GREEN SYNTHESIS OF CALCIUM AND PHOSPHATE COMPOUNDS BY VARYING pH VALUE AND CA/P ATOMIC RATIO USING AQUEOUS PRECIPITATIONS

#WEN CHENG CHEN*, CHIEN PING JU**, WEN HSIEN CHENG**, JIIN HUEY CHERNLIN**

**Advanced Medical Devices and Composites Laboratory, Department of Fiber and Composite Materials, College of Engineering, Feng Chia University, Taichung 40724, Taiwan*
***Department of Materials Science and Engineering, National Cheng Kung University, 70101 Tainan, Taiwan*

#E-mail: wencchen@fcu.edu.tw

Submitted September 4, 2012; accepted April 1, 2013

Keywords: Apatite, Precipitation, Biomaterials, Biphasic, Calcium carbonate

Using an aqueous synthesis with varying pH values and changing ion source of calcium-to-phosphorus atomic ratios to clarify precipitations was the purpose of this study. The precipitation procedures were processed in two ways; firstly, a pH range of 3.0 to 12.0 was controlled while the ion source of relative Ca/P ratio was kept at 2.0. Secondly, the reaction was kept alkaline with a pH of 12.0 and the value range of Ca/P ratios in the precursor ion-solutions was 1.0 to 3.0. The physicochemical properties were measured to characterize the various precipitates. Results showed that when the pH was varied, the morphology of the precipitates gradually changed to micrometer column-like shape under acidic condition and nano-scale globular-like shape in alkaline environments. By controlling the pH and Ca/P ratio of ion-sources in aqueous reactions, precipitates in biphasics of calcium carbonate/apatite and calcium hydroxide/apatite were obtained with nanometer-scale morphologies. The precipitation procedures that are used in the synthesis of the biphasics provide a simpler, safer, less expensive and more environmentally friendly than the co-precipitation by sintering processes.

INTRODUCTION

In recent years, calcium phosphates (CaP) as bone void fillers have been employed extensively in restorative dentistry and orthopedics. For examples, periodontal defect repair, as scaffolds for bone reconstruction, and in orthopedics. The mineral of bones and teeth is an impure form of hydroxyapatite (HA). When CaPs are used in the restoration accordingly, better osteoconductive properties are obtained than other ceramics (e.g. zirconia and alumina) [1-4]. Bone regeneration proceeds after implantation of fillers, as certain materials dissolve and subsequently activate mineralization process [5]. Bone formation on the surface of an HA ceramic has been studied as a typical example of a biological interface with a bone filler [6]. Among mechanisms of bone repair and regeneration, cell adhesion and remineralization primarily depend on the dissolution of materials. That allows the calcium (Ca^{2+}) and phosphate ($H_{3-x}PO_4^{x-}$, x = 1-3) ions to be released. Furthermore, CaPs have varying solubility ranging from high to low at neutral pH in the following order: monocalcium phosphate monohydrate and anhydrous (MCPM and MCPA), dicalcium phosphate dihydrate and anhydrous (DCPD and DCPA), octacalcium phosphate (OCP), α- and β-tricalcium phosphate (α- and β-TCP), tetracalcium phosphate (TTCP), HA, and fluorapatite [4].

There are various ceramic processing methods for CaPs synthesis such as precipitation, sol-gel, hydrothermal or sintering processing and etc [7-13]. Among which, the most popular process for the CaPs synthesis is the aqueous precipitation that could further reduce energy usage than sintering processes and the process produced precipitates are suitable for biomedical application. Filler particles used in biomaterials are generally made of single-phase and have undesirable rates of bioresorbability that are either too fast or too slow *in vivo* [14-16]. To retain the osteoconduction and avoid the undesirable bioresorption rate of CaP biomaterials, research has been conducted on the fabrication of co- or multiple-precipitation. As a result, many synthetic CaPs restoration particles have been proposed and applied for repairing bone damages, including a biphasic mainly composed of HA and b-TCP phases [17, 18]. Such materials have already proven their effectiveness in various clinical applications [19-22].

Calcium-rich compounds, such as calcium oxide (CaO), calcium hydroxide ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$), have faster bioresorption rates than CaP compounds. They create a higher Ca^{2+} concentration at the surrounding sites of implantation and enhance new bone regeneration, as it releases Ca^{2+} ions largely into the biological medium [23-25]. The formation of carbonate hydroxyapatite (CAP) at the bone-biomaterial

interface is thought to be a cell-mediated dissolution and precipitation process *in vivo* [26]. In addition, ions of Ca^{2+} and phosphate that incorporate into CAP have intimate association with an organic component; this process leads to bone formation [24-26]. This means that Ca^{2+}, phosphate and CO_3^{2-} ions dissolved from the implant materials play an important role in the formation of new bone. The present work aims to synthesis and characterize biphasics of $CaCO_3$/apatite and $Ca(OH)_2$/apatite through the controlled precipitation method.

EXPERIMENTAL

Calcium and phosphate chemicals

To measure the effects on the precipitates of varying the pH and the Ca/P atomic ratios, an ions-solution was prepared using the following chemicals: calcium oxide (CaO, purity 95 %), potassium hydroxide (KOH, 85 %) (Osaka Chemical Co., Japan); calcium chloride ($CaCl_2$, 95 %), potassium phosphate n-hydrate ($K_3PO_4 \cdot nH_2O$, 98 %), phosphoric acid (H_3PO_4, 85 %) (Katayama Chemical Co., Japan); calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$, 98 %), calcium acetate ($Ca(CH_3COO)_2 \cdot H_2O$, 98 %), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 97 %), ammonium phosphate ($(NH_4)_3PO_4$, 96 %) (Showa Chemical Co., Japan) and dicalcium phosphate anhydrous (DCPA, $CaHPO_4$, 97 %) (Aldrich Chemical Co., USA).

