

# Effects of Mixing Technique and Filler Content on Physical Properties of Bovine Bone-Based CHA/PLA Composites

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Received 6 February 2010; accepted 21 February 2011

DOI 10.1002/app.34422

Published online 17 June 2011 in Wiley Online Library (wileyonlinelibrary.com).

**ABSTRACT:** This study presented influence of mixing technique as well as filler content on physical and thermal properties of bovine bone-based carbonated hydroxyapatite (CHA)/poly(lactic acid) (PLA) composites. CHA/PLA composites at various contents of CHA were prepared by either melt-mixing or solution-mixing techniques. Thermal properties, morphologies, and mechanical properties of the CHA/PLA composites including molecular weight deterioration of PLA matrices were investigated. Average molecular weights of PLA in the composites prepared by both techniques decreased with increasing CHA content, whereas their molecular weight distributions (MWDs) increased. Nonetheless, average molecular weights of PLA in melt-mixed composites were lower than those of solution-mixed composites. With increasing CHA content, elongation at break, tensile

strength, and impact strength of the composites were decreased, whereas the tensile moduli of the composites were increased. In comparison between two mixing techniques, the melt-mixing distributed and dispersed CHA into PLA matrix more effectively than the solution-mixing did. Therefore, tensile moduli, tensile strength, and impact strength of the melt mixed composites were higher than those of the solution-mixed composites of the corresponding CHA content. Moreover, decomposition temperatures and % crystallinity of the melt-mixed composites were higher than those of the solution-mixed composites. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 122: 2433–2441, 2011

**Key words:** composites; mixing; mechanical properties; morphology; thermal properties

## INTRODUCTION

Since Thailand is an agricultural-based country, one of the abundant wastes is bovine bones. Normally, the bone is used in fertilizer, animal foods, and making porcelain (*i.e.*, bone china). To increase added value of the bovine bone and to reduce volumes of the livestock waste, bovine bone can also be used as a raw material for producing natural hydroxyapatite (HA). Generally, HA ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) has been investigated as a biomaterial and used in bone tissue engineering because of its excellent biocompatibility, osteoconductivity, and bone-bonding ability.<sup>1–3</sup> Although HA is considered as a good bone substitute, its chemical structure was rather different from the natural bone. Generally, natural bone is a non-stoichiometric HA, which contains several ions, *e.g.*,  $\text{CO}_3^{2-}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{HPO}_4^{2-}$ ,  $\text{F}^-$ , and  $\text{Cl}^-$ .<sup>4</sup> Among them, carbonate ions ( $\text{CO}_3^{2-}$ ) play an important role in the bone metabolism. The presence of  $\text{CO}_3^{2-}$  in carbonated hydroxyapatite (CHA) structure

influences the biocompatibility, osteoconductivity, decomposition, and solubility of CHA implanted material.<sup>5</sup> CHA can be obtained from chemical synthesis<sup>5–7</sup> or natural sources, *e.g.*, animal bones.<sup>8,9</sup> Nowadays, natural CHA is attracting much attention from points of natural and less expensive material. As another approach to utilize bovine bone, this research aimed to prepare bovine bone-based CHA powder and further used the powder as filler for producing a biodegradable polymer composite.

that occurred during mixing. Many kinds of biodegradable polymers have been investigated as materials for therapeutic approaches and tissue engineering applications. Among those polymers, poly(lactic acid) (PLA) is a good candidate due to its biodegradability and yielding nontoxic byproducts after hydrolysis reaction.<sup>10,11</sup> Hence, a composite between CHA and PLA is a good alternative for using as a biomaterial, since it combines strength and stiffness of CHA with flexibility and resorbability of PLA. This composite should guide osteoblast cell attachment thereby bone tissue regeneration when it is implanted in human body. Additionally, CHA/PLA composite is flexible to process into required shapes.

However, one of the major problems of the CHA/PLA composite manufacturing is agglomeration of

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the CHA powder in the PLA matrix. In general, fine CHA particles tend to combine together through electrostatic or van der Waals forces to form agglomerated particles. This effect leads to deteriorated mechanical properties of the composite. So, the mixing technique selected to prepare the CHA/PLA composites must have ability to break down agglomerated CHA and disperse the powder into the PLA matrix.<sup>12</sup> By an effective mixing process, homogeneous distribution of CHA powder in PLA matrix and enhancement of mechanical properties of the composite are obtained. The approaches currently used to prepare the composites are based on solution mixing,<sup>13</sup> forging,<sup>14</sup> and hot pressing.<sup>15</sup> Melt-mixing and solution-mixing are the most frequently used techniques for CHA/PLA composites preparation. On composite preparation, the melt-mixing technique generates higher shear force and higher temperature than the solution-mixing technique. The high shear force and the high mixing temperature would promote the distribution of CHA in PLA matrix. Nevertheless, melt-mixing technique would deteriorate molecular weight of the PLA, since PLA is a thermally sensitive polymer.<sup>15,16</sup> In contrast, solution-mixing technique with lower shear force and lower mixing temperature would protect PLA from thermal degradation. However, the low shear force and the low mixing temperature might affect the distribution of CHA in PLA matrix.

