Surface Modification of PHBV Films with Different Functional Groups: Thermal Properties and *In Vitro* Degradation

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ABSTRACT: Polyacrylamide was photografted on solution-cast poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (PHBV) films (amide-PHBV), on which amide groups were transformed into amine groups through Hofmann degradation reaction (amine-PHBV), followed by collagen coupling reaction to prepare collagen-modified PHBV (collagen-PHBV). Amide-, amine-, and collagen-PHBV had higher water absorption and *d*-spacing values than PHBV, and melting temperatures and enthalpies decreased in the order of collagen-PHBV < amine-PHBV < amide-PHBV < PHBV. Thermal decomposition kinetics of PHBV component in the films has been investigated by means of nonisothermal thermogravimetric and derivative thermogravimetric studies. Applying the Avrami-Erofeev equation with index of 2/5 as the probable kinetic function, the suitable activation energy was calculated by the Friedman method through linear fitting (correlation coefficient > 0.98). The activation energy of PHBV was lower than that of amide-PHBV but higher than that of amine- and collagen-PHBV. Being incubated in phosphate-buffered saline at 37°C, the modified PHBV films showed more weight loss than PHBV during 360 days; however, pH of degradation fluids was nearly neutral as the initial pH was recorded at 7.2. The modified PHBV films with different functional groups may provide an improved biodegradation rate for various cytocompatible biomaterials constructs. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 390–398, 2010

Key words: PHBV; surface modification; thermal properties; degradation; cytocompatible

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are natural biodegradable thermoplastics that are accumulated by a wide variety of microorganisms as a unique intracellular storage of carbon, among which poly(3-hydroxybutyric acid) (PHB) and poly(3-hydroxybutyric acid-*co*-3-hydroxyvaleric acid) (PHBV) are two main products.[1] Their inherent biodegradability, nontoxicity, piezoelectricity, and biocompatibility make them suitable for biomaterials in tissue engineering.[2–4]

Once biomaterial is implanted *in vivo*, its surface is initially exposed to body fluids. Surface properties, such as chemical functionality, roughness and morphology, electrostatic interaction, surface energy, and biological cues of the surface, influence cell–biomaterial interaction directly.[5–8] As this interaction is strongly dependent on chemical characteristics of polymeric surface, factors that influence cell proliferation, migration, and differentiation have been incorporated into the design of biomaterials' surface.

PHBV is hydrophobic polyester without bioactive fragments, which might limit its *in vivo* application. To achieve positive cell–biomaterial interaction, functional groups or biomacromolecules, including carboxyl, amine, methyl methacrylic, *N*-vinylpyrrolidone, glucosamine, and alkaline phosphatase, have been immobilized onto PHAs for direct tuning of surface without altering their bulk properties.[9–14] Our laboratory has obtained surface-modified PHBV films with amide, amine, or collagen fragments, which have good cytocompatibility with sheep chondrocytes and bone marrow stromal cells.[15–17]

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PHAs degrade *in vitro* and *in vivo* through hydrolysis, enzymolysis, or by microorganisms.[18–23] The mode and rate of degradation will influence biomaterials' service life, mechanical properties, and the response of the biological system toward them, so that optimize biodegradability is essential for biomaterials in medical application. PHAs blends with cellulose acetate butyrate, wollastonite, or poly(L-lactide) have been studied, with the aim to enhance hydrolytic or enzymatic biodegradation.[24–26] Biodegradability of surface-modified PHAs membranes or films has been attracting much attention in recent years. For example, PHB films with a functionalized surface have been prepared by alkali treatment so as to enhance hydrophilicity and biodegradability in soil.[27] Acrylic acid has been linked to PHB and PHBV membranes through radiation-induced graft polymerization, which has increased the enzymatic degradability. However, chitosan or chitooligosaccharide graft on acrylic acid-modified surface via esterification has lowered the degradable capacity.[28,29] Till now, little attention has been paid to the abiotic hydrolysis of PHAs grafts, perhaps owing to their very slow rate compared with enzymatic biodegradation.

In this work, we report thermal properties and *in vitro* degradation of the amide-, amine-, and collagen-modified PHBV films, with a final aim to construct degradable and cytocompatible biomaterials. Thermal properties were characterized by differential scanning calorimetry (DSC), wide-angle X-ray diffraction (WAXD), thermogravimetric (TG), and derivative thermogravimetric (DTG) analyses. The PHBV and modified PHBV films were immersed in phosphate-buffered saline (PBS) at 37°C for up to 360 days, and weight loss of the films was used to monitor *in vitro* degradability. The factors influencing the *in vitro* weight loss were also discussed.

