

# CHAPTER V

## THERMAL PROPERTIES AND *in vitro* DEGRADATION OF BOVINE BONE BASED HA/PLA COMPOSITES

### 5.1 Abstract

In this present study, effect of silane coupling on thermal properties of bovine bone based hydroxyapatite (u-HA)/PLA composites and on molecular weight of PLA upon processing of the composites were studied. TGA and GPC results showed that the incorporation of silane-treated HA into the PLA matrix significantly increased thermal stability of the composite and decreased the thermal degradation of PLA chains. In addition, the *in vitro* degradation of HA/PLA composites were analyzed. PLA and HA/PLA composites specimens were immersed in phosphate-buffered solution at 37°C for the periods of time up to 8 weeks. The changes in specimen weight, pH of PBS solution and PLA molecular weight were investigated. In addition, the changes in morphologies of the specimens were also examined. The results showed that the stronger interfacial bonding between silane-treated HA and PLA matrix significantly delayed the *in vitro* degradation rate of the PLA. In addition, the results of bioactive study showed that the incorporation of u-HA into the PLA matrix significantly induced the formation of calcium phosphate compound on the composite surface, after 3 days of immersion in SBF, and generously covered the surface with a fairly thick layer after 7 days as evaluated by means of SEM, EDX, FTIR and XRD.

## 5.2 Introduction

Numerous of bioresorbable and biodegradable materials have been studied and applied for biomedical applications, including those based on bioresorbable polymer such as poly(lactic acid) (PLA), poly(3-hydroxybutyrate) (PHB) (Wanga, Yang, Wu, Cheng, Yu, Chen, and Chen, 2005), poly(ethylene glycol) (Wan, Chen, Yang, Bei, and Wang, 2003). The most widely used bioresorbable polymer is PLA, from points of its biocompatibility, biodegradability and yielding nontoxic byproducts after hydrolysis reaction (Kothapalli *et al.*, 2005; Shikinami *et al.*, 2001; Russias *et al.*, 2006; Tsuji *et al.*, 2004). However, mechanical properties of PLA should be improved in order to achieve optimum performance for specific applications, *e.g.* bone substitute material. Preparation of composite between bioactive fillers and PLA matrix is a commonly used method to improve the mechanical properties of PLA. Furthermore, the incorporation of these fillers resulted in an increase of the bioactivity of the composites.

Hydroxyapatite [HA:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] is good candidate as the filler for preparing PLA composites due to its osteoconductivity for bone growth and bone bonding ability (Benmarouane *et al.*, 2004; Ruksudjarit *et al.*, 2008; Yoganand *et al.*, 2009). In addition, HA can be synthetically prepared or derived from natural sources, *e.g.* coral (Sivakumar, Kumart, Shantha, and Rao, 1996), bovine bone (Shikinami *et al.*, 2001; Ruksudjarit *et al.*, 2008), swine bone (Haberko *et al.*, 2006). The natural HA is less expensive material than synthetic HA, so, choosing natural HA is an alternative choice for preparing PLA composite. Natural HA/PLA composite is an interesting composite expecting to be used as a biomedical application.

Devices prepared from HA/PLA composites can be processed via various methods, such as forging (Shikinami *et al.*, 2001) and hot pressing method (Cam, Hyon, and Ikada, 1995). However, HA/PLA composite processing still has some problems, such as thermal stability, dispersion of HA and compatibility of HA-PLA. These problems need to be concerned in the search for property enhancement of the composite specimens. Various research works have used silane coupling agent to enhance the homogeneous dispersion and compatibility of HA in PLA matrix (Daglilar *et al.*, 2007; Wang *et al.*, 2001). In those research works, they found that the incorporating of silane-treated HA led to the higher mechanical properties of the composites.

HA/PLA composite is usually proposed as an implant material, so, the *in vitro* degradation behavior study has become an interesting area of the study. By the *in vitro* study, it would be possible to predict the degradation behavior of the composites during period of implant time. Moreover, the degradation behavior *in vitro* can be used as a preliminary result for searching the HA/PLA composites with a suitable degradation properties for an implant material.

It is known that there are numerous factors affecting the polymer degradation mechanisms such as chemical structure, crystallinity and molecular weight of PLA, *etc.* Besides, the addition of HA phase into the PLA matrix increases the complexity of the degradation pattern of the material due to various parameter, *e.g.* shape and size of HA particles, composite processing condition, (Russias *et al.*, 2006; Cam *et al.*, 1995; Stefani, Coudane, and Vert, 2006; Navarro *et al.*, 2005; Li *et al.*, 2006). Some studies have reported dramatic changes in PLA degradation with the incorporation of some calcium phosphate compounds (Navarro *et al.*, 2005).

The scopes of the present study were to explore influence of silane coupling agent on thermal properties of HA/PLA composites during processing and on molecular weight of PLA upon processing of HA/PLA composites. In addition, the *in vitro* degradation of HA/PLA composites in phosphate-buffered solution at 37°C for the periods of time up to 8 weeks was studied. Furthermore, the detailed degradation of the composites in a simulated body fluid (SBF) were also investigated.

## **5.3 Experimental**

### **5.3.1 Materials**

PLA (4042D) was purchased from NatureWorks LLC Co. Ltd.. Bovine bones were supplied by Limeiseng Co., Nakhon Ratchasima, Thailand. 3-aminopropyltriethoxysilane (APES) and 3-methacryloxypropyltrimethoxysilane (MPTS) were purchased from Optimal Tech Co., Ltd. and Aldrich, respectively. Dibasic sodium phosphate ( $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ), Potassium chloride (KCl) and dibasic potassium phosphate ( $\text{KHPO}_4 \cdot 2\text{H}_2\text{O}$ ) were purchased from Carlo Erba Reagent Spa. Sodium chloride (NaCl) was purchased from VWR International buba/sprl. Sodium hydrogen carbonated ( $\text{NaHCO}_3$ ), Sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and tris(hydroxymethyl) methylamine ( $\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$ ) were purchased from Fisher Scientific UK, Ltd. Magnesium dichloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) was purchased from Ajex chemicals. Calcium chloride ( $\text{CaCl}_2$ ) was purchased from APS Ajex finechem.



### 5.3.2 Preparation of bovine bone based HA powders

Bovine bones were burned in open air and were ground into powder using a ball milling machine. Then, the powder was heat treated at 1100°C for 3 h and the obtained powder was called u-HA. Next, the powder was modified by either APES (a-HA) or MPTS (m-HA) in acidic solution at pH of 3.5 for 3 h, subsequently, each solution was neutralized with 0.1 N NaOH solution. The content of silane based on weight of HA powder was 2.0wt%. After that, the silane-treated HA powders were washed and dried overnight in an oven at 80°C. APES treated HA and MPTS treated HA were called a-HA and m-HA, respectively.

### 5.3.3 Preparation of phosphate-buffered solution and simulated human body fluid

Gomori buffers, the most commonly used phosphate buffers, consist of a mixture of monobasic dihydrogen phosphate and dibasic monohydrogen phosphate. By varying the amount of each salt, a pH range of buffers can be prepared that buffer well between pH 5.8 and pH 8.0. To prepare 0.15 M phosphate buffered solution (PBS) with pH of 7.4, 23.4 g of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and 26.7 g of  $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$  were separately dissolved in 1 litre of distilled water. Then, 810 ml of  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  solution was mixed together with 190 ml of  $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$  solution.

Simulated body fluid (SBF) in which inorganic ion concentrations are similar to those of human extracellular fluid were prepared. This fluid was used to immerse the composite specimens in order to observe *in vitro* the formation of HA on HA/PLA composites. To prepare simultaneous human body fluid, 750 ml of distilled water was poured into a 1000 ml beaker and each chemical listed in Table 5.1 was added one by one in the 1<sup>st</sup>-8<sup>th</sup> order. The added chemical must be completely

dissolved before adding the other. Subsequently,  $\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$ , the 9<sup>th</sup> reagent, should be added little by little with less than about 1g, in order to avoid inhomogeneous increase in pH of the solution.

**Table 5.1** Reagents for preparation of SBF (pH 7.40, 1 L).

Order	Reagent	Amount
1	NaCl	8.036 g
2	NaHCO <sub>3</sub>	0.352 g
3	KCl	0.225 g
4	K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.230 g
5	MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.311 g
6	1.0 M HCl	40 ml
7	CaCl <sub>2</sub>	0.293 g
8	Na <sub>2</sub> SO <sub>4</sub>	0.072 g
9	$\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$	6.063 g
10	1.0 M HCl	Appropriate amount for adjusting pH

### 5.3.4 Preparation of HA/PLA composites

HA/PLA composites were prepared using an internal mixer (HAAKE/RHEOMIX). PLA and u-HA were mixed at 170°C with 70 rpm for 10 min. The weight ratios of HA/PLA in each composite are shown in Table 5.2. Each HA/PLA composite was left at room temperature for 24 h before grinding into small pieces. Subsequently, it was hot-pressed by a compression molding machine (CARBOLITE) for 10 min at 180°C under a pressure of 2000 psi and cooled to room temperature.

**Table 5.2** Composition of HA/PLA composite.

Designation	Filler	Silane coupling agent	Filler content (wt%)
1A	u-HA	-	10
2A	u-HA	-	20
3A	u-HA	-	30
4A	u-HA	-	40
1B	a-HA	APES	10
2B	a-HA	APES	20
3B	a-HA	APES	30
4B	a-HA	APES	40
1C	m-HA	MPTS	10
2C	m-HA	MPTS	20
3C	m-HA	MPTS	30
4C	m-HA	MPTS	40

### 5.3.5 Determination of thermal properties of HA and HA/PLA composites

Thermal degradation temperature and weight loss of untreated HA powder, silane-treated HA powders and HA/PLA composites were determined by a thermogravimetric analyzer (TGA) (TA INSTRUMENT/SDT2960). The sample was heated from room temperature to 600°C at a heating rate of 10°C/min under a nitrogen atmosphere.

### **5.3.6 Determination of molecular weight of neat PLA and HA/PLA composites**

Molecular weight of PLA in neat PLA and in HA/PLA composite were evaluated by a gel permeable chromatography (GPC) using chloroform as an eluent. The chromatographer consisted of a styrene-divinylbenzene copolymer column (PLgel Mixed-C, 300×7.5 mm, 5μm), differential refractometer detector (AGILENT/RI-G1362A), online degasser (AGILENT/G1322A), autosampler (AGILENT/G1329A), thermostatted column compartment (AGILENT/G1316A) and quaternary pump (AGILENT/G1311A). The eluent flow rate was kept constant at 0.5 ml/min. Temperature of the column and the detector were maintained at 40°C and 35°C, respectively. Polystyrene standards (SHODEK STANDARD) with molecular weights of  $3.90 \times 10^6$ ,  $6.29 \times 10^5$ ,  $6.59 \times 10^4$ ,  $9.58 \times 10^3$  and  $1.30 \times 10^3$  g/mol were used to generate a calibration curve. PLA and HA/PLA composites were dissolved and diluted using chloroform (2 mg/ml) and filtered before  $\overline{M}_w$  determination.

