized inTable 2. Drug loading content and entrapment efficiency are strongly affected by the water solubilities of the drugs. For

Table 1 Structure and molecular weight of PHEA-*g*-PDTC polymers [17]

Polymer	-OH:DTC feed ratio (mol:mol)	^{1}H-NMR			SEC-MALLS	
		HEA:DTC in polymers (mol:mol)	Unreacted -OH groups in PHEA (%)	Mean number of DTC units in each branch	M_w (g/mol)	M_w/M_n
PHEA	–	–	100	–	41600	1.27
PHEA-*g*-PDTC-1	50:50	80:20	85	1.7	38000	2.20
PHEA-*g*-PDTC-2	20:80	27:73	25	3.6	–	–
PHEA-*g*-PDTC-3	10:90	12:88	18	8.9	38500	1.42
PDTC	0:100	0:100	–	–	11400	1.67

In ^{1}H-NMR characterization, D_2O was used as a solvent for PHEA-*g*-PDTC-1, and $CDCl_3$ was used as a solvent for PHEA-*g*-PDTC-2 and PHEA-*g*-PDTC-3

In SEC-MALLS analysis, H_2O was used as an eluent for PHEA, PHEA-2 and PHEA-*g*-PDTC-1, and THF was used as an eluent for PHEA-*g*-PDTC-2, PHEA-*g*-PDTC-3 and PDTC. The M_w value of PHEA-*g*-PDTC-2 is not available because of the insufficient solubility of PHEA-*g*-PDTC-2 in THF, which causes difficulty in the measurement

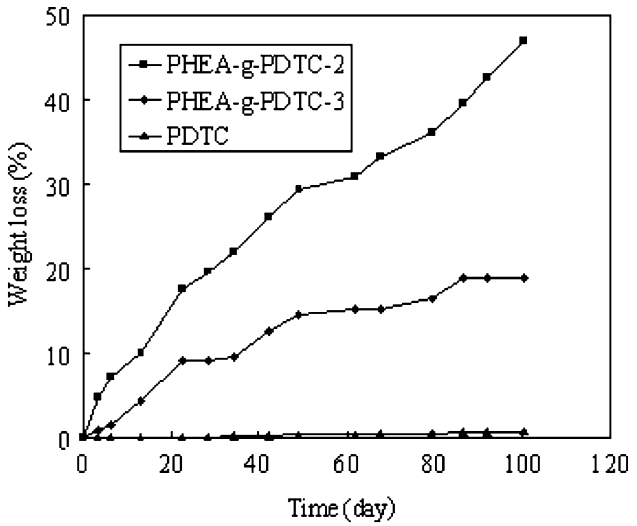


Fig. 1 Weight loss of PHEA-*g*-PDTC copolymers and PDTC during hydrolytic degradation

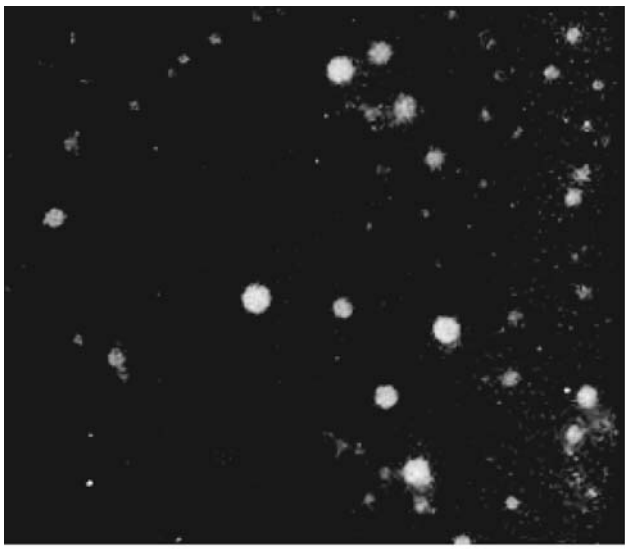


Fig. 2 TEM image of PHEA-*g*-PDTC-1 nanoparticles

the two drugs we studied, the drug prednisone acetate, which is poorly water soluble, is mainly encapsulated into the hydrophobic cores of nanoparticles, while the much more hydrophilic drug tegafur is entrapped in the outer hydrophilic layers of the nanoparticles and partially adsorbed on the nanoparticle surfaces. Therefore, both drug loading content and entrapment efficiency of prednisone acetate are higher than that of tegafur.