In this present study, we prepared CHA from bovine bones and used as the filler for PLA composites. CHA/PLA composites were prepared by either solution-mixing or melt-mixing techniques. Effects of preparation technique and filler content on morphological, mechanical, and thermal properties of the composites were determined. Furthermore, degradation of PLA chains in the composites prepared by those two techniques was also investigated.

## EXPERIMENTAL

### Materials

PLA (4042D) was purchased from NatureWorks LLC, Minnetonka, USA. The resin consists of 94% L-lactide and 6% D-lactide units with a weight average molecular weight of  $2.44 \times 10^5$  g/mol, a polydispersity index of 2.5, a density of  $1.24$  g/cm<sup>3</sup>, and a melting temperature of 145°C. Bovine bones were supplied by Limeiseng, Nakhon Ratchasima. Chloroform (both AR and HPLC grades) was purchased from Labscan, Gliwice, Poland.

### Preparation of bovine bone-based CHA powder

Bovine bones were burned in open air and were ground into powder using a ball milling machine. Then, the powder was calcined at 1100°C for 3 h.

According to the observed characteristics of the obtained powder, the powder after calcination was called CHA.

### Characterization of CHA powder

The CHA powder was characterized by an X-ray diffractometer (XRD; BRUKER AXS/D5005) with a Cu-K $\alpha$  as a radiation source. A step size of 0.02°, and a scan speed of 0.4°/min were used, whereas the voltage was held at 35 kV. In addition, fraction of crystalline phase ( $X_{c(\text{CHA})}$ ) in CHA powder can be calculated by the following equation:<sup>17</sup>

$$\%X_{c(\text{CHA})} = \left(1 - \frac{V_{112/300}}{I_{300}}\right) \times 100$$

where  $I_{300}$  is the intensity of (3 0 0) diffraction peak, and  $V_{112/300}$  is the intensity of the hollow between (1 1 2) and (3 0 0) diffraction peaks of the obtained powder.

Functional groups of CHA powder were identified by a Fourier transform infrared spectrometer (FTIR; BIO-RAD/FTS175C, KBr pellet technique). The spectrum was recorded in the 4000–400 cm<sup>-1</sup> region with 2 cm<sup>-1</sup> resolution.

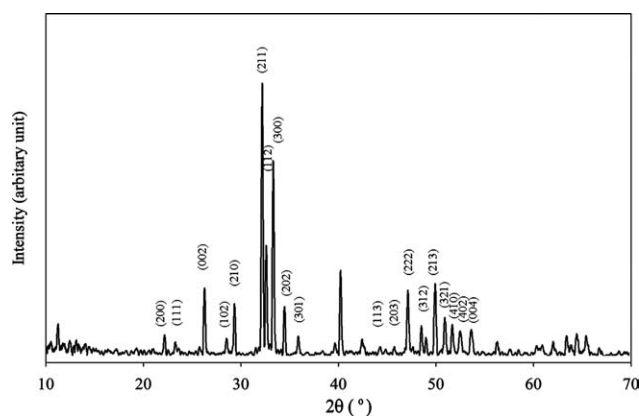
In addition, microstructure of the CHA powder was investigated by a scanning electron microscope (SEM; JOEL/JSM-6400) operating at 20 kV. Average particle sizes and density of CHA powder were determined by a diffraction particle analyzer (MATTERSIZER S, MSS) and a pycnometer, respectively.

### Preparation of CHA/PLA composites

CHA/PLA composites at various contents of CHA, i.e., 0, 20, and 40 wt %, were prepared via two mixing techniques. For solution-mixing technique, 20 g of PLA was dissolved in 100 mL of chloroform and left at room temperature for 24 h. Then, CHA was added to PLA solution, and the mixture was ball milled for 4 h. After that, the mixture was poured into clean Petri dishes and dried at room temperature for 48 h and at 70°C in a vacuum-oven for 24 h. Thickness of the obtained CHA/PLA composite films was approximately 0.10–0.15 mm. Then, each composite film was cut into small pieces (1 × 1 mm<sup>2</sup>).

For melt-mixing technique, PLA composites at various contents of CHA were prepared in an internal mixer (HAAKE/RHEOMIX) at 170°C with a rotor speed of 70 rpm for 10 min. Each CHA/PLA composite was left at room temperature for 24 h and cut into small pieces.

Various molding dies according to ASTM D638-03 and D256 standards were used to prepare composite specimens for mechanical testing. CHA/PLA composites were heated in the dies from room temperature to 180°C and maintained at that temperature for 15 min.



**Figure 1** XRD pattern of CHA powder.

Subsequently, it was hot-pressed at 180°C by a compression molding machine (GOTECH/GT-7014-A30) for 10 min under a pressure of 2000 psi and cooled to room temperature.

### Characterization of CHA/PLA composites

A SEM (JEOL/JSM-6400) operating at 15–20 kV was used to visualize fractures surfaces of the CHA/PLA composites. All samples were coated with a thin layer of gold before examining.

