Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates

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Abstract
Biogenic materials like corals, which are readily available, could be used to produce bioceramic materials and address significant advantages due to their unique structures and chemical compositions that contain Mg and Sr. Many conversion processes has been in the past proposed. In this work, a comparison study between the conversion of coral with orthophosphoric acid and ammonium dihydrogen phosphate was conducted. The resultant structures and compositions were studied using XRD, ICP-MS, SEM and FTIR. The results show that with phosphoric acid the coral was converted into mainly monetite (92%). The ammonium dihydrogen phosphate converted approximately 76% of the coral to hydroxyapatite through solid state reactions. The two routes proved to be effective in producing bioceramic materials from corals under moderate conditions of temperature with a basic condition favouring the yield of hydroxyapatite.

Keywords: hydroxyapatite, monetite, whitlockite, coral, orthophosphoric acid, ammonium dihydrogen phosphate

INTRODUCTION

Regenerative medicine and tissue engineering are research areas which deal with the functions and the recreation of substitute materials for tissue replacement and regeneration. Bone is one of the most complex living organic-inorganic composite systems known. It undergoes constant remodelling and can heal itself when fractured, usually to perfection [1]. Clinical investigations have focused on producing implant materials for the treatment of different kinds of bone problems such as fractures, bone cancer, and osteoporosis [2]. Currently, considerable attention has been focused on the synthesis of bioceramic materials like hydroxyapatite (HA) and tri-calcium phosphates (TCP) for use in maxillofacial and orthopaedic surgery and tissue regeneration and repair materials.

Biological natural materials like eggshells, sea urchins, coral, nacre, mussel and land snails [3 – 6] have been used to synthesize bioceramics due to their chemical composition and their unique architectural structure [7]. Coral consists mostly of calcium carbonate in the form of aragonite crystals ($CaCO_3$) which can easily be converted to calcium phosphate bioceramics. It has been reported that, coral shows cell ingrowth and bone formation similar to the host bone tissue [3].

Calcium phosphate materials are characterized by the structure and also by type of the phosphate anion, such as: ortho- (PO_4^{3-}), hydrogeno- (HPO_4^{2-}), pyro- ($P_2O_7^{4-}$) or meta- (PO_3^-) and poly- ($(PO_3)n^{n-}$). The driving force to produce and use these materials is based on the similarity -of these materials to the composition- of the major bone mineral, a calcium phosphate in the form of calcium-deficient carbonate apatite nanocrystals. Table 1 shows known calcium orthophosphates, including their mineral names and formulae.

Table 1: Existing calcium orthophosphates [6]

Mineral name		Short name	Empirical formulas	Ca/P
Dicalcium phosphate dihydrate	Brushite	DCPD	$CaHPO_4.2H_2O$	1.00
Dicalcium phosphate	Monetite	DCPA	$CaHPO_4$	1.00
Octacalcium phosphate		OCP	$Ca_8H_2(PO_4)_6.5H_2O$	1.33
β-Tricalcium phosphate	Whitlockite	β-TCP	β-$Ca_3(PO_4)_2$	1.50
Hydroxyapatite		HAp	$Ca_{10}(PO_4)_6(OH)_2$	1.67
Tetracalcium phosphate monoxyde		TTCPM	$Ca_4(PO_4)_2O$	2.0
Defect apatite		DA	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $0 < x < 2$	(10-x):6

Different synthesis methods have been employed to convert natural materials to calcium phosphates for a wide range of biomedical applications with the aim to get a pure phase material with a defined composition and morphology. The high price of bioceramics in the market reflects the significant costs of production entailed in the use of current, energy intensive synthesis methods.

In this research, the aim was focused to produce bioceramics from coral materials by using a simple, chemical conversion method and comparison of the two conversion methods under acidic and basic environments were made.

MATERIALS AND METHODS

2.1 Materials

Corals were obtained from the Great Barrier Reef, QLD. Ammonium dihydrogen phosphate dibasic ($NH_4H_2PO_4$, 98%) (ADP), hydrophosphoric acid (H_3PO_4, 85%) (HPO) and sodium hypochlorite (NaClO) were obtained from Sigma Aldrich Australia.

