

Development of a Biomaterial from Naturally Occurring Chloroapatite Mineral for Biomedical Applications

H. K. G. K. D. K. Hapuhinna, R. D. Gunaratne, H. M. J. C. Pitawala

Abstract—Hydroxyapatite is a bioceramic which can be used for applications in orthopedics and dentistry due to its structural similarity with the mineral phase of mammalian bones and teeth. In this study, it was synthesized, chemically changing natural Eppawala chloroapatite mineral as a value-added product. Sol-gel approach and solid state sintering were used to synthesize products using diluted nitric acid, ethanol and calcium hydroxide under different conditions. Synthesized Eppawala hydroxyapatite powder was characterized using X-ray Fluorescence (XRF), X-ray Powder Diffraction (XRD), Fourier-transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) in order to find out its composition, crystallinity, presence of functional groups, bonding type, surface morphology, microstructural features, and thermal dependence and stability, respectively. The XRD results reflected the formation of a hexagonal crystal structure of hydroxyapatite. Elementary composition and microstructural features of products were discussed based on the XRF and SEM results of the synthesized hydroxyapatite powder. TGA and DSC results of synthesized products showed high thermal stability and good material stability in nature. Also, FTIR spectroscopy results confirmed the formation of hydroxyapatite from apatite via the presence of hydroxyl groups. Those results coincided with the FTIR results of mammalian bones including human bones. The study concludes that there is a possibility of producing hydroxyapatite using commercially available Eppawala chloroapatite in Sri Lanka.

Keywords—Dentistry, eppawala chloroapatite, hydroxyapatite, orthopedics.

I. INTRODUCTION

ROCK phosphate deposit at Eppawala is one of the most valuable nonrenewable phosphate sources in Sri Lanka, located in the North Central Province part of Thalawa Divisional Secretariat within the Anuradhapura District. It is situated around 20 kilometers south of the ancient city of Anuradhapura and 150 kilometers north east of the Sri Lankan capital, Colombo [1].

It contains about 34-40% total phosphorus expressed as percentage of Phosphorus pentoxide (P_2O_5). Rock phosphate also contains CaF_2 or $CaCl_2$ and the chemical formula can be

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written as $Ca_5(PO_4)_3Cl$ or $3Ca_3(PO_4)_2$, $Ca(F,Cl)_2$. The presence of relatively high chlorine content in Eppawala apatite is a special feature in this mineral. Therefore, it is known as Eppawala Chloroapatite. However, its water solubility is about 0.5% and 2% solubility in citric acid, which measures the agronomic availability of phosphorus, is estimated to about 5%. Apatite has an extremely stable crystal structure, which can withstand soil weathering conditions. Chlorine positions in the structural framework of Eppawala apatite are under strain, and such that it is relatively easy to replace chlorine with other groups at high temperature. This enhances its reactivity at high temperature leading to more soluble products.

There are lots of industrial products that can be manufactured from rock phosphate include P-fertilizers, pharmaceuticals, biomaterials, analytical reagents, animal feeds, detergents, emulsifiers etc. [2].

Up to now Eppawala apatite has been considered only as a raw material for the fertilizer industry. It is essential that the production of biomaterials and other important industrial products with much higher value addition also should be given serious consideration.

Currently, Lanka Phosphate Ltd. produces two types of raw apatite as follows:

- High grade Eppawala Rock Phosphate (HERP) – 38% P_2O_5
- Eppawala Rock Phosphate (ERP) – 28% P_2O_5

Due to the commercial availability, highest purity and highest percentage of P_2O_5 , HERP was used as the raw apatite for the research [1], [3], [26].

Hydroxyapatite (HAp) is a calcium phosphate similar to human hard tissues in morphology and composition [4]. It has a hexagonal structure [5], [6] and a stoichiometric Ca/P ratio of 1.67, which is identical to bone apatite [5], [7], [8]. Its chemical formula can be mentioned as $Ca_5(PO_4)_3OH$ or $Ca_{10}(PO_4)_6(OH)_2$.