### **5.3.7 Determination of *in vitro* degradation of PLA and HA/PLA composites**

*In vitro* hydrolytic degradation of HA/PLA composites were determined by soaking PLA and HA/PLA composites in a phosphate-buffered solution (PBS) (0.15 M, pH 7.4). Three specimens ( $4 \times 12 \times 63$  mm<sup>3</sup>) of each sample were placed in a 100 ml test tube filled with 35 ml PBS solution. The immersed specimens were incubated at 37°C for 0, 1, 2, 3, 4, 6 and 8 weeks. The buffer solution in all test tubes was weekly replaced by fresh PBS solution. This was done in order to maintain a constant volume of PBS solution and to imitate, to some extent, the *in vivo* flow model of continuously refreshing extracellular fluids.

At the end of each period, pH of PBS solution in each test tube was measured by pH meter (JENWAY/3020). The specimens were removed from PBS and wiped with a filter paper to remove surface water. The wet weight ( $W_w$ ) and thickness of the samples ( $T_t$ ) were measured. Then, these specimens were rinsed by distilled water for 3 times and vacuum dried at a temperature of 45°C to a constant weight ( $W_d$ ). Water absorption of the HA/PLA composites from PBS solution was determined. The percentage increase in weight, thickness change and percentage decrease in weight of the specimen during the specimen immersion in PBS solution was calculated by the following equations:

$$\text{Increase in weight, \%} = \left[ \frac{(W_w - W_o)}{W_o} \right] \times 100 \quad (5.1)$$

$$\text{Thickness change, \%} = \left[ \frac{(T_t - T_o)}{T_o} \right] \times 100 \quad (5.2)$$

$$\text{Weight loss, \%} = \left[ \frac{(W_o - W_d)}{W_o} \right] \times 100 \quad (5.3)$$

Where  $W_o$  is an initial weight of the specimen;  $W_w$  is the wet weight of the specimen after removing from PBS;  $W_d$  is the weight of the specimen after removing from PBS and drying at 45°C;  $T_o$  is an initial thickness of the specimen;  $T_t$  is the immediately measured thickness of the specimen after removed from PBS, respectively.

Additionally, the change in PLA molecular weight and morphological properties of the PLA composites after PBS immersion were investigated using a gel permeation chromatograph (GPC) and a scanning electron microscope (SEM), respectively. To investigate surface of the HA/PLA composites, specimens were coated with a thin layer of gold before examination using the SEM (JOEL/JSM-6400) operating at 10 kV.

### **5.3.8 Determination of bioactive properties of PLA and HA/PLA composites**

Bioactive properties of HA/PLA composites were determined by immersing PLA and HA/PLA composites in a simultaneous body fluid (SBF) (pH 7.4). A specimen ( $4 \times 12 \times 10 \text{ mm}^3$ ) of each sample was placed in a 100 ml test tube filled with 35 ml SBF solution. The specimens were incubated at 37°C for 0, 1, 2 weeks. At the end of each period, the specimens were removed from PBS and, then, the morphological properties of PLA and HA/PLA composites were investigated using a SEM (JOEL/JSM-6400) operating at 10-15 kV. To investigate surfaces of the PLA and the HA/PLA composites, samples were coated with a thin layer of gold before examination.

The precipitates formed on the surface of the composite, after 1 week of immersion, were scratched and analyzed by X-ray diffractometer (XRD) (OXFORD/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 while the voltage was held at 35 kV.

Functional groups of the scratched precipitates were identified by a Fourier transform infrared spectrometer (FTIR) (BIO-RAD/FTS175C, KBr pellet

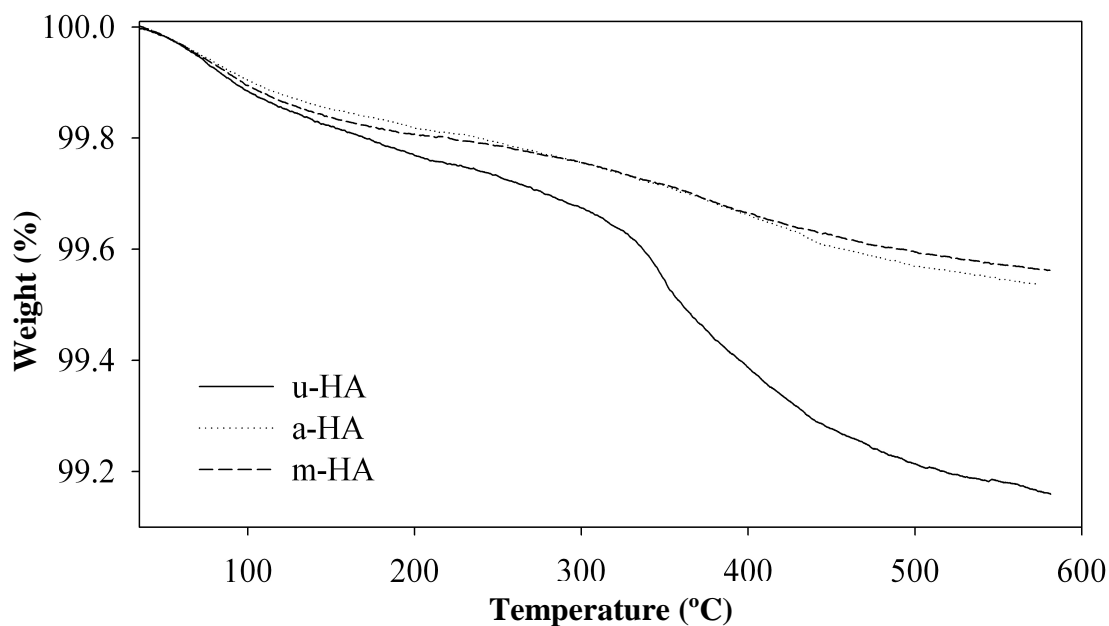
technique). The spectrum was recorded in the 4000-400  $\text{cm}^{-1}$  region with 2  $\text{cm}^{-1}$  resolution.

In addition, elemental compositions of the precipitates were analyzed by an energy dispersive x-ray spectrometer (EDX) (OXFORD INSTRUMENT/LINK ISIS6209). Each peak of the recorded spectrum is a characteristic of a particular element.

## **5.4 Results and Discussion**

### **5.4.1 Thermal properties of HA and HA/PLA composites**

Figure 5.1 shows TGA thermograms of u-HA, m-HA and a-HA. All of them were thermally stable during the given temperature range since their weight losses were less than 1wt% during the temperature range of 35-600°C. The m-HA and a-HA showed higher decomposition temperatures and less weight losses than u-HA indicating the appearance of deposited silane coupling agent on HA surface increased thermal stability of HA powder.



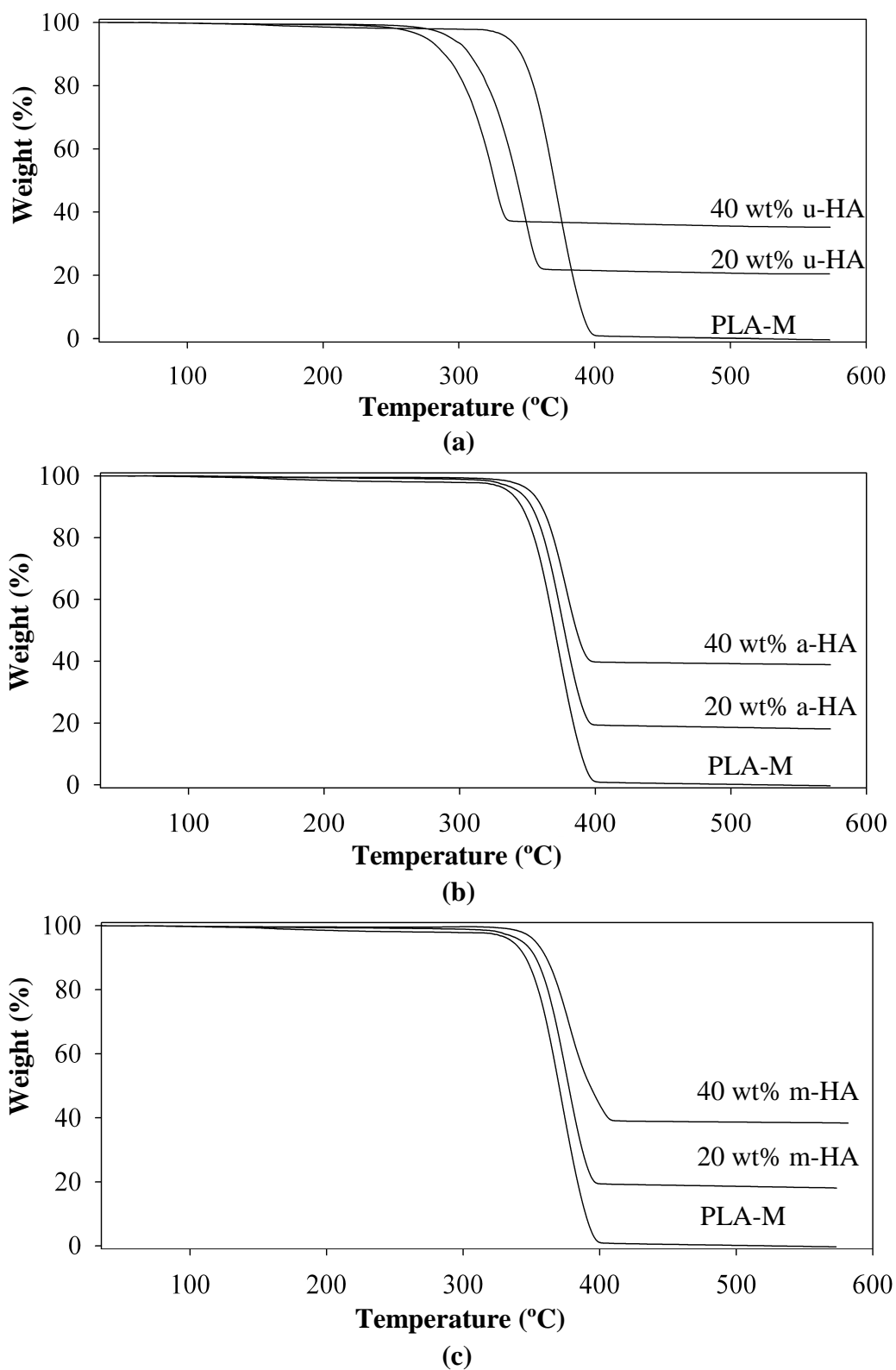
**Figure 5.1** TGA thermograms of u-HA, a-HA and m-HA.

Figure 5.2 shows TGA thermograms of neat PLA, u-HA/PLA, a-HA/PLA and m-HA/PLA composites at various contents of the HA. The neat PLA had higher thermal stability than u-HA/PLA composites as shown in Figure 5.2 (a). In addition, the composites containing u-HA exhibited significantly lower thermal stability with increasing filler content as compared between 20wt% and 40wt% of u-HA. In contrast, thermal stability of the composites containing silane-treated HA was slightly increased with increasing filler content, as shown in Figure 5.2 (b-c).