Variation in reaction processes

The procedures used were modified from the previous study [27]. To find the equilibration time as a function of precipitate's Ca/P atomic ratio, a control group was monitored over a range of reaction time and up to 24 h. A 0.67-M Ca^{2+} ion solution (300 ml) was mixed with a 0.14-M phosphate ion solution (700 ml) to make an ion solution with Ca/P ion-atomic ratio of 2.0. While the pH was controlled by monitoring the pH of the ion solution at 12.0, the reaction was noted after Ca^{2+} ion source had been added to the phosphate ion solution for different time period under continuous stirring. The precipitates were centrifuged, vacuum-filtered and then lyophilized at reaction times of 0, 0.5, 1, 2, 3, 6, 12 and 24 h. With the above-mentioned process as a control, two experimental processes were studied after 3 h reaction at 26°C. In one process, different ion source with a constant Ca/P ion-atomic ratio of 2.0 were prepared and the pH values were allowed to vary. As shown in Table 1, a range of pH values for various processes and different chemicals were used for the varied ion sources for specific pH ranges. Otherwise, different controlled pH values would have resulted in the dissolved ion chemicals. Under continuous stirring of phosphate ion solution (0.14-M), the Ca^{2+} ion source (0.67-M) was added over a 10-min period. The pH value of the solution was continuously monitored for 3 h. To control the pH of the solution, the pH was dynamically adjusted by adding 0.1-M KOH or 0.1-M H_3PO_4 through the whole process.

The other process involved alkaline ion solutions with a constant pH of 12.0, which had different Ca/P atomic ratios at 26°C were measured. The ion sources used were made of 0.67-M Ca^{2+}, to which solutions with varying phosphate ion concentrations were added and Ca/P ion-atomic ratios tested were 1.0, 1.5, 2.0, 2.5 and 3.0. The pH value of the reaction was controlled for 3 h. After the reaction time was reached, the solutions were immediately centrifuged and the precipitates were vacuum-filtered for 30 min, washed twice with 1 l of deionized water and lyophilization. Ten replicate specimens were prepared and analyzed for each process (n = 10).

Analysis of precipitates

Precipitates were analyzed via x-ray diffraction (XRD) at 30 kV, 20 mA, 1°/min (Rigaku D-max IIIV, Tokyo, Japan). The various phases were identified by matching them to the Joint Committee on Powder

Table 1. Phases of precipitates formed in the ion-source solutions with different pH values were reacted at 26°C for 3 h.

Symbol/ acidic (A) or basic (B) (initial/final pH value)	Ion source: relative Ca/P atomic ratio was equal to 2.0[c]	Phases of precipitates based on XRD patterns	Mean relative phase ratios (%)[d]
P1A (4.2/3.1)[a]	$CaCl_2/(NH_4)_2HPO_4$	DCPA	~100
P2A (5.0/5.5)[a]	$CaO/CaHPO_4$	DCPA/DCPD	50/50
P3A (5.5/5.9)	$Ca(NO_3)_2 \cdot 4H_2O/(NH_4)_2HPO_4$	DCPA/DCPD	11/89
P4B (7.5/7.4)	$CaCl_2/(NH_4)_2HPO_4$	DCPA/DCPD	15/85
P5BA (12.0/5.0)	$Ca(NO_3)_2 \cdot 4H_2O/(NH_4)_3PO_4$	DCPA/DCPD/Apatite	26/38/36
P6B (9.0/9.0)[b]	$Ca(CH_3COO)_2 \cdot H_2O/(NH_4)_2HPO_4$	Apatite	~100
P7B (10.0/10.0)[b]	$CaCl_2/(NH_4)_2HPO_4$	Apatite	~100
P8B (12.0/12.0)[b]	$Ca(CH_3COO)_2 \cdot H_2O/K_3PO_4 \cdot nH_2O$	$CaCO_3$/Apatite	17/83

[a] *Phosphoric acid was added to adjust the pH of the phosphate ion solution only.*
[b] *Potassium hydroxide was used to stabilize the pH during the reaction process.*
[c] *Ion source solutions generated by dissolving different amounts of calcium and phosphate chemicals; 300 ml of 0.67-M Ca^{2+} mixed with 700 ml of 0.14-M phosphate to make a total volume of 1 l ionic solution.*
[d] *The ratio calculated by relative XRD peak intensities I100 (n = 3).*

Diffraction Standards (JCPDS) files. A Fourier transform infrared spectroscopy (FTIR) system (Jasco, FT/IR-460 Plus, USA) in transmission mode with a spectral resolution of 2 cm^{-1} was used. To study the morphological, the dispersed samples were examined using a field emission scanning electron microscope (SEM) (Hitachi S-4100, Hitachi, Tokyo, Japan) equipped with an energy dispersive spectroscopy (EDS) system. To further study the precipitates, a JEOL JEM-3010 scanning transmission electron microscope (STEM) was used.

Statistical Analysis

One-way ANOVA was used to evaluate the statistical significance of the microchemical EDS analysis data. Scheffes' multiple comparison technique was used to determine the significance of the deviations in the measured properties. The results were considered to be statistically significant at $p < 0.05$.