Thermal decomposition temperatures and weight losses of the composites were determined by a thermogravimetric analyzer (TGA; TA INSTRUMENT/SDT2960). The sample was heated from room temperature to 600°C under a nitrogen atmosphere at a heating rate of 10°C/min.

Enthalpy of melting ( $\Delta H_m$ ), enthalpy of crystallization ( $\Delta H_c$ ), and percentage of crystallization ( $\%X_c$ ) of PLA and CHA/PLA composites were evaluated by a differential scanning calorimeter (DSC; PERKIN ELMER/DSC7). A dried sample (10–12 mg) was run under a nitrogen atmosphere from 30 to 180°C at a heating rate of 3°C/min using an empty aluminum pan as reference.  $\Delta H_m$  and  $\Delta H_c$  of the sample were calculated by integrating the areas under melting endotherm and crystallization exotherm, respectively. The percentage of crystallization ( $\%X_{c(PLA)}$ ) of PLA was calculated using the following equation:

$$\%X_{c(PLA)} = \left[ \frac{\Delta H_m - \Delta H_c}{\Delta H_m^0 \times \Phi_{PLA}} \right] \times 100$$

where  $\Phi_{PLA}$  is weight fraction of PLA in the sample and  $\Delta H_m^0$  is the melting enthalpy of 100% crystalline PLA which is approximately 93 J/g.<sup>18</sup>

Molecular weights of the PLA were evaluated using a gel permeable chromatograph (GPC). The GPC instrument was equipped with a universal styrene-divinylbenzene copolymer column (PLgel Mixed-C, 300 × 7.5 mm, 5 μm), a differential refrac-

tometer detector (AGILENT/RI-G1362A), an online degasser (AGILENT/G1322A), a thermostatted column compartment (AGILENT/G1316A), and a quaternary pump (AGILENT/G1311A). Chloroform (HPLC grade) was used as an eluent. The eluent flow rate was kept constant at 0.5 mL/min. Temperature of the column and the detector was maintained at 40°C and 35°C, respectively. Polystyrene standards (Shodek standard) with molecular weights of  $3.90 \times 10^6$ ,  $6.29 \times 10^5$ ,  $6.59 \times 10^4$ ,  $9.68 \times 10^3$ , and  $1.30 \times 10^3$  g/mol were used to generate a calibration curve. Compression-molded specimens of PLA and CHA/PLA composites were dissolved, diluted using chloroform (2 mg/mL), and filtered before injection.

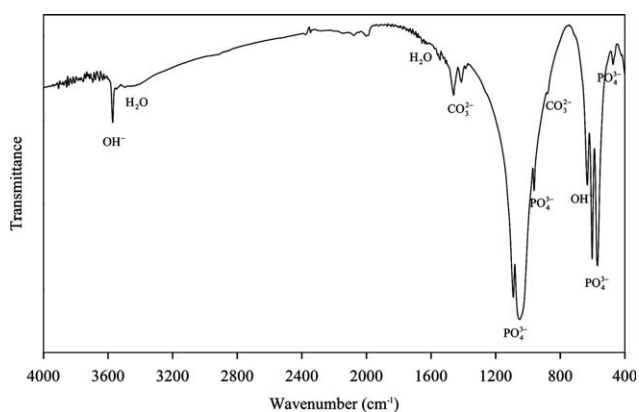
Tensile properties of the composites were investigated according to ASTM D638-03 using a universal testing machine (INSTRON/5569) with a load cell of 50 kN, a crosshead speed of 1 mm/min and a gauge length of 7.62 mm. Izod impact strength of unnotched CHA/PLA specimens were determined using an impact testing machine (ATLAS/BPI) according to ASTM D256.

## RESULTS AND DISCUSSION

### Characterization of CHA powder

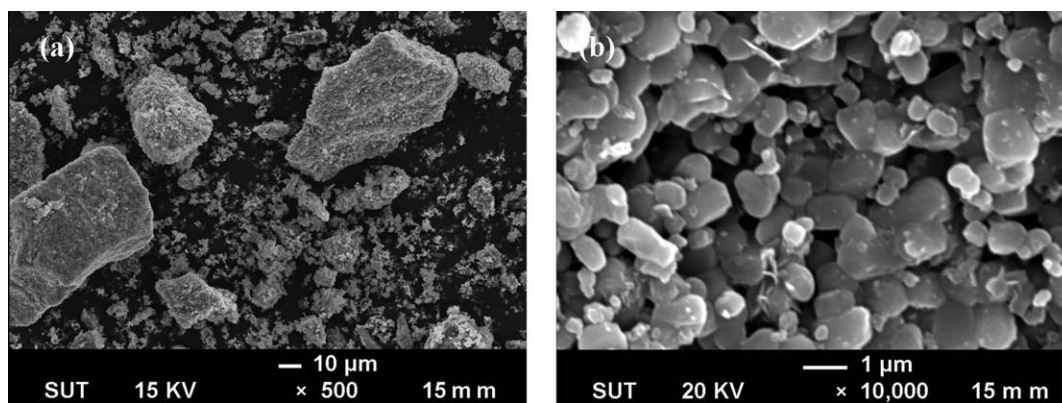
XRD pattern of CHA powder is illustrated in Figure 1. All peaks of the powder matched with XRD reference: JCPD card-PDF 9-432, the characteristic spectrum of pure HA, confirming that the bovine bone powder mainly composed of HA. The XRD pattern also indicated that the calcined bovine bone powder was in crystalline form, and the calculated crystalline phase fraction of the powder was around 90%.

