Drug Release from Electrospun Fibers of Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Grafted with Poly(*N*-vinylpyrrolidone)

Wei Wang, Jianda Cao, Ping Lan, Wen Wu

College of Materials and Textile Engineering, Jiaxing University, Zhejiang 314001, China

Received 16 December 2010; accepted 2 April 2011
DOI 10.1002/app.34617
Published online 25 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Poly(*N*-vinylpyrrolidone) (PVP) groups were grafted onto poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) backbone to modify the properties of PHBV and synthesize a new novel biocompatible graft copolymer. Based on these graft copolymers, electrospun fiber mats and commonly cast films were explored as drug delivery vehicles using tetracycline hydrochloride as a model drug. Toward that end, the fibers were electrospun and the films were cast from chloroform solutions containing a small amount of methanol to solubilize the drug. The Brookfield viscosities of the solution were determined to achieve the optimal electrospinning conditions. The vitro release of the tetracycline hydrochloride from these new drug delivery systems was followed by UV–vis spectroscopy. To probe into the factors affected on the release behavior of these drug delivery systems, their water absorbing abilities in phosphate buffer solution were investigated, together with their surface hydrophilicity, porosity and crystallization properties were characterized by water contact angles, capillary flow porometer, DSC, and WAXD, respectively. The morphological changes of these drug delivery vehicles before and after release were also observed with SEM. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 1919–1928, 2012

Key words: drug delivery systems; electrospun fibers; graft copolymers; poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV); poly(*N*-vinylpyrrolidone) (PVP)

INTRODUCTION

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a thermoplastic aliphatic polyester synthesized by bacterial fermentation, is known to degrade fully in the environment without forming any toxic products. This biodegradable nature is very important from the view of reducing plastic wastes. The synthesis of PHBV is very different from common oil-based polymers. PHBV can be produced by microorganisms with renewable nature materials such as starch, waste edible oils, waste vegetable, waste fruit and other organic wastes originated from industry or trade.[1,2] It can also be produced and extracted from plants, for example Arabidopsis, through transgenic technology.[3,4] PHBV has many excellent properties such as biodegradability, biocompatibility, piezoelectric property and optical activity, so it can be widely used as biodegradable packing materials, tissue engineering materials, drug delivery system and electric materials. However, the application of PHBV is very limited presently due to its inherent disadvantage like high cost, hardness and brittleness, low thermal decomposition temperature, narrow heat-processing window and absence of functional groups. To improve the chemical, physical and heating-processing properties of PHBV, various methods including chemical modification,[5–7] as well as blending with other polymers have been investigated.[8–11] Out of these modification methods, graft modification is a well-known method to modify the properties of the polymer as well as to introduce new functional groups to the same polymer backbone. Many studies on the graft modification of PHBV have also been reported.[12–17]

Poly(*N*-vinylpyrrolidone) (PVP), is a synthetically derived vinyl polymer with a unique combination of properties, such as good solubility in water and a range of organic solvents, remarkable capacity to interact with a wide variety of organic and inorganic compounds, good biocompatibility and nontoxicity to living tissues. PVP has been widely used in biomedical, cosmetic and edible fields which are closely related to the human health for decades. PVP has also been widely used as a medical additive or polymeric modifier.[18–20] Our research has already described a method by which PVP groups were

Correspondence to: P. Lan (lanping007@126.com).
Contract grant sponsor: Zhejiang Provincial Natural Science Foundation of China; contract grant number: Y4110606.
Contract grant sponsor: Key Scientific Research Project of Jiaxing University; contract grant number: 70110072.

successfully grafted onto poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) backbone to modify the properties of PHBV and synthesize a new novel biocompatible graft copolymer.[15] The effects of grafting modification on the properties of PHBV such as biodegradation, mechanical and thermal properties have been also carried out in our laboratory.[16] Due to their high costs and excellent biocompatible properties, these new novel graft copolymers have practical significance as candidate materials.

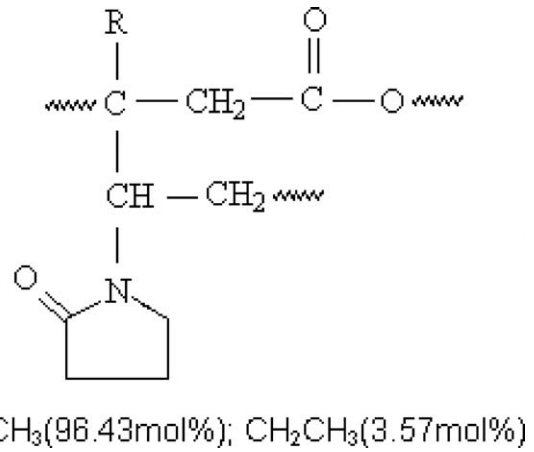
Electrospinning is a straightforward method of producing fibrous polymer sheets for control over morphology, porosity, and composition using simple equipment. Such materials may be useful for many applications in biomedical materials such as biosensor, wound dressings, tissue engineering and drug delivery systems.[21–25] The simplicity of the electrospinning process itself and the facility to incorporate therapeutic compounds into the electrospun sheets opens a new prospect in controlled drug delivery systems. A wide variety of biocompatible polymeric materials have been used as delivery carriers with electrospinning technology.[26–29]

We describe here a method of preparing a novel drug delivery system with common films and nanoscale fibers based on graft copolymers of PHBV and PVP. Tetracycline hydrochloride was selected as a model drug due to interest in the treatment of inflammation diseases.[30,31] Toward that end, the films were cast and the fibers were electrospun from chloroform solutions containing a small amount of methanol to solubilize the drug. The Brookfield viscosities of the solution were determined to achieve the optimal electrospinning conditions. The effects of graft modification on the vitro release behavior, surface hydrophilicity and crystallization properties of these new drug delivery systems were investigated by UV–vis spectroscopy, water contact angles, DSC and WAXD, respectively.

EXPERIMENTAL

Materials

PHBV (Tian'an Bioproducts Company, Hangzhou City, China; HV content: 3.57 mol %, determined by ^{1}H-NMR spectrum) was recrystallized from ethanol. PHBV-*g*-PVP(2.54), PHBV-*g*-PVP(6.98), PHBV-*g*-PVP(9.01), and PHBV-*g*-PVP(10.48), representing samples with graft yield (graft%) of 2.54, 6.98, 9.01, and 10.48%, respectively, were synthesized in our laboratory. The proposed mechanism for the graft reaction is a random hydrogen abstraction from PHBV backbone by direct attack of initiator radicals or chains transfer of PVP growing radicals onto PHBV backbone as reported in the literature.[15] Because the branching points of PHBV in free-radical reactions mostly occur at the tertiary carbons,[17] we speculate that the PHBV-*g*-PVP copolymers have the molecular structure as showed in Scheme 1.