To study the in vitro drug release property of PHEA-*g*-PDTC-1 nanoparticle drug delivery systems, the release profiles of the drugs were determined from UV absorbance. The results are shown inFig. 3. Generally, the drug release rate of nanoparticles is faster, relative to that of microsphere systems because of the high surface area and the small size of the nanoparticle delivery systems. From Fig. 3, it can be found that the release of prednisone acetate lasts for several days. While the release rate of tegafur is much faster, with more than 90% of tegafur being released in 10 h. The difference in release rates of these two drugs is mainly due to the different water solubilities of the drugs. Since prednisone acetate is highly hydrophobic, it is encapsulated in the hydrophobic cores of the nanoparticles and the release rate is slow. In the contrast, the hydrophilic

Table 2 Drug loading content, entrapment efficiency and particle yield of PHEA-*g*-PDTC-1 nanoparticle drug delivery systems

Drug	Loading content (wt%)	Entrapment efficiency (%)	Particle yield (%)
Prednisone acetate	1.2	15.8	83.4
Tegafur	1.1	10.7	79.5

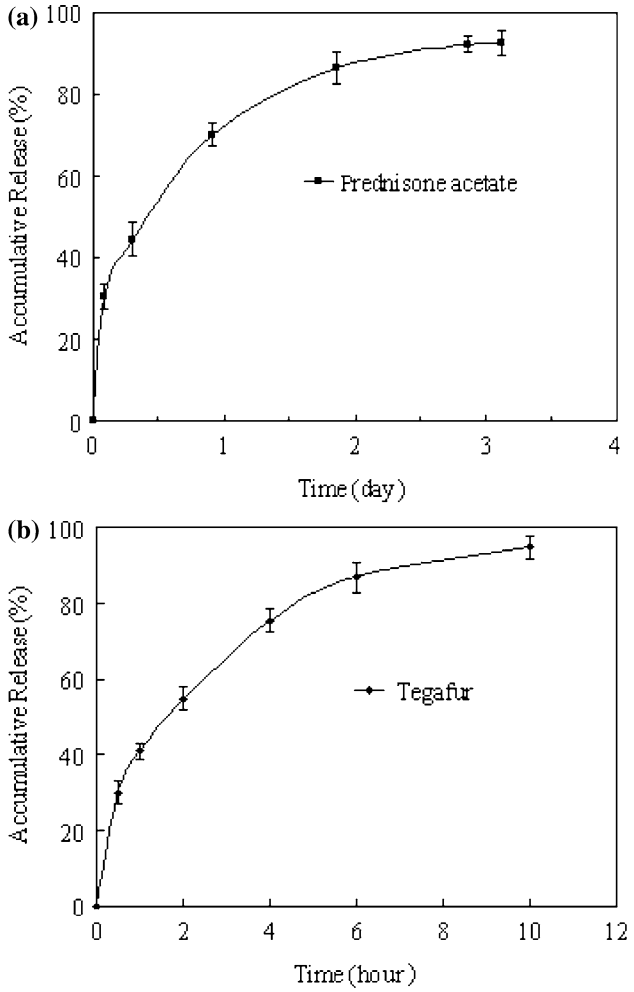


Fig. 3 In vitro release profiles of (**a**) prednisone acetate and (**b**) tegafur from PHEA-*g*-PDTC-1 nanoparticle drug delivery systems. Results are the mean ± SD of 3 independent experiments

tegafur is entrapped in or absorbed on the outer hydrophilic shells of the nanoparticles, so its release rate is much faster.

Conclusions

The PHEA-g-PDTC graft copolymers with different branch lengths were synthesized by bulk ring-opening polymerization with different feed ratios of the macroinitiator PHEA to the monomer DTC. PHEA-*g*-PDTC-1 forms self-assembling micelles in an aqueous solution, with a mean size of 64.8 nm in diameter. Nanoparticle drug delivery systems were successfully prepared by the direct dissolution method. The in vitro drug release profiles show the release rate increases with increasing hydrophilicity of the drug encapsulated.

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