MATERIALS AND METHODS

Materials

PHBV with 8% hydroxyvalerate (HV) content (number-average molecular weight = 1.85×10^5, polydispersity index = 2.2) was purchased from Aldrich Chemical, USA. Acetone, chloroform, sodium hydroxide, and sodium hypochlorite were purchased from Tianjin Chemical Reagent No. 1 Plant, China. Benzophenone (BP) and acrylamide (AM) were obtained from Shanghai Runjie Chemical, China. Formaldehyde was obtained from Shanghai Donghong Chemical, China. Acid-soluble bovine tendon collagen Type I (5.8 mg/mL) was supplied by Trauer Biotechnology, China. All reagents were analytical grade.

Preparation of the PHBV and modified PHBV films

According to our previous studies, PHBV films were prepared by casting PHBV solution onto a glass plate at a concentration of 60 mg/mL with chloroform as solvent. After being stationed at 25°C for 2 days, the PHBV films with dimension of 20×20 mm^2 were immersed into 5 wt % BP solutions with acetone as solvent for 24 h, dried, and dipped into 7 wt % aqueous acrylamide. The film-containing solution was then irradiated for 1 h with a Philips (the Netherlands) 400S high-pressure mercury lamp under a nitrogen atmosphere. After irradiation, the resulting amide-modified PHBV films (amide-PHBV) were purified by Soxhlet extracting with acetone and rinsed with deionized water. Then, a mixture of 90 mL sodium hydroxide (10%) and 53.8 g sodium hypochlorite (12%) was stirred vigorously and thermostated at 0°C. Twenty milliliters of mixture was applied to amide-PHBV in an ice bath for 30 min to obtain amine-modified PHBV films (amine-PHBV). Before preparing collagen-modified PHBV films (collagen-PHBV), amine-PHBV was treated with formaldehyde solution (37%) at 25°C for 4 h. The films were immersed into collagen acid solution for 24 h, followed by rinse and freeze-drying treatment.

Characterization

Weight loss of the films during *in vitro* incubation was studied according to the following procedure. The PHBV and modified PHBV films with average weight of 50 mg were dipped in a cap-sealed tube containing PBS (0.1*M*, pH 7.2). The volume of PBS (mL) to film mass (g) was 100 : 1. All films were incubated without agitation at 37°C during 360 days. At prescheduled time, films were washed, vacuum-dried, and weighed on a Sartorius (Germany) BS 223S electrobalance to assess weight loss as a function of incubation time. pH value of incubation fluid was recorded using a Jingke (China) PHS-3C pH meter. The results were the average of five replicates.

Water swelling percentage (WSP) was measured by gravimetric measurement. The preweighed (M_0) dry films were dipped in deionized water for 1 h. At the end of immersion, the swollen samples were removed from water, tapped with filter paper to dry the surface, and then weighed (M_1). WSP was calculated by the following formula:

$$\mathrm{WSP} = (M_1 - M_0)/M_0 \quad (1)$$

WSP was the average of six replicates.

DSC studies were performed on a Netzsch (Germany) 204 F1 DSC instrument under a nitrogen atmosphere. The films were heated from −50 to

200°C at a rate of 10°C/min. TG analyses were carried out using a Netzsch (Germany) 209 F1 thermogravimetric analyzer. TG curve was recorded from room temperature to 800°C under a nitrogen atmosphere at heating rates of 5, 10, and 15°C/min, respectively. WAXD patterns were recorded from 5° to 70° 2Θ in steps of 0.033° on a Philips (the Netherlands) X'Pert PRo X-ray diffractometer. The applied potential was 40 kV, and the corresponding current was 40 mA. The measuring time was 15.24 s per step.

RESULTS AND DISCUSSION

According to our previous studies, the illustrative structure of the PHBV and modified PHBV films is shown in Figure 1. Solution-cast PHBV films were photografted with polyacrylamide on the surface, where the amide groups were partially converted into highly reactive amine groups through Hofmann degradation reaction. The formation of collagen-PHBV involved hydroxylamine groups as an intermediate when formaldehyde was applied to amine-PHBV. The subsequent collagen coupling was achieved via condensation between the hydroxylamine groups and primary amine groups of collagen. X-ray photoelectron spectroscopy results illustrated that O/C ratio was 0.35, 0.44, 0.26, and 0.27, respectively, for PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV, and N/C ratio was 0, 0.06, 0.23, and 0.22 correspondingly. Fourier transformed infrared spectroscopy spectra with an attenuated total reflectance were used to characterize surface functionality. Amide-PHBV had the absorption peaks at 3340 and 3190 cm^{-1}, being assigned to asymmetric and symmetric stretching vibration of N—H, respectively. The bands at 1662 and 1610 cm^{-1} were attributed to the strong C=O stretching vibration (primary amide) and the medium N—H bending vibration (primary amide), respectively. The only difference between amine-PHBV and amide-PHBV was that the band at 1662 cm^{-1} broadened after Hofmann degradation reaction. Collagen-PHBV showed amide characteristic bands at 1653 and 1551 cm^{-1} and N—H stretching band at 3326 cm^{-1}, which belonged to a new secondary amide bond. The above results confirmed that amide-, amine-, and collagen-PHBV had been successfully achieved.[17]

