

PREPARATION OF CALCIUM PHOSPHATE BIOMATERIALS BY USING MICROWAVE-ASSISTED CO-PRECIPIATION

Seinn New Aung¹, Aye Aye Thant², Nwe Ni Hlaing³, Than Htike Win⁴

Abstract

The calcium phosphate-based biomaterials have been synthesized by microwave assisted co-precipitation method. The calcium nitrate tetrahydrate and diammonium hydrogen phosphate have been used as calcium and phosphorus sources. The pH regulation has been performed by using two types of inorganic bases; sodium hydroxide and ammonium hydroxide during processing. The phase formation of the synthesized biomaterials with two different inorganic bases has been compared in this work. The synthetic samples have been characterized by using X-ray diffractometry (XRD), Fourier Transform-Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). These results show that crystallized monetite phase and biphasic calcium phosphate (20% of Apatite & 80% of Beta tricalcium phosphate) have been obtained via sodium hydroxide assisted synthesis. Crystallized hydroxyapatite (HA) phase and biphasic calcium phosphate (23% of Apatite & 97% of β -tricalcium phosphate) have been obtained via ammonium hydroxide assisted synthesis at calcination temperature of 900°C.

Keywords: biomaterials, precipitation, microwave, XRD, FTIR, SEM.

Introduction

Calcium Phosphate based biomaterials play a pivotal role as scaffolds for tissue regeneration and drug delivery due to their chemical similarity to the inorganic phase of bone. Biomaterials are not only biocompatible but also non-toxic, immune agent, bioactive and osteoconductive. CaP family includes four main categories: Hydroxyapatite (HAP), calcium deficient Hydroxyapatite (CDHAP) (tetracalcium phosphate or octacalcium phosphate), biphasic calcium phosphate (BCP) and tricalcium phosphate (α or β -TCP). When precipitating CaP in basic aqueous solutions, three main different solid phases could be formed; either amorphous calcium phosphates, Octacalcium phosphates or HAP (Behzad and Gholam, 2015).

These phases are different by increasing in their thermodynamic order and decreasing on the time scale. Although being similar chemically, their crystal structure, mechanical, physical and biological differ according to the processing parameters temperature, pH, partial pressure of water, aging time and the presence of impurities) (I Y.J. Zhu, F, 2014).

Materials and Method

Materials

In this study, calcium nitrate tetrahydrate and ammonium hydrogen phosphate were used as calcium and phosphorus sources. The pH regulation has been performed by using two types of inorganic bases; sodium hydroxide and ammonium hydroxide during processing. Distilled water (H₂O) was used as solvent.

¹ PhD candidate, Assistant Lecture, Department of Physics, University of Dagon

² Dr, Professor, Department of Physics, University of Yangon.

³ Dr, Lecturer, Department of Physics, University of Taunggyi.

⁴ Dr, Lecturer, Department of Physics, University of Mandalay.

Method

There are various methods of CaP synthesis such as precipitation technique, sol-gel approach, hydrothermal technique, multiple emulsion technique, biomimetic deposition technique, electrode position technique, etc. Each employs various process parameters. In this research, microwave-assisted precipitation method has been used to provide enhanced reaction rate and improved product field in chemical synthesis (E.Lerner, S. Sarig, 1991)

Result and Discussion

The synthetic samples have been characterized by X-ray diffractometry (XRD), Fourier Transform-Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM).

XRD Analysis of Calcium Phosphate Sample by Using Sodium Hydroxide before and after calcination

The XRD pattern of Calcium Phosphate sample by using sodium hydroxide (inorganic base) has been shown in Figure 1. Figure 1 (a) shows the XRD pattern of synthetic Calcium Phosphate sample by using sodium hydroxide before calcination. The monetite (CaHPO_4) hexagonal, space group of P63/m (ICDD-00-009-0432) has been observed as a major phase. The sharp intensity peak at 26.45° indicated that monetite phase could be attained by simple precipitation method at room temperature.