Scheme 1 Proposed molecular structure of PHBV-*g*-PVP.

Tetracycline hydrochloride was purchased from Sigma and used as received. Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen ($Na_2HPO_4{\cdot}12H_2O$) were supplied by China Medicine Group and were used without further purification to prepare 0.1 *M* phosphate buffer solutions (PBS) of pH 7.35. Analytical grade methanol and chloroform were also supplied from China Medicine Group and used as received. Dialysis bag (molecular weight cut-off 7000, width 3.4 cm) was obtained from Sigma.

Preparation of drug loaded films and electrospun mats

Electrospinning was carried out using 4 wt % solutions of PHBV in chloroform. Tetracycline hydrochloride, which is insoluble in chloroform, was solubilized in a small amount of methanol (0.05 g/mL) and added to the polymer solution. The mass ratio of tetracycline hydrochloride to PHBV was 5%. The resulting solutions were yellow but clear, indicating that homogeneous solubilization of both the polymer and drug. A positive voltage (15 kV) was applied to the solution which was delivered at the stead flow rate of 1.5 mL/h when electrospinning. The resulting fibers were collected on a metal board which had the space about 15 cm from the needle tip with the aperture diameter of 0.4 mm for producing a sheet of nonwoven fabric.

For comparative purposes, cast films with the thickness from 35 to 40 μm were also made from the same solution. The PHBV solutions in chloroform were cast onto glass board and left at room temperature for evaporating the solvents. All resulting film and electrospun mat samples were dried at ambient temperature under vacuum for 3 h before investigation.

Measurements

Solution viscosities were measured using a Brookfield Model TC-502 R/S instrument.

DSC measurements were carried out on a Mettler-Toledo Star DSC822 System (Switzerland). The instrument was calibrated with an indium standard. The measurement was conducted under nitrogen atmosphere and the sample weight used in the DSC pan was kept within 6–8 mg. The samples were heated up from room temperature to 210°C at a rate of 10°C/min and the endothermic peaks of the thermograms were taken as the melting temperature (T_m).

WAXD experiments were performed with a Philips PW1700 X-ray diffractometer using Cu Kα X-rays at a voltage of 40 kV and a current of 30 mA.

The morphological analysis was performed with a scanning electron microscope (JSM-5600LV, Nikon, Japan) at an accelerating voltage of 20 kV after the sample was sputter-coated with gold for a period up to 120 s. The diameters of electrospun fibers were measured from SEM images, and five images were used for each electrospun fiber mat. From each image, at least 20 different fibers and 100 different segments were randomly selected and their diameters were measured using Photoshop 8.0 edition for statistical analysis.

Statistic water contact angles of the drug loaded films and electrospun sheets were measured by sessile drop method at ambient temperature using a contact angle goniometer (OCA20, Germany). For each contact angle reported, at least five readings from different parts of the sample surface were averaged.

Water absorptivity studies of the drug loaded films and electrospun sheets were carried out in PBS. Samples were gently taken out from the medium at certain time intervals and then hung for removing the surplus surface water. The samples were weighted at 3-minute intervals till the mass difference between two consecutive experiment results was less than 1% and the latter result was considered as the mass of samples after absorbing sufficient water. The water absorptivity was then taken as the percent of increased mass of samples with water retention to the original samples.

The pore size and distribution of electrospun fiber mats were analyzed with a capillary flow porometer (CFP-1100-A1, PMI) and the supporting soft ware. The standard Porewick® solution (PMI) with the adjusted surface tension of 16 dyne/cm was used as the wetting agent for porometry measurements.

UV-vis measurements were obtained using a TU-1901 Spectrophotometer (Beijing Purkinje General Instrument). The molar extinction coefficient for tetracycline hydrochloride in PBS was found to be 17,320 L/mol per cm from a linear Beer-Lambert plot of absorbance at 360 nm vs. concentration. The drug loading and loading efficiency of films and electrospun fibers was determined by dissolving samples (105 mg) in chloroform (15 mL) and then removing the solvents by air provided with an air pump after adding 50 mL PBS into the solution for extracting the tetracycline hydrochloride in the samples. This process was repeated at least three times until the polymer obtained as a white, tetracycline hydrochloride free, precipitate. The resultant tetracycline hydrochloride solutions in PBS were analyzed spectrophotometrically (at 360 nm) by TU-1901 Spectrophotometer and the data were used to calculate the drug loading and loading efficiency:

$$\text{Drug loading } \% = 100\,(\text{weight of drug measured})/(\text{weight of substrate polymer}) \quad (1)$$

$$\text{Loading efficiency } \% = 100\,(\text{weight of drug measured})/(\text{weight of drug added}) \quad (2)$$

The 105 mg samples were enclosed in dialysis bags and then introduced to 100 mL PBS which was continuously stirred by a magnetic stirrer at ambient temperature. At certain time intervals, samples (5.0 mL) were removed from the medium for release studies and then returned. Release of tetracycline hydrochloride was determined by monitoring the absorbance at 360 nm as a function of time. Results of triplicate tests were used to calculate the released tetracycline hydrochloride. For comparative purposes, a parallel study of release for 5 mg pure tetracycline hydrochloride enclosed in dialysis bag was also conducted.

RESULTS AND DISCUSSION

Viscosities characterization

Extensive chain entanglements are necessary to produce electrospun fibers, the consequence being that lower concentrations lead to electrospraying rather than spinning.[32,33] Toward that end, the viscosities of polymer solutions in chloroform containing tetracycline hydrochloride solubilized in methanol were determined to assist in choosing suitable concentrations for spinning. The mass ratio of the drug to substrate polymer was 5% and the concentration of drug solubilized in methanol was 0.05 g/mL. The solution viscosities are functions of polymer concentration and graft% as shown in Figure 1. Unpublished work in our laboratory suggests that about 3–4 wt % PHBV concentration is necessary to achieve electrospinning fibers, in agreement with the onset of significant chain

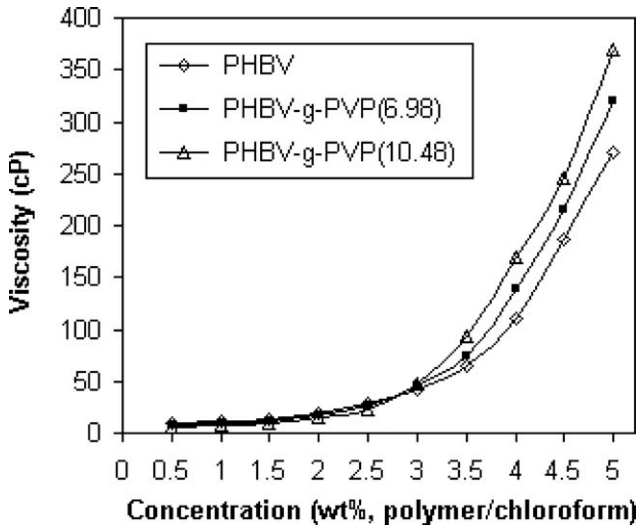


Figure 1 Brookfield viscosity data for PHBV and PHBV-*g*-PVP in chloroform.

entanglements in the viscosity data in Figure 1. Therefore, solution concentration of 4 wt % for all polymers in chloroform was selected to ensure electrospinning solution into fibrous mats.