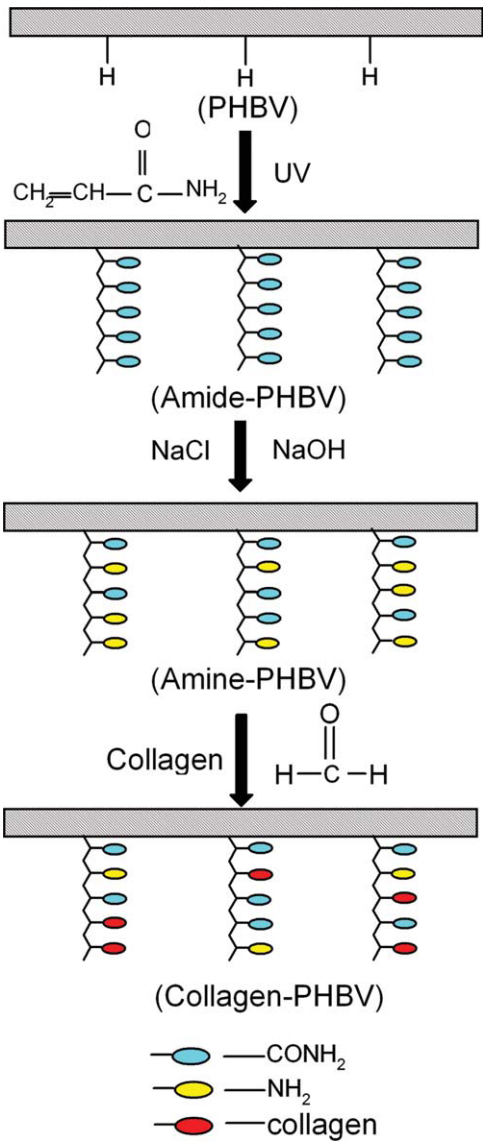

Figure 1 Schematic structure of PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Water absorption

Figure 2 presented wettability of the PHBV and modified PHBV films. PHBV was quite hydrophobic and did not absorb water during 1 h of immersion. WSP of amide-PHBV was 4.79% due to hydrophilic amide groups being introduced on the surface. It reached the highest value of 50.49% for amine-PHBV and then decreased to 27.39% for collagen-PHBV. On amide-PHBV macromolecular chains, primary amide groups attract water molecules strongly through H-bonding. When primary amide groups were partially transferred into amine groups, the H-bonding combination enhanced owing to the increasing density of electron cloud of nitrogen, so that water absorption of amine-PHBV improved significantly. Once the amine groups had been transferred into secondary amide groups after immobilization with collagen, WSP of collagen-PHBV decreased.

Melting properties

Melting behaviors of the PHBV and modified PHBV films were studied by DSC, as shown in Figure 3.

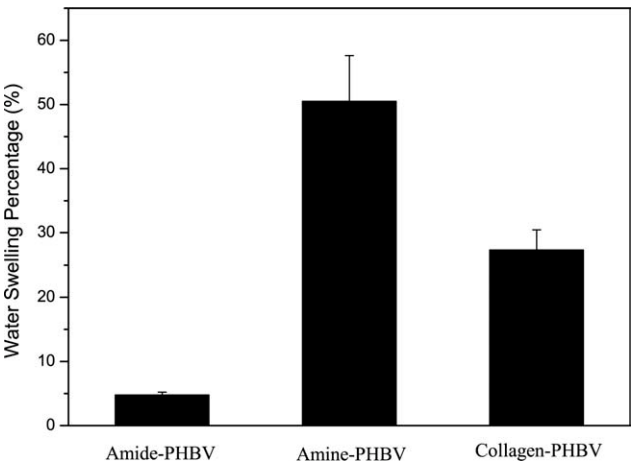

Figure 2 Water swelling percentage of amide-PHBV, amine-PHBV, and collagen-PHBV.