As reported by several research groups, an increase in thermal stability of a polymer composite can be explained in two aspects: either polymer decomposition products are blocked by a good distribution, good adhesion and good dispersion of better thermal stability of filler (Fukushima *et al.*, 2009) or the good distribution and good dispersion of filler in the composite acts as a barrier preventing heat transfer (Ignjatovic *et al.*, 2004). In addition, the results from chapter 3



suggested that u-HA/PLA composites occurred thermal oxidative upon processing and the OH groups on HA surface accelerated the reaction. By treating HA surface with a silane coupling agent, OH groups on the HA surface were less exposed to PLA matrix. Thermal oxidation of PLA in silane-treated HA/PLA composite was then slow down as confirmed by the initial  $\overline{M}_w$  of 4A, 4B and 4C in table 5.4. According to other reports the increase in thermal stability of the silane-treated HA/PLA composites with increasing HA content was due to the better distribution and adhesion between silane-treated HA and PLA matrix as well as the less exposition of OH groups on HA surface to the PLA matrix.



**Figure 5.2** TGA thermograms of (a) b-HA/PLA, (b) a-HA/PLA and (c) m-HA/PLA composites with HA contents of 20wt% and 40wt%.

#### **5.4.2 Change in molecular weight and molecular weight distribution of PLA after processing**

It is known that PLA chains can be easily broken at a particular temperature range. Subsequently, molecular weight of PLA is decreased; monomers and gas-products are formed (Ignjatovic *et al.*, 2004). Therefore, during the preparation of HA/PLA composite, the PLA chains could possibly be degraded by high processing temperature. This was further investigated by GPC technique. Effects of HA surface treatment and HA content on molecular weight of PLA are illustrated in Table 5.3. Based on GPC results, it was clearly shown that the degradation of PLA chains in the composites containing m-HA and a-HA was slow down.

The planar conformation of PLA basic structure is defined as  $H-[O-CH(CH_3)-CO-]_n-OH$ . One can predict where the breaking of bonds in the basic chain will occur. Gupta and Deshmukh (1982) reported that the thermal degradation of PLA chain is a one-step process with the first-order reaction kinetics. The OH groups at the end of PLA chain can destroy the basic chain under the influence of thermal energy. In each of these reactions, the OH group regenerates and the process continues. In addition, the OH groups on untreated HA surface can also directly destroyed the PLA basic chain (Ignjatovic *et al.*, 2004). According to their works, the appearance of OH groups in the composite system has an important influence on the PLA molecular weight. However, it is known that some silane coupling agents can be bonded with OH group on HA surface. Treating HA surface with a silane coupling agent, as done in this study, before incorporating into PLA matrix decreased amounts of OH groups on HA surface. Therefore, PLA chains in the composites were less attacked and their molecular weight were less degraded.

**Table 5.3** Effect of HA surface treatment and HA content on molecular weight of PLA in neat PLA and the composites.

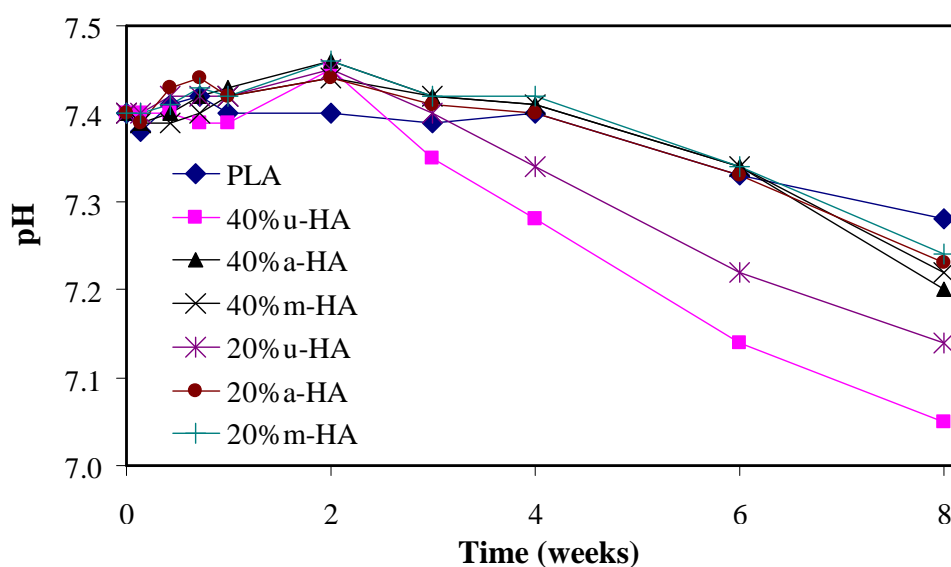
Designation	Average molecular weight		MWD
	$\overline{M}_w$	$\overline{M}_n$	
as-received PLA	$2.83 \times 10^5$	$1.31 \times 10^5$	2.16
PLA-M	$1.94 \times 10^5$	$0.76 \times 10^5$	2.55
1A	$0.67 \times 10^5$	$0.11 \times 10^5$	6.09
2A	$0.61 \times 10^5$	$0.10 \times 10^5$	6.10
3A	$0.56 \times 10^5$	$0.09 \times 10^5$	6.22
4A	$0.53 \times 10^5$	$0.08 \times 10^5$	6.63
1B	$1.62 \times 10^5$	$0.33 \times 10^5$	4.90
2B	$1.33 \times 10^5$	$0.18 \times 10^5$	7.38
3B	$1.29 \times 10^5$	$0.15 \times 10^5$	8.60
4B	$1.24 \times 10^5$	$0.13 \times 10^5$	9.53
1C	$1.52 \times 10^5$	$0.29 \times 10^5$	5.24
2C	$1.47 \times 10^5$	$0.23 \times 10^5$	6.39
3C	$1.37 \times 10^5$	$0.19 \times 10^5$	7.21
4C	$1.24 \times 10^5$	$0.15 \times 10^5$	8.27

### 5.4.3 *In vitro* degradation of PLA and HA/PLA composites

#### 5.4.3.1 Change in pH of PBS solution

Neat PLA and HA/PLA composite specimens were put into vials and immersed in PBS solution. Then, pH values of the PBS solution at various immersion time were measured as illustrated in Figure 5.3. For the neat PLA specimens at the immersion time up to 4 weeks, the pH of the PBS solution remained constant at about 7.4 and followed by a slight decrease.

In comparison between HA/PLA composites, the pH values of the PBS solution after immersing u-HA/PLA composites were lower than those of the PBS solution after immersing silane-treated HA/PLA composites (*i.e.* 2B, 4B, 2C and 4C). Additionally, the pH of the PBS solution after immersing u-HA/PLA composites decreased faster with increasing u-HA content.



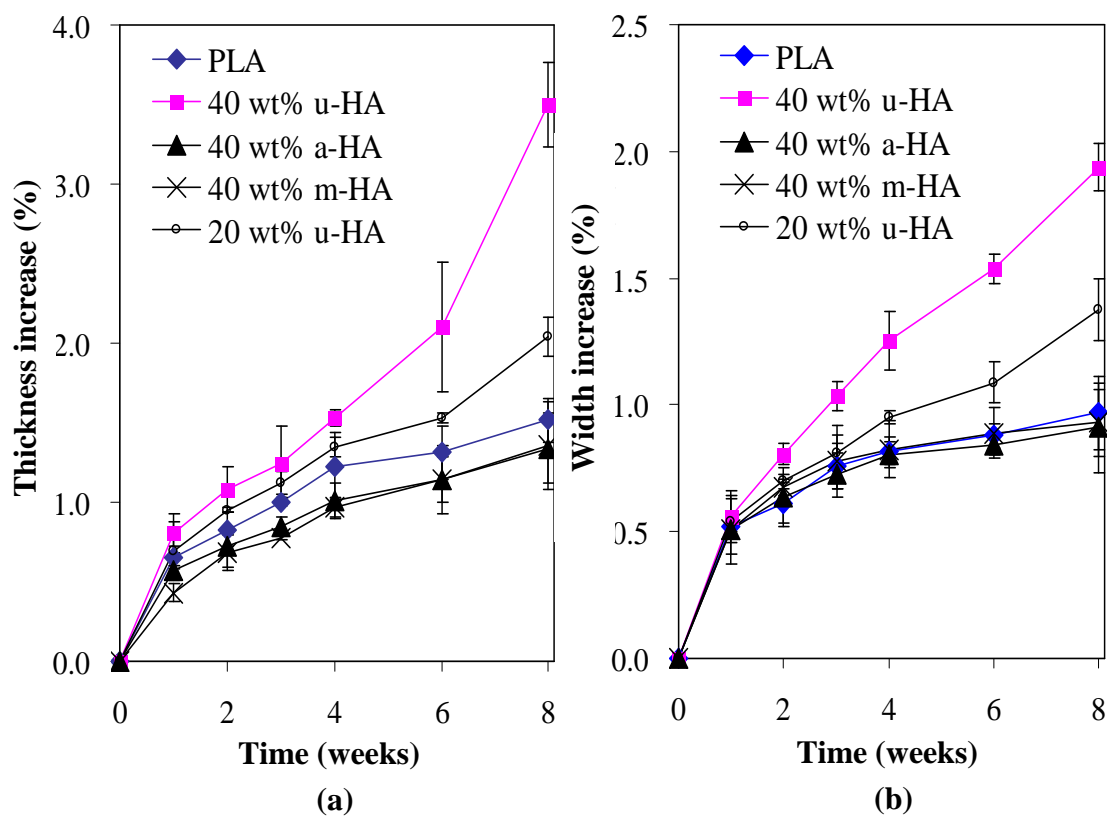
**Figure 5.3** Changes in pH of PBS solution after immersion of neat PLA, u- HA/PLA, a-HA/PLA and m-HA/PLA composites.

Generally, the decrease in the pH value of the PBS is due to the formation of the degraded acidic molecules and their release from the composite. However, in the present study, a part of the acidity of the degradation products could be neutralized by alkaline calcium phosphate in the composite. This observation was similar to other research works, Li *et al.* (2004) found that the basic ions released from the wollastonite powder neutralized the acidic degradation products of the polyhydroxybutyrate-polyhydroxyvalerate (PHBV) and compensated the pH decrease. Moreover, the compensated acidification of the PBS, due to acidic products of the biodegradable polyester degradation, by exchanging of protons in PBS for alkali in the Bioglass® particles were also reported (Stamboulis, Hench, and Boccaccini, 2002; Boccaccini and Maquet, 2003). Therefore, the pH value of the PBS solution slightly increased after the first two weeks of the immersion of HA/PLA composites. The rapid decrease in pH after 4 weeks of immersion is possibly caused by the more hydrolysis of PLA where H<sup>+</sup>-ions come into the solution, exceeding the buffering capacity of PBS. As the degraded acidic molecules accumulated and released from the composite, the pH values of PBS obviously decreased. In addition, the poor adhesion between u-HA and PLA and the poor distribution of u-HA in the matrix could release more acidic molecules from the composite into the PBS. In this case, the pH values of the PBS after immersing u-HA/PLA composites were lower than those of the PBS after immersing silane-treated HA/PLA composite.

#### 5.4.3.2 Effect of *in vitro* degradation on the size of composites

Figure 5.4 shows percentage of width and thickness changes of neat PLA, u-HA/PLA, a-HA/PLA and m-HA/PLA composites during *in vitro* degradation. As shown in Figure 5.4 (a), in the first four weeks of *in vitro* degradation a slight increase in thickness of the neat PLA specimens was observed as compared with those of the HA/PLA composite specimens. The increased in size at initial time of immersion should be attributed to the relaxation of stress of the u-HA/PLA, a-HA/PLA and m-HA/PLA composites generated in the fabrication when the specimens were incubated in the PBS at 37°C (Yang *et al.*, 2008).