2.2 Experimental Method

The coral samples were crushed in a mill and cleaned with 2% (v/v) NaClO, further ground within an aluminium oxide ball mill (46 rpm, 2 h), and then dried at 100˚C for 2 hours before use. Thermogravimetric analysis of the coral was performed (SDT Q600, TA Instruments, New Castle, DE, USA) to determine the total amount of calcium carbonate in the coral. About 3 – 6 mg of coral was used during analysis under a circulating air environment with a heating rate of 10 ^oC/min from room temperature to 1100^oC. Next the required amount of H_3PO_4 or $NH_4H_2PO_4$, to obtain HAp or TCP, was dissolved in 25 ml of distilled water. Then it was added, drop by drop, to 3 g of coral powder suspended in 150 ml of distilled water at 80 ^oC on a temperature control hot plate with magnetic stirrer. Water amount was controlled and measured to be constant under sealed condition.

Four experiments were set up with the aim to produce both HAp and TCP with phosphoric acid and ammonium dihydrogen phosphate respectively (Figure 1).

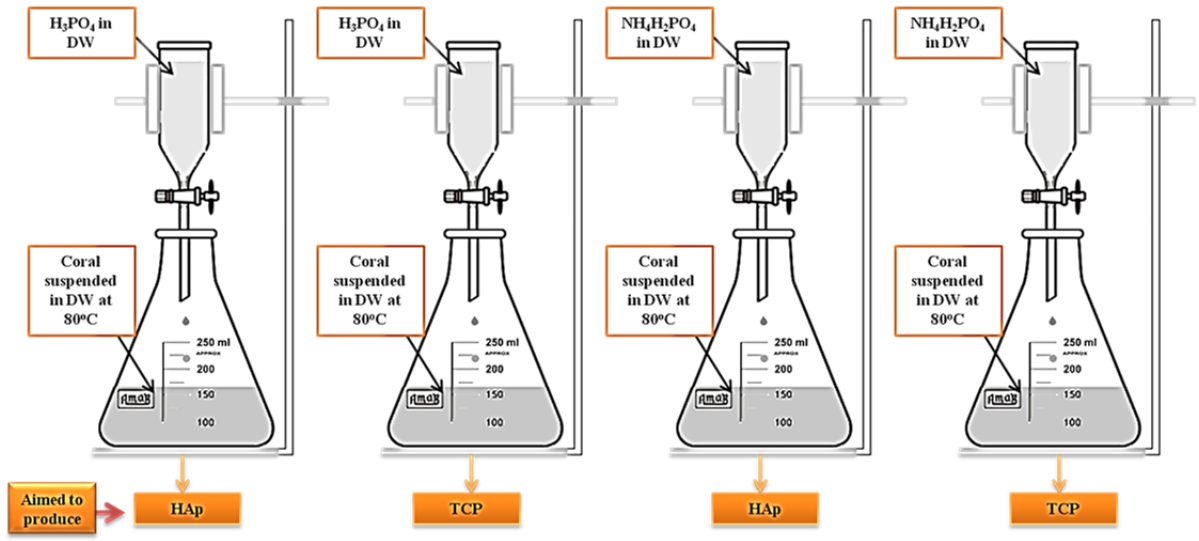

Fig. 1: Experimental set-up

The stirring rate was 200 rpm and the temperature was kept at 80 ˚C for 24 hrs. The pH values prior and after were monitored. Samples were taken every hour for ICP analysis. One and a half ml of the reaction mixture was pipetted into an Appendorf tube then separated by centrifuging (Eppendort, Centrifuge 5702) for 2 min at 3000 rpm. The supernatant liquid was pipetted and analysed for trace elements by ICP. The solids were washed twice with distilled water and separated by centrifuge. The samples were then dried in an oven at 80 ˚C for 24 hrs for further analysis.

2.2.1 Characterisation

2.2.1.1 X- Ray Diffraction

Phase analysis of the products were carried out by X-ray powder diffraction using Bruker d8 X-ray Diffractometer employing CuK_{α} radiation (λ=0.15418 nm). The diffractometer was operated at 40 kV, 0.02 step size from 20 – 70° with step time 0.5 s. The powder samples were loaded into a holder and flattened before analysis. The phase quantification was done using Xpowder 12 software on full profile quantitative analysis of components using 'Dirac' patterns and convolution and PIR scale factor.