PHBV exhibited two melting endotherm peaks at 152.27 and 166.27°C, respectively. The low-temperature endotherm is usually considered to be the true melting behavior of the as-formed crystals, whereas the high-temperature endotherm represents the melting of material that has undergone annealing upon heating.[30] The low and high melting temperatures and total enthalpies decreased in the order of collagen-PHBV < amine-PHBV < amide-PHBV. It was believed that the decrease of melting temperatures suggested the decreasing lamellar thickness of the films, whereas the decrease of enthalpies suggested the decreasing relative crystallinity of the films.[31,32] In addition, special melting endotherms appeared in the modified PHBV films, with the peak temperature at 72.97, 68.92, and 69.15°C for amide-, amine-, and collagen-PHBV, respectively. We presumed that these special endotherms might be the melting of imperfect PHBV crystals, and PHBV crystals became less perfect after surface modification.

Crystallization properties

Figure 4 illustrated WAXD spectra of the PHBV and modified PHBV films. Only PHB reflection could be found for PHBV because hydroxybutyrate and hydroxyvalerate units cocrystallized within the PHB subcell at HV contents below 30 mol %.[33] Compared with PHBV, *d*-spacing value of amide-PHBV increased, especially (020) and (110) faces, indicating that the grafted PAM chains had an impact on the crystal structure of PHBV. Among the three modified PHBV films, there was no significant difference in *d*-spacing value. However, the intensities of the peaks decreased following the order: collagen-PHBV < amine-PHBV < amide-PHBV. It might suggest that the Hofmann degradation and collagen coupling reaction did not change the crystal structure of PHBV in amide-PHBV evidently.

Thermal decomposition

Weight loss for pyrolysis of the films as a function of temperature is shown in Figure 5. At the heating rate of 10°C/min, PHBV showed a one-step thermal decomposition within 263.2–284.5°C. Usually, PHB and PHBV are known to degrade thermally in one step by a random chain scission process, which includes a six-member transition producing unsaturation at the chain ends. This type of scission reaction might cause a gradual decrease in molecular weight.[34,35] The thermal decomposition of amide-

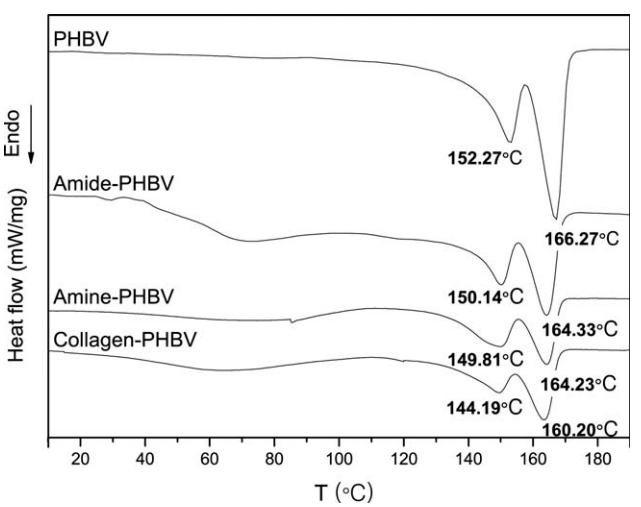

Figure 3 DSC thermograms of PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV, recorded at the heating rate of 10°C/min.

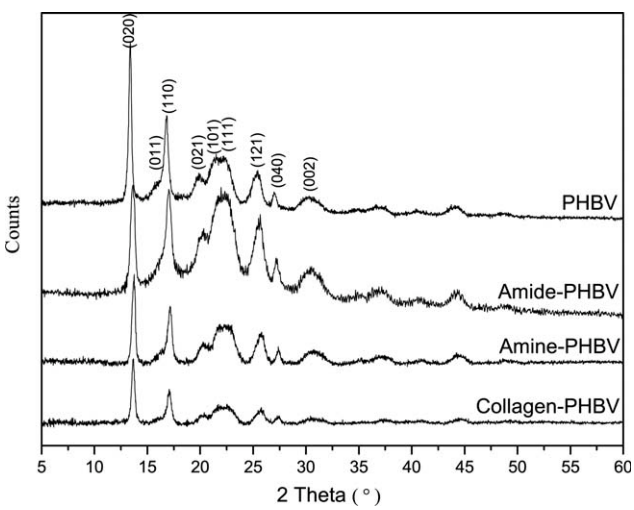

Figure 4 WAXD profiles of PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV.