After four weeks of immersion, the increases in thickness of the a-HA/PLA and the m-HA/PLA composites were less than that of u-HA/PLA composites. These phenomena were probably attributed to the hydrophilicity of the deposited silane coupling agent on the HA surface leading to the less swelling of the silane-treated HA composite. According to SEM micrographs of u-HA/PLA composites, it should be noted that u-HA is a hydrophilic material resulting in the gap occurrence at interface between u-HA and PLA matrix. These gaps would lead to the faster diffusion of PBS into inner site of the composite, then, the thickness swelling of u-HA/PLA composites was greater than those of silane-treated HA/PLA composites. As shown in Figure 5.4 (b), similar phenomena were found in width changes of all specimens during *in vitro* degradation.



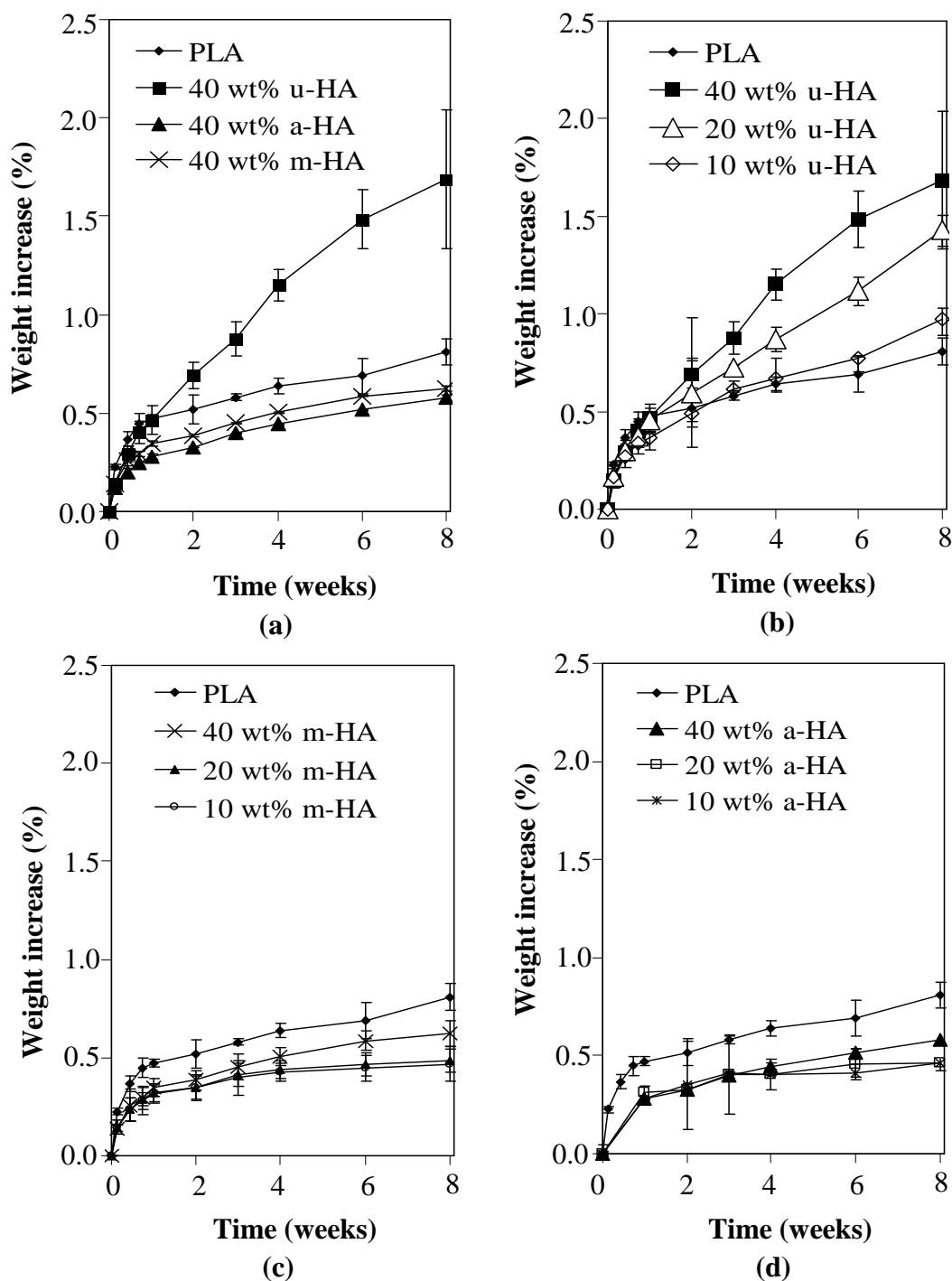
**Figure 5.4** Dimensional changes of the neat PLA and the composite specimens upon immersion in PBS solution: (a) thickness change and (b) width change.



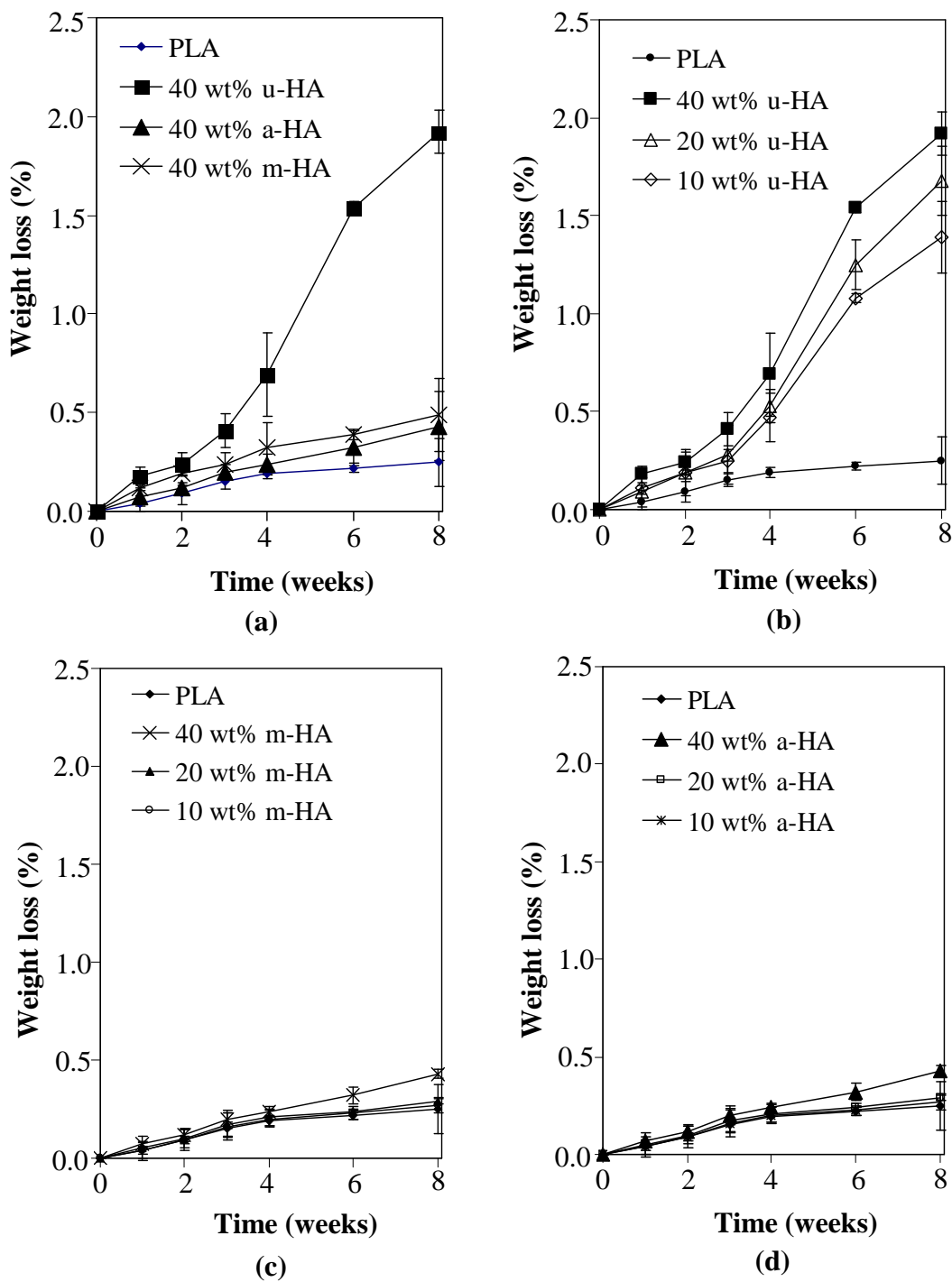
#### 5.4.3.3 Effect of *in vitro* degradation on the weight of composites

It was shown in Figure 5.5 that all specimens after *in vitro* experiment tended to increase their weight with increasing the immersion time. As seen, the weight of neat PLA increased slower than that of the composite. For the composites, at the equal content of filler, the u-HA/PLA composites showed higher increase in weight than that of silane-treated HA/PLA composite. The rapid increase in weight of u-HA/PLA composite indicated that water can easily diffuse through the matrix. This result was probably due to the porosity or gap in the composite matrix created by lacking of adhesion between u-HA and PLA matrix. Additionally, by increasing filler contents, the gradual increase in weight of the composites were also observed especially for the u-HA/PLA composites.

Subsequently, all specimens were dried at 45°C to constant weight before measuring the weight loss. The weight of the neat PLA, u-HA/PLA, a-HA/PLA and m-HA/PLA composites was measured at different immersion periods. As shown in Figure 5.6, the neat PLA showed a slight weight loss, less than 0.4%, during the experimental periods.



**Figure 5.5** Water absorption of the neat PLA and the composite specimens upon immersion in PBS: (a) effect of filler types, (b) effect of untreated HA content, (c) effect of MPTS-treated HA content and (d) effect of APES-treated HA content.



**Figure 5.6** Weight changes of the neat PLA and the composites specimens upon immersion in PBS: (a) effect of filler type, (b) effect of untreated HA content, (c) effect of MPTS-treated HA content and (d) effect of APES-treated HA content.

However, the weight loss profiles of the composites were different from that of neat PLA. For the u-HA/PLA, a-HA/PLA and m-HA/PLA composite specimens, after incubating in PBS, exhibited increases in weight loss as functions of HA content and types of surface modified HA as shown in Figure 5.6 (a-d). The weight loss of u-HA/PLA composite specimens was the highest, as compared with those of the silane-treated HA/PLA composite specimens. This was due to the higher chance of PBS penetration via the u-HA and PLA interface and leakage of the degradation products throughout surface cracks, created by low surface adhesion, and the solubility of the HA particles themselves (Navarro *et al.*, 2005). Moreover, the weight loss of all composite specimens increased with increasing HA content. It seemed that treating HA surface with silane coupling agent diminished those causes of weight loss of the composites mentioned previously.