2.2.1.2 Scanning Electron Microscope (SEM)

The SEM pictures were taken with a Zeiss Supra 55VP SEM with RAITH E-beam Lithography System & EBSD for the secondary electron imaging (SEI) the energy was kept at 20 kV, working distance and magnification were varied to obtain best possible pictures. The powder samples were fixed by mutual conductive adhesive tape on aluminium stubs and coated with carbon using a sputter coater (CEA 010, Balzers union FL-9496 Balzers, SCD 020.

2.2.1.3 Fourier Transform Infrared Spectroscopy (FTIR)

A spectrum in the range 400 – 40000 cm^{-1} was obtained with FTIR (Nicolet Magna 6700 FTIR spectrometer) for the product samples. The product powders were ground in an agate mortar and thoroughly mixed with KBr (FTIR Grade). For the analyses 3 mg of each sample was ground with 150 mg of KBr and pressed into a pellet (Carver press). KBr background was used.

2.2.1.4 Inductively Coupled Plasma- Mass Spectrometry (ICP- MS)

The converted samples were analysed in an Agilent Technologies ICP- MS (7500 Series). A set of standards were prepared. As the elements of interest are calcium and phosphate, 1 ml of each (High- Purity- Standards, Choice Analytical Pty Ltd - Thornleigh, Australia**;** 10 µg/ ml in 2 % HNO_3, Ca # 10- 9- 1, Lot 1229724; P # 10 39-1, Lot. 1016801) were pipetted into 8 ml of 1% nitric acid (HNO_3) for the 1 ppm standard. After that a set of dissolutions were prepared with HNO_3 for the 500 ppb, 100 ppb, 50 ppb, 10 ppb and 1 ppb also a blank of 1 % HNO_3 was used. The sample containers for the machine have 5 ml size, in this 4.75 ml of 1 % HNO_3 together with 0.25 ml sample were mixed.

RESULTS

3.1 X- Ray Diffraction

From the four experimental sets shown in Table2, samples were taken and analysed in the XRD after 24 hours. Figure 1 shows XRD patterns of the product samples after 24 hrs of reaction. The results provide strong evidence of the presence of monetite (JSPDS Card no. 089-5969) as the main phase when using phosphoric acid. For the HAp experiment a small amount of HAp (JSPDS Card no. 09-432) was shown in the product while whitlockite (JSPDS Card no. 09-0165) was shown as the second phase in the TCP samples. Other experiments aimed at producing HAp and TCP using ADP showed HAp (JSPDS Card no. 09-432) as the main phase with small amounts of retained calcium carbonate (JSPDS Card no. 85-1108) remaining as unconverted coral. Whitlockite is the mineral name for (TCP) [4]. Phase quantification analysis of the XRD results suggested that by using phosphoric acid (aimed at producing HAp) all coral was converted into monetite (92.1 %) and calcium deficient carbonate HAp (7.9 %). For TCP synthesis product samples, monetite (93.4%) and whitlockite (6.6 %) were the phases produced. On the other hand, 76 % coral was converted into hydroxyapatite when reacted with ammonium dihydrogen phosphate.

Running the experiment for further 24 hr showed no other phase development, (Table 2). For the acid based conversions, the amount of monetite increased at the expense of HAp and whitlockite for the HAp and TCP experiments respectively. For the ammonium dihydrogen phosphate (ADP) route, the amount of HAp increased for both sets of experiments with the decrease of calcium carbonate.

3.2 Scanning Electron Microscope

The morphology of the products, after 24 hours, were analysed and is presented in Figure 3. Figure 4 shows the morphology of coral powder before conversion. The morphology of the final products from phosphoric acid reactions, Figures 3a and 3b, is a mixture of rectangular blocks and reformed platelets of various sizes, consistent with the shapes of monetite and small amount of hydroxyapatite respectively. However the ADP route, shown in Figures 3c and 3d, yielded mainly platelet morphology. The plates in 3d are smoother and look better defined with platelet sizes ranging from 0.06 x 1.3 µm to approximately 0.002 x 1.5 µm.

