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Synthesis of Biodegradable Poly(butylene terephthalate)/poly(ethylene glycol) (PBT/PEG) Multiblock Copolymers and Preparation of Indirubin Loaded Microspheres +

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Summary

Biodegradable PBT/PEG copolymers were synthesized by macromolecular transesterification method, and were characterized by ^{1}H-NMR and DSC. Alkaline degradation behavior was studied too. Indirubin loaded microspheres were prepared by O/W solvent evaporation method.

Keywords:

multiblock copolymer, PBT/PEG, poly(ether ester)s, microspheres

1. Introduction

Poly(butylene terephthalate)/poly(ethylene glycol) (PBT/PEG) multiblock copolymer is a kind of biocompatible and biodegradable copolymer, which was studied extensively [1-3]. J. Feijen et al have studied its potential application in drug delivery system [2]. PBT/PEG and PET/PEG copolymers were mainly synthesized from ethylene diol (or: butylene diol), dimethyl terephthalate, and poly(ethylene glycol) [2-4]. But during the process, the poisonous methanol would be released. In this paper, PBT/PEG copolymer was prepared by macromolecular transesterification method from PBT and PEG macromonomers. And there is no poisonous byproducts produced during this synthesis process. Indirubin is a new kind of anticancer drug, which is extracted from Chinese Traditional Medicine. In this letter, we synthesized a kind of amphiphilic PBT/PEG multiblock copolymer, and attempted to encapsulate indirubin in such microspheres. This drug delivery system might have potential application in human cancer therapy.

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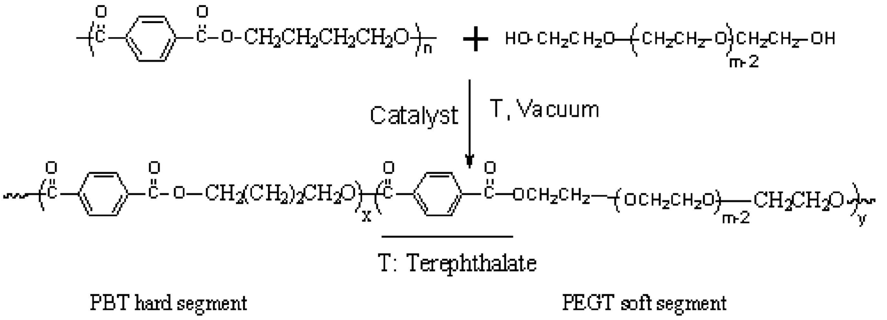
2. Experimental

2.1 Materials

PEG (M_n=600-4000), PBT ([η]=0.81 measured in *m*-cresol, Yizheng Chemical Company, P.R. China), and all the other reagents were analytical pure agents. In this work, the chemicals were used as received.

2.2 Synthesis of PBT/PEG copolymers

PBT/PEG copolymer was synthesized by the macromolecular transesterification method shown in Scheme 1, which was similar to the synthesis of PET/PEG copolymers [1]. The mixture of calculated amount of PBT, PEG (M_n=600-4000), and tetrabutyl titanate was added into a flask under nitrogen atmosphere, and then was gradually heated to 230°C. At this temperature, the reaction mixture underwent a transesterification reaction for 30 min with constant stirring. The temperature was then elevated to 270°C under high vacuum for 1-2 h. At the end, the resultant melt was poured into cold water and then dried at 70°C under vacuum for 12 h. All the samples prepared in this work were listed in Table 1.



Scheme 1 Synthesis of PBT/PEG multiblock copolymers

Table 1 The PBT/PEG copolymers prepared in this work

Sample	Weight fraction of PBT (%Wt) in feed	Molecular Weight of PEG (g/mol)	Intrinsic Viscosity ([η], dl/g)
PBT50/PEG600	50	600	0.58
PBT50/PEG1000	50	1000	0.70
PBT50/PEG1540	50	1540	0.84
PBT50/PEG4000	50	4000	0.70
PBT40/PEG1000	40	1000	0.74
PBT60/PEG1540	60	1540	0.71

2.3 ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR)

^{1}H-NMR spectra (in $CDCl_3$) were recorded on Bruker 300 spectrometer (Bruker, Rheinstetten, Germany) at 300MHz using trimethylsilane (TMS) as internal standard.