#### **5.4.3.4 Changing in molecular weight of PLA molecules**

The decrease in molecular weight of PLA chains with immersion time are shown in Table 5.4 and Table 5.5. As seen, the molecular weight of PLA in all specimens gradually decreased with increasing immersion time. At 1, 4, and 8 weeks after immersion in PBS, the PLA molecular weights in the u-HA/PLA composite specimen were decreased about 38.65, 49.48 and 52.06% of their initial values, respectively, while those in the a-HA/PLA and m-HA/PLA specimens were 3.22, 7.25, 13.71, 6.45, 25.81 and 26.61%, respectively. After 2 weeks of immersion, the molecular weight of PLA in the composites drastically decreased at faster rate than that of the neat PLA. In comparison, the PLA molecular weight in u-HA/PLA specimens decreased faster than those in the m-HA/PLA and a- HA/PLA specimens.

The possible reason of degradation of PLA chains is the random scissions of the ester linkages in their backbone. Generally, the rate of non-enzymatic degradation of poly( $\alpha$ -hydroxy acid) attributed to the diffusion rate of water and the concentration of OH<sup>-</sup> ions which catalyse the hydrolysis of ester bonds of poly( $\alpha$ -hydroxy acid) (Cam *et al.*, 1995; Yuan *et al.*, 2002). Hence, an important factor affecting hydrolysis degradation mechanism of biodegradable polymer via hydrolysis reaction attributed to the uptake of water (Gopferich, 1996).

**Table 5.4** Changes in molecular weight of PLA in neat PLA and the composites after immersion in PBS.

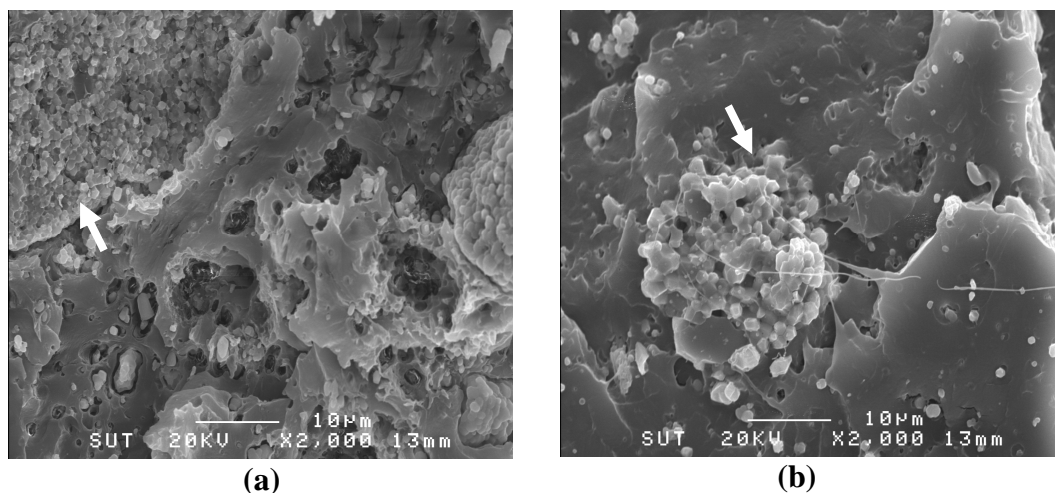
Immersion time (weeks)	$\bar{M}_w$			
	PLA	4A	4B	4C
0	$1.94 \times 10^5$	$0.53 \times 10^5$	$1.24 \times 10^5$	$1.24 \times 10^5$
1	$1.19 \times 10^5$	$0.42 \times 10^5$	$1.20 \times 10^5$	$1.16 \times 10^5$
2	$1.18 \times 10^5$	$0.42 \times 10^5$	$1.18 \times 10^5$	$1.07 \times 10^5$
4	$0.98 \times 10^5$	$0.41 \times 10^5$	$1.15 \times 10^5$	$0.92 \times 10^5$
6	$0.95 \times 10^5$	$0.34 \times 10^5$	$1.10 \times 10^5$	$0.91 \times 10^5$
8	$0.93 \times 10^5$	$0.30 \times 10^5$	$1.07 \times 10^5$	$0.91 \times 10^5$

**Table 5.5** Percentage of changes in molecular weight of PLA in neat PLA and the composites after immersion in PBS.

Immersion time (weeks)	%Change in $\overline{M}_w$			
	PLA	4A	4B	4C
0	-	-	-	-
1	38.65	20.75	3.22	6.45
2	39.17	20.75	4.84	13.71
4	49.48	22.64	7.25	25.81
6	51.03	35.85	11.29	26.61
8	52.06	43.39	13.71	26.61

As shown in Table 5.4, the molecular weight of PLA in the u-HA/PLA composites decreased faster than those in the neat PLA, a-HA/PLA and m-HA/PLA composites. This was because of the lacking of adhesion between u-HA particles and the PLA matrix indicating by the appearance of gap at interface between u-HA particles and the PLA matrix as shown in SEM micrograph of tensile fracture surface of 20wt% u-HA and 20wt% a-HA composite (Figure 5.7). Hence, phosphate-buffered solution can easily penetrated into the inner site of u-HA/PLA composites though the interface between PLA and u-HA particles and initiate hydrolysis reaction in this composite at a faster rate than it does to other composite systems. On the other hand, the decrease in PLA molecular weight in the composite seemed to be slow down by treating HA surface with a silane coupling agent. This can be explained as follows. Silane-treated HA has more hydrophobic and more compatible to PLA matrix than untreated HA. The incorporated silane-treated HA distribute homogeneously in PLA matrix and provide good adhesion between the two phases as

shown in Figure 5.7 (b). So, PBS solution need long time period to penetrate in and to hydrolyze the silane-treated HA/PLA composites, *i.e.* a-HA/PLA and m-HA/PLA composites.

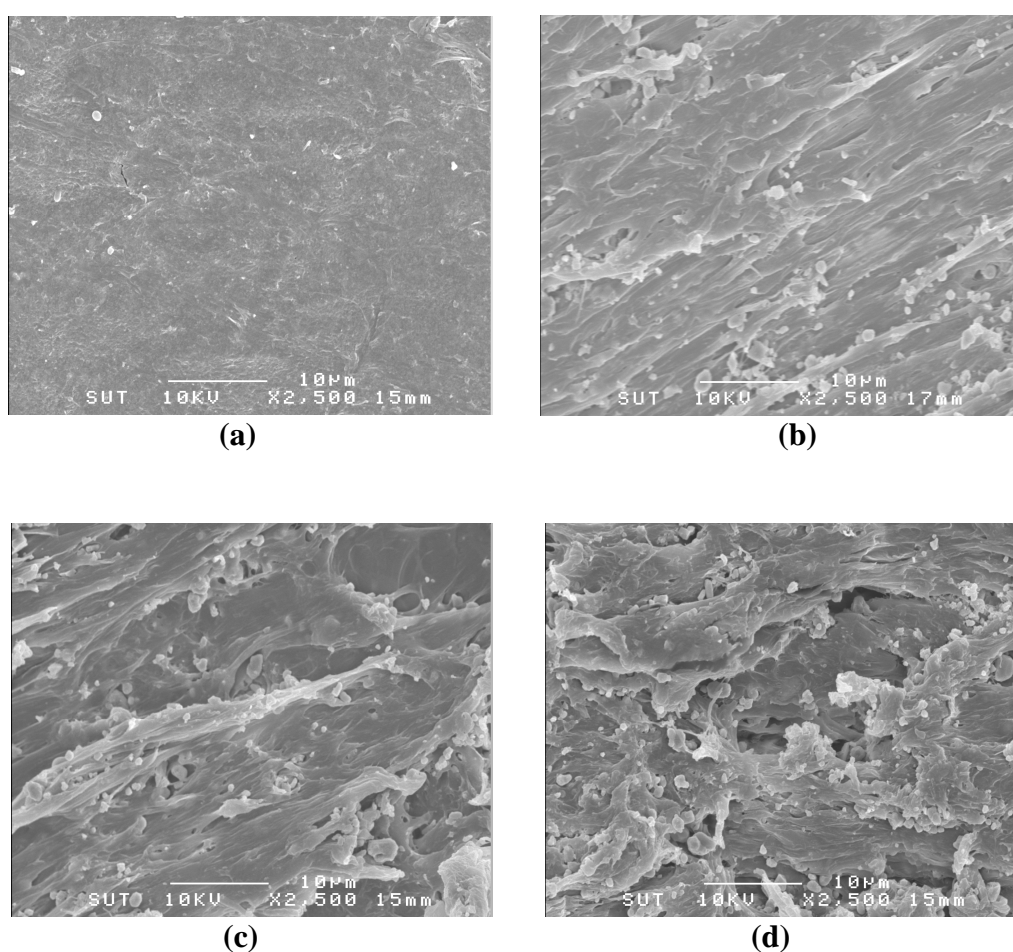


**Figure 5.7** SEM micrographs of tensile fracture surfaces of PLA composites at 20wt% of (a) u-HA, (b) a-HA.

#### 5.4.3.5 Change in surface morphologies of the HA/PLA composites

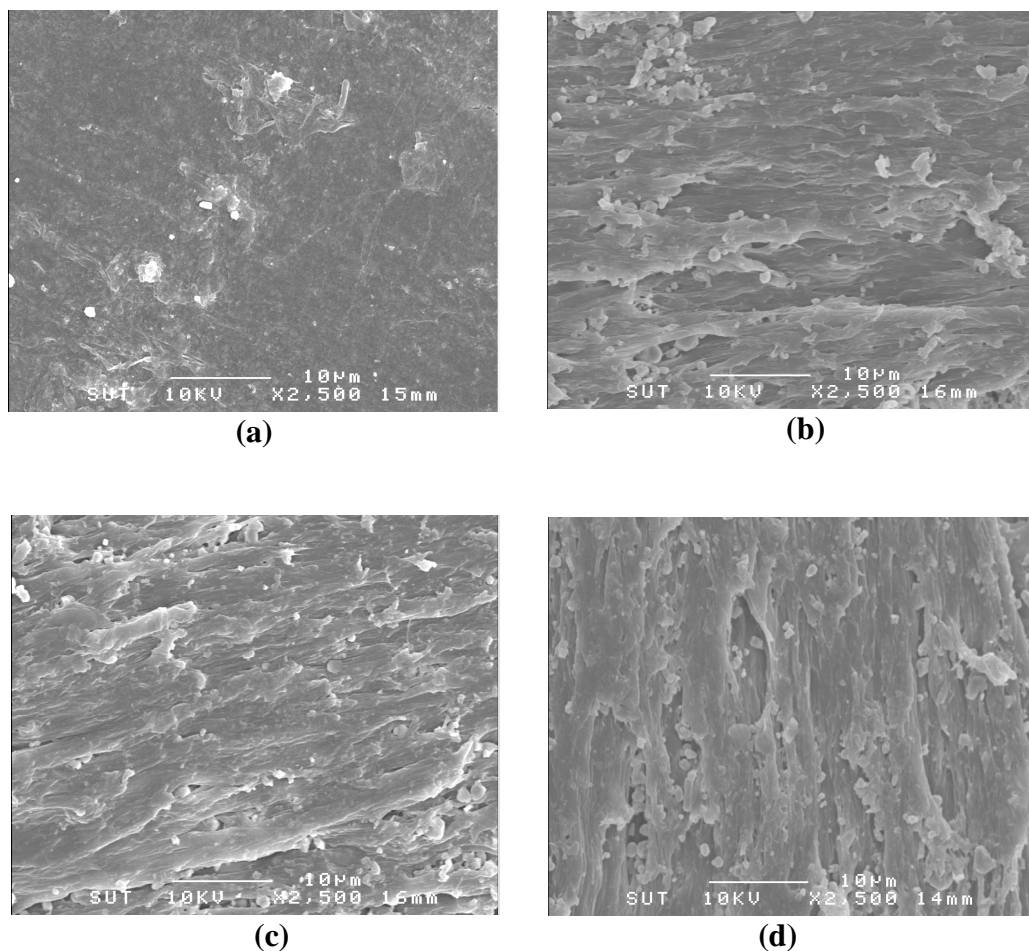
Figure 5.8 and 5.9, respectively, show the SEM micrographs of 40wt% u-HA/PLA and a-HA/PLA composite surfaces at various immersion periods in PBS. As seen, the morphologies of all composites were altered with increasing immersion time, especially those of the u-HA/PLA composite. At the initial time, all composites showed flat composite surfaces. After 2 weeks of immersion, the surface of the composite began to erode because of reactivity between PLA and PBS. After 4 weeks of immersion, the PLA part continued degrading and created the scratch on composite surface, further, some of the cracks became larger. At the immersion time of 8 weeks, the larger hole on the composite surface was observed. This was because

of the degradation of PLA and the release of the degradation products; some of HA particles were exposed out of the composite surface. A large amounts of u-HA particles were observed and accumulated on the u-HA/PLA composite surface. This was due to the faster degradation of PLA in the composite than that of a-HA/PLA and m-HA/PLA composites.