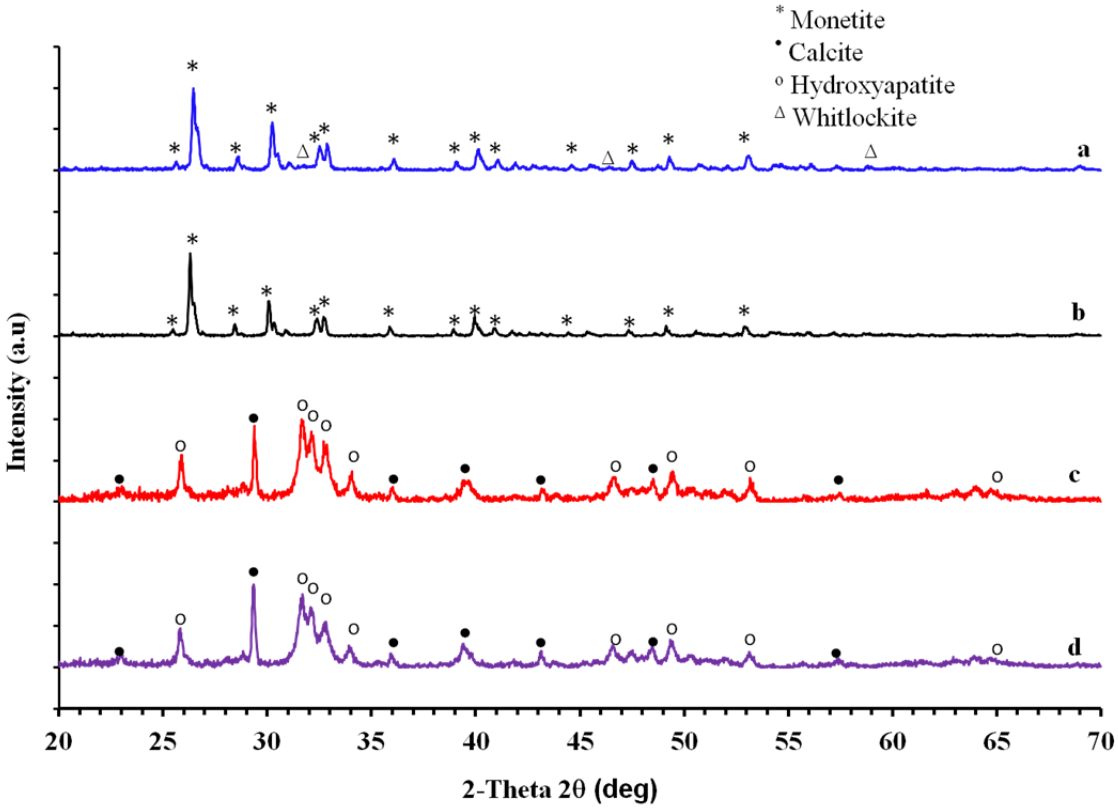

Fig. 2: XRD patterns of the four experiments; **a)** TCP/HPO **b)** HAp/HPO **c)** TCP/ADP **d)** HAp/ADP