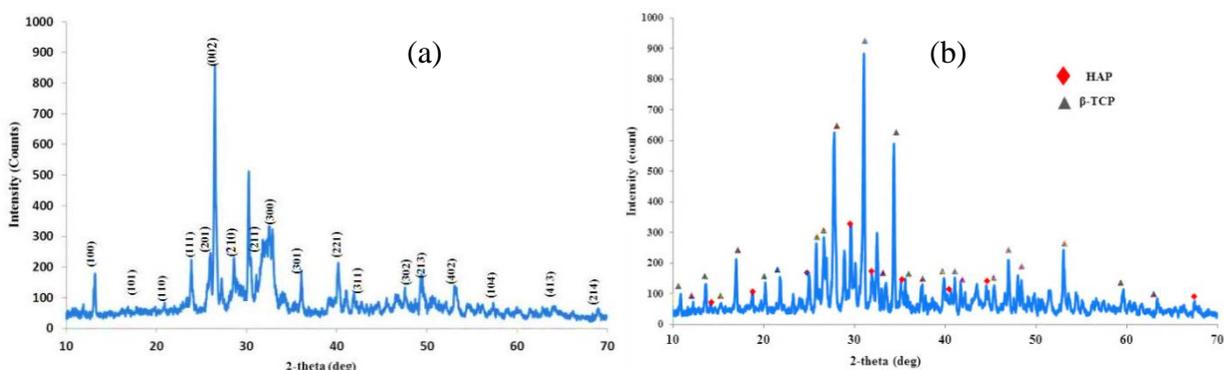


Figure 1 XRD pattern of synthetic Calcium Phosphate sample by using sodium hydroxide (a) before calcination (b) after calcination at 900°C

Figure 1 (b) shows the XRD pattern of synthetic Calcium Phosphate sample by using sodium hydroxide at 900°C . The formation of biphasic hydroxyapatite (trigonal (H), R3c) with beta tricalcium phosphate with (hexagonal, P63/m) have been reported in (ICDD-00-009-0432).

XRD Analysis of Calcium Phosphate Sample by Using Ammonium Hydroxide before and after calcination

The XRD pattern of calcium phosphate sample by using Ammonium hydroxide (inorganic base) is shown in Figure 2. Figure 2 (a) shows the hydroxyapatite (hexagonal, P63/m) reported in (ICDD-00-009-0432). The sharp intensity peak at 2θ value of 32.22° corresponding to (211) plane indicated that hydroxyapatite phase could be attained by simple precipitation method at room temperature.

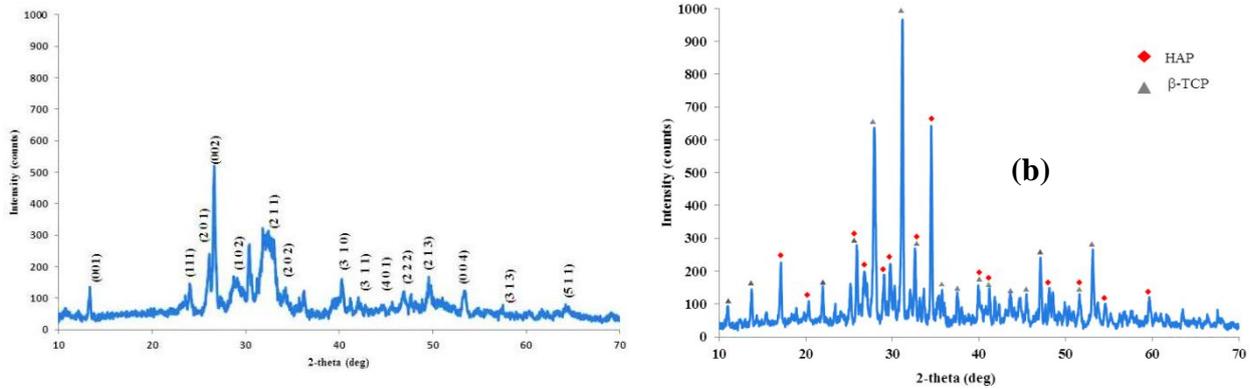


Figure 2 XRD pattern of synthetic Calcium Phosphate sample by using ammonium hydroxide (a) before calcination (b) after calcination at 900°C

XRD Analysis of Calcium Phosphate Sample by Using ammonium hydroxide after calcination

The XRD pattern of calcium phosphate sample at 900°C is shown in Figure 2 (b), the formation of biphasics; hydroxyapatite (trigonal, R3c) with betatricalcium phosphate (hexagonal, P63/m) reported in ICDD-00-009-0432 (Baradaran S, 2013).