**Figure 5.8** Changes in morphologies of 40wt% u-HA/PLA composites upon immersion in PBS for: (a) 0 week, (b) 2 weeks, (c) 4 weeks and (d) 8 weeks.



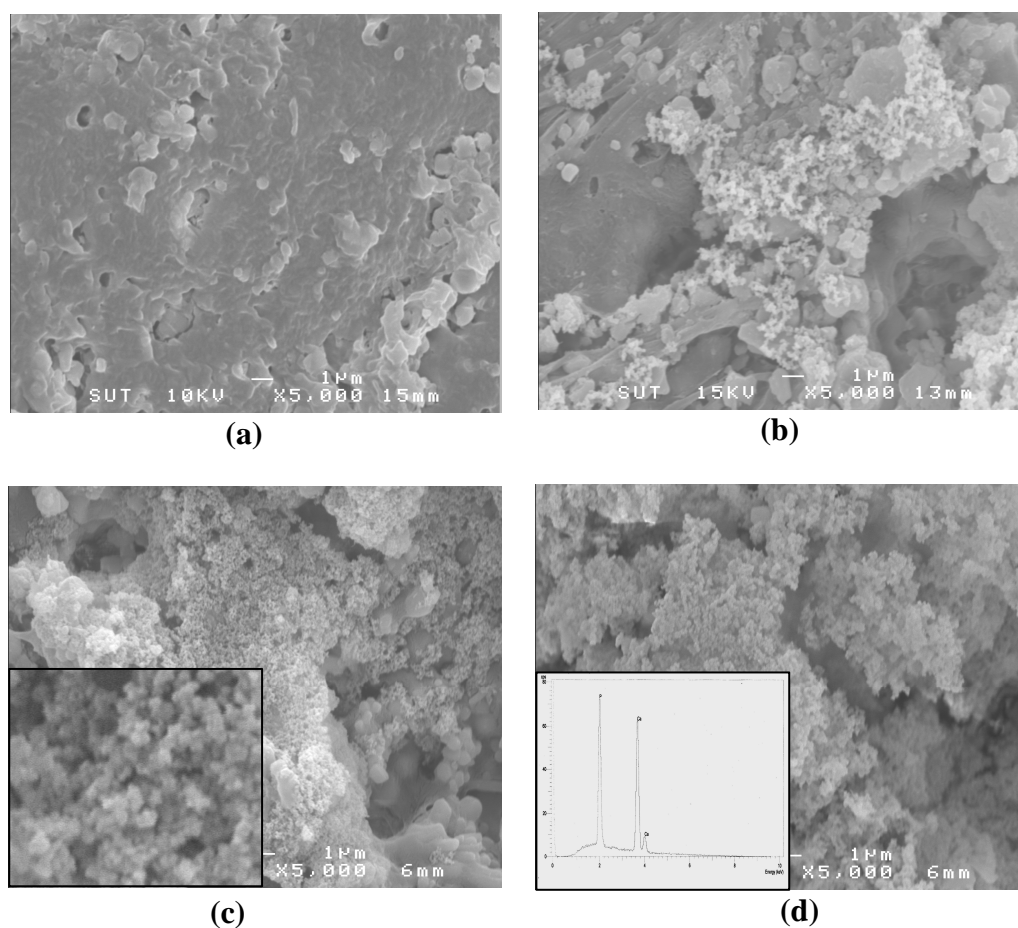


**Figure 5.9** Changes in morphologies of 40wt% m-HA/PLA composites upon immersion in PBS for: (a) 0 week, (b) 2 weeks, (c) 4 weeks and (d) 8 weeks.

#### 5.4.4 Investigation of bioactivity of HA/PLA composites

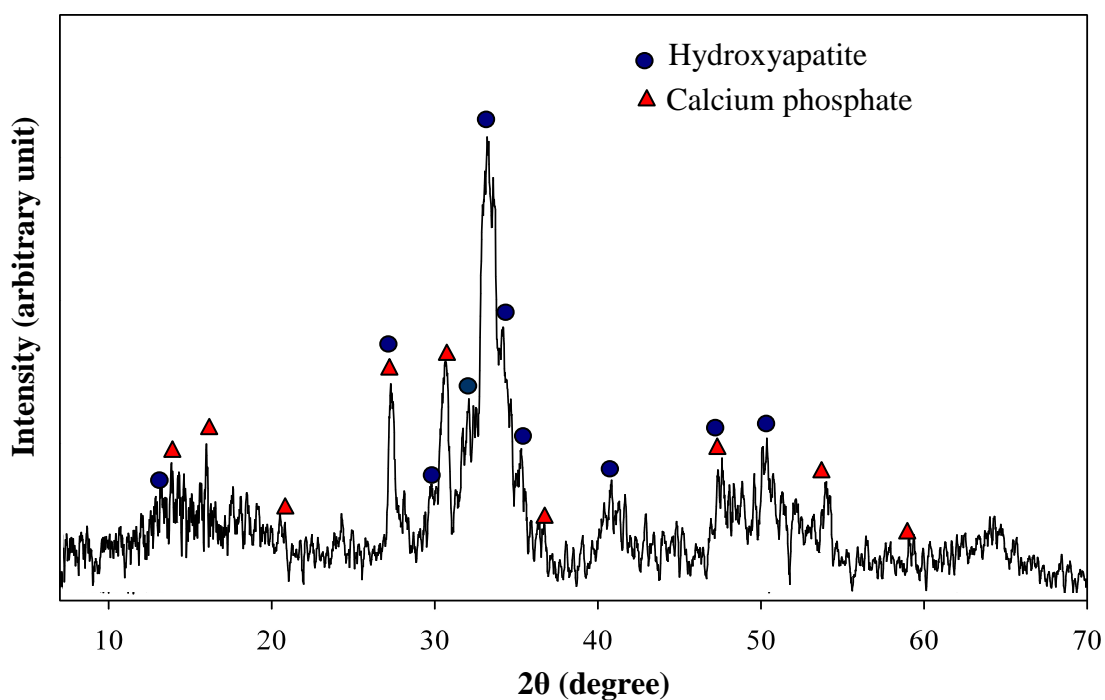
Figure 5.10 (a-d) shows SEM micrographs of the outer surfaces of PLA composites containing 40wt% of u-HA at various immersion period in SBF. At the initial time (Figure 5.10 (a)), a flat composites surface was observed. However, after 3 days of immersion in SBF at 37°C, the surface of the composite began to degrade while precipitated particles were observed on the composite surface, as

shown in Figure 5.10 (b). Further, after 1 week of immersion, the amounts of the precipitates were obviously increased (Figure 5.10 (c)). After 2 weeks of immersion, the composites specimen surface was covered with the precipitates (Figure 5.10 (d)). The EDX attached to the SEM indicated that the elemental composition in the precipitates were calcium and phosphorus. The precipitated powder was scratched out from the composite surface for further investigation by an XRD and a FTIR spectroscopy.



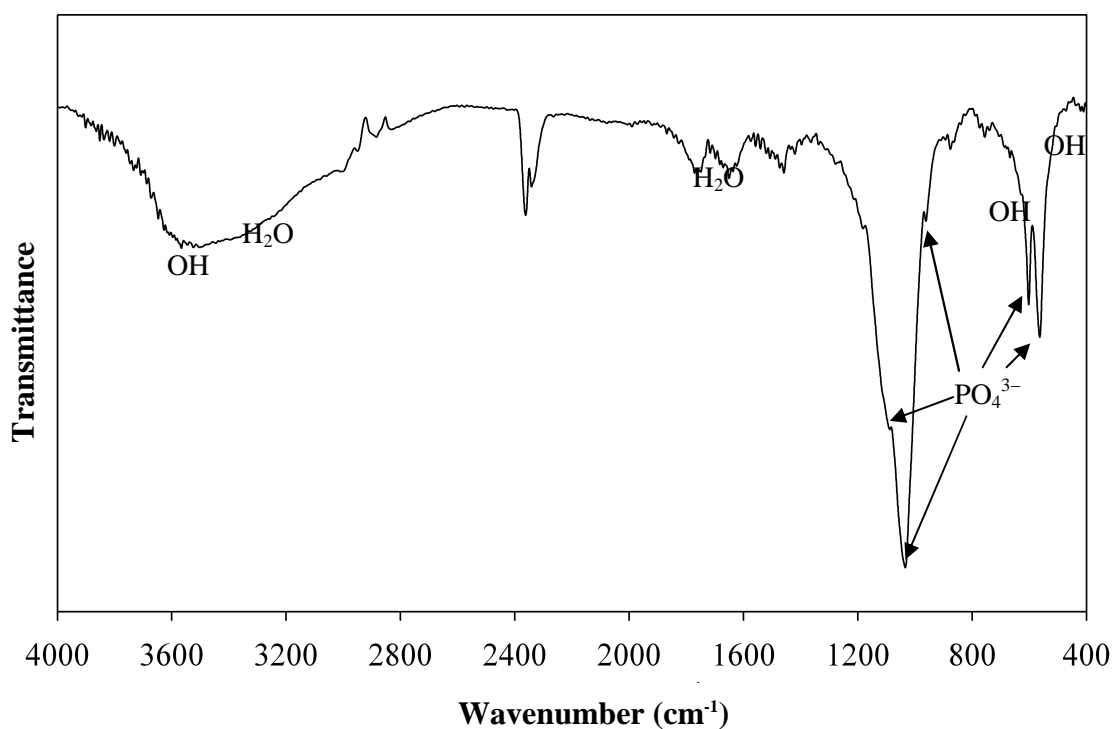
**Figure 5.10** SEM micrographs of composite surfaces and the precipitated layer formed after immersion in SBF for: (a) 0 week, (b) 3 days, (c) 1 week and (d) 2 weeks.

