

## **DISSOLUTION/PRECIPITATION BEHAVIOR OF HYDROXYAPATITES PREPARED FROM CATTLE BONE ASH**

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### **ABSTRACT**

The dissolution/precipitation behaviors of hydroxyapatites (HA) derived from calcined cattle bone with and without chemical treatments (MP and TP respectively) were studied under human physiological condition. Both specimens were incubated in simulated body fluid (SBF) at 37°C with a sample surface area to solution volume ratio of 0.1 cm<sup>-1</sup>, 5%CO<sub>2</sub> was used to adjust pH of this solution to 7.40±0.05. The characteristics of MP and TP specimens were examined before and after incubation in SBF. The phase present and functional group of both specimens did not change after incubation for 90 days but the Ca:P ratio and bulk density decreased, hence the porosity increased. Furthermore, the newly formed precipitates appeared on the surface of both specimens after incubation for 30 days and covered all over the surface in 90 days. From the chemical analyses, it was found that this newly formed precipitates were calcium phosphate compound containing carbonate group in phosphate site structure, the phase of this compound was similar to natural cattle bone.

**KEYWORDS:** Hydroxyapatite, Dissolution, Precipitation, *In vitro* study, Cattle bone

### **INTRODUCTION**

Because of its crystallographical similarity to various calcified tissue of vertebrates, hydroxyapatite has attracted much more attention as a substitute material for damaged teeth or bone over the past two decades. Hydroxyapatite is very close to our life as the main component of bone and tooth minerals. Many apatite compounds, including fluoroapatite, chlorapatite, carbonate-apatite and hydroxyapatite are being used in the industrial field as fertilizers, catalysts, sensors, etc. In the medical and dental fields, hydroxyapatite is being utilized as artificial bones, tooth roots, toothpaste, percutaneous devices,

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blood vessels, and materials for drug delivery systems.

The surface structure of HA has a significant influence on surface-related phenomena such as adsorption, dissolution, ion exchange and precipitation. Dissolution/precipitation study of HA will contribute much more understanding *in vivo* phenomena related to calcified tissue. Some previous studies on those phenomena have demonstrated that dissolution behaviors are influenced by not only chemical composition but also surface and internal structures. Thus, dissolution behaviors must be studied carefully in relation with the starting materials and preparation processings.

The present research, dissolution behavior of two kinds of HA derived from cattle bone ash<sup>1</sup> was studied under simulated physiological condition. The *in vitro* study data could be related to the bone growth enhancement and bone bonding because apatite dissolution might promote enhanced bone bonding.<sup>2</sup>

## MATERIALS AND METHODS

We have used two kind of starting powder samples<sup>3</sup>: (1) MP was cattle bone ash calcined at 700°C in air for 3 hrs. and then ground in a mortar and (2) TP was a chemically treated of MP by dissolution in nitric acid, reprecipitated in ammonia solution, calcined at 500°C in air for 0.5 hr. and then ground. MP and TP powders were fabricated by cold isostatic pressing (CIP) into disc-shaped specimens (15 mm. in diameter, 12 mm. in thickness). Green specimens of MP and TP samples were heated to remove PVA binder, then sintered at 1200°C for 2 hrs. The sintered samples were cut by low speed refined saw machine into a specimen of 15 mm. in diameter, 1.2 mm. in thickness, polished and cleaned using acetone and ethyl alcohol in ultrasonic bath.

Dissolution testing of HA was designed by incubating of HA specimens; MP and TP in simulated body fluid (SBF) of which composition was shown in Table 1<sup>4</sup>. The fluid was prepared by dissolving analytical grade chemicals into deionized-distilled water and buffered at physiological pH 7.4 at 37°C with 50mM trishydroxymethyl aminomethane [(CH<sub>2</sub>OH)<sub>3</sub>CNH<sub>2</sub>] and 45mM HCl. After being soaked in SBF at 37°C for every 7 days, specimens were taken out of the fluid and cleaned in deionized-distilled water, and finally dried at ambient temperature for further characterization.