Crystallite Size

The crystallite size can be estimated using the Scherer's equation:

$$D = \frac{0.9\lambda}{B \cos \theta}$$

where, B = full width at the half maximum (radian)

λ is the wavelength (Å), θ is diffraction angle

Table 1 Results of nano-sized Calcium Phosphate powders from(XRD)

Inorganic bases	Condition	Crystallite size (nm)
NaOH	before calcination	33.24
	after calcination	41.98
NH ₄ OH	before calcination	17.18
	after calcination	36.09

Reference Intensity Ratio (RIR) Value and Weight Percentage

Reference Intensity Ratio (RIR) value and weight percentage of constituent of the sample has been calculated by using following formula(I Y.J. Zhu, F.)

$$RIR_{HAP} = I_{HA} / (I_{HAP} + I_{\beta-TCP})$$

$$RIR_{\beta-TCP} = I_{\beta-TCP} / (I_{\beta-TCP} + I_{HAP})$$

$$\text{Weight \%} = (RIR \times 100)\%$$

where, I_{HAP} = maximum intensity of HAP phase

$I_{\beta-TCP}$ = maximum intensity of β -TCP phase

Comparison of RIR Value and Weight % of Calcium Phosphate by Using Sodium Hydroxide and Ammonium Hydroxide after Calcination at 900°C

Table 2 Comparison of RIR Value and Weight % of Calcium Phosphate by using sodium hydroxide and ammonium hydroxide after calcination

Inorganic bases	Phase	2 (deg)	Plane	Relative Intensity	RIR value	Weight(%)
NaOH	β-TCP	31.50	(0210)	149	0.80	80
	HAP	33.00	(112)	38.30	0.20	20
NH ₄ OH	β-TCP	27.87	(214)	168	0.97	97
	HAP	34.50	(202)	38.30	0.03	3.0

FT-IR analysis

FT-IR Analysis of Calcium Phosphate by using sodium hydroxide before and after calcination

The functional groups generally observed in the FT-IR spectra of calcium phosphate based materials are PO_4^{3-} , OH^- , CO_3^{2-} , P_2O_7 and HPO_4^{2-} groups in the range around 4000–400 cm^{-1} .

Figure 3 shows FTIR spectrum of Calcium Phosphate by using sodium hydroxide (before calcination). The characteristic bands of phosphate groups have been appeared around 560–613 cm^{-1} and 1100–1000 cm^{-1} . There are sharp and well-separated peaks at 431.1 cm^{-1} , 560.97 cm^{-1} , 601.49 cm^{-1} and 1026 cm^{-1} have been all attributed to the PO_4^{3-} group of monetite. The peak 3571 cm^{-1} has been attribute to OH^- group and 861.80 cm^{-1} has been attribute to CO_3^{2-} .

Figure 4 shows FTIR spectrum of Calcium Phosphate by using sodium hydroxide (after calcination). The sharp and well-separated peaks at 542.61 cm^{-1} , 589.85 cm^{-1} , 945.25 cm^{-1} , 971.61 cm^{-1} and 1023.63 cm^{-1} have been assigned to biphasics (β -TCP and HAP) groups. These results are in good agreement with the X-ray diffraction analysis.