Crystallization properties

Figure 2 presents the DSC traces of samples on heating. The melting endothermic peak (T_m) can be detected for all samples. It can be seen that the T_ms are almost not changed for all drug loaded films, but the melting range increases and the initial slope of T_m peak decreases with increasing graft% of polymer, implying the higher crystallites size distribution and more imperfect crystallites formed during crystallization from solution under atmosphere for PHBV after graft modification. This is because that the introduction of PVP side groups into PHBV backbone hinders the regular fold of macromolecule chains due to the steric effects as reported in our previous study.[16] As for ungrafted PHBV, the drug loaded electrospun fibers exhibit a higher T_m, narrower melting range and sharper melting peak in comparison with the common PHBV films cast from solution, indicating that the crystallization process is enhanced, thus more perfect and uniform crystallites are formed during electrospun for ungrafted PHBV. However, the melting behavior of PHBV-*g*-PVP electrospun fibers and films is very different as shown in Figure 2. All the PHBV-*g*-PVP electrospun fibers exhibit a very weak T_m peak, indicating their low crystallization degrees. It is generally believed that there are two main factors affecting the crystallization behavior for polymer during electrospinning: one is that the static electric field with high voltage induces a high stress on the electrospun jet, enhancing the macromolecule stretched and crystallites formed, the other is that the thin fluid jet with high stretch rate leads to accelerated solidification and deposition on the collector during the later stages of electrospinning, retarding the crystalline microstructure formed and well developed.[25,34–36] PHBV exhibited a quite high crystallization ability and crystallization rate, thus the polymer chains during electrospinning probably had sufficient time to organize themselves into crystal structures. Moreover, the crystallites of electrospun fibers may be more perfect than those of cast films because of the high stress and high elongational rate on the electrospun jet induced by static electric field with high voltage. Although the drug loaded films cast from PHBV-*g*-PVP solution showed the similar degree of crystallization, the retardation effect on crystallization caused by the rapid evaporation of solvents and rapid solidification of the stretched chains during electrospinning was still present. This is due to the fact that the introduction of PVP side groups into PHBV backbone would suppress the crystallization process and decrease the crystallization rate because of the steric effects as revealed by our previous study.[16] In other words, the stretched chains of electrospinning jets for all PHBV-*g*-PVP did not have enough time to organize into 3D ordered crystal structures before they were solidified. Hence the crystalline microstructure for all PHBV-*g*-PVP electrospun fibers could not be well developed, or even

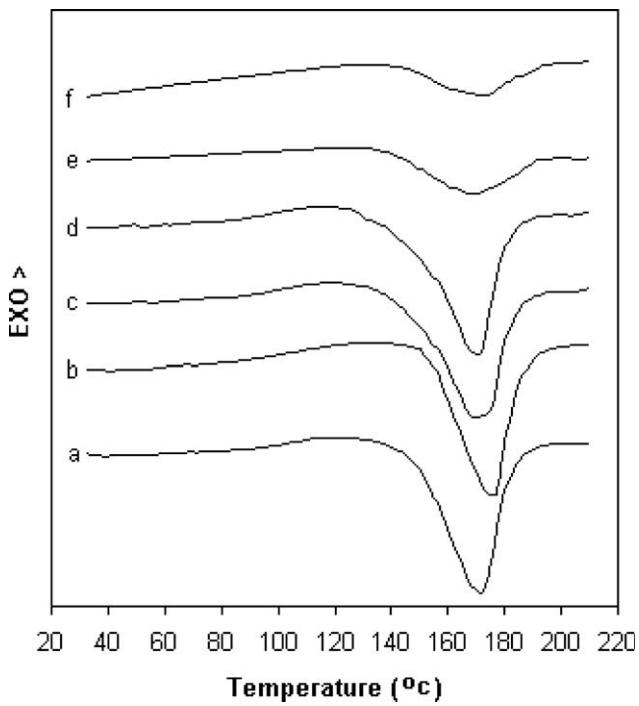


Figure 2 DSC traces of drug loaded polymer films and electrospun fibers on heating: (a) PHBV film, (b) PHBV electrospun fibers, (c) PHBV-*g*-PVP(2.54) film, (d) PHBV-*g*-PVP(9.01) film, (e) PHBV-*g*-PVP(2.54) electrospun fibers, (f) PHBV-*g*-PVP(9.01) electrospun fibers.

TABLE I
Thermal Parameters Derivable from DSC Heating Traces for Drug Loaded Films and Electrospun Fibers of PHBV and PHBV-*g*-PVP

	T_m (°C)		ΔH_f (J g^{-1})		X_c (%)	
Substrate polymers	Casted films	Electrospun fibers	Casted films	Electrospun fibers	Casted films	Electrospun fibers
PHBV	172.6	177.1	69.8	68.4	52.6	51.5
PHBV-*g*-PVP (2.54)	173.7	172.6	70.9	14.3	53.4	10.8
PHBV-*g*-PVP (6.98)	174.3	171.4	70.4	12.7	53.0	9.6
PHBV-*g*-PVP (9.01)	173.7	170.8	70.7	11.0	53.2	8.3
PHBV-*g*-PVP (10.48)	173.2	170.3	69.5	10.8	52.3	8.1

could not be formed at all, accounting for the very weak T_m peak in DSC traces (Fig. 2).

The parameters derivable from the DSC traces are summarized in Table I. T_m and ΔH_f were taken as the peak temperature and the area of the melting endothermic respectively. X_c, registered as the crystal degree, was calculated by X_c (%) = $100\Delta H_f / \Delta H_o$, where ΔH_o is the melting enthalpy per gram of perfect crystal (100% crystalline). With method of interpolation, ΔH_o = 132.8 J/g for PHBV containing 3.57 mol % used in this study could be calculated adopting the reported value of ΔH_o = 146 J/g for the PHB and ΔH_o = 109 J/g for the PHBV containing 10 mol % 3HV units.[37,38] It can be seen that the crystallization degrees of all drug loaded films are almost constant regardless of the different graft%. As for ungrafted PHBV, their drug loaded films and electrospun fibers present the near crystallization degree, however as for PHBV-*g*-PVP, their drug loaded electrospun fibers exhibit the very lower crystallization degree than the corresponding films. Taking into account that the T_m of tetracycline hydrochloride crystals is also at about 172.5°C, it is reasonable to believe that all PHBV-*g*-PVP electrospun fibers were almost noncrystalline.