2.4 Differential Scanning Calorimetry (DSC)

The nonisothermal crystallization behavior of PET/PEG copolymers was characterized on a DSC instrument (NETZSCH DSC 204, Deutsch). The specimens were first heated to 220°C from room temperature, then were cooled to -55°C, and then were reheated to 220°C. The heating rate and cooling rate are both 10°C/min.
The crystallinity (X_c) of the sample was calculated according to the heat of fusion of the second heat run:

$$Xc_{,\,PBT}\,(\%) = \frac{\Delta H_{m-PBT}}{\Delta H^{\circ}_{m-PBT}} \times 100 \tag{1}$$

Where $\Delta H^{o}_{m\text{-}PBT}$ = 144.5J/g is for the 100% crystalline PBT according to Zhang Y. et al [5].

$$Xc_{,\,PEG}\,(\%) = \frac{\Delta H_{m-PEG}}{\Delta H^{\circ}_{m-PEG}} \times 100 \tag{2}$$

Where $\Delta H^{o}_{m\text{-}PEG}$ =197J/g is for the 100% crystalline PEG according to Hu Y. et al [6].

2.5 Water absorption behaviour

The copolymer samples were immersed in distilled water at 37°C for predetermined period, and then were taken out [4]. The surplus surface water was removed by a filter paper. The water absorption was calculated according to equation (3):

$$\text{Water absorption (\%)} = \frac{W_{ht} - W_d}{W_d} \times 100 \tag{3}$$

Where W_d is the initial dry weight, W_{ht} is the humid weight at time t.

2.6 Hydrolytic degradation of PBT/PEG copolymers

The copolymer chips were immersed in 0.01mol/L NaOH aqueous solution and phosphate buffer solution (with pH=4.0, 6.88, 9.23 respectively) at 37°C for predetermined time. The weight loss was calculated according to Equation (4):

$$\text{Weight loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100 \tag{4}$$

Where W_0 is the dry weight before degradation, W_t is the dry weight at time t.

2.7 Preparation of indirubin loaded PBT/PEG microspheres

In this letter, PBT50/PEG1540 copolymer was used to prepare microspheres due to its great solubility in chloroform (TCM). Indirubin loaded PBT/PEG microspheres were prepared by oil-in-water (O/W) emulsion evaporation technique. The indirubin and PBT50/PEG1540 copolymer were dissolved in 10ml TCM, then the resultant solution was added into 200ml 4%W/V aqueous polyvinyl alcohol (PVA) solution with continuous stirring at a predetermined adding rate. The O/W emulsion was formed by extensive stirring with a four-blade stirrer for 5 hours at a predetermined rate. After 5 hours, the evaporation of organic solvent was complete. The obtained microspheres were washed with distilled water for three times and then dried in *vacuo*.

2.8 Surface morphology

The surface morphology of polyetherester microspheres was observed on SEM (Hitachi, Japan).

2.9 Determination of particle size distribution

These microspheres were first dispersed in distilled water by ultrasound dispersion, and the particle size distribution was determined on particle sizer (SALD-2001, shimadzu, Japan).

3. Results and Discussion

3.1Characterization of PBT/PEG copolymers

The as-polymerized PBT/PEG copolymer was characterized by ^{1}H-NMR. A typical ^{1}H-NMR spectrum of the PBT50/PEG1540 copolymer was shown in Fig. 1. Three of them were for the protons of the PEG blocks: at 3.68ppm (peak “c”), 3.83ppm (peak “d”’), and 4.69ppm (peak “e”). The peaks at 1.96ppm (peak “a”) and 4.46ppm (peak “b”) were for the methylene protons of the PBT block respectively, the peak at 8.1ppm (peak “f+g”’) was for the aromatic protons of PBT and PEGT blocks. The ^{1}H-NMR spectrum confirmed the production of PBT/PEG copolymers.