Functional groups of CHA powder were identified using FTIR and shown in Figure 2. Peaks at 1085, 1036, 963, 600, and 575  $\text{cm}^{-1}$  were assigned to different vibration modes of  $\text{PO}_4^{3-}$  groups. The stretching and the bending vibration of structural OH groups in the apatite lattice were observed at 3571  $\text{cm}^{-1}$  and 632  $\text{cm}^{-1}$ , respectively. Additionally, vibration peaks



**Figure 2** FTIR spectrum of CHA powder.





**Figure 3** SEM micrographs of CHA powder: (a) CHA at low magnification ( $\times 500$ ) and (b) CHA at high magnification ( $\times 10^4$ ).

corresponding to  $\text{CO}_3^{2-}$  groups were also observed at 1457, 1411, and  $878\text{ cm}^{-1}$ .<sup>17,19</sup> These vibration peaks indicated that the powder was CHA. The appearance of carbonate functional groups on surface of the obtained powder can be explained as follows: (1) During heating process, adsorbed carbon dioxide from atmosphere substituted the  $\text{PO}_4^{3-}$  groups of the HA or (2) The incompletely pyrolyzed carbon dissolved into the HA crystal.<sup>7,20,21</sup>

SEM micrographs of CHA powder are shown in Figure 3(a-b). The micrograph at low magnification (Fig. 3(a)) illustrated agglomeration of the powder. At higher magnification (Fig. 3(b)), the micrograph revealed that the agglomerated powder composed of irregular shaped particles.

These results confirmed that the powder prepared from bovine bone was crystalline CHA. The agglomerated powder consisted of irregular shaped particles. Therefore, the bovine bone powder was called CHA in this present study. The additional information on average particle size, density, and calculated crystalline phase fraction ( $X_{c(\text{CHA})}$ ) of CHA powder is illustrated in Table I.

### Characterization of CHA/PLA composites

#### Morphological properties of CHA/PLA composites

Figure 4 shows SEM micrographs of tensile fracture surfaces of CHA/PLA composites prepared by either solution-mixing or melt-mixing techniques. In Figure 4(a), agglomeration of CHA in the solution-mixed composite was clearly observed. Also, a gap was noticeable at the interface between CHA and

PLA matrix. This indicated that there was no adhesion between CHA surface and PLA matrix. On the other hand, CHA in the melt-mixed composite exhibited more homogenous distribution and less agglomeration in the PLA matrix, as shown in Figure 4(b). However, size of CHA agglomeration seems to increase with increasing CHA content in the PLA composites as shown in Figure 4(c,d).

From the micrographs, it was clearly shown that the melt-mixing technique led to a homogeneous distribution of CHA particles in the PLA matrix, whereas the solution-mixing technique gave more agglomerated CHA, isolating PLA rich zones and less intimate contacting of the two components. According to the less homogeneous distribution of CHA in the solution-mixed composites, the filamentous structure of PLA was observed on the tensile fracture surface [Fig. 4(a)], indicating the higher ductility of the composites as mentioned in the mechanical properties section (elongation at break).