Studies on the structure of crystals were carried out with WAXD and the results were presented in Figure 3. It had been reported that the original PHBV and PHBV-*g*-PVP films without drug loaded had the same WAXD spectra indicating that the crystal structure were almost not changed by graft modification with PVP.[16] From Figure 3, it can be seen that several new diffraction peaks appear at about 11°, 16.5°, and 20° corresponding to the crystalline characteristics of tetracycline hydrochloride particles in all drug loaded films and electrospun fibers. All the drug loaded films have the same characteristic peaks, indicating that the crystalline unit cell is almost not changed. Furthermore, the intensity of each corresponding peaks is also constant, indicating that the crystallization degree of all drug loaded films are almost not changed after graft modification, being in agreement with the DSC results. As for PHBV drug loaded vehicles, their electrospun fibers have almost the same characteristic peaks and corresponding peak intensity with commonly cast films, indicating that both crystalline structure and crystallization degrees are not changed during electrospinning. However, the main characteristic diffraction peaks of PHBV drug loaded electrospun fibers at about 13.3°, 16.8° become a little sharper than those of drug loaded films, indicating that more perfect and uniform crystallites were formed during electrospinning, being in agreement with the DSC traces (Fig. 2). It is very interesting to find out that all PHBV-*g*-PVP drug loaded electrospun fibers

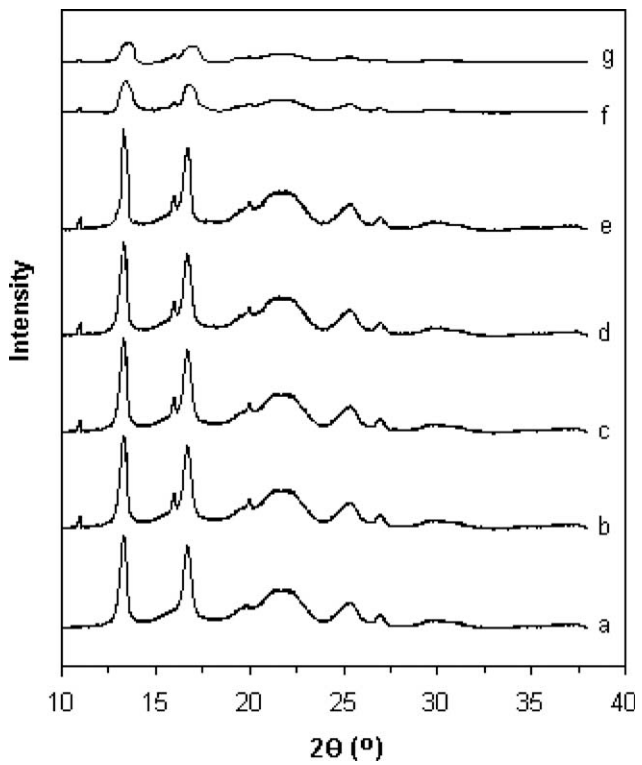


Figure 3 WAXD patterns of polymer films and electrospun fibers: (a) PHBV films without drug loaded, (b) drug loaded PHBV film, (c) drug loaded PHBV-*g*-PVP(2.54) film, (d) drug loaded PHBV-*g*-PVP(9.01) film, (e) drug loaded PHBV electrospun fibers, (f) drug loaded PHBV-*g*-PVP(2.54) electrospun fibers, (g) drug loaded PHBV-*g*-PVP(9.01) electrospun fibers.

TABLE II
Contact Angles of Drug Loaded Polymer Films and Electrospun Fiber Sheets

Substrate polymers	Contact angles on casted films (°)	Contact angles on electrospun fiber sheets (°)
PHBV	57.7	112.8
PHBV-*g*-PVP (2.54)	48.5	113.7
PHBV-*g*-PVP (6.98)	45.3	114.6
PHBV-*g*-PVP (9.01)	39.6	109.8
PHBV-*g*-PVP (10.48)	37.2	113.1

have the wide and very weak characteristic diffraction peaks, indicating their very low crystallization degrees. All the results are well in agreement with the DSC measurements.

Hydrophilicity and porosities

The surface hydrophilicity of samples, as characterized by the static water contact angle, is listed in Table II. The water contact angle decreases with increasing the graft% of drug loaded film, indicating that the surface hydrophilicity is enhanced through incorporation of the PVP side chains. The improved hydrophilic properties for PHBV-*g*-PVP films are due to the fact that the PVP is a polar macromolecule and has good solubility in water. All electrospun fiber mats exhibit the superhydrophobic surface with contact angles ranging from 109.8 to 113.7°, but there is not functional relationship between the contact angle and graft% of sample, indicating that the incorporation of PVP contents could not change the hydrophobic property of sample surface in essence. This is because that the superhydrophobic property was mainly created by the micro or nanostructure surface of electrospun fiber mat.[39]

Figure 4 shows that the water absorptivities of sample are functions of immersing time. It is can be observed that the drug loaded PHBV films have poor water retention, due to that the films cast from solution were compact and possessed very few porosities for water uptake. The similar phenomena also appeared in PHBV-*g*-PVP films with different graft%. All eletrospun fiber mats retain a small amount of water during the initial immersing time within 20 minutes because of their superhydrophobic surface. With the increase of immersing time after 20 minutes, a great amount of water could overcome the surface tension and then fill the pores of elctrospun fiber mat, causing the water absorptivity increased remarkably and thereafter almost level off after 60 minutes of immersing time. The saturated water absorptivity of elctrospun fiber mat is ranged from 200 to 220% and has not functional relationship with the graft% of sample (Table III). This is due to the fact that the high porosities are most important to create the high water absorptivity for elctrospun fiber mats and the water retained in pores is mainly physical adsorption because of capillary effect other than chemical effect.

The porous properties of electrospun fiber mats, as characterized by a capillary flow porometer, are summarized in Table III. It can be seen that both the average and maximum diameters of pore are very different but have no functional relationship with the graft% of samples. This is probably caused by the experimental errors due to the fact that the aggregation behavior of fiber during electrospinning is difficult to control. Drug loaded mats electrospun from different graft% polymer have the porosity percent from 66.5 to 68.5% in agreement with their saturated water absorptivity percent.