RESULTS

Two conspicuous findings of EDS analysis of the precipitates with Ca/P atomic ratio of 2.0 and varied pH values were shown (Figure 1a). When the pH of the ion solutions was below 8.0, DCPA or DCPA/DCPD dominated the precipitated phases. Higher pH levels showed a precipitate of apatite only or $CaCO_3$/apatite biphasic. Detailed element analysis (EDS) of precipitates with ion source (Ca/P of 2.0) and stabilized pH value at 12.0 was evaluated over different times in our preliminary study (Figure 1b). The precipitate with Ca/P ratio ~ 2.5 was found in the early stage of reaction and the precipitated Ca/P ratios declined to the original ion source solution of ~ 2.0 at 3 h and after which the decline began to slow and reached a plateau. The time at 3 h reaction was selected to examine the precipitates in other groups in this study.

The phases of the precipitates with varied pH values were found to differ, even though the ion source Ca/P atomic ratio was kept at 2.0 (Table 1). When pH value was not above than 5.0 (P1), the single phase of DCPA was dominant (Figure 2). When pH values gradually increased and were ranging from 5.0 - 7.5, the DCPD was formed, the intensities of the DCPA peaks were reduced comparatively (P2-4) and the amount of DCPD was gradually increased to 89 % (Figure 2 and Table 1). When reaction kept alkaline (pH 9.0 - 10.0), the main phase of apatite was precipitated. In highly alkaline ion-solution at pH 12.0 (P8B), the XRD pattern showed developed a biphasic of $CaCO_3$/apatite.

The FTIR spectra obtained were shown in Figure 3. The 3535 and 3400 cm^{-1} bands of precipitates formed at processes (P1-4) was due to the OH^- bonds of DCPD, DCPA [28]. The bands at 2365, 1644, 1340, 890 cm^{-1} and 600-570, 1200-1000 cm^{-1} were attributed to the respective HPO_4^{2-} and PO_4^{3-} groups that resulted from the overlapping DCPA, DCPD and apatite phases [29, 30]. Despite the weak absorption in the P5-8 precipitates, the band of the OH^- at 630 cm^{-1} (bending mode) and 3570 cm^{-1} (stretching mode) for apatite were evident. The spectra of the precipitates (P6-8) formed at pH levels of 9.0 - 12.0 had CO_3^{2-} bands in the range of 870 and 1570 - 1450 cm^{-1} [31-34].

Figure 4 clearly showed the detailed precipitated phases formed at controlled pH 12.0 with different ion sources of varied Ca/P ratios at 3 h of the reaction. For the ion source solutions with Ca/P < 1.5, only a single apatite phase showed in Figure 4b and with Ca/P atomic

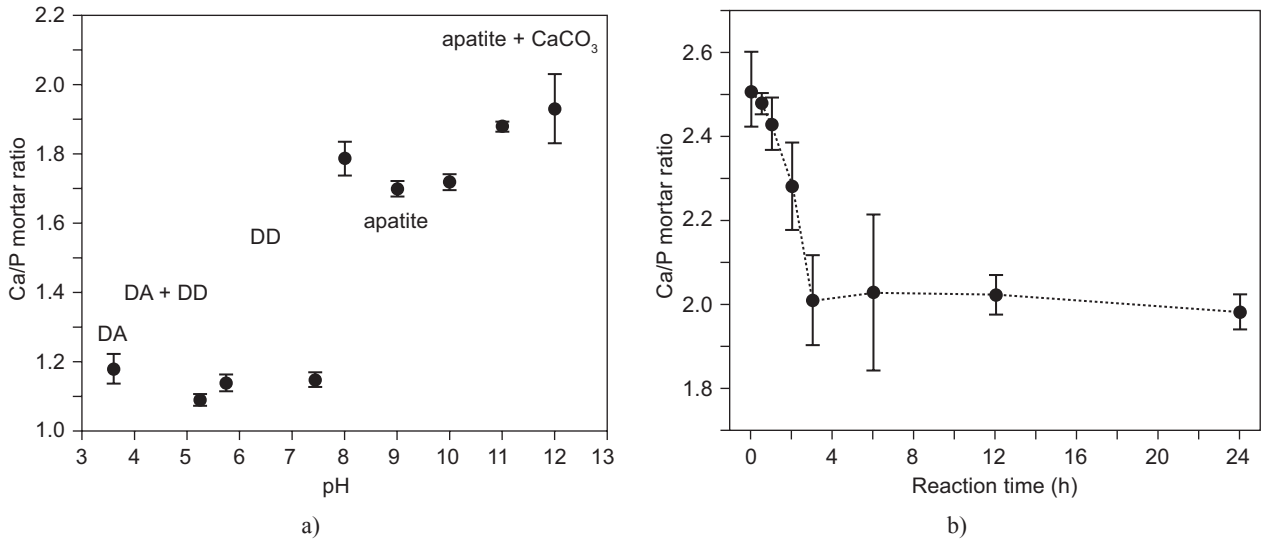


Figure 1. EDS analyses of the precipitate produced by allowing variation in the pH for 3 h (a), and as a function of reaction time while the pH was kept at 12.0 (b). The ion source of Ca/P atomic ratio was 2.0, and the reaction was maintained at 26°C. DA: DCPA; DD: DCPD. (n = 10; p < 0.05).

ratios 2.5 and higher, a precipitated biphasic of $Ca(OH)_2$/apatite was showed more often than a precipitate of $CaCO_3$/apatite.

