Synthesis and Properties of Collagen/Polylactic Acid Blends

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ABSTRACT: Polylactic acid (PLA) is a biodegradable aliphatic polyester that is suitable for use in many fields related to medical treatment. This article reviews the glass transition temperature (T_g), the biodegradable rate of PLA blended with collagen (Col), and its mechanical properties through tensile, bending and impact testing, thermal analysis (DSC), scanning electron microscopy (SEM), and enzymatic hydrolysis. The results of the mechanical and SEM examination demonstrated partial biocompatibility. The T_g and crystallinity (x_c) of the blends decreased with increasing collagen content. The tensile strength and bending strength changed from 53.826Mpa and 102.261Mpa to 11.707Mpa and 24.994Mpa, respectively. It was also found that the enzymatic hydrolysis rate of PLA increased with increasing collagen content. The weight of the blends decreased to half of the original weight after more than five weeks. Viscosity($[\eta]$) and molecular weight (M_v) changed slowly in the period of enzymatic hydrolysis. It was concluded that the introduction of the collagen phase clearly diminished the mechanical properties of PLA, but the biodegradable property was improved. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 1670–1675, 2004

Key words: polylactic acid; collagen; blends; strength; hydrolysis

INTRODUCTION

Much attention has been paid to polylactic acid (PLA) in the research of alternative biodegradable polymers.[1–3] PLA degrades to lactic acid and low molecular-weight oligomers by hydrolysis of ester bonds, which can be metabolized by both soil and marine organisms. PLA is a linear aliphatic thermoplastic polyester, produced from renewable resources and readily biodegradable.[4,5] PLAs are produced by ring-opening polymerization of lactides, and the lactic acid monomers used are obtained from the fermentation of sugar feed stocks.[6] Generally, commercial PLAs are copolymers of poly (L-lactic acid) (PLLA) and poly(D,L-lactic acid) (PDLLA), which are produced from L-lactides and D,L-lactides, respectively. The ratio of L- to D,L-enantiomers influences the properties of PLA, such as melting temperature and crystallinity. Recently, PLA resins have been used in biomedical applications such as drug delivery systems.[7] However, brittleness and cost limits the applications of PLA. A number of studies have focused on the blending of PLLA with poly(D,L-lactic acid) (PDLLA), or copolymers containing D,L-lactide units, and stereocomplexation occurs between the L-lactide and D,L-lactide monomer sequences.[8–10] Blends of PLLA, with aliphatic polyesters or copolyesters, have been well documented; these polyesters include PDLLA,[11–16] poly-ε-caprolactone (PCL), Poly-L-lactide-co-ε-caprolactone,[17,18] polylactide-*co*-glycolide (PLGA),[19,20] and bacterial polyesters such as poly-3-hydroxybutyrate (PHB)[21] and poly-3-hydroxybutyrate-*co*-3-hydroxy-valerate (PHBV).[22] Blends of PLLA with polyethylene oxide (PEO) have also been investigated by several authors.[23–26] Recently, polyvinyl-acetate (PVAc) and clay were used to modify the properties of PLLA.[27,28] Blends of PLA with various nonbiodegradable polymers have been also investigated[29–30]; however, some of these blends were immiscible, resulting in poor mechanical properties. Up to now, no study has been conducted to investigate the possibility of using collagen as a biodegradable blend component. Collagen is a connective tissue protein with a triple-stranded helical structure. Each strand is about 100KD and composed mainly of glycine, praline, and hydroxyproline.[31] As a representative proteinous biomaterial,[32–34] collagen has been applied as a cultivation substratum for various cells, including fibroblast and epidermal cells[35–38] as well as the preparation of artificial skins,[39–43] membranes,[44] lens,[45,46] fibers,[47] and others.[48]

In this study, the influence of collagen on the physical properties of PLA was investigated. Moreover, to

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obtain fundamental information concerning enzymatic hydrolysis, the properties of the subsequent materials were characterized through tensile, bending and impact testing, thermal analysis, microscopy, and enzymatic hydrolysis.

METHODS

PLA and collagen preparation

Collagen (type I) from calf-skin was prepared according to patent (CN 1110284A).[49] Briefly, the method of collagen preparation included the following steps:

a. Clean calf-skin was degreased by cyclohexane.
b. Extracted collagen from tissue with acetic acid.
c. Precipitated collagen by adding inorganic salt.
d. Gelated with acetic acid.
e. Filtered and freeze-dried.