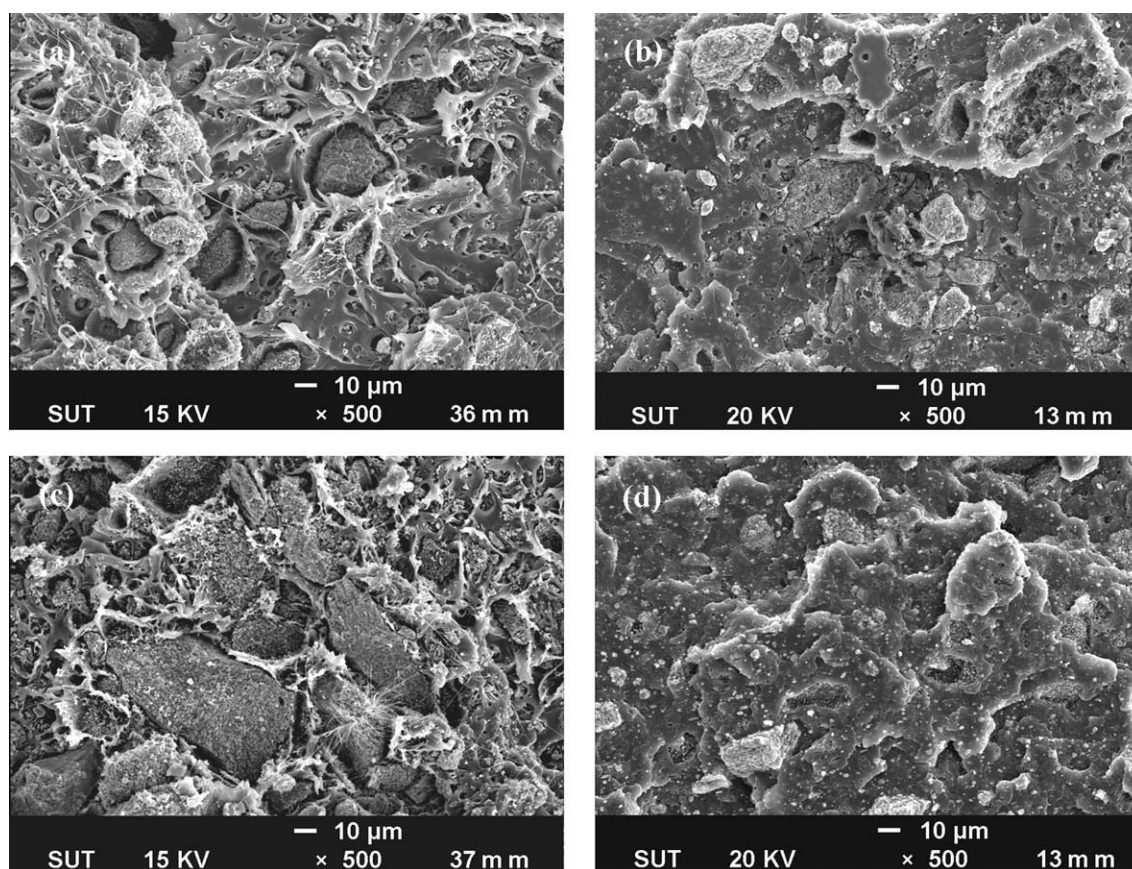
#### Thermal properties of CHA/PLA composites

TGA and the derivative of TGA (DTG) thermograms of as-received PLA, processed PLA, and CHA/PLA composites are illustrated in Figure 5. According to the onset and the peak of decomposition temperatures, as-received PLA had higher thermal stability than the processed PLA. These results implied that the chain scissions of PLA occurred during preparation process.

Several researchers have studied thermal properties of polymer composites. They found in their systems that the thermal decomposition temperatures of the polymer composites increased as compared with that of the neat polymer.<sup>15,22</sup> From their investigations, an increase in thermal stability of a polymer composite could be explained in two aspects: either decomposition products are blocked by thermally stable filler or the filler in the composite acts as a barrier preventing heat transfer. This was in contrast to the present results. All processed samples, including processed

**TABLE I**  
Physical Properties of CHA Powder

Properties	Value
Crystalline phase fraction (%)	$89.32 \pm 5.02$
Average particle size ( $\mu\text{m}$ ) (D[3,2])	$9.22 \pm 0.24$
Density ( $\text{g}/\text{cm}^3$ )	$3.154 \pm 0.002$

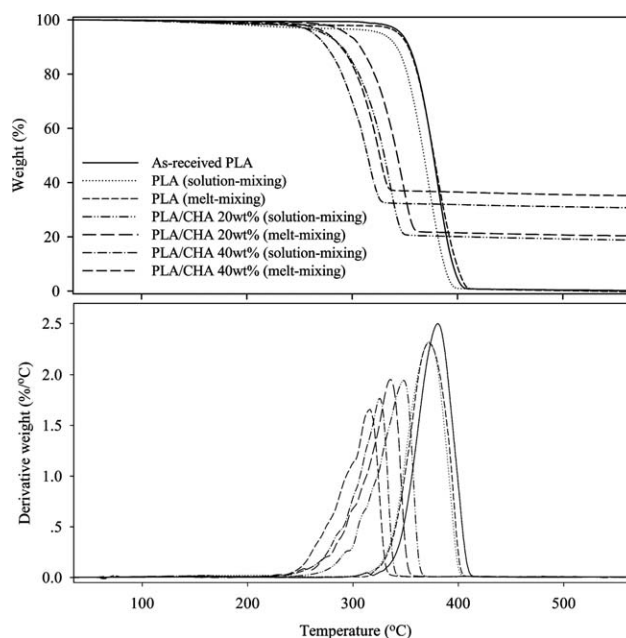


**Figure 4** SEM micrographs of fracture surfaces of CHA/PLA composites: (a) solution-mixed composite at 20 wt % of CHA, (b) melt-mixed composite at 20 wt % of CHA, (c) solution-mixed composite at 40 wt % of CHA, and (d) melt-mixed composite at 40 wt % of CHA.