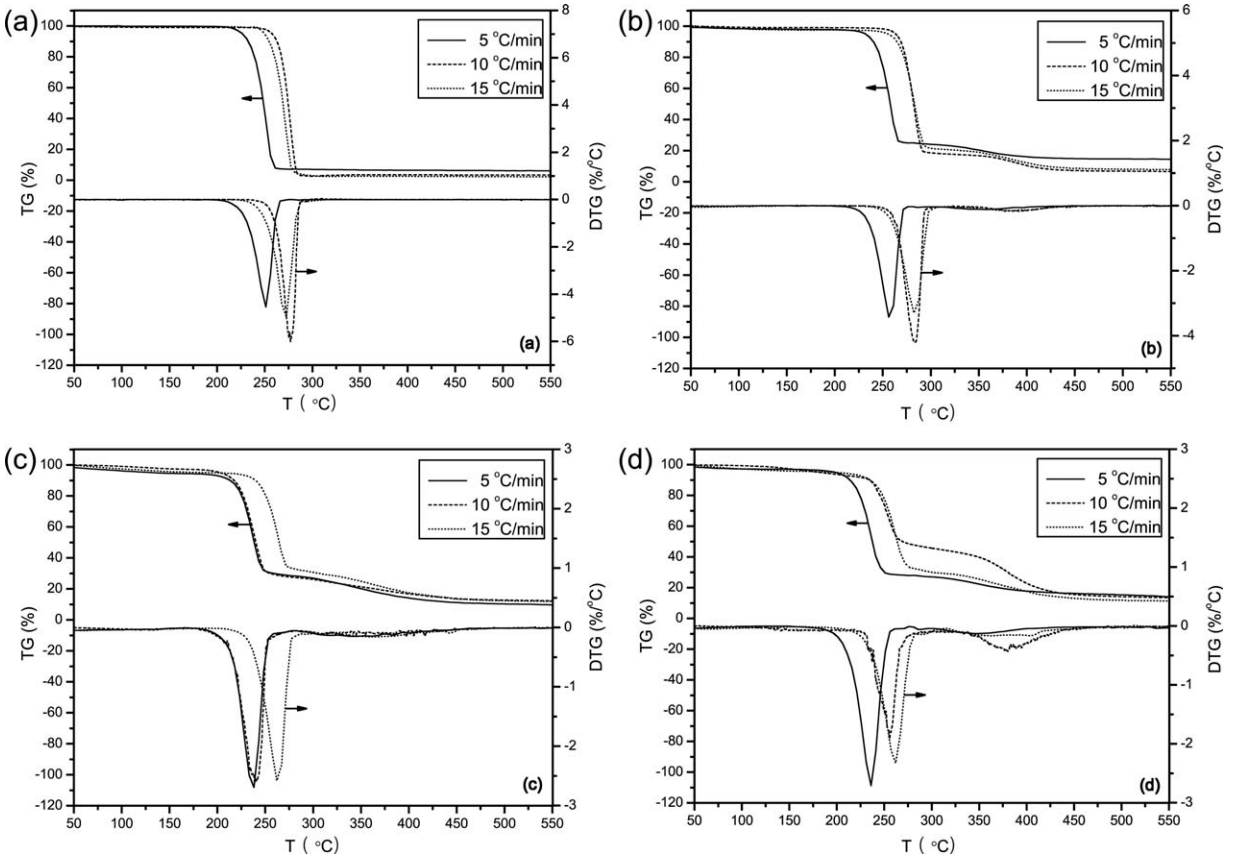

Figure 5 TG-DTG curves for thermal decomposition of the films: (a) PHBV; (b) amide-PHBV; (c) amine-PHBV; and (d) collagen-PHBV, recorded at the heating rates of 5, 10, and 15°C/min.

PHBV presented two main stages. It underwent massive thermal degradation with about 78% weight loss below 300°C, mainly due to the degradation of PHBV; within 370–430°C, the weight loss might be the decomposition of neighboring acrylamide groups to nitrile and final carbonization of PAM. There was no more distinct weight loss above 430°C. Amine-PHBV showed weight loss from the beginning because of its strong water absorption. In the range of 223.3–247.8°C, nearly 67% of total weight loss was observed, which might be the thermal decomposition of PHBV. The weight loss continuously increased in linear below 440°C, perhaps owing to the main thermal degradation of polyvinylamine and unreacted PAM.[36] There was not any distinct weight loss above 440°C. Collagen-PHBV showed about 8% weight loss below 140°C. Usually, collagen denatures above 40°C, where H-bonds and hydrophobic bonds collapse and the triple-helix fails. When the temperature increases up to 60°C, some covalent bonds collapse and collagen starts thermal degradation. Over 125°C, amino acid residues destroy with release of ammonia and water.[37] Within 239.2–266.3°C PHBV decomposed quickly with weight loss of 42%, and the remaining polyvinylamine degraded below 360°C. The second distinct weight loss (nearly 24% weight loss) fell in the range of 360–430°C, and no more significant weight loss was observed above 430°C. The residual weight percentage at 800°C was 3.20, 6.10, 11.20, and 10.20% for PHBV, amide-, amine-, and collagen-PHBV, respectively.