Polylactic acid (PLA) was polymerized in a way similar to that described by other authors.[50] Briefly, PLA was obtained by ring-opening polymerization of L-lactide using tin octoate and lauryl alcohol, as initiator and polymerization modulator, respectively, followed by extracting with ethyl acetate.

The viscosity-average molecular weight (M_v)of PLA powder was 7.7×10^4 before blending with collagen. The PLA powder was purified a few times by precipitating from a polymer/dichloromethane solution through dropwise addition of ethanol. The residual monomer and tin were 0.05 wt % and 10ppm, respectively. After ultra-sonic dispersion, the PLA powder was examined by SEM, and the particle size was found to be in the range 2–3μm.

PLA/collagen blend preparation

The PLA powder and collagen were blended, by a single-screw machine, in varying proportions; the proportion of collagen was 0 wt %, 10 wt %, 20 wt %, and 30 wt %, and the samples were marked as 1#, 2#, 3#, and 4#, respectively. Then the blended powder was compressed to a standard plate by a compression molding machine at 130°C and 3.5Mpa.

Characterization

Tensile strength testing

Tensile testing was carried out on an Instron tensile testing machine (Canten, MA, USA model 4206) according to the ASTM D88291, with a crosshead speed of 5mm/min. The width and thickness of each sample were measured before testing. Five samples were tested for each blend ratio; Young modulus and maximum tensile strength were calculated by using Instron series 4 software.

$$\sigma_t = P_{max}/bd \quad E_t = (\Delta P/bd)/(\Delta l/l_0)$$

where P_{max}: maximum force,
ΔP = the force of sample deform hardly,
b: width of samples,
Δl = the difference between length before and after,
d: thickness of samples, and
l_0: the original length of sample.

Bending strength and modulus

Three-point bending strength was determined by the same testing machine. The crosshead speed was 2mm/min, the span was 2mm. The temperature was 25°C.

$$\sigma b = (1.5P\max\ l0)/bd2 \qquad Eb = (\Delta P\ l03)/(4bd3\delta)$$

where P_{max} = maximum force,
l_0 = span,
b: width of samples,
d: thickness of samples, and
δ = displacement of sample when compressed.

Impact resistance

The impact resistance was determined by an impact tester (Chapy XCJ-4). Five samples, similar to the tensile test specimens, were cut out from the central part of the dumbbell specimens and were tested by the impact tester. The impact resistance was determined according to the following formula:

$$\sigma_i = W/bd$$

where W: consumed work,
b: width of samples, and
d: thickness of samples.

Thermal analysis

Differential scanning calorimetry (DSC) was used for thermal analysis of PLA and blends. Pure PLA and blends were sealed in hermetic aluminum pans to prevent water evaporation. The samples were first heated to 100°C from 25°C at a rate of 10°C/min. The samples were kept at 100°C for 10 min, cooled to 25°C, and heated again from 25 to 200°C at a rate of 10°C/min. A nitrogen flow (35 mL/min) was maintained throughout the test. The glass transition temperature(T_g), melting temperature(T_m), and crystalline degree (x_c) were determined in the second heating scan.

Scanning electron microscopy (SEM)

The blend samples were cut with a diamond saw, then polished with a nonaqueous solvent, and finally etched with chloroform for 24 h to remove the PLA

TABLE I
Mechanical Strengths of Col/PLA Biomaterials Prepared by Compression Molding at 130°C

Sample	σ_t(MPa)	E_t(MPa)	σ_b(MPa)	E_b(MPa)	E_i(J/m^2)
PLA : Col(100 : 0)1#	53.826	1405.458	102.261	2941.588	6943.982
PLA : Col(90 : 10)2#	35.764	1432.377	67.989	2599.227	1809.824
PLA : Col(80 : 20)3#	23.605	1326.276	46.223	2356.268	892.284
PLA : Col(70 : 30)4#	11.707	1005.070	24.994	2284.299	588.054

phase to reveal the morphology of the blend. SEM observation on selected collagen/PLA blends was performed with a Philips SEM515 scanning electron microscope. Low voltage (2.1 kv) was used, and the samples were coated.