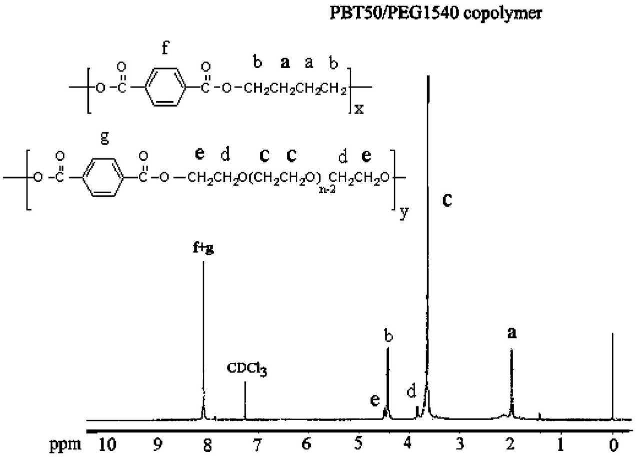


Fig. 1 ^{1}H-NMR spectrum of PBT50/PEG1540 copolymer

3.2 Thermal properties of PBT/PEG copolymers

The thermal properties of PBT/PEG copolymers were studied by DSC, and the results were shown in Table 2.

For these semicrystalline PBT/PEG copolymers, when the weight fraction of PBT was kept constant, the melting temperature, crystallization temperature and heat of fusion of PBT and PEG increased accordingly with the increase in PEG molecular weight. When the molecular weight of PEG was kept constant, with the increase in PBT content, the melting temperature, crystallization temperature, and the heat of fusion of PBT increased then, but the melting temperature and heat of fusion of PEG segment decreased accordingly.

The increase in PEG molecular weight and PBT content enhanced the microphase separation between PBT and PEG segment, as a result, the thermal property of this copolymers changed accordingly, which is very similar to multiblock PET/PEG copolymer [1].

Table 2 Thermal properties of PBTx/PEGy copolymers*

	PBT50 /PEG600	PBT50 /PEG1000	PBT50 /PEG1540	PBT50 /PEG4000	PBT40 /PEG1540	PBT60 /PEG1000
$T_{c,\,PEG}$ (^oC)	--	-22.69	-9.69	16.92	-27.77	--
$T_{c,\,PBT}$ (^oC)	109.02	134.81	166.11	181.89	141.61	160.60
$T_{m,\,PEG}$ (^oC)	-6.99	1.75	17.57	35.48	27.08	-3.58
$\Delta H_{m,\,PEG}$ (J/g)	0.3736	6.19	17.74	26.84	33.49	0.8694
$T_{m,\,PBT}$ (^oC)	127.07	168.29	190.49	201.40	172.29	190.29
$\Delta H_{m,\,PBT}$ (J/g)	5.301	7.799	9.351	16.22	4.359	11.15

* The data of heating run was obtained according to the 2nd heating scan of PBT/PEG copolymers.

3.3 Water absorption of PBT/PEG copolymers

PBT is a kind of hydrophobic polyesters, but its hydrophilicity increased dramatically with the incorporation of hydrophilic PEG segment into the PBT main chain. For these amphiphilic hydrolytic degradable PBT/PEG copolymers, the hydrophilicity was greatly affected by the PBT and PEG segment. So, the water absorption of this copolymer was affected by two parameters: the weight fraction of PBT segment and PEG molecular weight.