Table 1 Composition of simulated body fluid

Chemicals	NaCl	NaHCO <sub>3</sub>	KCl	K <sub>2</sub> HPO <sub>4</sub>	MgCl <sub>2</sub> •6H <sub>2</sub> O	CaCl <sub>2</sub>
Ingredients (mmol/l)	137.8	4.2	3.0	1.0	1.5	2.5

The SBF solution was put into the HDPE brown bottle and the volume of solution in each bottle was limited by the factor of sample surface area to leachant volume (S/L) = 0.1 cm.<sup>-1</sup>. The mixture of gas N<sub>2</sub>:CO<sub>2</sub> (95:5%)<sup>5</sup> was bubbled into the incubating bottles in order to maintain pH at 7.40±0.05. These bottles were sealed and kept in incubator at 37±1°C. After various incubation periods, the samples were taken to examine and evaluated by XRD, SEM and IR.

## RESULTS AND DISCUSSION

### Characteristics of the Specimens Before Incubation

From the chemical analysis of HA specimens, it was indicated that both MP and TP hydroxyapatites were non-stoichiometric having Ca-deficient structures as shown in Table 2.

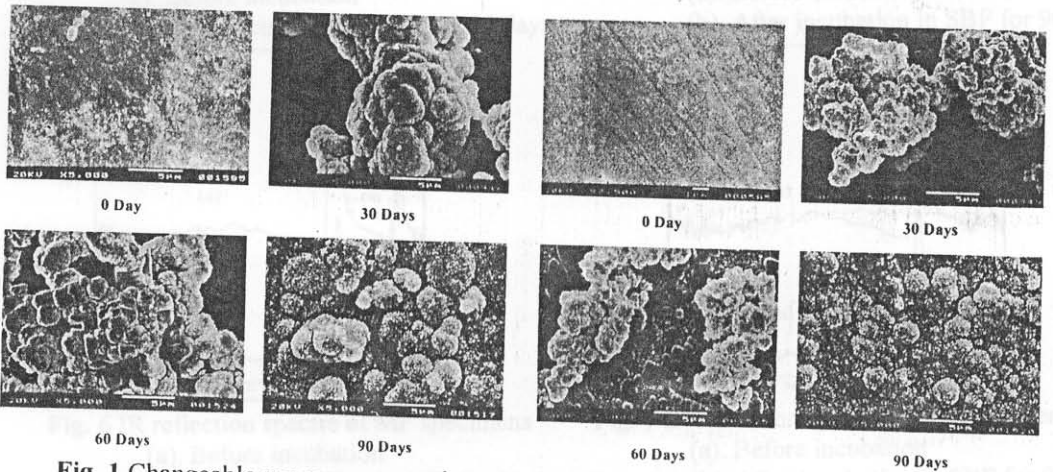
Table 2 Chemical composition of MP and TP hydroxyapatites before incubation in SBF

	Ca (%)	P (%)	Ca:P	Mg (%)	Fe (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)	As (ppm)	Cd (ppm)	Pb (ppm)	Hg (ppm)
MP	38.2	18.3	1.63:1	1.08	0.05	143	2	5	1.7	<0.5	<5	<1
TP	39.2	18.3	1.66:1	0.93	<0.02	42	2	5	<0.5	<0.5	<5	<1

After comparison of impurities in MP and TP, it was found that MP contained higher impurities, especially zinc. The phases present before incubation were well crystallized HA [see Figure 4(a) and 5(a)]. The IR spectra indicated that there were no OH<sup>-</sup> loss in the HA structure. The bulk densities of MP and TP were 93.2%TD and 96.0%TD and the porosities 0.74% and 0.60%, respectively [water replacement method (ASTM C 373-72)].

### Characteristics of the Specimens After Incubation

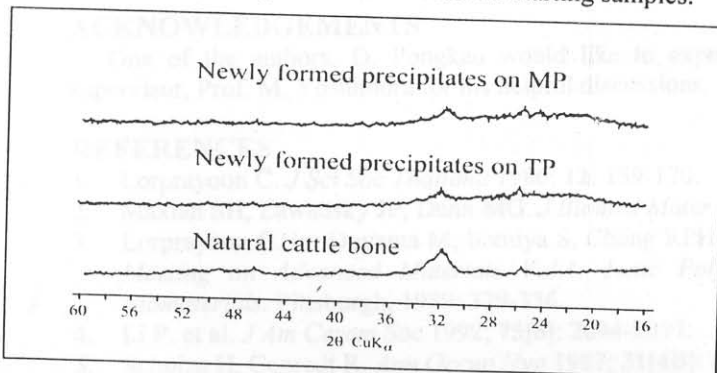
The SEM observation in Figure 1 and 2 showed the changeable appearances on the MP and TP surface. After 30 days in SBF, the newly formed precipitates appeared on the surface of both specimens and increased with longer period of incubation, finally it covered all over the surface after exposure in SBF for 90 days. The precipitates were composed of many agglomerates with the size of 1-8 $\mu$ m. In each agglomerate, the primary particles were observed especially at 90 days of incubation. Those primary particles had the characteristics of acicular shape of 0.3-0.4  $\mu$ m, with aspect ratio 3.75.