Hydrolysis

The samples of collagen/PLA blends marked 1#, 2#, and 4# were employed in the test. All samples were placed in a vial filled with 10 mL of Tris-HCl buffer (pH 8.6) containing 1.0mg of proteinase K and 1.0mg of sodium azide. The solution pH was kept in the range of 8.6–8.0 within 20 h during the enzymatic hydrolysis. Within this pH range the enzyme activity was practically constant.[51] The hydrolyzed samples were washed thoroughly with 4°C distilled water and dried under vacuum for at least 24 h. The viscosity for average molecular weight determination (M_v) in chloroform was evaluated at 25°C according to the Ubbelohde model. The calculation formula was $[\eta] = 5.45 \times 10^{-4} M_v^{0.73}$.[52]

RESULTS AND DISCUSSION

Collagen and PLA properties

The color of PLA powder used in the experiment was white. Several physical and chemical parameters of collagen were characteristic and constant in the experiment. The physical parameters included:

a. Soaking time of unit weight was not less than 15 min.
b. Absorbing coefficient of unit weight water was not below 25g.
c. Shearing resistance was not below 2000g/cm^2.
d. The digesting time was more than 20 min in HCl(10*N*) containing enzyme.

The chemical parameters included:

a. The content of hydroxyproline exceeded 13% according to spectrophotometric tests.
b. The content of nitrogen exceeded 17.5%.
c. The residual humidity was less than 20%.

FT-IR discovered that absorption bands of the samples included 1760cm^{-1}, 2950cm^{-1}, 1457cm^{-1}, and 3350cm^{-1}, representing the existence of C = O group and CH_3 group. This indicated that the opened polymerization ring of lactide existed because the polylactic acid molecule has only –OH end; generally these absorption bands were not obvious.

Mechanical properties and SEM observation

Measurements were repeated 4 times. All the data presented in Table I were the average values of each sample. With pure PLA as a control, the effect of collagen content on mechanical properties of collagen/PLA compounds was clearly revealed by the results. Unlike other thermoforming methods, such as the conventional injection molding, molding under relatively lower temperature and higher pressure did not cause any apparent decrease of M_v.

Table I shows that, with increasing collagen content, the tensile strength decreased from 53.826Mpa to 11.707 Mpa, the bending strength decreased from 102.261Mpa to 24.994Mpa, and the impact strength decreased from 6943.982J/m^2 to 588.054J/m^2. It seemed that mechanical strength decreased linearly with increasing collagen content. From the point of molecular structure of high polymers, resistance to external force mainly depends on molecular bonding, intermolecular Van der Waals forces, and hydrogen bonds. The covalent link bond energy of the main chain in most polymers is 350KJ/mol. The dissociation energy of hydrogen bonds is 20KJ/mol, and the effective domain is 0.3nm. The dissociation energy of the Van der Waals force is 8KJ/mol, and the effective domain is 0.4nm. Increasing polarity, hydrogen bonds, and adequate crosslinking can enhance the strength of polymers. These forces could prevent molecular chains from gliding. But in this experiment, it seemed that hydrogen bonds or crosslinks were not produced between PLA molecules and collagen molecules. In Figures 1 and 2, it was found that, although notches were not formed on the surface of collagen and PLA, the collagen particles didn't uniformly distribute in the composite material. As a powder premix, it seemed that hydrophilic collagen and lipophilic PLA particles demonstrated poor mutual compatibil-

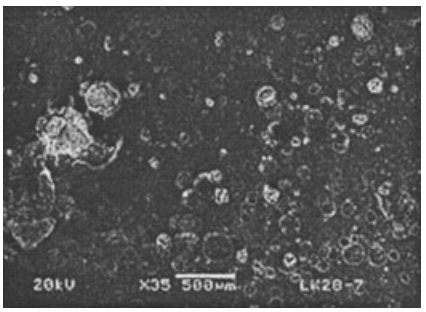

Figure 1 SEM photo of cross section, 2# sample.