SEM morphologies

Figure 5 presents the SEM morphologies of drug loaded PHBV-*g*-PVP(6.98) film cast from solution before and after release test. Many small crystals, presumably tetracycline hydrochloride, are observed in the SEM photograph of freshly cast film [Fig. 5(a)], which are not present after completion of the release experiment [Fig. 5(b)]. The same morphological characteristics are also observed in the PHBV films and other PHBV-*g*-PVP films with different graft%. The enrichment of tetracycline hydrochloride

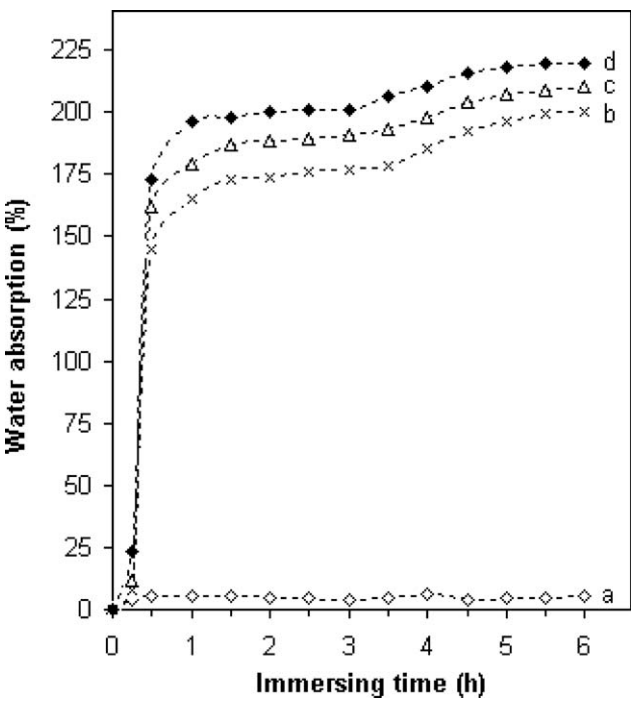


Figure 4 Water absorption of drug loaded vehicles versus immersing time: (a) PHBV film, (b) PHBV-*g*-PVP(6.98) electrospun fibers, (c) PHBV-*g*-PVP(10.48) electrospun fibers, (d) PHBV electrospun fibers.

TABLE III
Saturated Water Absorptivities and Porous Properties of Electrospun Fiber Mats

Substrate polymers	Saturated water absorptivities (%)	Maximum pore diameters (μm)	Minimum pore diameters (μm)	Average pore diameters (μm)	Porosity percent (%)
PHBV	220.16	8.62	2.14	4.72	68.67
PHBV-*g*-PVP (2.54)	206.65	6.56	1.62	2.67	66.58
PHBV-*g*-PVP (6.98)	200.82	5.83	1.55	2.43	66.26
PHBV-*g*-PVP (9.01)	218.73	7.81	1.94	3.94	67.63
PHBV-*g*-PVP (10.48)	210.43	7.02	1.82	3.25	67.21

on the film surface may be due to the fact that chloroform and methanol possesses the different boiling point and surface tension, causing chloroform evaporates before methanol thus the polymer solidifies in advance and then tetracycline hydrochloride crystals sequester at its surface.

It can be seen from the SEM micrograph [Fig. 6(a)] that the freshly electrospun fiber mats of PHBV g-PVP(6.98) exhibit the high porosities with the random array superfine fibers as expected. This is due to the fact that the formation of electrospun fiber results from "bending instability", making the travel path/trajectory of electrospinning jet/fiber completely random.[39]

All fibers are smooth with bead-free and there are no any visible tetracycline hydrochloride crystals appeared on the fiber surface. PHBV g-PVP(6.98) electrospun fibers after release experiment [Fig. 6(b)] do not change their surface morphology essentially in comparison with the freshly fibers [Fig. 6(a)]. The

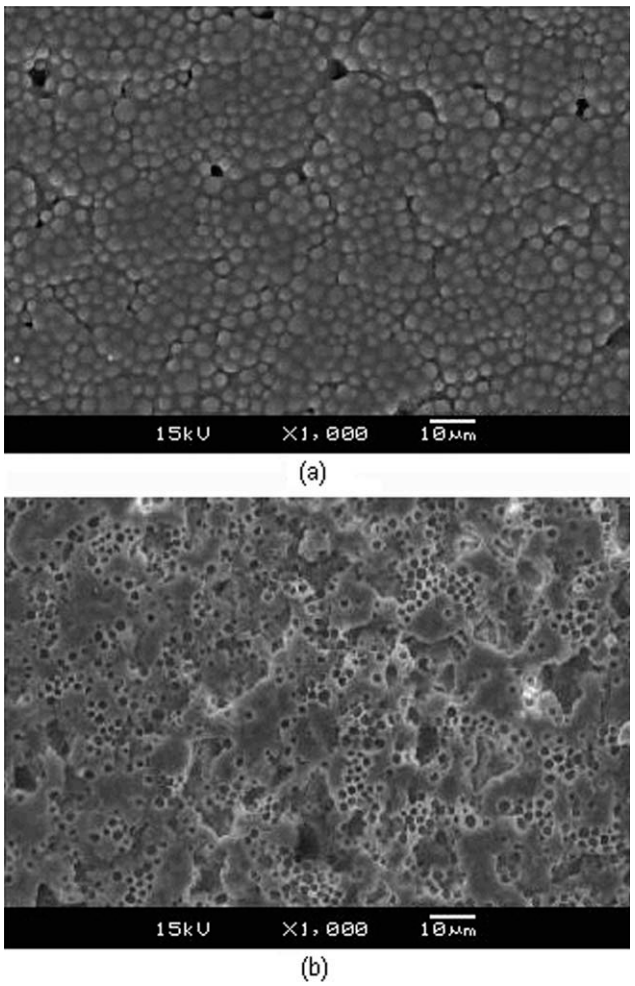


Figure 5 SEM surface micrographs of drug loaded PHBV-*g*-PVP(6.98) films before (a) and after (b) release experiment.