PLA and CHA/PLA composites, exhibited lower thermal stability than the as-received PLA. This was probably due to two factors: (1) the limit in the distribution and dispersion of CHA in PLA matrix and (2) the acceleration of PLA degradation by OH functional groups on CHA surface.<sup>15,23</sup>

In comparison at an equal content of CHA, the melt-mixed composites had higher thermal stability than the solution-mixed composites. In the composites prepared by melt-mixing technique, the volatile products were probably blocked by the good distribution of CHA in PLA matrix [Fig. 4(b)]. In contrast, the composites prepared by solution-mixing technique exhibited more agglomeration of CHA [Fig. 4(a)], therefore, the composites would lose their volatilization blocking ability. However, the thermal stability of the composites prepared by both techniques decreased with increasing CHA content. This may be because of the poor distribution of CHA in PLA [Fig. 4(c,d)]. At higher content of CHA, CHA tended to agglomerate in PLA matrix much more than it did at lower content of CHA, since there was polarity difference between CHA surfaces and PLA matrix.

Crystallization temperature ( $T_c$ ), melting temperature ( $T_m$ ), melting enthalpy ( $\Delta H_m$ ), crystallization



**Figure 5** TGA and DTG thermograms of PLA and PLA composites prepared by various mixing techniques.



**TABLE II**  
 $T_c$ ,  $T_m$ ,  $\Delta H_c$ ,  $\Delta H_m$ , and  $\%X_{c(PLA)}$  of As-received PLA, Processed PLA, and CHA/PLA Composites Prepared by Various Mixing Techniques

Material	$T_c$ (°C)	$\Delta H_c$ (J/g)	$T_m$ (°C)	$\Delta H_m$ (J/g)	$\%X_c$
Solution-mixing					
PLA	98.4	23.51	145.3	27.95	4.75
PLA/CHA (20 wt %)	88.4	17.29	137.5	24.51	12.91
PLA/CHA (40 wt %)	84.9	10.81	137.4	19.87	17.55
Melt-mixing					
PLA	92.4	26.43	140.1	30.55	4.41
PLA/CHA (20 wt %)	85.1	17.27	139.2	27.10	13.14
PLA/CHA (40 wt %)	75.9	1.99	136.4	23.22	37.80

enthalpy ( $\Delta H_c$ ), and percent of crystallinity ( $\%X_c$ ) of as-received PLA, processed PLA, and CHA/PLA composites are summarized in Table II. A decrease in  $T_c$  and  $T_m$  of the composites with increasing CHA content was observed. These were due to the decrease in molecular weight of PLA chain occurred during mixing. In contrast,  $\%X_{c(PLA)}$  of both types of composites increased with increasing CHA content. In comparison at the equal CHA content,  $T_c$  and  $T_m$  of the melt-mixed composite were lower than those of the solution-mixed composites. As shown in Table II, the decrease in  $T_c$  and the increase in  $\%X_{c(PLA)}$  of the melt-mixed composites were more pronounced than those of solution-mixed composites. These were because of crucial degradation of PLA chain in melt-mixed composites, as reported in next section, further, these degraded PLA chains might be easily rearranged into crystalline forms.

#### Molecular weight and MWD of PLA in CHA/PLA composites

It was known that PLA chains can be easily broken at a particular temperature range resulting in lower molecular weight PLA, oligomers, monomers, and gas-products.<sup>15</sup> Therefore, during preparation of CHA/PLA composites, the PLA chains could possibly be degraded by the high processing temperature. Table III illustrates molecular weights ( $\overline{M}_n$  and  $\overline{M}_w$ ) and molecular weight distributions (MWDs) of PLA in processed PLA and CHA/PLA composites.

Based on GPC results,  $\overline{M}_n$  and  $\overline{M}_w$  of solution-mixed PLA and melt-mixed PLA were lower than those of as-received PLA. Also, their MWDs were increased. The decrease in molecular weight and the increase in MWD of the processed PLA revealed the occurrence of PLA chain scission. As compared between two mixing techniques,  $\overline{M}_w$  and  $\overline{M}_n$  of solution-mixed PLA were higher than those of melt-mixed PLA, whereas their MWDs were insignificantly different. It was due to the thermal oxidative degradation of PLA chain during melt-mixing pro-

cess. These results were similar to the research work investigated by Signori et al.<sup>16</sup> They have found an increase in MWD of PLA after thermal degradation and explained that it was the result of a random rearrangement of PLA chains on chain scission and chain recombination. Additionally, Perego et al.<sup>24</sup> reported a decrease in PLA molecular weight, about 14–40%, after injection molding at 195°C.

Furthermore, PLA chains in the melt-mixed composites had lower  $\overline{M}_w$ , lower  $\overline{M}_n$ , and higher MWDs than those in the solution-mixed composites. As observed from the SEM micrographs [Fig. 4(a,b)], the melt-mixed composites had better CHA distribution in PLA matrix than the solution-mixed composites. This enhanced the chance of interaction between OH groups on CHA surface and PLA chains and the breaking of PLA chains leading to the more decrease in the molecular weight of PLA. This result was similar to the research work done by Ignjatovic et al.<sup>15</sup> They have investigated effect of hot pressing on structures and characteristics of HA/PLLA composites and found that the OH groups at the end of PLA chains can destroy the PLA basic chains under the influence of thermal energy.