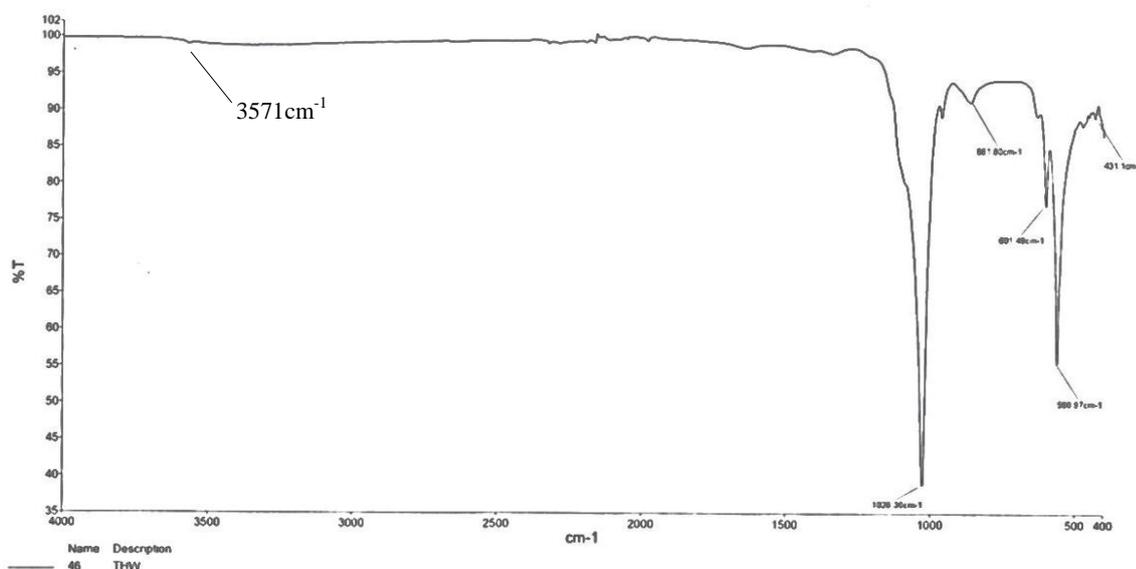


Figure 3 FT-IR spectrum of CaP sample by using sodium hydroxide (before calcination)

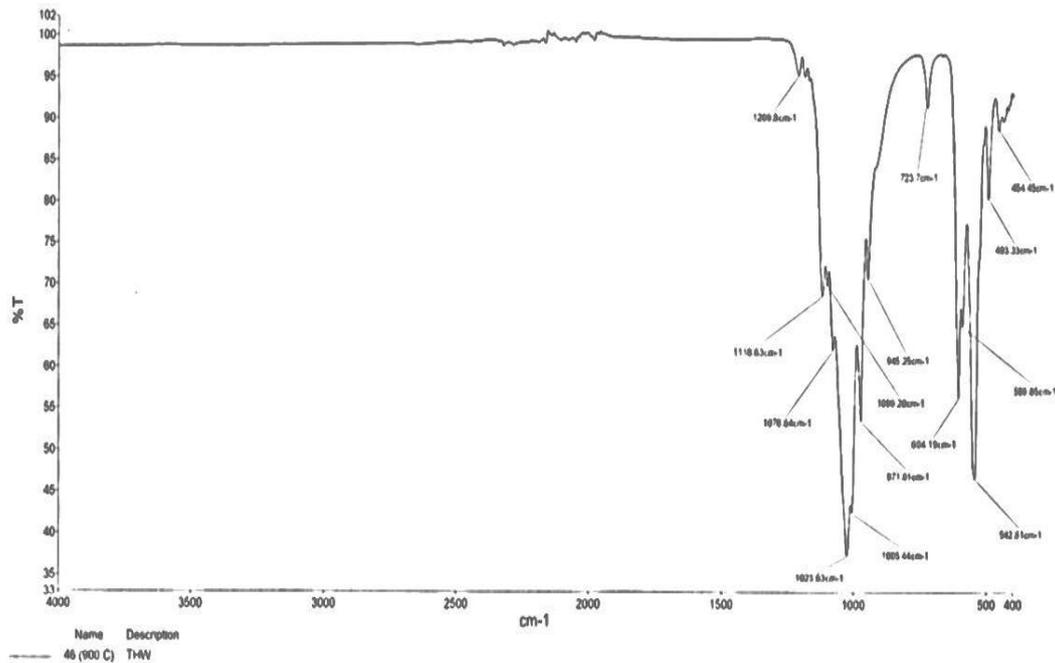


Figure 4 FT-IR spectrum of CaP sample by using sodium hydroxide (after calcination at 900°C)

Table 3 The FT-IR transmission bands of CaP sample using sodium hydroxide before and after calcination

Calcination condition	Transmission Bands(cm^{-1}) of Chemical groups					
	PO_4^{3-}				OH-	CO_3^{2-}
	ν_1 symmetric (P-O) stretching	ν_2 (O-P-O) bending	ν_3 asymmetric (P-O) stretching	ν_4 (P-O) bending	Stretching	
before calcination	-	431.10	1026.30	560.97 691.40	3571.00	861.80
900°C	493.83 542.61 589.85 945.25 971.61	451.45	1018.63 1005.44 1118.63 1209.80	- - 604.19	-	-

FT-IR Analysis of Calcium Phosphate by using ammonium hydroxide before and after calcination

Figure 5 shows FT-IR spectrum of Calcium Phosphate by using ammonium hydroxide (before calcination). There are sharp and well-separated peaks at 560.94 cm^{-1} , 601.01 cm^{-1} , 962.45 cm^{-1} and 1025.23 cm^{-1} have been all attributed to the hydroxyapatite group.