Figure 5.11 displays the XRD pattern of the scratched precipitated powder from the composite surfaces. The scratched powder showed mixed phases of tri-calcium-di-phosphate (TCP) and HA. The characteristic peak of HA at  $31.30^\circ$  was observed and the characteristic peak of TCP at  $30.44^\circ$  was also observed. In addition, the presence of broad peak between  $20^\circ$  and  $38.5^\circ$  indicates the formation of an amorphous phase. Therefore, it can be inferred that the mixture of an amorphous TCP and HA apatite was formed on the HA/PLA composite surface after 3 days of immersion in SBF.



**Figure 5.11** XRD pattern of scratched powder from the composite surface after 2 weeks of immersion.

FTIR spectrum of the scratched precipitated powder is shown in Figure 5.12. The peaks at 1085, 1036, 963, 600 and 575  $\text{cm}^{-1}$  were assigned to different vibration modes of  $\text{PO}_4^{3-}$  group in the precipitated powder. Moreover, The stretching and the bending vibration of structural OH groups in the apatite lattice were also observed at 3571  $\text{cm}^{-1}$  and 632  $\text{cm}^{-1}$ , respectively. According to these results, an amorphous calcium phosphate was formed under this experimental condition (SBF, 37°C, pH 7.4).



**Figure 5.12** FTIR spectrum of the precipitate powder on the composite after 1 week of immersion in SBF at 37°C.

## 5.5 Conclusions

The TGA results of HA/PLA composites revealed that thermal properties of HA/PLA composite were crucially depended on surface properties of HA particle. Silane-treated HA/PLA composites showed an increasing in thermal stability as compared with the untreated HA/PLA composites. Moreover, the deposit of silane coupling on HA surface in silane-treated HA/PLA composites delayed the PLA chains scission, which were confirmed by GPC results. Additionally, the *in vitro* degradation behaviors of all HA/PLA composites were investigated. Results showed that the untreated HA/PLA composites exhibited more change in pH stability of PBS, mass, thickness, width, and morphologies than those of silane-treated HA/PLA composites. Moreover, the molecular weight of PLA in the untreated HA/PLA composites were higher changed, after immersion in PBS, than silane-treated HA/PLA composites. The porosity and gap in the composite matrix created by lacking of adhesion between u-HA and PLA matrix led to rapid diffuse of water through the matrix. These was the main reason of the faster degradation of PLA in u-HA/PLA composites. In contrast, silane-treated HA/PLA composite had more adhesion between two phases leading to the less penetrate of water into inner side, therefore, lower degradation of PLA was obtained. Additionally, the bioactive characterization showed the positive result in the formation of a calcium phosphate on the composite surface, after immersion in SBF, which could enhance the interaction between the material and the bone tissue.

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## CHAPTER VI

### CONCLUSIONS

Preparation of biomedical materials from bovine bone is an alternative approach to obtain a suitable bone replacement material with an inexpensive expense. In this study, HA powder was prepared from natural source, *i.e.* bovine bone, and used as a filler for preparing PLA composites. Processing technique and u-HA content were the factors that influenced mechanical properties of u-HA/PLA composites. To form HA/PLA composites, u-HA was incorporated into PLA matrix by either melt-mixing or solution mixing techniques. With increasing u-HA content, tensile strength and impact strength of the composites were decreased while the tensile moduli of the composites were increased. In comparison between two preparation techniques, the composites prepared by melt-mixing technique have higher tensile strength, tensile modulus and impact strength than those prepared by solution-mixing technique. However, 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. According to mechanical properties, the melt-mixed process was selected to prepare HA/PLA composite.

To decrease the degradation of PLA chains and increase the adhesion between HA and PLA matrix, the silane coupling agents, *i.e.* APES and MPTS, were selected to modify HA surface. Tensile strength, tensile modulus, elongation at break and impact strength of the PLA composites can also be improved by modifying u-HA surface with either APES or MPTS. The enhancement of mechanical properties of

silane-treated HA/PLA composites was caused by the good dispersion of silane-treated HA in PLA matrix and the good interfacial interaction between the two phases. However, the mechanical properties of silane-treated HA/PLA composites still need to be further enhanced or adjusted in order to meet requirement for a specific medical application. The TGA results of HA/PLA composites revealed that thermal properties of HA/PLA composite were crucially dependent on surface properties of HA particle. Silane-treated HA/PLA composites showed an increasing in thermal stability as compared with the untreated HA/PLA composites. Moreover, the deposit of silane coupling on HA surface in silane-treated HA/PLA composites delayed the PLA chains scission, which were confirmed by GPC results.

Additionally, the *in vitro* degradation behaviors of all HA/PLA composites were investigated. Results showed that the untreated HA/PLA composites exhibited more change in pH of PBS, mass, thickness, width, and morphologies than the silane-treated HA/PLA composites. Moreover, the molecular weights of PLA in the untreated HA/PLA composites decreased faster than those of silane-treated HA/PLA composites after immersion in PBS. The porosity and gap in the composite matrix created by lacking of adhesion between u-HA and PLA matrix led to rapid diffuse of water through the matrix. These was the main reason of the faster degradation of PLA in u-HA/PLA composites. In contrast, silane-treated HA/PLA composite had more adhesion between two phases leading to the less penetration of water into inner side, therefore, lower degradation of PLA was obtained. Additionally, the bioactive characterization showed the positive result in the formation of calcium phosphate compound on the composite surface, after immersion in SBF, which could enhance the interaction between the material and the bone tissue. In addition, the results from

*in vitro* cytotoxicity test suggested a potential of using bovine bone based HA/PLA composite as a biomaterial.

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**APPENDIX A**  
**PUBLICATION**

## CHARACTERIZATION OF SILANE-TREATED NATURAL HYDROXYAPATITE/POLY (LACTIC ACID) NANOCOMPOSITES

Suriyan Rakmae<sup>1,2</sup>, Yupaporn Ruksakulpiwat<sup>1,2</sup>, Wimolnak Sutapan<sup>1,2</sup> and Nitinat Suppakarn<sup>1,2\*</sup>

<sup>1</sup> Suranaree University of Technology /Institute of Engineering /School of Polymer Engineering, Nakhon Ratchasima, 30000 Thailand

<sup>2</sup>Center of Excellence for Petroleum, Petrochemical, and Advanced materials /Chulalongkorn University, Bangkok, 10330 Thailand

\*E-mail:nitinat@sut.ac.th, Tel: +66-44-224439

**Abstract:** In this work, bovine bone based HA was prepared by thermal treatment and then incorporated into PLA matrix. The 3-aminopropyltriethoxysilane (APES) and 3-methacryloxypropyltrimethoxysilane (MPTS) were used to modify HA surface in order to improve the compatibility between HA and PLA matrix. FTIR spectrum of untreated HA revealed that the obtained HA was a highly crystalline carbonated hydroxyapatite. SEM micrograph showed that the obtained HA after calcination at 1100°C was composed of agglomerated hydroxyapatite particles. The agglomeration of particles was reduced by treating HA surface with silane coupling agent. Tensile modulus of all HA/PLA composites increased with increasing HA content. This was in contrast to the tensile and impact strength of the composites which decreased with increasing HA content. In addition, mechanical properties of HA/PLA composites can be improved by treating HA surface with a silane coupling agent.

### Introduction

Hydroxyapatite (HA) has been investigated as a biomaterial and used in various medical applications, e.g. spacers and bone graft substitutes in orthopaedic and maxillofacial applications. This is because of its excellent biocompatibility and its osteoconductivity [1, 2]. HA can be synthetically prepared or derived from natural sources, e.g. coral, bovine bone. However, the major drawbacks of using HA as a biomaterial are its brittleness and the difficulty of processing. One attempt to solve these problems is mixing HA with a flexible and bioresorbable polymer.

Many kinds of bioresorbable polymers have been developed and used in medical applications. Among those polymers, poly (lactic acid) (PLA) is a good candidate due to its biodegradability and nontoxic byproducts yielded after degradation [3]. A composite between PLA and HA is a good alternative for using as a biomaterial since it is combining strength and stiffness of HA with flexibility and resorbability of PLA. However, the major drawbacks found in HA/PLA composites are the agglomeration of HA and the failure at the interface between HA and the polymeric matrix. These lead to the decreasing in mechanical properties of HA/PLA composites. Therefore, an improvement of the interfacial adhesion between particles and matrix has become an important

area of studies of HA/PLA composites [4, 5]. The HA surface can be treated with a coupling agent, such as organofunctional silanes, by which the interfacial adhesion between filler and polymer matrix is effectively improved [6].

In recent years, natural HA has been attracting much attention since it is natural and less expensive sources for producing HA [7, 8]. In addition, there are high volumes of bovine bone as a livestock waste in Thailand. So, this work attempted to produce natural HA powders from bovine bones and to use the powder as a filler for PLA. The bovine bone based HA was treated with silane coupling agents, i.e. 3-aminopropyl triethoxysilane and 3-methacryloxypropyltrimethoxy silane. The properties of untreated HA and silane-treated HA were characterized. In addition, effects of filler characteristics and filler content on morphological and mechanical properties of HA/PLA composites were determined.

### Materials and Methods

**Materials:** PLA (4042D) was purchased from NatureWorks, LLC. Bovine bones were supplied by Limeiseng Co., Nakhon Ratchasima. 3-aminopropyl triethoxysilane (APES) and 3-methacryloxypropyl trimethoxysilane (MPTS) were purchased from Optimal Tech Co., Ltd. and Aldrich, respectively.

**Preparation of HA powders:** Bovine bones were burned in open air and were then ground into powders, using a ball milling machine. Then the HA powders were heat treated at 1100°C for 3 h, call HA. After that, the powders were modified by either APES or MPTS. The content of silane used was 2.0 wt% based on weight of HA powders. To prepare silane solution, the APES was dissolved in an aqueous solution whereas MPTS was dissolved in 30 vol% of alcoholic solution. The pH of each silane solution was adjusted to 3.5 using acetic acid. HA powders were soaked in each silane solutions and left under agitation at room temperature for 3 h. Then the silane-treated powders were washed and dried at 80°C overnight in an oven.

**Preparation of HA/PLA composites:** HA/PLA composites were prepared through melt-compounding technique using an internal mixer (HAAKE/Rheomix). PLA and HA were mixed at 170°C with 70 rpm for 10



min. The weight ratios of HA/PLA were 9/1, 8/2, 7/3, and 6/4. Each HA/PLA composite was left at room temperature for 24 h. then it was ground into small pieces. To prepare composite specimens for mechanical tests, the ground HA/PLA composite was heated in dumbbell-shaped and rectangular-shaped molds from room temperature to 180°C and maintained at that temperature for 10 min. Subsequently, it was hot-pressed vertically for 5 min at 180°C under a pressure of 2000 psi and cooled to room temperature.

**Characterization of HA powders:** Functional groups of the untreated and silane-treated HA powders were identified by a Fourier transform infrared spectrometer (FTIR) (BIO-RAD/FTS175C, KBr pellet technique). The spectrum was recorded in the 400-4000  $\text{cm}^{-1}$  region with 2  $\text{cm}^{-1}$  resolution. The thermal behaviors of silane-treated and untreated HA powders were determined by thermogravimetric analyzer (TGA) (TA Instrument/SDT2960). The powders were heated from room temperature to 600°C at a rate of 20°C/min under a nitrogen atmosphere. In addition, a scanning electron microscopy (SEM) (Jeol/JSM-6400) operating at 15-20 kV was used to reveal microstructure of the HA powders.