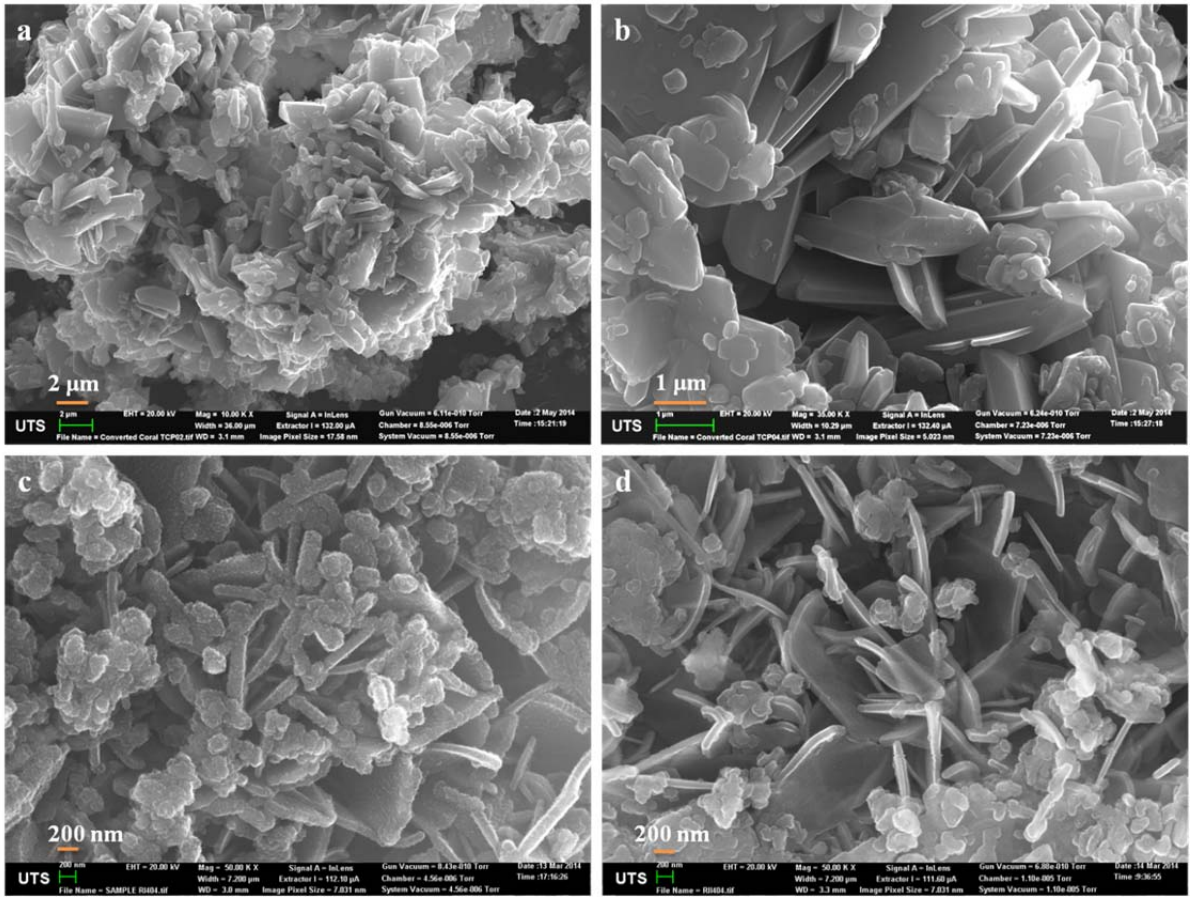

Fig. 3: Morphology of converted product samples after 24 hrs **a)** and **b)** Monetite with HAp by HPO, **c)** and **d)** HAp with retained calcite by ADP.

Table 2. Shows in percentage the quantity of the phases present, after 24 hours

Experiment	Phosphoric acid, H_3PO_4				Ammonium dihydrogen phosphate, $(NH_4)_2HPO_4$			
	Aim to produce HAp		Aim to produce TCP		Aim to produce HAp		Aim to produce TCP	
Phase	HAp [%]	Monetite [%]	Whitlockite [%]	Monetite [%]	HAp [%]	$CaCO_3$ [%]	HAp [%]	$CaCO_3$ [%]
24 hrs	7.9	92.1	6.6	93.4	76.2	23.8	76.1	23.9

After 48 hours

Experiment	Phosphoric acid, H_3PO_4				Ammonium dihydrogen phosphate, $(NH_4)_2HPO_4$			
	Aim to produce HAp		Aim to produce TCP		Aim to produce HAp		Aim to produce TCP	
Phase	HAp [%]	Monetite [%]	Whitlockite [%]	Monetite [%]	HAp [%]	$CaCO_3$ [%]	HAp [%]	$CaCO_3$ [%]
48 hrs	5.3	94.7	5.9	94.1	77.9	22.1	81.7	18.3