An important characteristic of hydroxyapatite is its stability when compared to other calcium phosphates. Under physiological conditions such as temperature, PH and composition of the body fluids, HAp is the most thermodynamically stable calcium phosphate compound [5]. Microstructure, pore size, pore volume, chemical composition and phase composition are the factors relate with the biological behavior of HAp ceramics.

As a bioceramic, hydroxyapatite performs many outstanding properties such as biocompatibility, bioactivity,

osteoconductivity, non-toxicity, etc. [6].

Due to that, hydroxyapatite has a variety of applications, mainly in the fields of orthopedics and dentistry. When considering orthopedics, HAp is widely used in bone implants, implant coatings for metallic implants to trick the body and to reduce implant rejection rate, as well as in bone tissue engineering and as bone void fillers, while the process is carried out through the use of powders, blocks or beads placed in the affected areas of bone. When considering the dentistry field, HAp is used in several ways such as dental implants, implant coatings, maxillofacial and dental surgeries, restoration of periodontal defects, Edentulous ridge augmentation, Endodontic treatments, as dental filler materials, a remineralizing agent in toothpastes and a desensitizing agent in post teeth bleaching [6]-[10].

There are lots of methods to synthesize Hydroxyapatite such as wet precipitation technique [11]-[13], sol-gel approach [14], hydrothermal technique [15], multiple emulsion technique [16], biomimetic deposition technique [17], [18], electro deposition technique [12], etc. Among them sol-gel approach and wet precipitation technique are used for this research.

The sol-gel process is a widely used wet-chemical technique to fabricate an integrated network (so-called gel) of metal oxides or hybrid polymers. This method offers a molecular level mixing of the calcium and phosphorous, which leads to improvement of the chemical structure of resulting HAp to a significant extent. Also, it is an effective method for synthesizing nanophasic HAp, due to the possibility of a strict control of process parameters [13], [19]. Only limited attempts have been taken to synthesis HAp using sol-gel process [19]-[21]. Researchers have reported that the HAp materials synthesized by this route can be used under both in vitro and in vivo environment to improve the contact and stability at the artificial/natural bone interfaces [21]-[23].

The solid state sintering techniques are widely use for the preparation of polycrystalline solids. In those methods, solids are taken as the starting materials due to its unreactive property at room temperature over normal time scales. In order to have an appreciable rate raw materials necessary to heat up to higher temperatures, often to 1000°C to 1500°C. Under this technique reaction conditions, mainly structural properties of reactants, surface area, reactivity and thermodynamic free energy change associated with the reactions have been focused.

Accordingly, objectives of present work are to synthesize HAp using commercially available Sri Lankan rock phosphate as the raw material using sol-gel approach and solid state sintering method to characterize synthesized HAp powder using different techniques such as XRF, XRD, FTIR, SEM, TGA and DSC etc., and to improve the mechanical properties of products by controlling its composition, morphology and particle size.

II. EXPERIMENTAL PROCEDURE

A. Powder Preparation

Two different methods were used under the sol-gel approach. In the first method, High Grade Eppawala Rock Phosphate (HERP) was reacted with Absolute Ethanol drop wise, and then the mixture was stirred slowly for 4 hrs using a magnetic stirrer until formation of a gel. Further, the gel was dried in an electric oven at temperature lower than 100°C in air for 15 hrs, followed by two-stage heat treatment in stagnant air, starting from 400°C to 750°C for 8 hrs. In the second method, dil.HNO₃ was added to HERP 1:1 ratio. The mixture was dissolved in Absolute Ethanol and was stirred well for 4 hrs using a magnetic stirrer until formation of a gel. Further, the gel was dried in an oven at a temperature lower than 100°C in air for 15 hrs, followed by two-stage heat treatment in stagnant air, starting from 400°C to 750°C for 8 hrs. When considering the solid state sintering method, 100 g of HERP was mixed with amount of Ca(OH)₂ and was oven dried at 1000°C for 3 hrs.

All methods were carried out varying particle size range of HERP powder as less than 60 microns, 60-125 microns and 125-250 microns.