Thermal decomposition temperatures at 5% (T_5), 10% (T_{10}), and 50% (T_{50}) weight loss of the films are listed in Table I. At the same heating rate, amide-PHBV presented an increasing T_5, T_{10}, and T_{50} compared with PHBV, perhaps due to the fact that acrylamide may hinder the formation of six-membered ring ester during thermal degradation through decreasing the inductive effect of the neighboring β-methylene groups to the ester oxygen.[38] The values of amine- or collagen-PHBV were even less than those of PHBV, suggesting the lower thermal stability of polyvinylamine and collagen macromolecules.

DTG curves of the films are also shown in Figure 5. At the heating rate of 10°C/min, the thermal degradation of PHBV proceeded by a one-step process with a peak decomposition temperature at 277.3°C. There were two peak decomposition temperatures for the modified PHBV films, the first temperature

TABLE I
Thermal Decomposition Temperatures of the PHBV and Modified PHBV Films

Sample	Heating rate (°C/min)	T_5[a] (°C)	T_{10}[b] (°C)	T_{50}[c] (°C)	T_{p1}[d] (°C)	T_o[e] (°C)	T_e[f] (°C)	$T_e - T_o$ (°C)
PHBV	5	226.9	232.5	248.3	251.5	236.7	258.4	21.7
	10	257.9	263.0	274.7	277.3	263.2	284.5	21.3
	15	249.8	260.7	269.7	273.1	257.5	279.3	21.8
Amide-PHBV	5	232.0	239.6	257.9	255.8	239.6	264.9	25.3
	10	263.4	268.9	282.1	283.4	269.5	291.3	21.8
	15	255.4	265.8	283.3	282.1	266.1	293.2	27.1
Amine-PHBV	5	143.5	213.5	239.1	237.6	219.8	244.5	24.7
	10	203.2	217.5	240.3	239.8	223.3	247.8	24.5
	15	197.5	239.7	264.8	262.2	242.0	272.1	30.1
Collagen-PHBV	5	198.5	213.9	237.5	235.9	215.3	244.9	29.6
	10	182.6	234.4	269.5	255.8	239.2	266.3	27.1
	15	197.9	235.0	263.9	261.9	237.7	271.3	33.6

[a] Thermal decomposition temperature at 5% weight loss of the films.
[b] Thermal decomposition temperature at 10% weight loss of the films.
[c] Thermal decomposition temperature at 50% weight loss of the films.
[d] The first peak temperature in DTG of the films.
[e] Onset thermal decomposition temperature of the first peak in DTG of the films.
[f] End thermal decomposition temperature of the first peak in DTG of the films.

(T_{p1}) and the second temperature (T_{p2}). Amide-PHBV had T_{p1} at 283.4°C, higher than that of PHBV. Because the weight loss at this stage is mainly associated with the ester cleavage of the PHBV component by elimination reaction, this retarded degradation might suggest certain bonding between PHBV and PAM. T_{p2} at 384.6°C of amide-PHBV was believed to thermal degradation of PAM. Amine-PHBV presented T_{p1} at 239.8°C, much lower than the thermal decomposition temperature of PHBV. It was supposed that PHBV chains might be scissored during Hofmann degradation reaction with a decreasing molecular weight, so that thermal decomposition temperature decreased markedly. Collagen-PHBV showed T_{p1} and T_{p2} at 255.8 and 382.2°C, respectively, confirming two noticeable stages of weight loss. It could be seen from Table I that T_{p1} showed an increasing tendency as the heating rate increased, probably due to temperature-hysteresis effect.

Onset decomposition temperature (T_o) and end decomposition temperature (T_e) of the first peak in DTG curves that might be the thermal decomposition of PHBV component are summarized in Table I. T_o and T_e of amide-PHBV increased slightly compared with those of PHBV. However, amine- and collagen-PHBV showed lower values than PHBV. We presumed that T_o and T_e were the decomposition of PHBV with low and high molecular weight, respectively. Amide-PHBV might form a branching or/and crosslinking structure because of chain transfer reaction of PHBV or PAM during UV polymerization, so that T_o and T_e increased. In the following Hofmann degradation reaction, PHBV chains might be scissored by alkali; therefore, T_o and T_e of amine-PHBV decreased evidently. Collagen macromolecules had a hysteresis effect on PHBV chains' movement. As the heating rate increased, there was an increasing tendency for T_o and T_e of the films. It is known that polymer chains move rapidly and can be scissored upon heating. Once relaxation time of polymer chains cannot follow observing time when the heating rate increases, the thermal decomposition temperature may shift to higher value. In addition, the difference between T_o and T_e at the same heating rate increased following the order: collagen-PHBV > amine-PHBV > amide-PHBV > PHBV, which might be associated with functional groups being introduced and molecule weight and its distribution of PHBV.