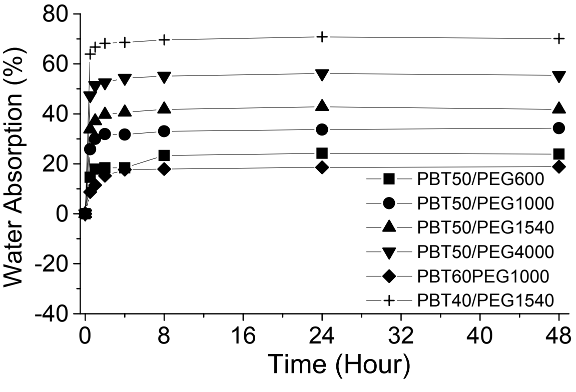


Fig. 2 Water absorption of PBT/PEG copolymers at 37°C in distilled water

According to Fig. 2, the water absorption increased quickly with the decrease in PBT content, which is consistent with the results obtained from DSC. With the decrease in PBT content, the crystallinity decreased then, so the amorphous region increased. As a result, the water absorption increased. On the other side, with the increase in molecular weight of PEG segment, the microphase separation between the PBT and PEG segments enhanced, then the water absorption increased.

3.4 Hydrolytic degradation behaviour of PBT/PEG copolymers

The hydrolytic degradation behavior of PBT/PEG copolymers was shown in Fig. 3 and Fig. 4.

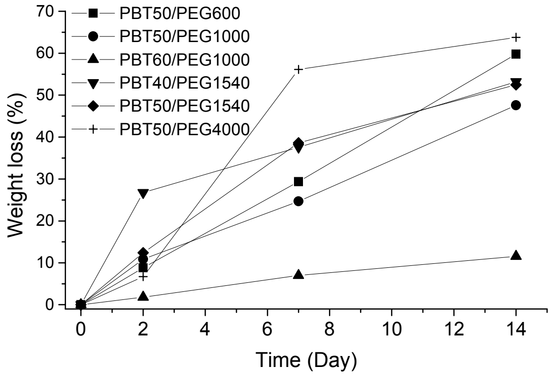


Fig. 3 Degradation behavior of PBTx/PEGy copolymers in 0.01mol/L NaOH solution at 37°C

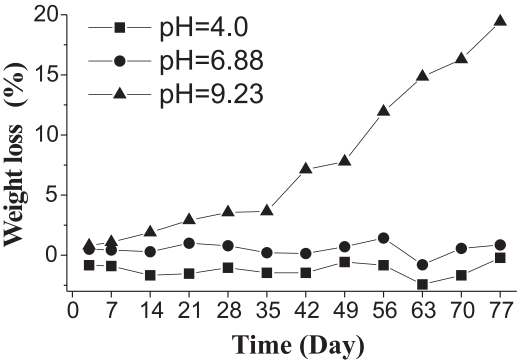
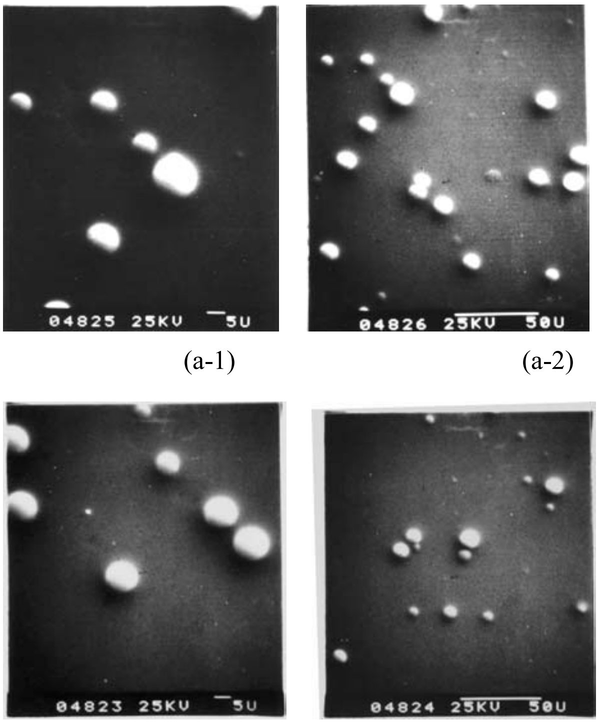


Fig. 4 Effect of pH value of PBS degradation medium on the degradation profile of PBT50/PEG1540 copolymers at 37°C

From Fig. 3, we could find that the degradation rate increased with the decrease in PBT weight content. But the effect of PEG molecular weight on the degradation rate is very complicated. For the PBT50/PEG4000 sample, it degraded more rapidly than the other ones, which might be due to its great water absorption. This phenomenon is similar to PET/PEG copolymer [1]. From Fig. 4, the degradation rate increased accordingly with the increase of pH value of the degradation medium. The copolymer degraded very fast in the alkaline medium.