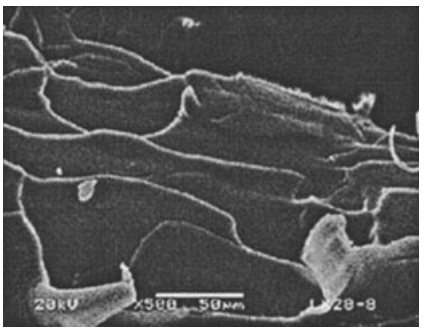

Figure 3 SEM photo of cross section, 1# sample.

ity during mechanical blending. Collagen particles seemed effective as "defects" or "impurity particles." When force was loaded on the material, the stress distribution changed: stress around the "defects" drastically increased, exceeding the average stress and giving stress concentration. These defects caused a sharp reduction of the material strength and became the main factor of material destruction. Due to the continuous phase of PLA, the strength and the elastic modulus of materials could be maintained. The collagen microparticles, scattered in the continuous phase of PLA, weakened the continuous phase, became stress concentration areas, and caused the material to break. On the other hand, particle size and proportion of the two phases also influenced the strength of the composites. In Figures 3 and 4, the surface of the cross section of pure PLA kept a regular structure. In blended samples, collagen particles destroyed the regular structure and caused disorder. This might be the main reason for the strength decrease.

The melting point and crystallinity under molded forge

Figure 5 shows the results of DSC analysis on the pure PLA sample and PLA/collagen composites. Three different parts of each sample were measured. The average value of crystallinity was obtained by measuring three times. The results demonstrated that the glass state temperature (T_g) of the PLA powder was 65.532°C, exceeding reported temperature. It was the high molecular weight of PLA that caused the increase of T_g. Because the decrease of molecular weight resulted in the increase of terminal chains, the activity of these chains increased, so the glass state temperature decreased while the molecular weight increased, but the decrease of terminal chains resulted in the increase of T_g. When molecular weight reached a certain value, the proportion of terminal chains could be ignored, and the relationship between T_g and molecular weight nearly disappeared. The melting point (T_m) was 122.662°C, and crystallinity (X_c%) was 5.48%; these results were lower than those reported by previous authors. PLA can partly change to polymeso-lactide by a different polymerization method and reaction situation. This may also cause reduction of crystallinity.

Recently, L. Mandelkern et al.[53] discovered that there was a regular relationship between molecular weight and the aggregated structure of crystalline polymer, and that crystallinity decreased with increasing molecular weight. A lower crystalline temperature resulted in lower melting point in the process of polymerization. When polymer was crystallized at a lower temperature, the activity of the molecular chain became weak. Under this condition, the crystals became imperfect and would be destroyed. It was these changes that primarily resulted in a lower melting point. Another reason for low melting point was the

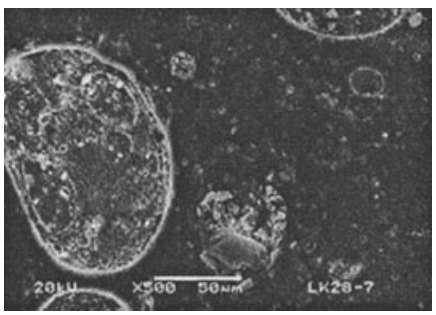

Figure 2 SEM photo of cross section, 2# sample.

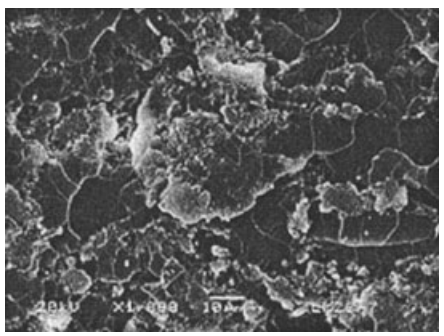

Figure 4 SEM photo of cross section, 2# sample.