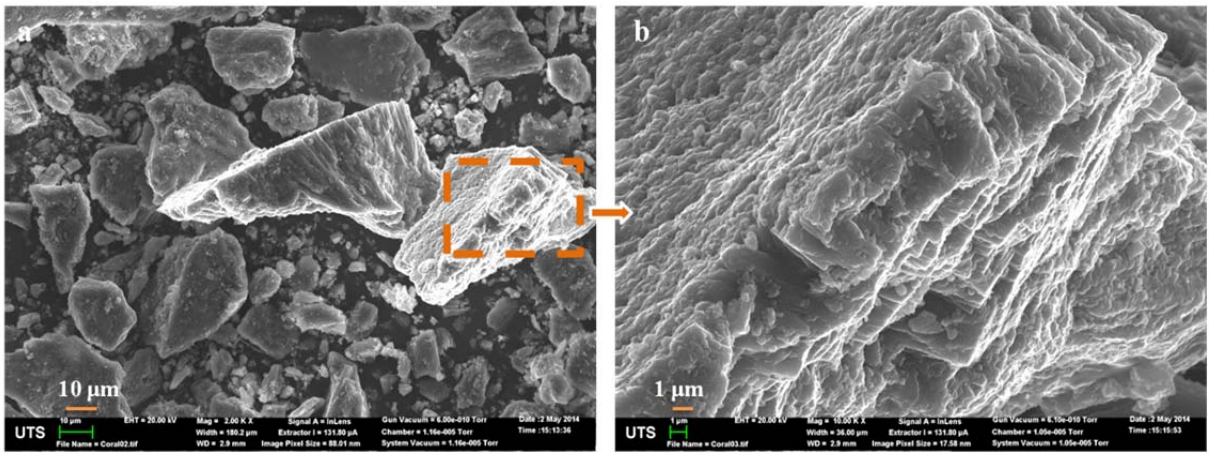

Fig. 4: **a)** SEM picture of coral powder before conversion **b)** SEM picture of selected area at a higher magnification

Table 3: ICP-MS results of Ca and P elements in the remaining liquid of reaction mixture

Time (h)	Phosphoric acid, H_3PO_4				Ammonium dihydrogen phosphate, $(NH_4)_2HPO_4$			
	HAp		TCP		HAp		TCP	
	P [ppm]	Ca [ppm]	P [ppm]	Ca [ppm]	P [ppm]	Ca [ppm]	P [ppm]	Ca [ppm]
4	21.3	13.1	48.6	36.3	307.7	0.30	349.9	0.10
24	21.3	12.8	49.7	37.0	291.6	0.20	326.5	0.10
48	22.8	13.3	49.4	37.0	331.9	0.10	273.9	0.20