Table II illustrates kinetic parameters of thermal decomposition of PHBV component in the films, being calculated according to TG and DTG curves. Usually, thermal decomposition under nonisothermal condition could be expressed by the general equation[39]:

$$\frac{d\alpha}{dT} = (A/\beta) e^{-E/RT} f(\alpha), \tag{2}$$

where the variable α is degree of conversion, β is heating rate, A is preexponential factor, E is the activation energy, R is the gas constant, T is temperature, and $f(\alpha)$ is the reaction model. Knowing that the Avrami-Erofeev with index (n) of 2/5 is available to represent the thermal decomposition of PHBV within 15–83% weight loss,[40] its differential equation [eq. (3)] was used to understand the random nucleation and growth mechanism.

TABLE II
Kinetic Parameters of Thermal Decomposition of PHBV Component in the Films at Three Heating Rates

Sample	Heating rate (°C/min)	E (KJ/mol)	ln A (min^{-1})	Correlation coefficient	Variance
PHBV	5	123.15	29.83	0.9981	0.0006
	10	133.61	31.06	0.9982	0.0006
	15	122.78	28.76	0.9975	0.0005
Amide-PHBV	5	154.34	36.39	0.9945	0.0074
	10	161.99	36.64	0.9916	0.0048
	15	151.30	34.06	0.9965	0.0039
Amine-PHBV	5	99.27	24.51	0.9903	0.0047
	10	97.89	24.15	0.9803	0.0060
	15	102.66	24.12	0.9848	0.0077
Collagen-PHBV	5	89.47	22.17	0.9998	0.0001
	10	96.73	22.67	0.9860	0.0031
	15	95.90	22.50	0.9987	0.0009

$$f(\alpha) = 2.5(1-\alpha)[-\ln(1-\alpha)]^{-3/5}. \quad (3)$$

The suitable E and ln A can be calculated applying the Friedman method. Based on eq. (2), a plot of $\ln(d\alpha/dT)$ vs. $1/T$, within 15–80% of the weight loss of the first distinct decomposition stage, was drawn. E and ln A were then estimated by the linear fitting (correlation coefficient > 0.98), and shown in Table II. The values of PHBV were lower than those of amide-PHBV but higher than those of amine- and collagen-PHBV. It was believed that the films with higher E value presented better thermal stability. However, the films with lower E value possessed more rapid decomposition speed.

In vitro weight loss

The rate and extent of degradability of biomaterials is critical for their assigned function. For example, biomaterial as fracture fixation is needed for a limited duration, where the ideal rate of degradation should not exceed the rate of bone formation. A long-term study is required to illustrate the degradation properties.

As shown in Figure 6, PHBV weight did not change significantly during the initial 90 days. A decrease was observed from 120 days onward with a final weight loss of 1.8%. No extensive weight loss was found even at 550 days of incubation (data not shown), confirming the slow degradation of PHBV. The results were in accordance with the previous degradation studies in PBS or neutral deionized water, which proved that PHB or PHBV underwent a simple hydrolytic degradation with a random chain cleavage of ester bonds along the main chains. Majid et al. have suggested that weight loss of PHB film in buffer solution at 37°C followed surface erosion mechanism by a zero-order pattern.[41–44] The modified PHBV films had a higher weight loss than PHBV. Weight loss of amide-PHBV increased with time before 90 days of incubation and then reached a plateau between 5 and 7.5% with the maximum value at 240 days. Amine-PHBV exhibited a steep increase in weight loss during the first 50 days and reached the maximum weight loss of 12.5%. During the rest 310 days, weight loss changed irregularly. Collagen-PHBV showed a gradually increasing weight loss with a final value at 13.5%. The rate constants of degradation, deriving from the gradient of the slope,[45] were 0.80, 1.15, and 3.01% day^{-1} for PHBV, amide-, and collagen-PHBV, and that of amine-PHBV was absent owing to the poor line fitting.