B. Powder Characterization

Synthesized HAp powder was characterized using X-ray fluorescence Spectroscopy (Rigaku XRF Spectrometer) to state its elementary composition and presence of impurities. The crystallographic phases of synthesized HAp powder were determined by X-ray diffractometer (Rigaku – Ultima. IV diffractometer) in reflection mode with Cu K α 1: 0.154 nm radiation. A 1.5° min⁻¹ scanned speed was used to collect data within a 2 θ range from 15° to 80°. The presence of functional groups was confirmed by using Fourier Transform Infrared Spectroscopy (Bruker – Alpha FTIR Spectroscopy). The FTIR spectra were obtained over the region 400-4000 cm⁻¹ using the KBr pellet technique. The resolution of spectrometer was 4 cm⁻¹. The surface morphology and microstructural features of the synthesized HAp powder was studied using Scanning Electron Microscopy (LEO-1420P, SEM). Furthermore, Thermo gravimetric analysis (TGA) was done using a Thermal Analyzer (SDT Q600) with N environment, 10°C min⁻¹ heating rate, and 1450°C maximum temperature to find out the thermal stability of synthesized HAp powder.

III. RESULTS AND DISCUSSION

A. XRD Analysis

XRD results interpret all characteristic peaks [24] related to the crystallographic phases 002, 210, 211, 112, 300, 202, 310, 222, 213 and 004 of hexagonal HAp. Therefore, it can be concluded that, all synthesized products reveal the formation of hexagonal hydroxyapatite.

B. XRF Analysis

Ca, P and O include in higher weight percentages. Fe, Al and Si include as the impurities in the synthesized products.

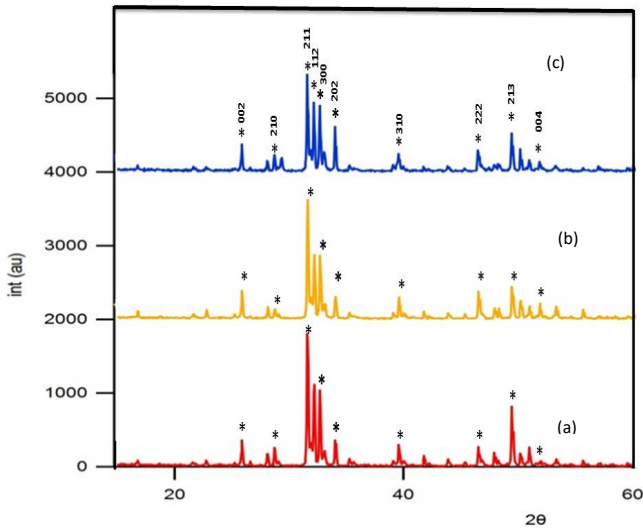


Fig. 1 XRD patterns of the synthesized HAP powder; (a) HERP + Ethanol, (b) HERP + dil. HNO₃ + Ethanol, (c) HERP + Ca(OH)₂

TABLE I
 XRF RESULTS FOR HERP AND SYNTHESIZED HAP POWDERS

Element or Ligand	Weight % (nearly)
CaO	67
P ₂ O ₅	22
Fe ₂ O ₃ , SiO ₂ , Al ₂ O ₃	10- 1
Zr, Sr, TiO ₂ , MnO ₂ , NaO	1-0.1
SO ₃ , Ce, Ba, La, Y	0.1 – 0.01
Cr, Co, As, Rb, Th, U, Cu, Zn	0.01- 0.001
K ₂ O, V, Ni, Ga, Nb, Mo, Ag, Cd, Hf, Ta, W, Pt, Au, Hg, Bi, U	ND (Not Detective level)

C. FTIR Analysis

1. FTIR Spectra for the Sol-Gel Synthesized HAP Materials of (HERP and Ethanol) Mixtures

Fig. 2 shows there is a broad envelop between the 3200 cm⁻¹ to 3700 cm⁻¹ wave number range for all synthesized HAP