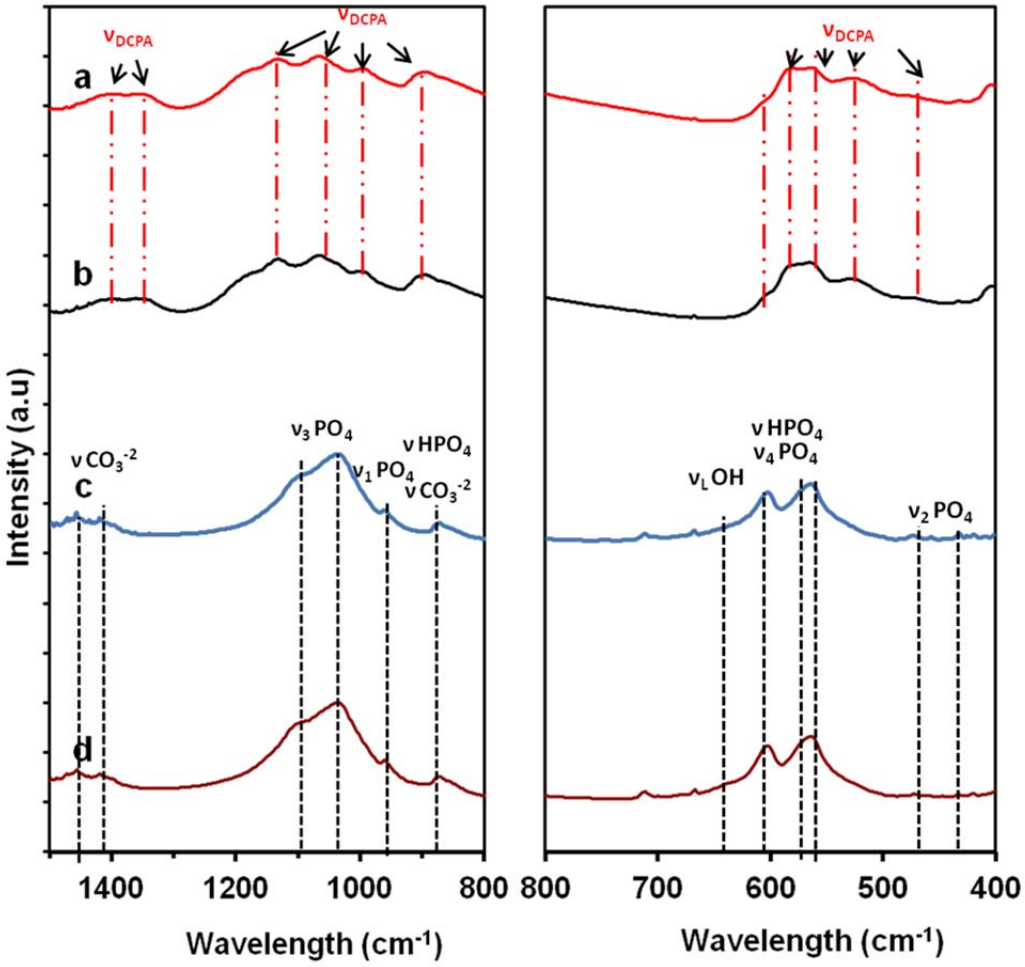

Fig. 5: FT-IR spectra of the 24th hr samples **a)** TCP/HPO **b)** Hap/HPO **c)** TCP/ADP **d)** HAp/ADP

3.3 FTIR

The FT-IR spectra of the product samples from the four experiments after 24 hrs are shown in Figure 5. The results confirm the presence of calcium phosphate in the product samples by characteristic vibrations of the PO_4 tetrahedral (ν1occurs at 962cm^{-1} and ν2 470 cm^{-1}; ν3, occurs at 1047 and 1087 cm^{-1}; ν4, occurs at 601 and 571 cm^{-1}). These IR bands observed were matching very well with those reported in previous literature [8, 9].

3.4 ICP-MS

ICP was run on several, samples to monitor the content of phosphorus and calcium in the solutions retained from the experiments. In Table 3 the results are shown for three different samples of each experiment set, samples 4 hr, 24 hr and 48 hr.

Presence of calcium in the remaining solution indicates the dissolution process of this element from coralline solid materials during reaction with the acid addition.

Measuring pH levels prior to H3PO4 addition showed pH levels of 8.5 to 8.6. After the acid addition, pH level immediately dropped to 2.2 and then to 0.98. For ADP process pH level was 8.5 – 8.6 initially but it dropped to 7.4 upon addition of ADP.

The results show that the samples with phosphoric acid as the source of phosphorous have lower phosphorus contents than the ADP samples due to the dissolution and reformation process. But the ADP samples have lower amounts of calcium in the liquid phase, indicating that not a dissolution process but solid state reaction. It should be mentioned that the ICP- MS is most accurate at lower ppb counts. The level of calcium in the H_3PO_4 samples stays more or less the same during the 48 hrs of experimental run.

DISCUSSIONS

The ICP-MS results and SEM pictures suggested that the reactions took place in two different conversion processes. The H_3PO_4 can be described as a dissolution and precipitation process indicated by the higher amount of calcium in the liquid during reaction period. Moreover, the present of the monetite phase in the XRD can be explained by the pH and by the intermediate reactions during the dissolution and precipitation of the conversion. On the other hand, the ADP shows very small amount

of calcium in the liquid and direct surface conversion into hydroxyapatite showing the attributes of interfacial conversion reactions. The SEM pictures show the same features, samples from ADP transform/grow from small rice shaped crystals, into the platelets (Figure 3b and 3c), that is different from the starting material (Figure 4). The H_3PO_4 samples have fine reformed crystals at the surface of the particles and platelets. Viewing the different analysis methods it is clear, that the monetite phase is the main phase in the conversion through the H_3PO_4 process from the dissolution and reassembling (nucleation and growth) process. Whereas the ADP is basic pH condition and a solid state exchange (diffusion) process and therefore no monetite formation, but direct conversion from calcium carbonate into HAp.

It was postulated that the production process of monetite, hydroxyapatite and whitlockite possibly follows the reaction scheme 1. Note that there must be several reaction intermediates not noted in this suggested scheme.