3.5 Preparation of indirubin loaded PBT/PEG microspheres

Indirubin is a very good anticancer drug, which was confirmed to have ability to cure leukemia. But unfortunately its side effect is serious too, and indirubin is not stable during storage. So, we attempted to encapsulate indirubin in PBT/PEG microspheres to improve its stability and decrease its side effect. In this article, we prepared indirubin loaded biodegradable PBT/PEG microspheres by oil in water (O/W) emulsion evaporation method. And the microspheres were observed on SEM, as was shown in Fig. 5.



(a-1) (a-2)

(b-1) (b-2)

Fig. 5 The surface morphology of indirubin loaded PBT/PEG microspheres and blank PBT/PEG microsphers observed on SEM(a-1, Indirubin loaded PBT/PEG microsphers, the scale bar is 5μm; a-2, Indirubin loaded PBT/PEG microsphers, the scale bar is 50μm; b-1, PBT/PEG microsphers, the scale bar is 5μm, b-2, PBT/PEG microsphers, the scale bar is 50μm)

From Fig. 5, there is no obvious difference in surface morphology between the PBT/PEG microspheres and indirubin loaded microspheres. The particle size is about 10μm, and the particle size distribution was shown in Fig. 6. The drug release behavior from this indirubin loaded microspheres will be studied in detail elsewhere.

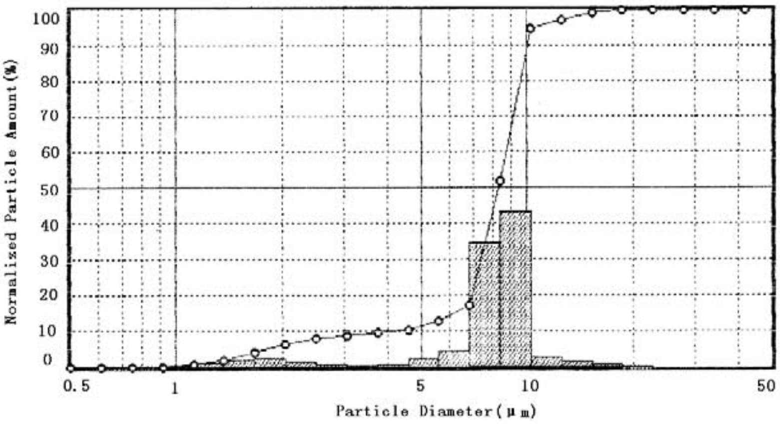


Fig. 6 The particle size distribution of indirubin loaded PBT/PEG copolymers

4. References

[1] Z.Y. Qian, S.Li, Y.He, X.B.Liu. Polym. Degrad. Stabil. 2004, 83: 93
[2] A.A. Deschamps, D.W. Grijpma, J. Feijen. Polymer. 2001, 42: 9335
[3] J.M.Bezemer, R.Radersma, D.W.Grijpma, et al. J Controlled Rel. 2000, 67(2-3): 233
[4] Nagata M. Kiyotsukuri T., Minami S., Tsutsumi N., Sakai W. Polym Int. 1996, 39: 83
[5] Zhang Y, Zhang AY, Fen ZG, Ye L, Xu RX. Acta Polym Sinica. 2002, (2): 167-171
[6] Hu Y, Rogunova M, Topolkaraev V, Hiltner A, Baer E. Polymer. 2003, 44: 5701-5710