Figure 6 shows FT-IR spectrum of Calcium Phosphate by using ammonium hydroxide (after calcination). The sharp and well-separated peaks at 493.83 cm^{-1} , 542.65 cm^{-1} , 603.98 cm^{-1} , 970.56 cm^{-1} and 1015.68 cm^{-1} have been assigned to biphasic (hydroapatite and β - TCP) group. These results are in good agreement with the X-ray diffraction analysis (M. Sadat-Shojai, M.T, 2013).

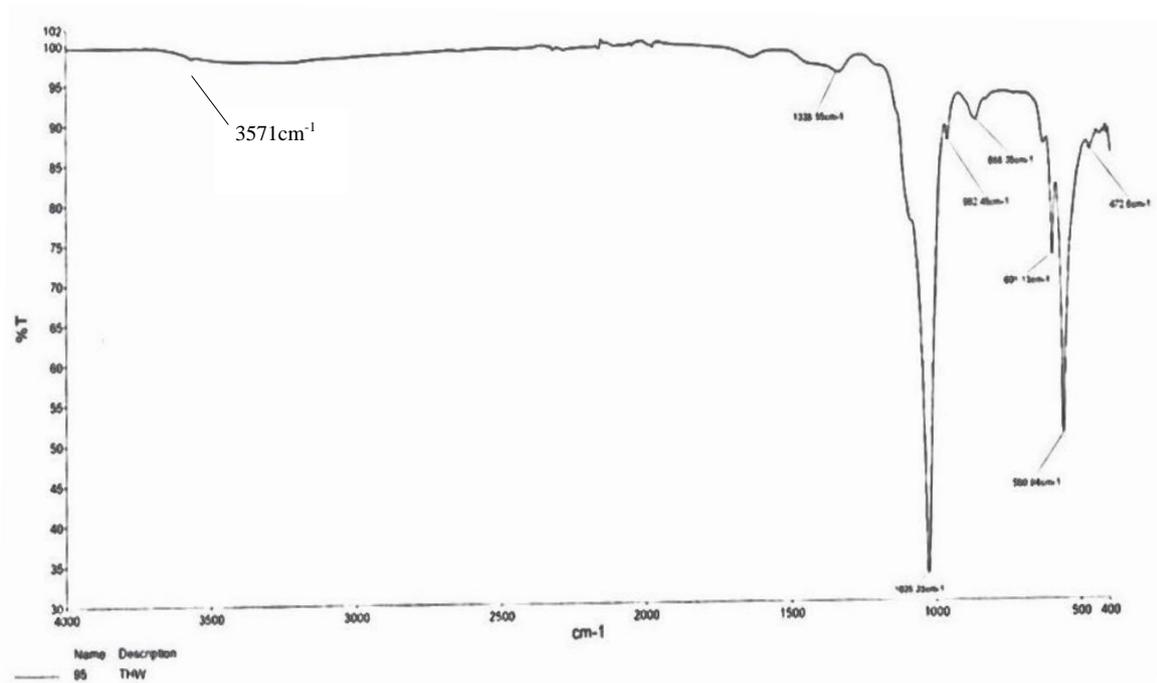


Figure 5 FT-IR spectra of CaP sample by using Ammonium hydroxide (before calcination)