In addition, the average molecular weight of PLA in the composites prepared by both techniques decreased with increasing CHA content, whereas their MWDs increased. These results indicated that PLA chain scission increased with increasing CHA content. With increasing CHA content in the composites, the PLA matrix was more exposed to OH-containing surface of CHA, by which PLA molecules underwent more chain scission.

As reported by several research works, there were various factors affecting the decrease in molecular weight of PLA during melt-mixing process.<sup>15,16</sup> The

**TABLE III**  
Molecular Weight of PLA in As-Received PLA and CHA/PLA Composites Prepared by Various Mixing Techniques

Material	Average molecular weight		
	$\overline{M}_w$	$\overline{M}_n$	MWD
Solution-mixing			
PLA	$2.02 \times 10^5$	$0.79 \times 10^5$	2.56
PLA/CHA (10 wt %)	$1.87 \times 10^5$	$0.61 \times 10^5$	3.07
PLA/CHA (20 wt %)	$1.71 \times 10^5$	$0.53 \times 10^5$	3.17
PLA/CHA (30 wt %)	$1.58 \times 10^5$	$0.49 \times 10^5$	3.22
PLA/CHA (40 wt %)	$1.44 \times 10^5$	$0.44 \times 10^5$	3.27
Melt-mixing			
PLA	$1.94 \times 10^5$	$0.76 \times 10^5$	2.55
PLA/CHA (10 wt %)	$0.67 \times 10^5$	$0.11 \times 10^5$	6.09
PLA/CHA (20 wt %)	$0.61 \times 10^5$	$0.10 \times 10^5$	6.10
PLA/CHA (30 wt %)	$0.56 \times 10^5$	$0.09 \times 10^5$	6.22
PLA/CHA (40 wt %)	$0.53 \times 10^5$	$0.08 \times 10^5$	6.63

**TABLE IV**  
**Mechanical Properties of Processed PLA and the CHA/PLA Composites Prepared by Various Mixing Techniques**

Material	Tensile modulus (MPa)	Tensile strength (MPa)	Elongation at break (%)	Impact strength (kJ/m <sup>2</sup> )
Solution-mixing				
PLA	607.8 ± 60.8	65.1 ± 1.5	17.5 ± 3.0	19.5 ± 1.5
PLA/CHA (10 wt %)	628.5 ± 61.9	46.2 ± 1.3	14.4 ± 1.4	15.9 ± 0.5
PLA/CHA (20 wt %)	673.6 ± 48.9	41.5 ± 0.8	11.2 ± 0.7	13.5 ± 0.9
PLA/CHA (30 wt %)	830.2 ± 75.1	35.2 ± 0.6	9.3 ± 0.4	9.1 ± 0.4
PLA/CHA (40 wt %)	1088.3 ± 76.5	25.5 ± 1.5	6.7 ± 1.1	5.2 ± 1.5
Melt-mixing				
PLA	586.0 ± 88.6	64.5 ± 0.8	16.4 ± 2.5	18.7 ± 0.8
PLA/CHA (10 wt %)	860.0 ± 47.6	51.1 ± 1.2	9.5 ± 0.8	16.5 ± 0.6
PLA/CHA (20 wt %)	1065.4 ± 81.7	47.8 ± 1.0	6.8 ± 0.3	14.3 ± 0.6
PLA/CHA (30 wt %)	1150.5 ± 19.2	41.9 ± 0.8	6.1 ± 0.2	10.7 ± 0.5
PLA/CHA (40 wt %)	1320.0 ± 31.0	35.2 ± 0.2	4.9 ± 0.9	7.0 ± 0.6

mechanisms leading to breakdown of PLA chains have been proposed as follows. First, hydrolysis of ester bond on PLA backbone is potentially occurred due to the presence of water absorbed on filler surface. Second, zipper-like depolymerization is occurred when traces of catalyst still presented in the system. Third, oxidative random chain scission is occurred if melt-mixing processing is done under air atmosphere. The last two mechanisms are intermolecular transesterification to monomer or oligomers and intramolecular transesterification to form monomers or oligomers. For this present study, according to our results, it should be noted that the oxidative random chain scission of PLA was the reasonable mechanism for the decrease in  $\overline{M}_n$  and  $\overline{M}_w$  and the increase in MWD of PLA chains in the CHA/PLA composites. Also, this mechanism was accelerated in the presence of OH-containing surface of CHA.