It is believed that hydrolysis reaction is programed via selection of the detailed environment around the ester bonds, such as side groups,

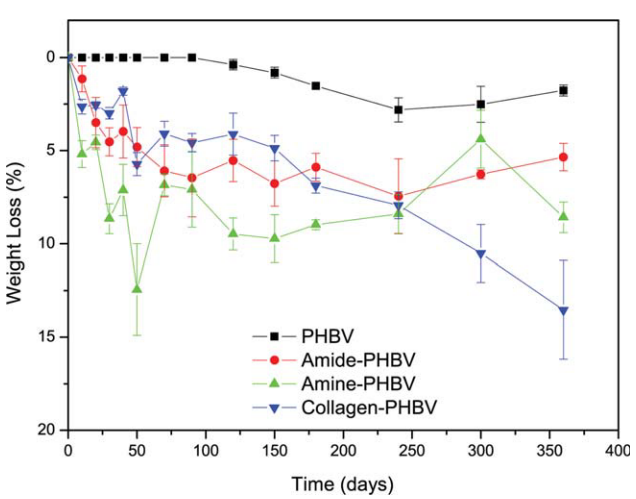

Figure 6 Weight loss of PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV during the 360 days of incubation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

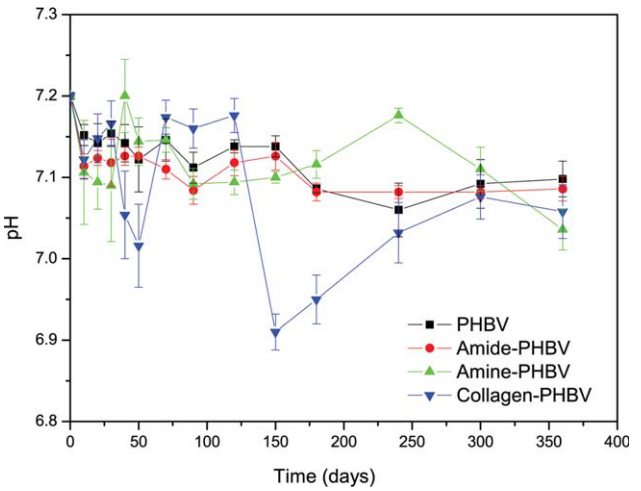

Figure 7 pH of degradation fluids during the 360 days of incubation in PBS for PHBV, amide-PHBV, amine-PHBV, and collagen-PHBV. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

crystallinity, hydrophilicity, or pH value. Acid or alkali could accelerate the hydrolysis of ester bond. For example, hydrolysis of poly(L-lactide) could be catalyzed because of carboxylic acid byproducts with pH approaching 3.0.[46] However, the acidic environment would limit its *in vivo* application. pH value of degradation fluid versus time is illustrated in Figure 7. pH of PHBV and amide-PHBV fluctuated between 7.1 and 7.2, as the initial pH was recorded at 7.2. Amine- and collagen-PHBV presented an increasing fluctuant extent, with the maximum pH of 7.18 for amine-PHBV at 240 days and the minimum pH of 6.91 for collagen-PHBV at 150 days. The approximately neutral pH suggested that the degradation products would not cause an acidic or alkaline environment, so that the hydrolysis reaction would not be autocatalyzed.

Degradation of PHBV involves chain scission of ester bond through hydrolytic attack by water molecules and shortening the main chains into oligomer fragments and soluble monomers. During this process, aliphatic polyester is degraded faster in amorphous domains than crystalline ones.[47] Because of hydrophobic nature and high degrees of crystallinity, it is difficult for water molecules to diffuse into PHBV freely, so that its hydrolytic degradation is a very slow process. The modified PHBV films had increasing wettability with hydrophilic chains on the surface, through which water molecules can be absorbed to accelerate *in situ* degradation. The weight loss should increase as the relative crystallinity being estimated from the enthalpies of the melting endotherms decrease. Amine-PHBV had the maximum weight loss at 50 days of incubation, perhaps mainly owing to its highest WSP among the modified PHBV films. After 50 days of incubation, the irregular weight loss may relate to chelation formation between nitrogen of amine groups and electron-deficient metal ions, which might further attract counterions of PBS to initiate inorganic nucleation.

CONCLUSIONS

The thermal properties and *in vitro* degradation of amide-, amine-, and collagen-PHBV were studied. The thermal decomposition temperatures of PHBV were lower than amide-PHBV but much higher than amine- and collagen-PHBV. The Avrami-Erofeev equation with $n = 2/5$ was the probable kinetic function (correlation coefficient > 0.98) while using the Friedman method to study thermal decomposition kinetics of PHBV component in the films, and the corresponding mechanism was controlled by random nucleation and growth process. E of PHBV was lower than that of amide-PHBV but higher than that of amine- and collagen-PHBV. The weight loss of the modified PHBV films increased compared with that of PHBV, which was influenced by films' wettability and crystallinity. The approximately neutral pH of the degradation fluids will not catalyze the hydrolysis degradation. With an initial aim to construct cytocompatible surface, the modified PHBV films with different functional groups offered biomaterials with various biodegradation.

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