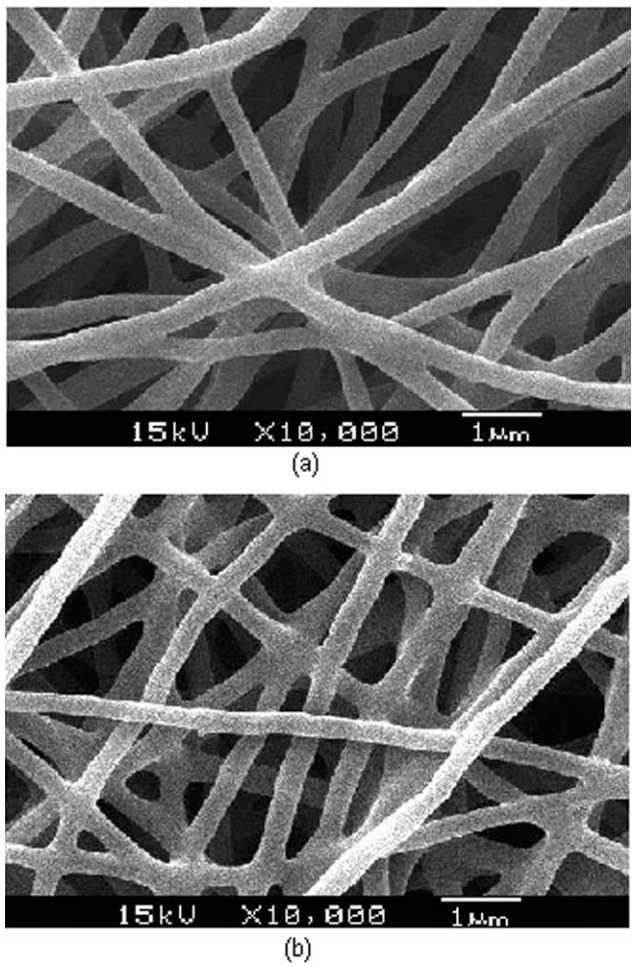


Figure 6 SEM micrographs of drug loaded PHBV-*g*-PVP(6.98) electrospun fiber mats before (a) and after (b) release experiment.

TABLE IV
Average Diameters and Standard Deviations of Polymer Electrospun Fibers

Substrate polymers	Average fiber diameters (μm)	Standard deviation of fiber diameters
PHBV	301	0.446
PHBV-*g*-PVP (2.54)	312	0.417
PHBV-*g*-PVP (6.98)	371	0.369
PHBV-*g*-PVP (9.01)	432	0.278
PHBV-*g*-PVP (10.48)	448	0.248

same morphological characteristics are also observed in the PHBV electrospun fibers and other PHBV-*g*-PVP electrospun fibers. The free of drug enrichment on the surface of electrospun fiber mats is probably because that the solvents evaporated very quickly during electrospinning process, thus the polymer and tetracycline hydrochloride could solidify from the solution almost in synchronal regardless the different volatility between chloroform and methanol.

The average diameters and standard deviations of electrospun fiber are summarized in Table IV. It can be seen that the fiber diameters are high polydisperse with the average from 300 to 450 nm. The average diameters of electrospun fibers increase, while their standard deviations decrease with increasing graft% of substrate polymers. This is maybe due to the fact that the viscosities of electrospinning solution increased with the increase in polymer graft% as revealed by Figure 1. The increased viscous resistance may bring negative effects on the course of stretching polymer fluid jet to thin under the high electric field. The decrease in distribution of fiber diameters is probably caused by that the increased viscosities of electrospinning solution may decrease the axisymmetric instability and whipping instability of polymer fluid jet after electrically charged.[39,40]

Drug release

The drug loading of films and electrospun fibers was spectrophotometrically analyzed by TU-1901 Spectrophotometer after extracting the tetracycline hydrochloride with PBS from the samples in chloroform and the results for all samples are almost at 5%, implying the almost 100% loading efficiency. It is due to the fact that the drug loaded samples were prepared with mild conditions and free of drug loss or denaturation. For this reason we decided to confine our experiments to 5% drug loading for all samples in the following release investigation.

The release profiles, reported as the accumulated percent tetracycline hydrochloride released from the cast films are shown in Figure 7. It can be seen that pure tetracycline hydrochloride enclosed in dialysis bag gave nearly 100% drug release within 10 h, but the drug loaded films only gave about the drug release from 30 to 40% over the same time period depend on the graft% of polymer substrate, exhibiting the excellent sustained release behavior. In general, the initial rates of release for many drug delivery systems are high during the first period, most likely due to the release of drug enriched on the sample surfaces.[31] The same behavior, defined as the initial burst release, is also presented in PHBV and PHBV-*g*-PVP drug loaded films during the first 20 h. This is due to the fact that all freshly cast films are enriched by many small tetracycline hydrochloride crystals as revealed by SEM [Fig. 5(a)]. When these drug particles on the films surface delivered completely after 20 h, the films would release at very low rate as shown in Figure 7. This is most likely due to the high crystallization degree of film samples, as revealed by DSC or WAXD, which limits the diffusion of aqueous environment into the polymer inner layers and consequently limits the desorption of the drug from the films. The times scale of our release experiments is too short to expect significant release of drug resulting from hydrolysis of PHBV and PHBV-*g*-PVP. We believe this is linked to the high crystallization degree of PHBV and PHBV-*g*-PVP films which inhibits release over short period. Long term release from PHBV and PHBV-*g*-PVP films via hydrolytic degradation and biodegradation is still under investigation. It is interesting that the drug release from PHBV-*g*-PVP films is increased with increasing the graft% of samples during the

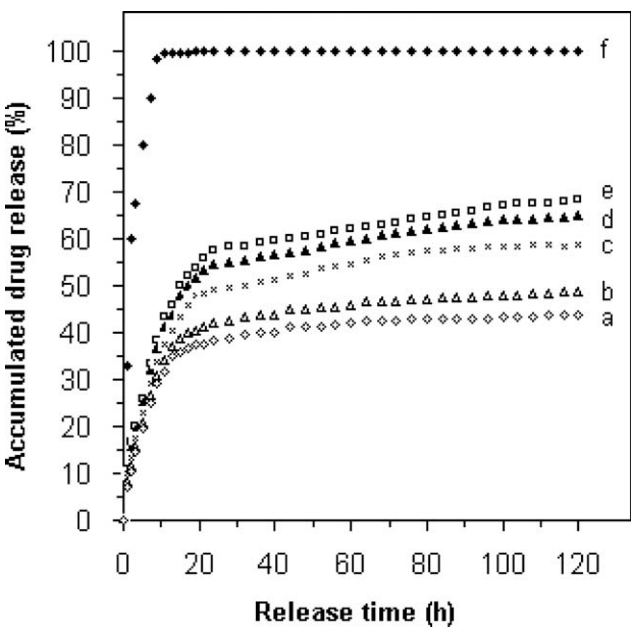


Figure 7 Accumulated release of tetracycline hydrochloride from cast films versus release time: (a) PHBV film, (b) PHBV-*g*-PVP(2.54) film, (c) PHBV-*g*-PVP(6.98) film, (d) PHBV-*g*-PVP(9.01) film, (e) PHBV-*g*-PVP(10.48) film, (f) pure tetracycline hydrochloride.