#### Mechanical properties of CHA/PLA composites

Tensile modulus, elongation at break, tensile strength, and impact strength of melt-mixed PLA, solution-mixed PLA, and CHA/PLA composites are summarized in Table IV. In comparison at the equal filler content, the composites prepared by melt-mixing technique had higher tensile moduli than the composites prepared by solution-mixing technique. The higher stiffness of the melt-mixed composites was because of the good distribution and dispersion of CHA in PLA matrix [Fig. 4(b,d)]. Nonetheless, the tensile moduli of the composites prepared by both techniques increased with increasing CHA content, since the rigid CHA filler restricted the molecular motion and the deformation of the PLA chains. Moreover, the incorporated CHA decreased the mobility of PLA chains and led to the decrease in ductility of the composites. In comparison, the solu-

tion-mixed composite had slightly higher elongation at break than the melt-mixed composite of the corresponding CHA content. According to the SEM micrographs, the less homogeneous distribution of CHA in the solution-mixed composites created the filamentous structure of PLA indicating the higher ductility of the composites. In addition, tensile strength of the composites was lower than that of processed PLA. This was because CHA as foreign inclusion disrupted the interaction between PLA molecules.

Moreover, tensile strength of the composites prepared by both techniques decreased with increasing CHA content. This indicated the weak interaction between the two phases. However, the tensile strength of the composites prepared by melt-mixing technique was higher than that of the composites prepared by solution-mixing technique. The CHA agglomeration and the poor adhesion between agglomerated CHA and PLA matrix illustrated in Figure 4(a,c) could be the main reason that was responsible for the obvious reduction in the tensile strength of the solution-mixed composites. On the other hand, the melt-mixed composites with the more homogeneous distribution and the less agglomeration of CHA created smaller size of voids leading to the higher tensile strength as compared with the solution-mixed composites of the corresponding CHA content.

Based on tensile modulus and tensile strength results, it should be noted that the difference between two techniques was much more emphasized with increasing CHA content. This could be explained as follows. At low content of CHA, the CHA could be easily distributed into PLA matrix by both preparation techniques. On the other hand, at high content of CHA, the melt-mixing technique with high shear force and high temperature could distribute CHA into PLA matrix better than the

solution-mixing technique. This led to the more effective enhancement of tensile modulus and tensile strength of the melt-mixed CHA/PLA composites.

In addition, impact strength of all CHA/PLA composites was lower than that of the processed PLA. This was because CHA disturbed matrix continuity and limited the ability of polymer chains to absorb impact energy. In addition, the large agglomerates and a large gap around agglomerated CHA were the site of stress concentration, which could act as a microcrack initiator. However, the composites prepared by melt-mixing technique showed less reduction in impact strength than the composites prepared by solution-mixing technique. This result was because the melt-mixing process dispersed CHA into PLA matrix better than the solution-mixing process. Therefore, the melt-mixed composites with smaller sizes of agglomerated CHA could dissipate impact energy better than the solution-mixed composites.

In comparison, the results showed that the composites prepared by melt-mixing technique had higher tensile modulus, tensile strength, and impact strength than those of the composites prepared by solution-mixing technique. The high mixing temperature and the high shear force generated during the melt-mixing process was the causes of the better distribution and dispersion and the smaller agglomeration size of CHA in PLA matrix.<sup>23</sup> The degradation of PLA chains at high mixing temperature was observed. Nonetheless, the degradation of PLA chains was in an acceptable level as seen from the mechanical properties of the melt-mixed composites. Moreover, the increase in PLA crystallinity after melt-mixing process, as confirmed by DSC results, affected the mechanical properties of the composites, since the optimum increase in PLA crystallinity could enhance the strength and stiffness of the composites.

Moreover, the major drawback found in the composite prepared by solution-mixing technique is the use of an organic solvent. Mathieu et al.<sup>25</sup> reported the presence of residual chloroform in PLA composites, determined by nuclear magnetic resonance (<sup>1</sup>H-NMR). It was well known that organic solvents are potentially toxic for living organisms. Therefore, the chloroform traces detected in the composites, that are expected to be used in biomedical applications, may cause adverse effects to biocompatibility of the materials.

According to the results, it should be noted that the homogeneously distributed CHA in PLA matrix had more influence on the mechanical properties of the composites than the degradation of PLA chains that occurred during mixing. To obtain CHA/PLA composites with good mechanical properties and avoid the use of an organic solvent, the melt-mixing technique was preferred to the solution-mixing technique.

## CONCLUSIONS

In this study, CHA powder was prepared from bovine bone and used as the filler for preparing PLA composites. Mixing technique and CHA content were the factors that influenced mechanical properties of CHA/PLA composites. Average molecular weights of PLA in the composites prepared by both mixing techniques decreased with increasing CHA content, whereas their MWDs increased. The PLA chains in the composites prepared by melt-mixing technique degraded much more than those in the composites prepared by solution-mixing technique as confirmed by GPC results. With increasing CHA content, elongation at break, tensile strength, and impact strength of the composites were decreased, whereas the tensile moduli of the composites were increased. In comparison between two preparation techniques, the melt-mixed composites had higher tensile strength, tensile moduli, and impact strength than those prepared by solution-mixing technique. Moreover, decomposition temperatures and % crystallinity of the melt-mixed composites were higher than those of the solution-mixed composites.

The authors are pleased to acknowledge the financial supports from National Research Council of Thailand, Suranaree University of Technology and Center for Petroleum, Petrochemical and Advanced materials, Chulalongkorn University, Thailand.

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