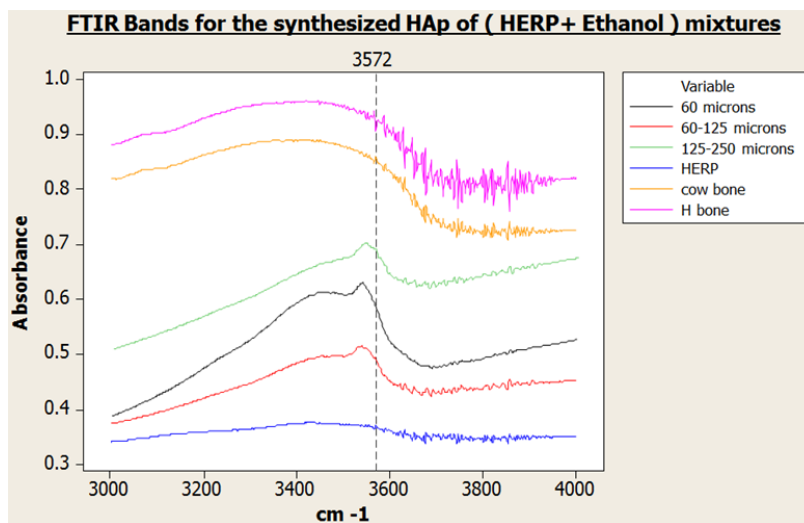


Fig. 2 FTIR spectra of the synthesized HAP materials of (HERP +Ethanol) mixtures, 3000 cm⁻¹ to 4000 cm⁻¹

materials as well as for human bone and cow bone except for the HERP, confirming the presence of hydroxyapatite peak [6]. As a result it can be concluded that, all products, human bone and cow bone consist of hydroxyapatite, except in HERP. Fig. 3 confirms the presence of phosphate groups (PO₄³⁻) for all products, human bone, cow bone and the raw HERP by showing peaks at (560–640) cm⁻¹, 963 cm⁻¹ and (1028-1110) cm⁻¹.

2. FTIR Spectra for the Sol Gel Synthesized Materials of (HERP, dil.Nitric and Ethanol) Mixtures

Fig. 4 shows there is a broad envelop between the 3200 cm⁻¹ to 3700 cm⁻¹ wave number range for all synthesized HAP materials as well as for human bone and cow bone, except for the HERP, confirming the presence of hydroxyapatite peak. As a result, it can be concluded that all products, human bone and cow bone, consist of hydroxyapatite except in HERP. Fig. 5 confirms the presence of Phosphate groups (PO₄³⁻) for all products, human bone, cow bone and the raw HERP by showing peaks at (560–640) cm⁻¹, 963 cm⁻¹ and (1028-1110) cm⁻¹.

3. FTIR Spectra for the Solid State Sintered HAP Materials of (HERP and Ca(OH)₂) Mixtures

Fig. 6 shows there is a broad envelop between the 3200 cm⁻¹ to 3700 cm⁻¹ wave number range for all synthesized HAP materials as well as for human bone and cow bone except for the raw HERP, confirming the presence of a hydroxyapatite peak. As a result it can be concluded that, all products, human bone and cow bone consist of hydroxyapatite except in HERP. Fig. 7 confirms the presence of Phosphate groups (PO₄³⁻) for all products, human bone, cow bone and the raw HERP by showing peaks at (560–640) cm⁻¹, 963 cm⁻¹ and (1028-1110) cm⁻¹.