**Characterization of HA/PLA composites:** A scanning electron microscopy (SEM) (Jeol/JSM-6400) operating at 20 kV was used to visualize tensile fracture surfaces of the HA/PLA composites. All samples were coated with a thin layer of gold before examining. Tensile properties of HA/PLA composites were investigated according to ASTM method D638-03 using a universal testing machine (Instron/5569). Moreover, Izod impact strength of unnotched HA/PLA specimens were determined according to ASTM D256 using an impact testing machine (Atlas/BPI).

## Results and Discussion

**Characterization of HA powders:** The FTIR spectrum of bovine bone based HA is shown in Figure 1. The peaks at 3420 and 1630  $\text{cm}^{-1}$  were attributed to the vibration bending mode of adsorbed water. Two absorption bands at 602 and 571  $\text{cm}^{-1}$  were ascribed to the bending modes of  $\text{PO}_4^{3-}$ . The peaks at 1092 and 1053  $\text{cm}^{-1}$  were assigned to the stretching vibration of  $\text{PO}_4^{3-}$ . The stretching vibration band of  $\text{OH}^-$  and the  $\text{OH}^-$  bending vibration were observed at 3570  $\text{cm}^{-1}$  and 632  $\text{cm}^{-1}$ , respectively [8-9]. The peaks appeared at 1412 and 1459  $\text{cm}^{-1}$  were related to  $\text{CO}_3^{2-}$  groups. The appearance of carbonate functional group on FTIR spectrum of the obtained powders could be explained as follows: 1) carbon was adsorbed from atmosphere during heating process and substituted the  $\text{PO}_4^{3-}$  groups of the HA or 2) the carbon from the organics did not pyrolyze completely and may instead dissolve into the hydroxyapatite crystal. As a result, the FTIR spectrum revealed that the powders obtained from bovine bone were carbonated hydroxyapatite with highly crystalline structure.

Figure 2 shows FTIR spectra of the silane-treated HA powders. The additional peaks compared to the FTIR spectrum of untreated HA were observed around

2850-2950  $\text{cm}^{-1}$ . The peaks were attributed to C-H stretching of carbon chains of APES and MPTS on HA surfaces [10]. Additionally, MPTS-treated HA showed the peak of C=O stretching vibration of MPTS molecules at 1712  $\text{cm}^{-1}$ .

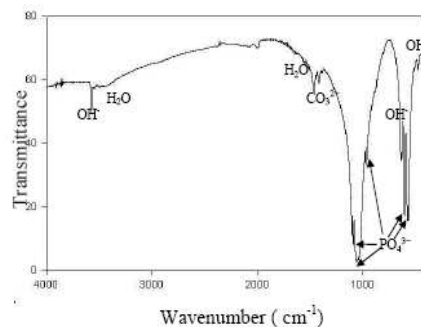


Figure 1. FTIR spectrum of bovine bone based HA (sintered at 1100 °C).

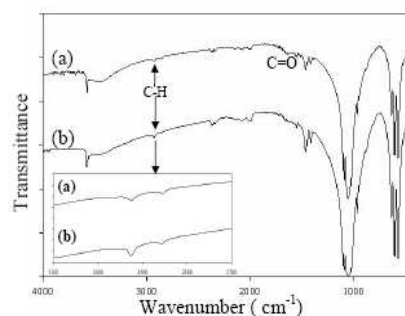


Figure 2. FTIR spectra of silane-treated HA : MPTS treated HA(a), APES treated HA(b).

SEM micrographs of untreated HA and silane-treated HA are shown in Figure 3 (a-d). The micrographs in Figure 3 (a) shows the agglomeration of HA powders. The micrograph at higher magnification revealed that the untreated HA powders were composed of particles with irregular shape as shown in Figure 3 (b). On the other hand, the micrographs of silane-treated HA in Figure 3 (c) and (d) show the smaller size of agglomerated HA powders compared to those of untreated HA. The results indicated that silane treatment tended to reduce particle agglomeration. To prepare HA/PLA composites, the less HA powder agglomeration would lead to better processibility and enhance mechanical properties of the composites.

The thermal behaviors of silane-treated and untreated HA were determined by TGA. As shown in Figure 4, the untreated HA displayed higher weight loss than silane-treated HA. This implied that treating HA surface with a silane coupling agent slightly increased thermal stability of HA.

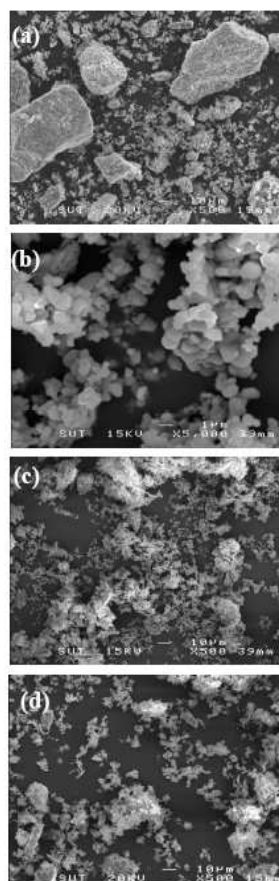


Figure 3. SEM micrographs of HA powders: untreated HA at low magnification (X 500) (a), untreated HA at high magnification (X 5000) (b), APES treated HA (X 500) (c) and MPTS treated HA (X 500) (d)

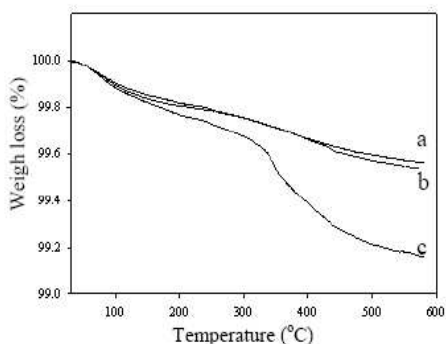


Figure 4. TGA curves of HA powders: MPTS treated HA (a), APES treated HA (b) and untreated HA (c)

*Characterization of HA/PLA composites:* Tensile strength, tensile modulus and impact strength of

HA/PLA composites at various HA contents are illustrated in Figure 5 (a-c). Tensile strength and impact strength of the composites were decreased with increasing HA content. In contrast, tensile modulus of the composites was increased with increasing HA content. The increase of the tensile modulus implied that HA is a reinforcing filler for PLA matrix.

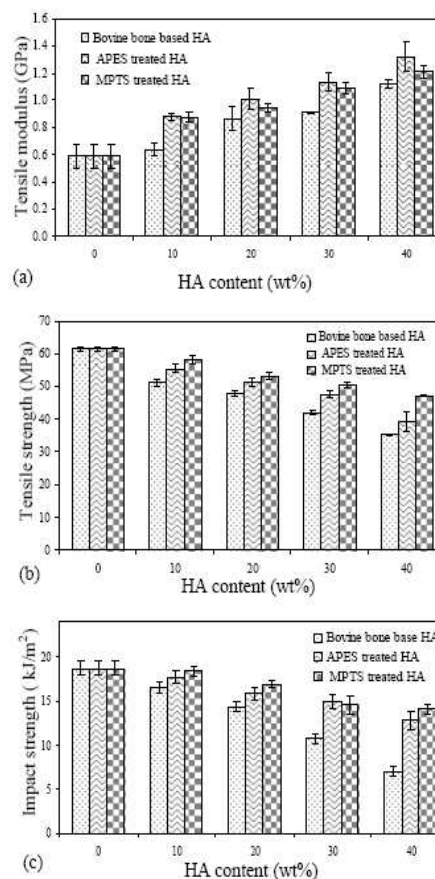


Figure 5. Tensile modulus (a), Tensile strength (b) and Impact strength (c) of HA/PLA composites at various HA contents

In comparison, the composites containing silane-treated HA had higher tensile strength, tensile modulus and impact strength than those containing untreated HA. This was probably attributed to good adhesion between two phases and good dispersion of silane-treated HA powders in PLA matrix. In addition, the composites containing MPTS treated HA exhibited the highest tensile strength and impact strength while those containing APES treated HA showed the highest tensile modulus.

The SEM micrographs of tensile fracture surfaces of the HA/PLA composites containing 20 wt% of HA are presented in Figure 6 (a-c). The agglomeration of HA powders were observed in HA/PLA composite

with untreated HA powders, as shown in Figure 6(a). In addition, a gap at the interface between untreated HA and PLA matrix was observed. From figure 6 (b-c), on the other hand, the microstructure of PLA composite containing silane-treated HA illustrated more homogenous distribution of HA powders in the PLA matrix. However, some HA agglomeration was still observed. This result was consistent with the morphology of silane-treated HA as shown in Figure 3 (c-d). Moreover, the micrograph shown in Figure 6 (b-c) elucidated that the gap between silane-treated HA and PLA interface became smaller than that between untreated HA and PLA interface. This implied that treating HA surface with a silane coupling agent can improve interfacial adhesion between PLA and HA.

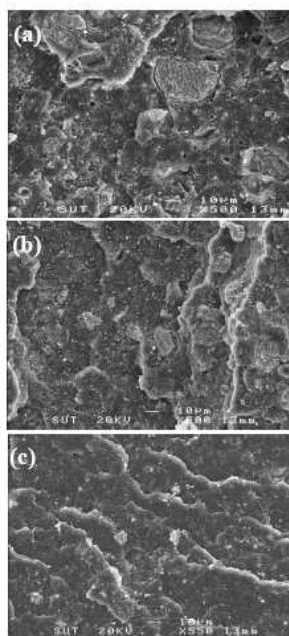


Figure 6. SEM micrographs of tensile fracture surfaces of HA/PLA composites at 20 wt% content of HA: untreated HA (a), APES-treated HA (b) and MPTS-treated HA (c)

### Conclusions

In this study, HA powders were prepared from natural source, *i.e.* bovine bone. The results from FTIR revealed that the obtained HA was a highly crystalline carbonate hydroxyapatite. HA surface treatment and HA content were the key factors that influenced mechanical properties of HA/PLA composites. SEM micrographs revealed the good distribution of silane-treated HA in PLA matrix and good adhesion between the HA powders and the polymer matrix. Tensile strength and impact strength of the composites were decreased with increasing HA content while the tensile

modulus of the composites was increased with increasing HA content. In comparison, the composites containing silane-treated HA had higher tensile strength, tensile modulus and impact strength than those containing the untreated HA composites. In addition, the composites containing MPTS treated HA exhibited the highest tensile strength and impact strength while those containing APES treated HA showed the highest tensile modulus.

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## **BIOGRAPHY**

Mr. Suriyan Rakmae was born on January 23, 1981 in Chiangrai Province, Thailand. He graduated from Maejo University in 2004 with the Bachelor of Science in Chemistry (2<sup>nd</sup> class honors). During his undergraduate study, he has taken a position of leader of chemistry club, Maejo University. After graduation, he has been employed as a pharmaceutical chemist at Better Pharma Co., Ltd., Betagro group, for 3 years. He then pursued his Master's degree in Polymer Engineering at school of Polymer Engineering, Institute of Engineering, Suranaree University of technology. During his master's degree study, he presented one paper entitled of **“Characterization of Silane-treated Natural Hydroxyapatite/Poly(lactic acid) nanocomposites”** in Pure and Applied Chemistry International Conference 2009 (PACCON2009) in Phitsanulok, Thailand.