The morphologies of various precipitates were shown in SEM images (Figure 5). The morphologies of precipitates formed in the acidic ion solutions changed from dull-edged (P1) and column-like (P2) to sharp-edged and petal-like (P3) shape, and then clusters of particles were formed (P4). For the processes of P4-6, clusters of micrometer-scale precipitates were predominantly formed. It was obvious that the sizes of the precipitates formed in an alkaline ion solution were much smaller than those obtained from the other processes [33]. The sizes of these globular $CaCO_3$/apatite biphasic were generally in the range of 50 - 60 nm (Figure 6). As shown in the dark field (DF) and high-resolution (HR) TEM micrographs (Figure 6a and b), the clustered and whisker like nano-crystallites were observed. The selected-area diffraction (SAD) pattern and its index (Figure 6c) clearly showed that the nano-crystallites have structure of apatite-dominant.

DISCUSSION

The crystallization, phases and size distributions would affect the degradation rate of bioresorbable biomaterials. For example, if the solubility of filler was too low, diffusion of ions such as Ca^{2+}, PO_4^{3-}, HPO_4^{2-} and CO_3^{2-} into the deeper regions of the lesion would be prevented, thus thwarting the full bonding or remineralization to the natural tissues [34-36]. The high ion concentrations present in solutions could be expected to result in the formation of substantial precipitation. In this study, varying the pH from 5.0 to 12.0 resulted in precipitates that were composed of PO_4^{3-}, HPO_4^{2-}, CO_3^{2-} and OH^- ion groups. This allowed the synthesis of the following substances: apatite or CAP, $CaCO_3$ or $Ca(OH)_2$, and DCPA or DCPD [29, 37]. With the exception of P8, the quantities of $CaCO_3$ were too low for detection by XRD but as shown in FTIR spectra, CO_3^{2-} band clearly formed in the P5-8 processes. Notably, EDS analysis gave information that only showed the microns depth under the surface of the specimen and bulk information

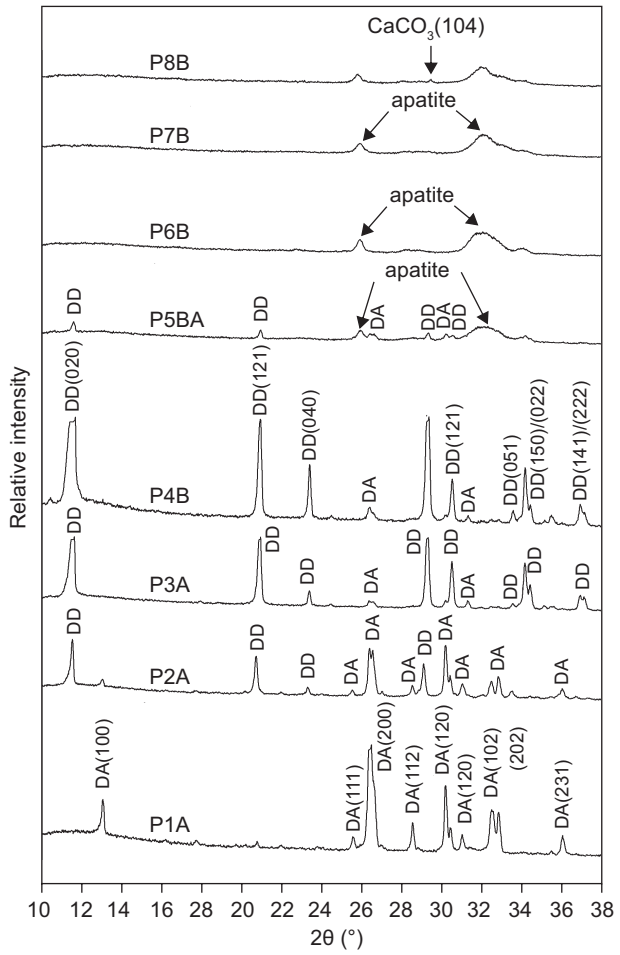


Figure 2. Variation of the XRD patterns of the precipitates in an aqueous chemical synthesis method for acidic (A) and basic (B) ion solutions. The ion source of Ca/P ratio was 2.0, and the reaction was maintained at 26°C for 3 h. DA: DCPA; DD: DCPD.