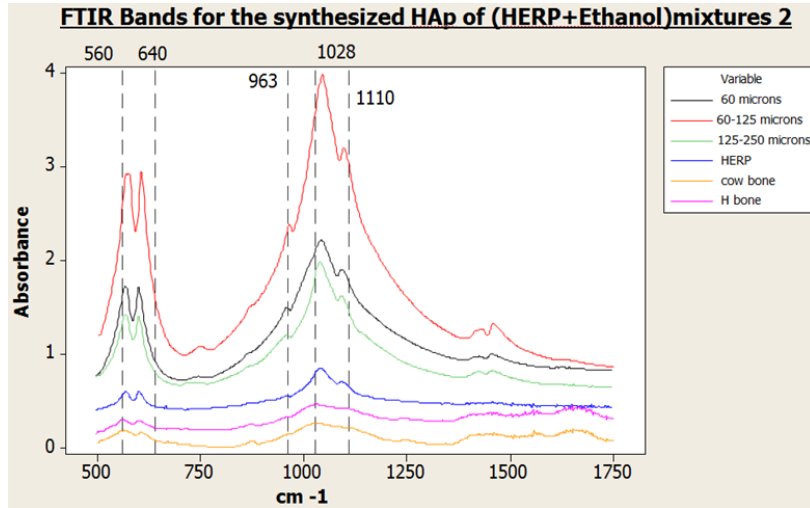


Fig. 3 FTIR spectra of the synthesized HAp materials of (HERP +Ethanol) mixtures, 500 cm^{-1} to 1750 cm^{-1}

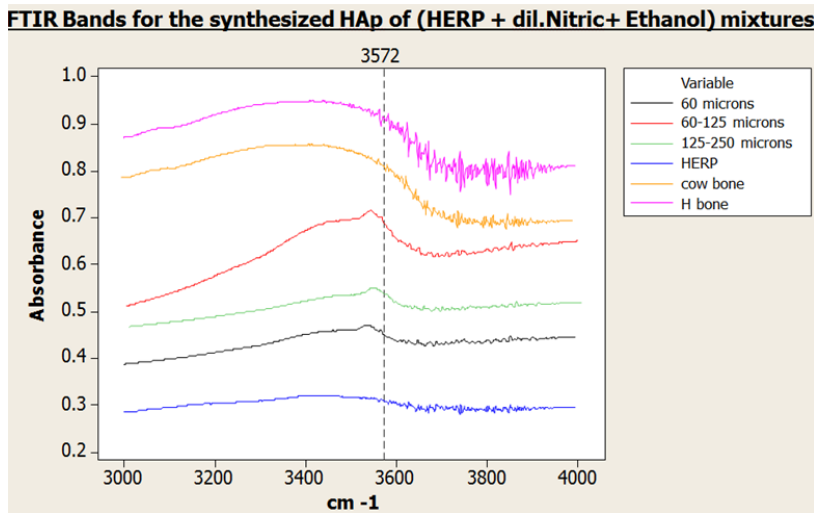


Fig. 4 FTIR spectra of the synthesized HAp materials of (HERP + dil.Nitric + Ethanol) mixtures, 3000 cm^{-1} to 4000 cm^{-1}

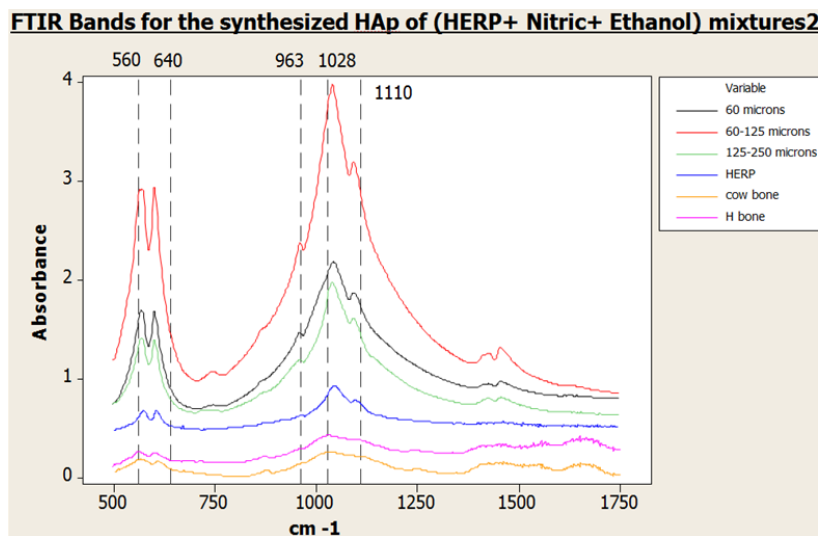


Fig. 5 FTIR spectra of the synthesized HAp materials of (HERP + dil.Nitric + Ethanol) mixtures, 500 cm^{-1} to 1750 cm^{-1}