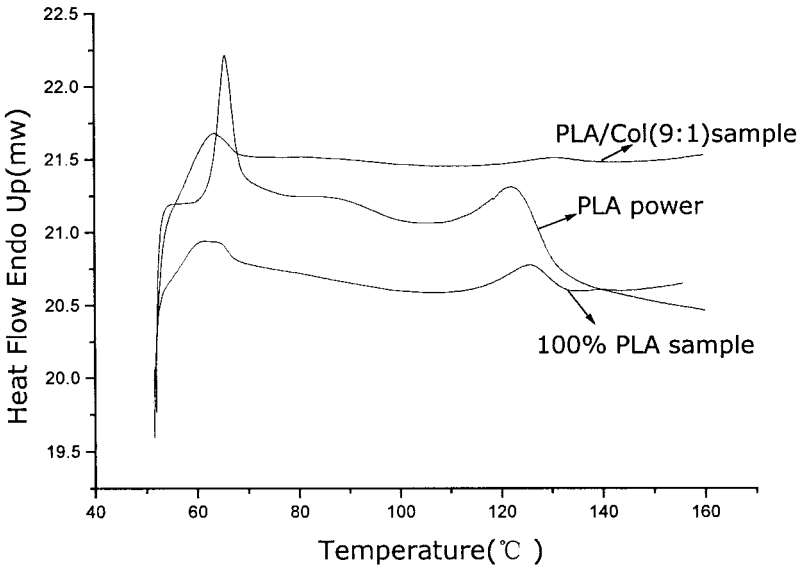

Figure 5 The normalized DSC cures of samples.

high melting entropy of ester bonds, because the internal rotated steric hindrance of carbon–oxygen bonds was smaller than that of carbon–carbon bonds and increased the mobility of the chains. Lower melting point is valuable for processing and cost reduction. In Figures 2 and 3, the crystallinity of the composite materials almost disappeared after the molding process. Possibly the collagen destroyed the aggregated structure. Moreover, the processing condition and temperature might also influence the crystallinity of the composite material.

Enzymatic hydrolysis of PLA/collagen composites

Table II shows the results of weight loss of 1#, 2#, and 4# samples. It demonstrates that the weight loss of samples increased with increasing collagen content. The weight loss of the composites was faster than that of the pure PLA sample. It seemed that the biodegradation rate increased with increasing collagen content. The weight decreased to half of the original weight of 4# sample after five weeks, but 1# and 2# samples did so after eight weeks and six weeks, respectively. These samples took twelve weeks or more to degrade completely. It seems that the degradation time decreased with increasing collagen. Due to the hydrophilic property of collagen, the amorphous regions of PLA degraded quickly when using proteinase K. This property is valuable for improving the long degradable time of high molecular weight polymers to conform to the healing period of bone fractures. During the hydrolysis experiment, the change of viscosity and molecular weight were not so great, and the composites maintained mechanical strength for a significant time.

TABLE II
Characteristics of Samples Before and After Enzymatic Hydrolysis

	Before hydrolysis				After hydrolysis		
Code	Weight (g)	$[\eta]$ (cm^3/g)	M_v ($\times 10^4$)	Hydrolysis time (Week)	Weight (g)	$[\eta]$ (cm^3/g)	M_v ($\times 10^4$)
1#	1.0	9.255	62.3		0.856	9.201	61.8
2#	1.0	8.617	56.5	3	0.710	8.517	55.6
4#	1.0	7.848	49.7		0.676	7.755	48.9
1#	1.0	9.255	62.3		0.623	8.960	59.6
2#	1.0	8.617	56.5	6	0.507	8.156	52.4
4#	1.0	7.848	49.7		0.484	7.239	44.5
1#	1.0	9.255	62.3		0.497	7.334	45.3
2#	1.0	8.617	56.5	9	0.365	5.283	28.9
4#	1.0	7.848	49.7		0.206	4.697	24.6
1#	1.0	9.255	62.3		0.224	4.257	21.5
2#	1.0	8.617	56.5	12	0.256	3.845	18.7
4#	1.0	7.848	49.7		0.009	2.363	9.6

Of course, this property is very important for degradable polymers used in biomedical materials.

CONCLUSION

We have investigated the properties of PLA/collagen blends. The properties and the compatibility of these biodegradable polymer blends were determined through mechanical, thermal testing, SEM observation, and enzymatic hydrolysis. A dependence of PLA glass transition temperature and crystallinity on the blends was observed by DSC. Because of the high molecular weight, the glass transition temperature exceeded reported values, while crystallinity and melting point were lower than reported values. PLA removed areas appeared as dark holes under the scanning electron microscope. The large and nonuniform holes suggested that PLA formed a continuous phase and collagen formed a separated coarse phase; this might be classified as partly compatible. SEM results showed that the morphological changes correlated well with the mechanical behavior. The introduction of the collagen phase drastically reduced the mechanical properties. The biodegradability rate increased with increasing collagen content, but the change of viscosity and molecular weight were not so clear.

Finally, despite the interest in developing PLA blends as biomedical materials, some difficulties have to be overcome, such as the lack of affinity between each component and weak mechanical strength. The compatibility of collagen and PLA obtained by reactive blending will be one of the leading directions to be investigated.

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