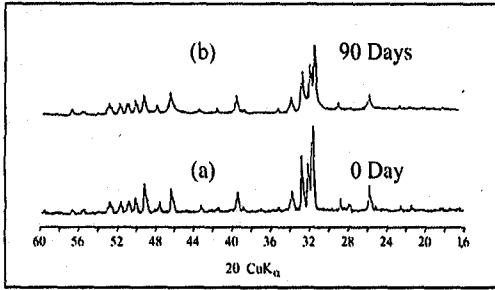
**Fig. 1** Changeable appearances on the surface of MP specimen

**Fig. 2** Changeable appearances on the surface of TP specimen

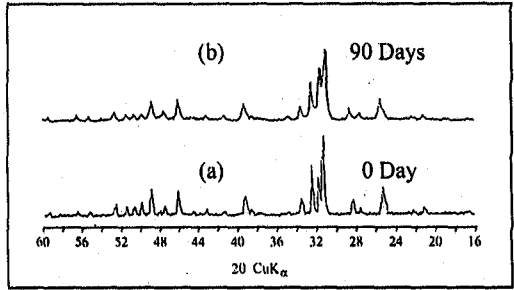
After incubation in SBF for 90 days, specimens were removed and dipped in deionized-distilled water, and the newly formed precipitates were scraped out and identified by XRD. As shown in Figure 3, the newly formed precipitates showed broad peaks which were similar to natural cattle bone. It indicates that the precipitates were not well-crystallized HA. Since the precipitates were difficult to separate completely from the bulk, the whole specimens were crushed into powder to analyze. The XRD patterns in Figure 4 and Figure 5 indicate that the crystallinity of HA decreased after incubation for 90 days in comparison with sample before incubation. It seems to indicate that the newly formed precipitates were less crystallized-HA than the starting samples.



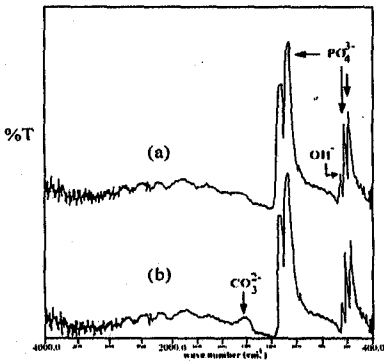
**Fig. 3** XRD patterns of newly formed precipitates on MP and TP specimens after incubation in SBF for 90 days



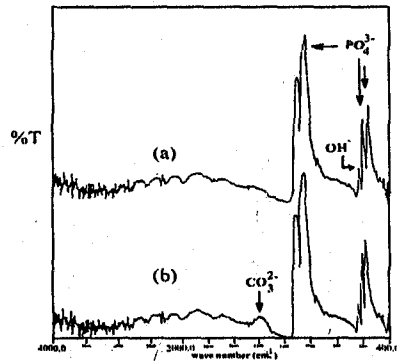
**Fig. 4** XRD pattern of MP specimens  
(a). Before incubation  
(b). After incubation in SBF for 90 days



**Fig. 5** XRD pattern of TP specimens  
(a). Before incubation  
(b). After incubation in SBF for 90 days



**Fig. 6** IR reflection spectra of MP specimens  
(a). Before incubation  
(b). After incubation in SBF for 90 days



**Fig. 7** IR reflection spectra of TP specimens  
(a). Before incubation  
(b). After incubation in SBF for 90 days

From the infrared reflection spectra of both specimens as shown in Figure 6 and 7, the newly formed precipitates on the surface of two types of bulk specimens after incubation in SBF for 90 days showed a peak at  $1410\text{ cm}^{-1}$  which was assigned to  $\text{CO}_3^{2-}$  in phosphate site.<sup>6</sup> This peak revealed the  $\text{CO}_3^{2-}$  group in the newly formed precipitates but it did not appear in the bulk of both specimens.

From above characterization results, we could summarize that the newly formed precipitates on the bulk of both MP and TP specimens was a less-crystallized HA phase which was similar to natural cattle bone with carbonate group in phosphate site structure and it was composed of many primary particles in acicular shape.

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