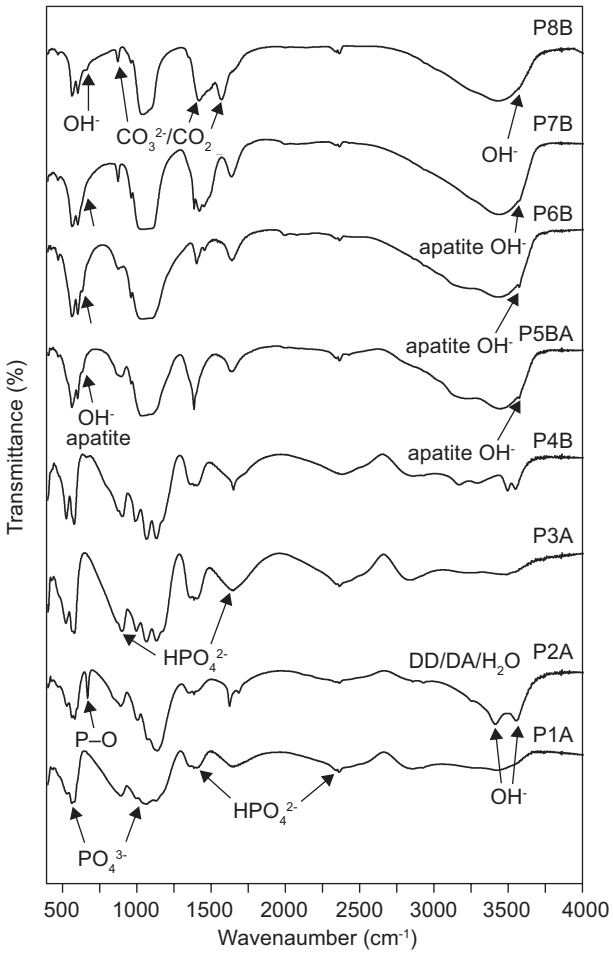
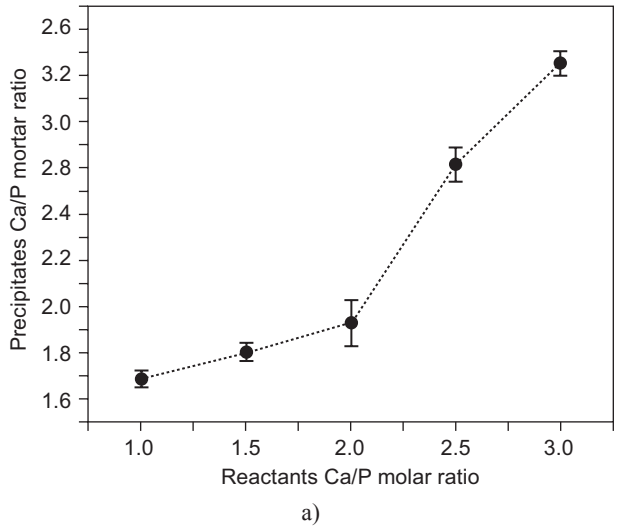
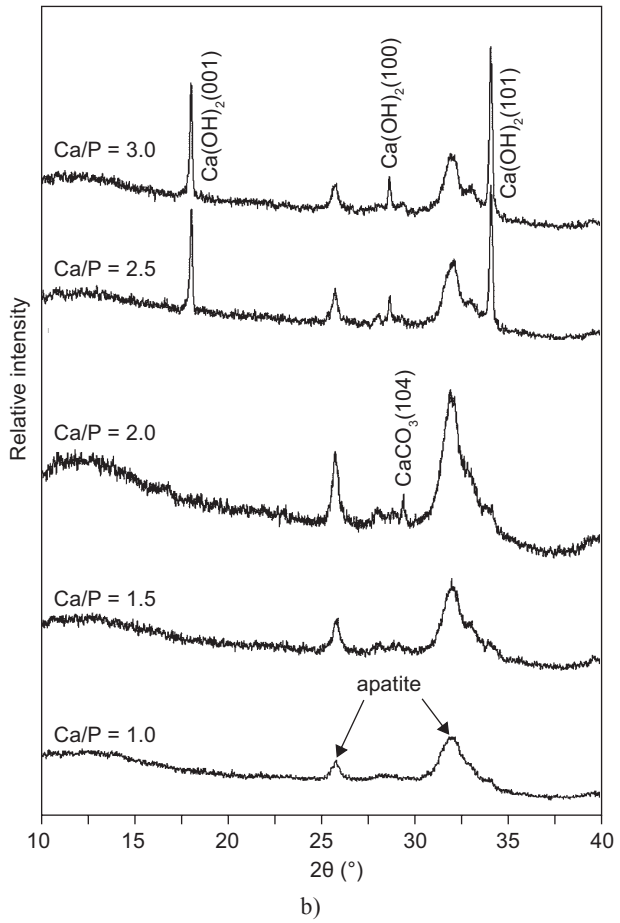


Figure 3. FTIR absorption spectra of precipitates formed by an aqueous chemical synthesis method in acidic (A) and basic (B) ion solutions. The ion source of Ca/P ratio was 2.0, and the reaction was maintained at 26°C for 3 h. DA: DCPA; DD: DCPD.

of a comparatively reduced Ca and P elements intensity [30]. The data clearly showed that precipitates from ion solutions with highly alkaline pH (12.0) had a Ca/P ratio larger than apatite was due to an increased calcium-rich precipitate of $Ca(OH)_2$ or $CaCO_3$ in the early stage of reactions within 3 h in Figure 1.



a)



b)

Figure 4. Ca/P molar ratios of the precipitates (a), (n = 10; $p < 0.05$); XRD patterns of precipitates (b) with different ion source Ca/P ratios. The reaction pH was 12.0, and was maintained at 26°C for 3 h.

By comparing the results (Figure 1 and Figure 4), it suggested that precipitate phases were more dependent on the pH levels than the ion sources of different Ca/P atomic ratios. The precipitate reaction can be generally divided into three stages: ion diffusion, nucleation and growth [9, 33]. The purities, morphologies and granulometrics of the precipitates should be dependent on the least soluble component (invariant points). Solubility isotherms of CaP showed that the invariant points at 25°C between $Ca(OH)_2$/HA and DCPD/HA corresponded to pH of 8.5 and 5.0, respectively [38]. In other studies, the least soluble phase was comprised of DCPA and HA at pH values below 4.5 and in a range of 4.5 - 14.0, respectively [39-42].