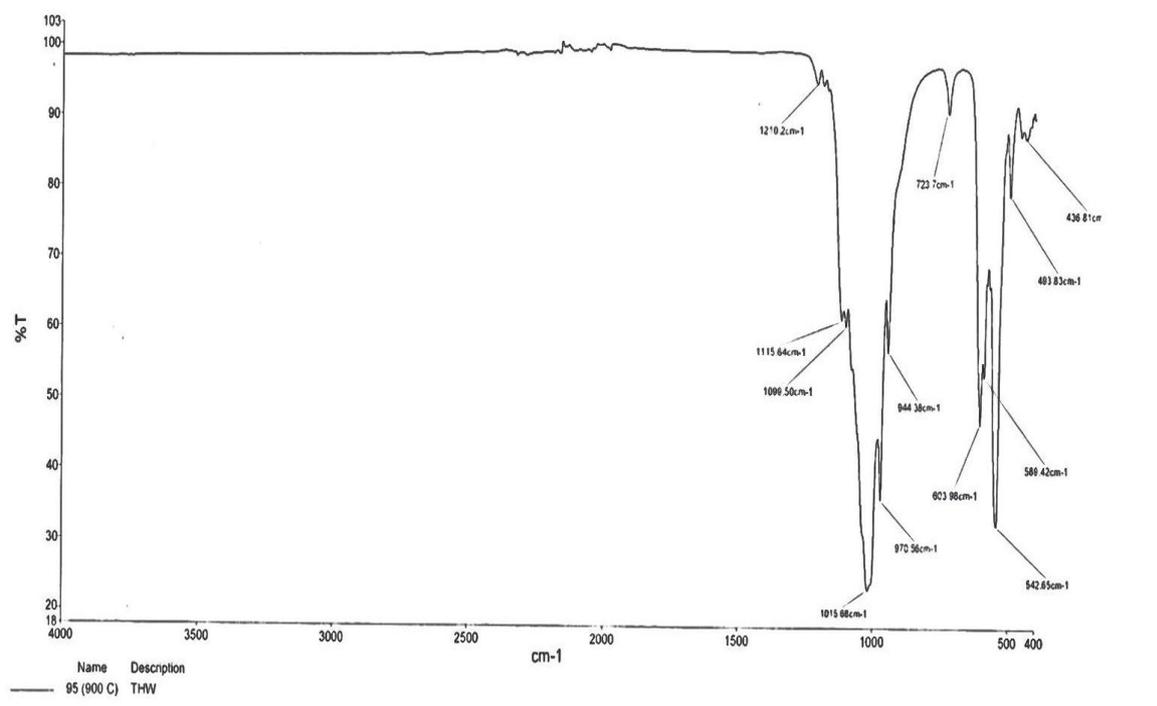


Figure 6 FT-IR spectra of CaP sample by using Ammonium hydroxide (after calcination at 900°C)

Table 4 The FT-IR transmission bands of CaP sample by using ammonium hydroxide before and after calcination

Calcination condition	Transmission Bands(cm^{-1}) of Chemical groups					
	PO_4^{3-}				OH-	CO_3^{2-}
	ν_1 symmetric (P-O) stretching	ν_2 (O-P-O) bending	ν_3 asymmetric (P-O) stretching	ν_4 (P-O) bending	Stretching	
before calcination	962.45	472.6	1025.23	560.94 601.13	1338.55 3571.00	868.05
900°C	493.83 542.62 589.42 944.38 970.56	436.81	1015.98 1099.50 1115.64 1210.2	- - 603.98 723.7	-	-

Scanning Electron Microscopy (SEM) Analysis

(SEM) Analysis of Calcium Phosphate powders by using sodium hydroxide and Ammonium hydroxide before and after calcination

Figure (7) shows SEM micrographs of the CaP powders by using sodium hydroxide before and after calcination. Figure 7(a) shows the monetite microparticles which were highly agglomerated. Figure 7(b) shows biphasics microsturcutre has been found. The grain size for the samples (after heating) have been estimated by using image J software and it is found to be 1.20 μm (E. Lerner, S. Sarig) .

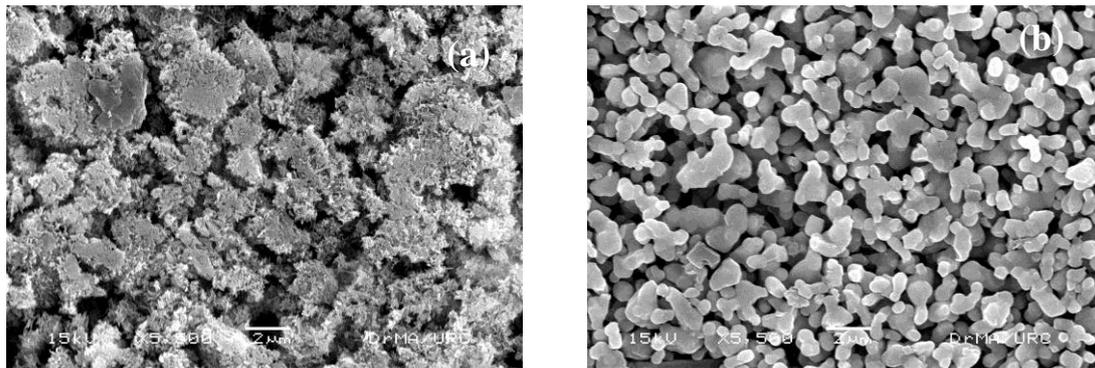


Figure7 SEM micrographs of CaP powders by using sodium hydroxide: (a) before calcination (b) after calcination at 900°C.