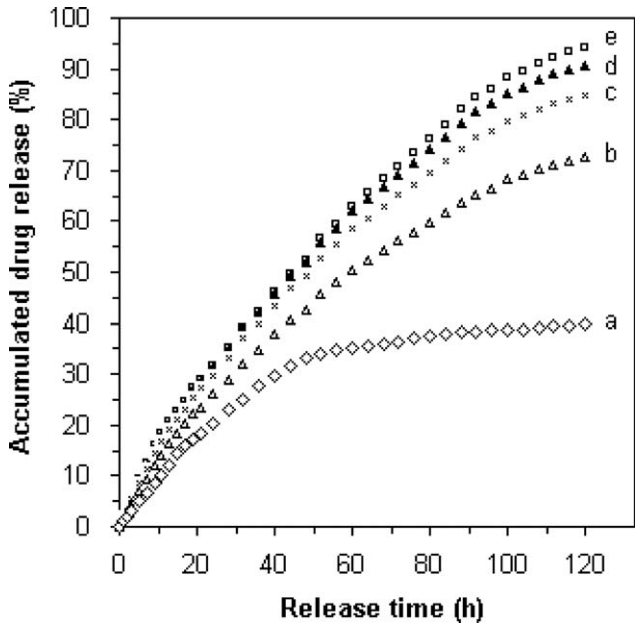


Figure 8 Accumulated release of tetracycline hydrochloride from electrospun fiber sheets versus release time: (a) PHBV electrospun fibers, (b) PHBV-*g*-PVP(2.54) electrospun fibers, (c) PHBV-*g*-PVP(6.98) electrospun fibers, (d) PHBV-*g*-PVP(9.01) electrospun fibers, (e) PHBV-*g*-PVP(10.48) electrospun fibers.

same period. PHBV-*g*-PVP(10.48) films deliver about 68.2% drug within 120 h, while PHBV films only deliver about 43.9% drug within the same period. This is probably because that the PHBV-*g*-PVP films with higher graft% have more imperfect crystallites as revealed by DSC and exhibit higher surface hydrophilicity as revealed by static water contact angle, thus enhancing the diffusion of aqueous environment into the polymer inner layers and the desorption of the drug from the films.

Figure 8 shows the release profiles of the accumulated percent drug from the electrospun fiber sheets. In general, the increase in diameters may decrease the specific surface area of electrospun fibers and result in decreased drug release. However the drug release from electrospun fiber sheets is increased with increasing the graft% of samples during the same period as shown in Figure 8. PHBV-*g*-PVP(10.48) electrospun fiber sheets deliver about 94.0% drug content within 120 h, while PHBV electrospun fiber sheets only deliver about 40.0% drug content over the same period. Moreover PHBV electrospun fiber sheets present a relative high drug release rate within the first about 50 h and thereafter give a very limited drug release. The PHBV electrospun fiber sheets give about 33.5% drug release within the first 50 h, but only give about 6.5% drug release during the later 70 h. This is probably caused by the high crystallization degree of PHBV electrospun fibers as revealed by DSC and WAXD. The higher drug release within the first period is mainly from amorphous region, whereas the limited drug release during the later period is mainly from crystalline region which limits the diffusion of aqueous environment into the polymer inner layers. However, all PHBV-*g*-PVP electrospun fibers give smooth and well-regulated drug release at a higher rate during the experiment period because of their low crystallization degree as revealed by DSC and WAXD. For comparative investigation, the drug release profiles from the electrospun fibers and cast films with PHBV, PHBV-*g*-PVP(2.54) and PHBV-*g*-PVP(10.48) substrate are also presented in Figure 9. It can be seen that all the electrospun fiber sheets give the approximately smooth drug release without any obviously initial burst release. This is due to the fact that there is not any drug particles presented on the surface of freshly electrospun fiber mats as observed in SEM micrographs [Fig. 6(a)]. In general, drug loaded electrospun fiber mats may give the higher release than the corresponding films because of their very higher surface area and polyporous morphology, which is very benefit for the diffusion of aqueous environment into the inner sheets and the drug from the fibers. Interestingly, all PHBV-*g*-PVP samples exhibit the expected release behavior, whereas PHBV electrospun fibers give a little low drug release than the corresponding cast films. A reasonable explanation lies in the fact that PHBV-*g*-PVP electrospun fibers presented very lower crystallization degrees than their cast films, whereas PHBV electrospun fibers presented the similar high

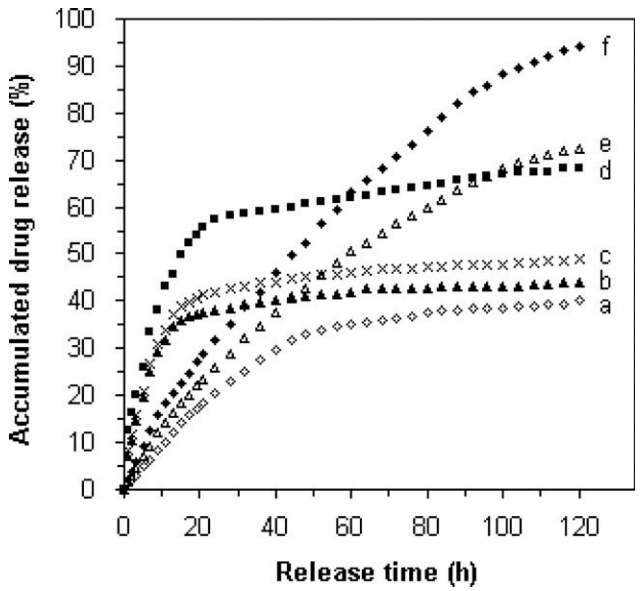


Figure 9 Comparative analysis on release behavior of drug loaded films and electrospun fiber sheets: (a) PHBV electrospun fibers, (b) PHBV film, (c) PHBV-*g*-PVP(2.54) film, (d) PHBV-*g*-PVP(10.48) film, (e) PHBV-*g*-PVP(2.54) electrospun fibers, (f) PHBV-*g*-PVP(10.48) electrospun fibers.

crystallization but their crystallites were a little more perfect and uniform in comparasion with corresponding cast films as revealed by DSC and WAXD. Taking into account the fact that all PHBV-*g*-PVP electrospun fibers exhibited the similar morphological properties, for example surface hydrophobicity, porosity, all the result data give us confidence that the drug release behavior of electrospun fibers is affected by their crystallization properties greatly in present study.