The homogeneous precursor for co-precipitation by calcining the mixed calcium and phosphate compounds to a compressing powder into a green compact, sintering at a temperature of at least 800°C is an highly energy required for processing. The advantage for the corporation of $Ca(OH)_2$ in the fillers is an increase in the amount of free hydroxide ions which has the abilities to increase pH, reduce bacteria growth and enhance osseointergration [10]. In the present study, biphasic of $CaCO_3$ or $Ca(OH)_2$ with apatite was formed from reactions with ion sources of Ca/P ration 2.0 and higher (Figure 4). Their formation mainly caused from the fact that supersaturated ions in the alkaline rapidly result in $Ca(OH)_2$ precipitation originally and such phase of $Ca(OH)_2$ tended to get easily converted into $CaCO_3$ and made the biphasic of $CaCO_3$/apatite instead of $Ca(OH)_2$/apatite after the 3 h reaction [30, 43]. The reactions with initial Ca/P ion sources in a range of 2.5 - 3.0 at pH 12.0 produced too much precipitate of $Ca(OH)_2$ for them to be totally converted into the phase of $CaCO_3$. According to the study, the morphologies originating with different Ca/P ion sources were formed of two crystallite populations: needlelike crystallites and globular elongated crystallites [44]. It was also showed that the dimensional reduction of the apatite crystal size was contributed to the incorporation of carbonate in apatite [13, 30, 45, 46], and another study showed when $CaCO_3$ was added to the DCPA-α-TCP system, the size and shape of the crystallites changed from long needles to smaller rods and then to tiny spheroids [47]. In this study, there was an obvious morphological changing from column-like to globular-like crystallites (Figure 5). However, the tiny whiskers like nano-crystallites were still shown on the surfaces of precipitate especially in the TEM image (Figure 6b). The altered CO_3^{2-} absorption band at 870 cm^{-1} showed that there were $CaCO_3$ formations in highly pH level (Figure 3) and the induction of carbonate was expected to induce the crystallites clusters and grew to form spheroids. As discussed in the literatures [34, 48-51], a significant amount of $CaCO_3$ was present in enamel and bone mineral (2 - 4 wt. % and 5 - 6 wt. %, respectively). This indicated that the carbonate and phosphate ion groups could play an important role by increasing the

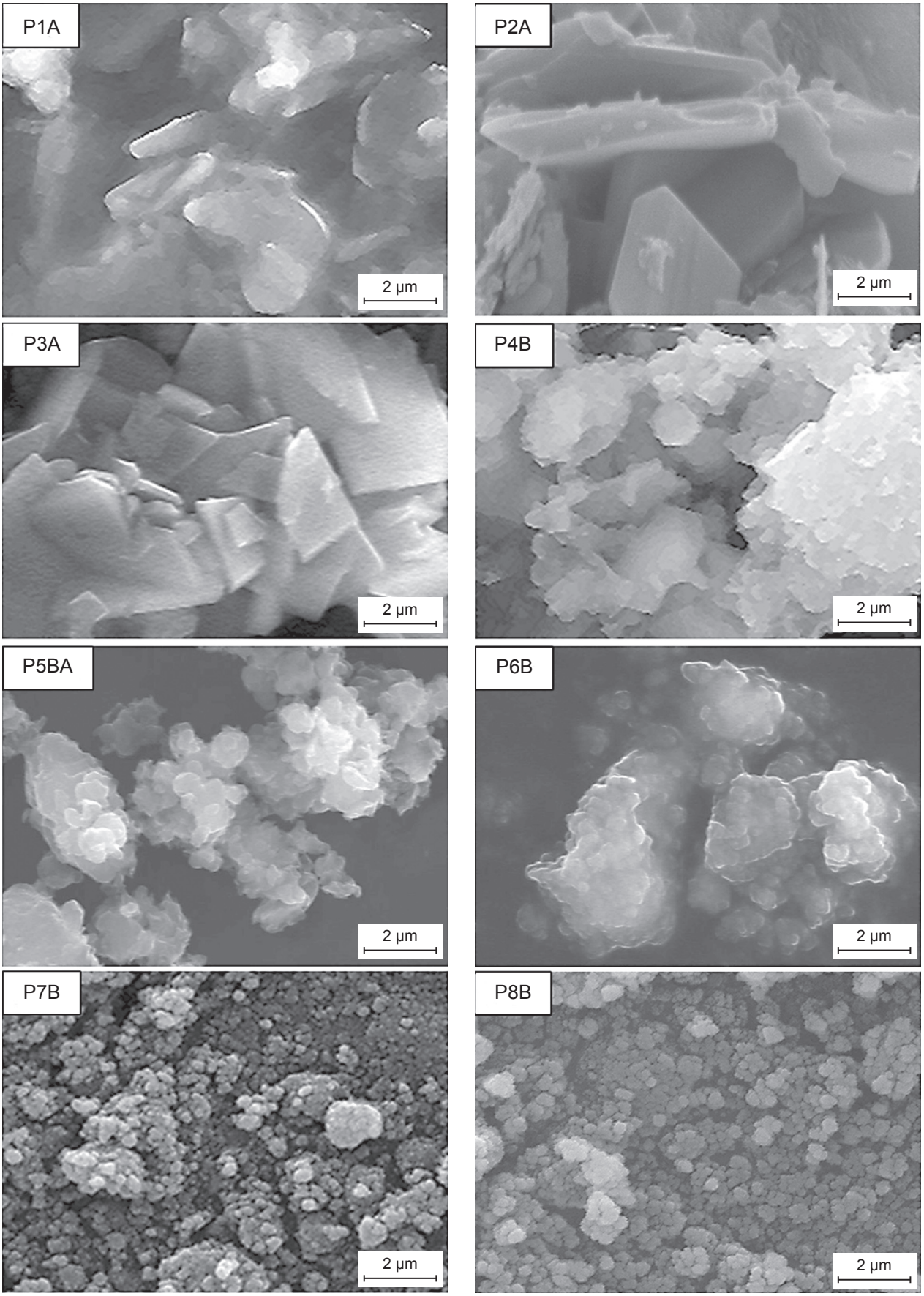


Figure 5. Variation in precipitate morphologies from reactions in aqueous chemical synthesis processes ranging from acidic (A) to basic (B) ion solutions. The ion source of Ca/P ratio was 2.0, and the reaction time was 3 h at 26°C.

surface energy of the apatite and by promoting protein attachment [13, 44]. The results of the present green synthetic process for the homogeneous co-precipitated biphasics of $CaCO_3$ or $Ca(OH)_2$ with apatite could be useful in applications and has a variety of applications for practicing clinician. The influences of different amounts of $CaCO_3$ and $Ca(OH)_2$ with apatite in the osteoregeneration *in vitro* and *in vivo* testing are the research limits in this study that were not investigated. Therefore, additional studies based on well-controlled studies evaluating the use of different phase composites are needed.