Figure (8) shows SEM micrographs of the CaP powders by using ammonium hydroxide before and after calcination. Figure 8 (a) shows the formation and agglomeration of small irregular particles in the sample. Figure 8(b) shows homogenous microstructure and interconnected micropores. The grain size for the sample after calcination have been estimated by using image J software and it is found to be 0.56 μm .

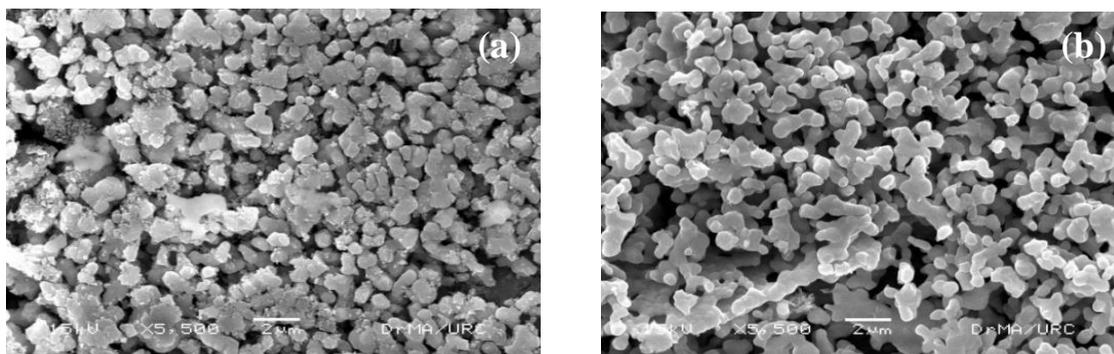


Figure 8 SEM images of CaP powders by using Ammonium hydroxide: (a) before calcination (b) after calcination at 900°C.

Conclusions

The synthesis of calcium phosphate biomaterials powder via microwave-assisted precipitation method has been reported. Two types of inorganic bases; Sodium hydroxide and Ammonium hydroxide have been used for the pH regulation. The XRD analysis proved that the well crystallized monetite phase was formed with sodium hydroxide where as hydroxyapatite phases with Ammonium hydroxide before calcination. The formation of biphasics (hydroxyapatite & beta-tricalcium Phosphate) after calcination has been identified by XRD and FTIR analyses. The biphasic biomaterials with different phases involving different RIR values could be obtained by assisting different types of inorganic base. Note worthily, Observation on morphology proved that the formation of agglomeration of small particles (before calcination) and microstructures (after calcination) have been obtained by using sodium hydroxide. The formation of irregular particles (before calcination), homogenous microstructure and interconnected micropores could be obtained by using ammonium hydroxide in this research. In this study, when comparison of sodium hydroxide and ammonium hydroxide, smaller crystalline size, more percentage weight of TCP groups and homogeneous microstructure have been obtained via ammonium hydroxide.

Acknowledgements

The authors are grateful to the Department of Physics, University of Yangon and University of Taunggyi for the supports with research facilities.

References

- Asmae Massit et al (2014) "Synthesis and characterization of nano-sized β -Tricalcium phosphate: Effects of the aging time", *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 7(7), pp. 57-61.
- Baradaran S. (2013). "Synthesis and Characterization of Monetite Prepared Using a Sonochemical Method in a Mixed Solvent System of Water/Ethylene Glycol/N,N-Dimethylformamide", *The Minerals, Metals & Materials Society and ASM International*.
- Behzad Mehdikhania, and Gholam Hossein Borhani. (2015) "Synthesis nano bio-ceramic powder β - $\text{Ca}_2\text{P}_2\text{O}_7$ ", *Journal of Ceramic Processing Researchy*, 16(3), pp. 308-312.
- E. Lerner, S. Sarig, Enhanced maturation of hydroxyapatite from aqueous solutions using microwave irradiation, *J. Mater. Sci. Mater. Med.* 2 (1991) 138–141.
- I Y.J. Zhu, F. (2014). "Microwave-assisted preparation of inorganic nanostructures in liquid phase, *Chem. Rev.* 114, pp.6462–6555.
- M. Sadat-Shojai, M.-T. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nano-sized hydroxyapatite with diverse structures, *ActaBiomater.* 9 (2013) 7591–7621, <http://dx.doi.org/10.1016/j.actbio.2013.04.012>.