CONCLUSIONS

Release behavior of tetracycline hydrochloride from commonly cast films and electrospun fibers prepared with PHBV and PHBV-*g*-PVP substrates was studied. It was found that all drug loaded films exhibited the initial burst release because of drug enriched on the films surface as revealed by SEM, but all drug loaded electrospun fibers were absent from drug enriched on their surface and gave the approximately smooth release. DSC and WAXD investigation showed that all drug loaded films and PHBV electrospun fibers presented the similar high crystallization degree, but PHBV-*g*-PVP electrospun fibers presented the very low crystallization degree. With the increase of graft% of substrate polymers, drug loaded films gave higher release because of their more imperfect crystallites as revealed by DSC and improved surface hydrophilicities as revealed by static water contact angle. The average diameters of electrospun fibers increased and their standard deviation decreased with graft% of substrate polymers increased, but the change of average diameters and their distributions made less susceptible to the hydrophobicity and porosities of electrospun fiber sheets in present study. All PHBV-*g*-PVP electrospun fibers presented higher drug release because of their higher specific surface area and much lower crystallization degree than the corresponding drug loaded films, whereas PHBV electrospun fibers presented a little lower drug release than the corresponding drug loaded films. Furthermore, the drug released from PHBV-*g*-PVP electrospun fibers increased with the increase in graft% of PHBV-*g*-PVP substrates. All the result data showed that the drug release behavior of PHBV and PHBV-*g*-PVP electrospun fibers was affected by their crystallization properties greatly in present study.

References

1. Liu, X. W.; Wang, H. H.; Chen, J. Y.; Li, X. T.; Chen, G. Q. Biochem Eng J 2009, 43, 72.
2. Chen, C. W.; Don, T. M.; Yen, H. F. Process Biochem 2006, 41, 2289.
3. Poirier, Y.; Dennis, D. E.; Klomparens, K.; Somerville, C. Science 1992, 256, 520.
4. Wróbel, M.; Zebrowski, J.; Szopa, J. J Biotechnol 2004, 107, 41.
5. Ferreira, B. M. P.; Pinheiro, L. M. P.; Ferreira, M. J.; Duek, E. A. R. Mat Sci Eng C 2009, 29, 806.
6. Guerrouani, N.; Baldo A.; Maarouf, T.; Belu, A. M.; Mas, A. J Fluorine Chem 2007, 128, 925.
7. Fei, B.; Chen, C.; Chen, S.; Peng, S. W.; Zhuang, Y. G.; An, Y. X.; Dong, L. S. Polym Int 2004, 53, 937.
8. Tan, S. M.; Ismail, J.; Kummerlöwe, C.; Kammer, H. W. J Appl Polym Sci 2006, 101, 2776.
9. Wang, X.; Chen, Z.; Chen, X.; Pan, J.; Xu, K. J Appl Polym Sci 2010, 117, 838.
10. Ma, P. M.; Wang, R. Y.; Wang, S. F.; Zhang, Y.; Zhang, Y. X.; Hristova, D. J Appl Polym Sci 2008, 108, 1770.
11. Cabedo, L.; Plackett, D.; Giménez, E.; Lagarón, J. M. J Appl Polym Sci 2009, 112, 3669.
12. Ke, Y.; Wang, Y.; Ren, L.; Wu, G.; Xue, W. J Appl Polym Sci 2010, 118, 390.
13. Ke, Y.; Wang, Y.; Ren, L.; Lu, L.; Wu, G.; Chen, X.; Chen, J. J Appl Polym Sci 2007, 104, 4088.
14. Lao, H. K.; Renard, E.; Langlois, V.; Vallée-Rehel, K.; Linossier, I. J Appl Polym Sci 2010, 116, 288.
15. Wang, W.; Zhang, Y.; Chen, Y. M. Iran Polym J 2007, 16, 195.
16. Wang, W.; Zhang, Y.; Zhu M. F.; Chen, Y. M. J Appl Polym Sci 2008, 109, 1699.
17. Fei, B.; Chen, C.; Chen, S.; Peng, S. W; Zhuang, Y. G.; An, Y. X.; Dong, L. S. Polym Int 2004, 53, 937.
18. Siepmann, F.; Eckart, K.; Maschke, A.; Kolter, K.; Siepmann, J. J Controlled Release 2010, 141, 216.
19. Yang, Q.; Chung, T. S.; Weber, M. J Membr Sci 2009, 326, 322.
20. Neelakandan, C.; Kyu, T. Polymer 2009, 50, 2885.
21. Kim, Y. J.; Bae, H. I.; Kwon, O. K.; Choi, M. S. Int J Biol Macromol 2009, 45, 65.
22. MacNeil, S. Nature 2007, 445, 874.
23. Zhang, X. H.; Reagan, M. R.; Kaplan, D. L. Adv Drug Delivery Rev 2009, 61, 988.
24. Moroni, L.; Schotel, R.; Hamann, D.; De Wijn, J. R.; Van Blitterswijk, C. A. Adv Funct Mater 2008, 18, 53.
25. Zhou, Y. S.; Yang, D. Z.; Chen, X. M.; Xu, Q.; Lu, F. M.; Nie, J. Biomacromolecules 2008, 9, 349.
26. Chew, S. Y.; Mi, R.; Hoke, A.; Leong, K. W. Adv Funct Mater 2007, 17, 1288.
27. Chew, S. Y.; Wen, J.; Yim, E. K. F.; Leong, K. W. Biomacromolecules 2005, 6, 2017.
28. Wang, M.; Wang, L. G.; Huang, Y. J Appl Polym Sci 2007, 106, 2177.
29. Qi, H. X.; Hu, P.; Xu, J.; Wang, A. J. Biomacromolecules 2006, 7, 2327.
30. Gurselt, I.; Yagmurlu, F.; Korkusuz, F.; Hasirci, V. J Microencapsul 2002, 19, 153.
31. Kenawy, E. R.; Bowlin, G. L; Mansfield, K.; Layman, J.; Simpson, D. G.; Sanders, E. H.; Wneka, G. E. J Controlled Release 2002, 81, 57.
32. Son, W. K.; Youk, J. H.; Lee, T. S.; Park, W. H. Polymer 2004, 45, 2959.
33. Deitzel, J. M.; Deitzel, J.; Harris, D.; Beck Tan, N. C. Polymer 2001, 42, 261.
34. Deitzel, J. M.; Kleinmeyer, J. D.; Hirvonen, J. K.; Beck Tan, N.C. Polymer 2001, 42, 8163.
35. Zong, X.; Ran, S; Fang, D.; Hsiao, B. S.; Chu, B. Polymer 2003, 44, 4959.
36. Stephens Jean, S.; Chase, D. B.; Rabolt John, F. Macromolecules 2004, 37, 877.
37. Zhang, L. L.; Goh, S. H.; Lee, S. Y.; Hee, G. R. Polymer 2000, 41, 1429.
38. Li, J.; Lai, M. F.; Liu, J. J. J Appl Polym Sci 2005, 98, 1427.
39. Zhu, M. F.; Zuo, W. W.; Yu, H.; Yang, W.; Chen, Y. M. J Mater Sci 2006, 41, 3793.
40. Shin, Y. M.; Hohman, M. M.; Brenner, M. P.; Rutledge, G. C. Polymer 2001, 42, 9955.