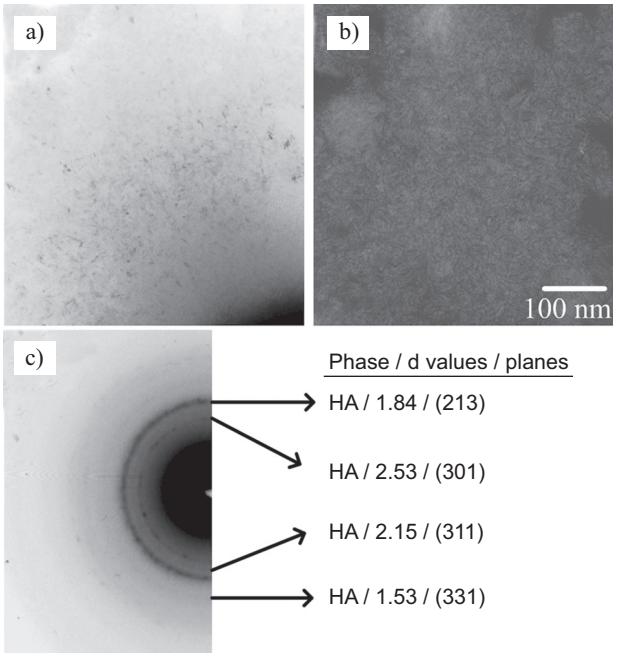


Figure 6. Morphologies of precipitates while the pH was kept at 12.0 and the ion source of Ca/P ratio was 2.0 for 3 h reaction: (a) dark field and (b) high-resolution TEM images of the same areas, (c) SAD pattern with its index.

CONCLUSIONS

The results showed that, when a Ca/P ion-source ratio of 2.0 was same, reactions carried out in a pH range of 8.0 or above resulted in the precipitates of biphasics with apatite. Also, for the case of an alkaline solution (pH value of 12.0), a precipitate of $CaCO_3$/apatite biphasic was found. By controlled the pH at 12.0 while allowing the ion-sources of Ca/P atomic ratio to vary, it was found that high Ca/P ratios of 2.5 and 3.0 led to the biphasic of $Ca(OH)_2$/apatite. High bioresorbabe materials of the homogeneous biphasics of $CaCO_3$ or $Ca(OH)_2$ with apatite are advantageous for bone regeneration and reconstruction processes and could potentially be applied as restorative materials based on the filler type, shape and size.

Acknowledgment

The authors acknowledge, with appreciation, support for this research from the National Science Council of the Executive Yuan, Taiwan. (NSC91-2320-B-006-049-M08; 97-2221-E-037-006).

REFERENCES

1. Malard O., Bouler J.M., Guicheux J., Heymann D., Pilet P., Coquard C., Daculsi G.: J. Biomed. Mater. Res. *46*, 103 (1999).
2. Dorozhkin S.V.: J. Mater. Sci. *42*, 1061 (2007).
3. Bermudez O., Boltong M.G., Driessens F.C.M., Ginebra M.P., Fernandez E., Planell J.A.: Biomaterials *15*, 1019 (1994).
4. Knaack D., Goad M.E.P., Aiolova M., Rey C., Tofighi A., Chakravarthy P., Lee D.D.: J. Biomed. Mater. Res. *43*, 399 (1998).
5. Nery E.B., Lynch K.L., Hirthe W.M., Mueller K.H.: J. Periodontol. *46*, 328 (1975).
6. Okumura M., Ohgushi H., Dohi Y., Katuda T., Tamai S., Koerten H.K., Tabata S.: J. Biomed. Mater. Res. *37*, 112 (1997).
7. Zou Q., Li Y., Zhang L., Zuo Y., Li J., Li X.: J. Biomed. Mater. Res., B *90*, 156 (2009).
8. Olderøy M.Ø., Xie M., Strand B.L., Flaten E.M., Sikorski P., Andreassen J.P.: Crystal Growth & Design *9*, 5176 (2009).
9. Wang J.C., Ko C.L., Hung C.C., Tyan Y.C., Lai C.H., Chen W.C., Wang C.K.: J. Dent. *38*, 158 (2010).
10. Ansari M., Naghib S.M., Moztarzadeh F., Salati A.: Ceramics-Silikáty *55*, 123 (2011).
11. Zhao L., Wang J.: Collids and Surf. A *393*, 139 (2012).
12. Sariibrahimoglu K., Leeuwenburgh S.C.G., Wolke J.G.C., Yubao L., Jansen J.A.: J. Biomed. Mater. Res. A *100*, 712 (2012).
13. Wang L., Ruiz-Agudo E., Putnis C.V., Menneken M., Putnis A.: Envir. Sci. & Tech. *46*, 834 (2012).
14. Chen W.C., Ju C.P., Tien Y.C., ChernLin J.H.: Acta Biomaterilia *5*, 1767 (2009).
15. Legeros R.Z.: Adv. Dent. Res. *2*, 164 (1988).
16. Chen W.C., Ju C.P., Wang J.C., Hung C.C., ChernLin J.H.: Dent. Mater. *24*, 1616 (2008).
17. Legeros R., Parsons J.R., Daculsi G., Driessens F., Lee D., Liu S.T., Metsger S., Peterson D., Walker M.: Ann. N. Y. *Acad.* Sci. *523*, 268 (1998).
18. Bouler J.M., LeGeros R.Z., Daculsi G.: J. Biomed. Mater. Res. *51*, 680 (2000).
19. Daculsi G., Passuti N., Martin S., Deudon C., LeGeros R.Z., Raher S.: J. Biomed. Mater. Res. *24*, 379 (1990).
20. Nery E.B., Eslami A., Van S.R.: J. Periodontol. *61*, 166 (1990).
21. Piattelli A., Scarano A., Mangano C.: Biomaterials *17*, 1767 (1996).
22. Daculsi G., Laboux O., Malard O., Weiss P.: J. Mater. Sci. Mater. Med. *14*, 195 (2003).
23. Teng F. Y., Ko C.L., Kuo H.N., Hu J.J., Lin J.H., Lou C.W., Wang Y.L., Cheng C.Y., Chen W.C.: Bioinorg. Chem. Appl. *2012*, 687291 (2012).