However, reaction of coral with ammonium dihydrogen phosphate to produce hydroxyapatite can be shown as, scheme 2 [10]. It was suggested that the reaction between ammonium dihydrogen phosphate with coral under hydrothermal conditions follows the reaction:

$$Ca^{2+} + (HPO4)^{2-} \longrightarrow CaHPO_4 \quad (1)$$

$$(10\text{-}x)Ca^{2+} + (6\text{-}x)PO_4^{3-} + xHPO_4^{2-} + (1\text{-}x)OH^- \longrightarrow Ca_{(10\text{-}x)}(PO_4)_{(6\text{-}x)}(HPO_4)_x(OH)_{(2\text{-}x)} \quad (2)$$

$$3Ca^{2+} + 2PO_4^{3-} \longrightarrow Ca_3(PO_4)_2 \quad (3)$$

Scheme 1: Global reactions of coral with orthophosphoric acid to produce 1) monetite 2) Ca deficient hydroxyapatite and 3) whitlockite

$$10CaCO_3 + 6NH_4H_2PO_4 + 2H_2O \longrightarrow Ca_{10}(PO_4)_6(OH)_2 + 3(NH_4)_2CO_3 + 7H_2CO_3 \quad (4)$$

Scheme 2: Reaction of coral with ammonium dihydrogen phosphate to produce hydroxyapatite under hydrothermal conditions

$$H_3PO_4(aq) \longrightarrow H^+(aq) + H_2PO_4^-(aq) \qquad K_{a1} = 7.5 \times 10^{-3}$$

$$H_2PO_4^-(aq) \longrightarrow H^+(aq) + HPO_4^{2-}(aq) \qquad K_{a2} = 6.2 \times 10^{-8}$$

$$HPO_4^{2-}(aq) \longrightarrow H^+(aq) + PO_4^{3-}(aq) \qquad K_{a3} = 1.7 \times 10^{-12}$$

Scheme 3: Dissociation of phosphoric acid in solution

Lynn and Bonfield and Drouet [11, 12] reported that pH affects the synthesis of calcium phosphates materials and is integrally linked to the properties of phosphate-containing solutions. In solution, phosphoric acid behaves as a triprotic acid, having three ionizable hydrogen atoms (Scheme 3). The hydrogen ions are lost sequentially with increasing pH. It was also suggested that variations in pH alter the relative concentrations of the phosphoric acid and thus both the chemical composition and the amount of the CaP that forms especially by direct precipitation [13, 14]. In addition, pH can also affect the solubility of species during synthesis, a property particularly important for distinguishing between systems in which calcium phosphates are precipitated. Dissolution of calcium carbonate is feasible in acidic condition which explains why H_3PO_4 process was dissolution and precipitation of calcium phosphate. In basic conditions like in ADP, direct conversion of coral to calcium phosphate is more likely to take place.

Dissolution of calcium carbonate for H_3PO_4 samples increases reaction rate due to an increase of reactants surface area. We envisage that changes in pH in the two production routes, influences the structure and water uptake of the final calcium phosphates materials. Care must be taken not to introduce additional contaminant ions into the reactant system that may alter both calcium phosphate precipitation behavior and the conformational structure. Careful monitoring and maintenance of the pH during reaction period may lead to the production of a single and pure phase with defined morphology at higher mass yield.

4.1 Mechanisms of conversion

The two methods used clearly identified separate conversion mechanisms based on the pH of the added solutions. Under acidic conditions calcium carbonate –in this case coral- is dissolved and enter in to the acidic solution. Once supersaturation is achieved the crystals of calcium phosphate based on the chemistry (Ca/P ratio) starts to nucleate and grow. This growth will depend on a number of factors including temperature, chemistry, acid addition rate, and stirring or agitation and the length of the reaction time.

On the other hand under basic conditions calcium carbonate cannot be dissolved but ion exchange occurs at the surface of the coral and around the macro pores with Ca^{2+} and PO_4^{-3} ions. Dependent on the surface area and the reaction conditions calcium deficient apatite is formed. The amount of the reaction in coralline structures which includes inter connected pores, increases this transformation efficiency.

CONCLUSIONS

The two synthesis routes employed in this article are simple and can be easily executed with laboratory conditions that are inexpensive. Production of calcium phosphate materials, like monetite, hydroxyapatite and whitlockite using biogenic raw materials have been proven to be feasible using this technique at moderate conditions of temperature. Alteration in pH seems to influence both the reaction mechanism and structure of final products. Treatment under low pH conditions favour dissolution of the coral and precipitation and crystal growth as a function of time, and supersaturation. However under basic conditions the mechanism is simple ion exchange and interfacial conversion mechanism based on chemical supersaturation and diffusion. The rate of reactant addition and the length of time in saturated solution is thought to influence the crystal size formed.

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