24. Daculsi G., LeGeros R.Z., Heughebaert M., Barbieux I.: Calcif. Tissue Int. *46*, 20 (1990).
25. Lin D.J., Ju C.P., Huang S.H., Tien Y.C., Yin H.S., Chen W.C., ChernLin J.H.: J. Mech. Behav. Biomed. Mater. *4*, 1186 (2011).
26. LeGeros R.Z., Orly I., Gregoire M., Daculsi G. in: *The bone biomaterial interface*, p. 76-88, University of Toronto Press, Toronto 1991.
27. Honda T., Takagi M., Uchida N., Saito K., Uematsu K.: J. Mater. Sci. Mater. Med. *1*, 114 (1990).
28. Hofmann M.P., Young A.M., Gbureck U., Nazhat S.N., Barralet J.E.: J. Mater. Chem. *16*, 3199 (2006).
29. Raynaud S., Champio E., Bernache-Assollant D., Thomas P.: Biomaterials *23*, 1065 (2002).
30. Arends J., Christoffersen J., Christoffersen M.R., Eckert H., Fowler B.O., Heughebaert J.C., Nancollas G.H., Yesinowski J.P., Zawacki S.J.: J. Cryst. Growth *84*, 515 (1987).
31. Murugan R., Ramakrishna S.: Biomaterials 25, 3829 (2004).
32. Murugan R., Ramakrishna S., Rao K.P.: Mater. Lett. *60*, 2844 (2006).
33. Zhao H., Dong W., Zheng Y., Liu A., Yao J., Li C., Tang W., Chen B., Wang G., Shi Z.: Biomaterials *32*, 5837 (2011).
34. Spanos N., Koutsoukos P.G.: J. Phy. Chem. B *102*, 6679 (1998).
35. Vallet-Regí M., González-Calbet J.M.: Prog. Solid State Chem. *32*, 1 (2004).
36. Bohner M., Gbureck U., Barralet J.E.: Biomaterials *26*, 6423 (2005).
37. Barradas A.M.C., Yuan H., van Blitterswijk C.A., Habibovic P.: Eur. Cells Mater. *21*, 407 (2011).
38. Heslop D.D., Bi Y., Baig A.A., Otsuka M., Higuchi W.I.: J. Coll. Inter. Sci. *289*, 14 (2005).
39. Lebugle A., Sallek B.: *Hydroxyapatite and related materials*, p. 319-328, CRC Press, Boca Raton, FL, 1994.
40. TenHuisen K.S., Brown P.W.: J. Biomed. Mater. Res. *36*, 233 (1997).
41. Chow L.C., Takagi S., Constantino P.D., Friedman C.D.: Mater. Res. Soc. Symp. Proc. *179*, 3 (1991).
42. Chow LC: J. Dent. Res. *69*, 595 (1990).
43. Takagi S., Chow L.C., Ishikawa K.: Biomaterials *19*, 1593 (1998).
44. Jung W.M., Kang S.H., Kim W.S., Choi C.K.: Chem. Eng. Sci. *55*, 733 (2000).
45. Midy V., Rey C., Bres E., Dard M.: J. Biomed. Mater. Res. *41*, 405 (1998).
46. Jarudilokkul S., Tanthapanichakoon W., Boonamnuayvittaya V.: Colloids Surf. A *296*, 149 (2007).
47. LeGeros R.Z., Trautz O.R., LeGeros J.P., Klein E.: Science *155*, 1409 (1967).
48. Fernández E., Gil F.J., Best S.M., Ginebra M.P., Driessens F.C.M., Planell J.A.: J. Biomed. Mater. Res. *41*, 560 (1998).
49. Chen W.C., ChernLin J.H., Ju C.P.: J. Biomed. Mater. Res. A *64*, 664 (2003).
50. Fox J.L., Yu D., Otsuka M., Higuchi W.I., Wong J., Powell G.L.: J. Dent. Res. *71*, 1389 (1992).
51. Baig A.A., Fox J.L., Hsu J., Wang Z., Otsuka M., Higuchi W.I., LeGeros R.Z.: J. Coll. Inter. Sci. *79*, 608 (1996).