FTIR Bands for the synthesized HAp of (HERP+ Calcium Hydroxide) mixtures

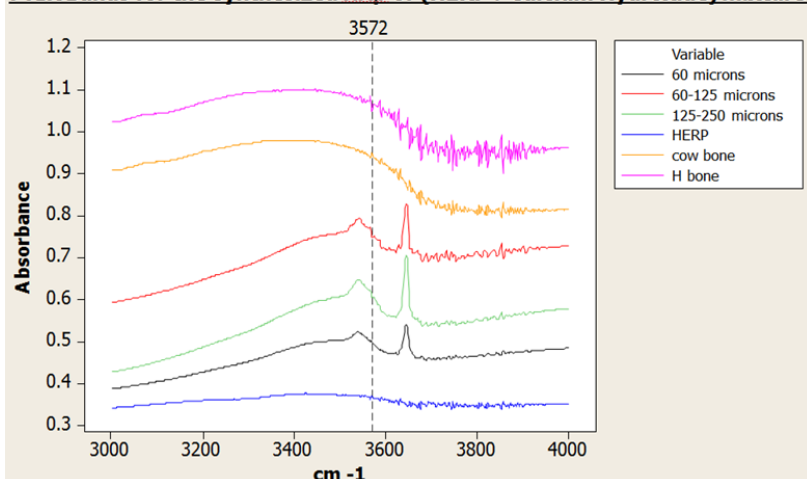


Fig. 6 FTIR spectra of the synthesized HAp materials of (HERP + Ca(OH)₂) mixtures, 3000 cm⁻¹ to 4000 cm⁻¹

FTIR Bands for the synthesized HAp of (HERP + Calcium Hydroxide) mixtures 2

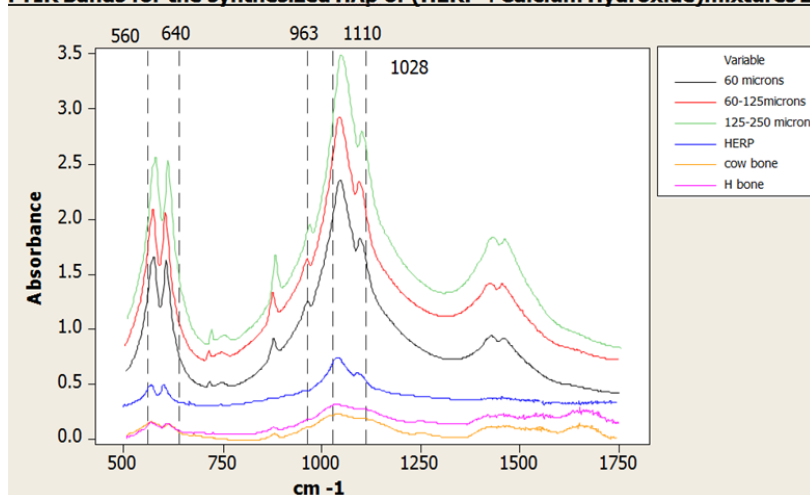


Fig. 7 FTIR spectra of the synthesized HAp materials of (HERP + Ca(OH)₂) mixtures, 500 cm⁻¹ to 1750 cm⁻¹

D. SEM Analysis

1. SEM Analysis for the Sol-Gel Synthesized HAp Materials of (HERP and Ethanol) Mixtures

Micro pores with less than 50 microns in diameter present as black holes, as indicated by white arrows. They can be considered as micro pores. As mentioned in the literature, it helps to improve the osteoconductivity of bioceramics.

Most of the particles are irregular in shape and there are particles less than 1 micrometer.

2. SEM Analysis for the Sol Gel Synthesized HAp Materials of (HERP, dil.Nitric acid and Ethanol) Mixtures

Highly Agglomerated particles are present in the image. Agglomerations show good correlation of particles.

Special cylindrical form/needle shaped particles with very less diameters are present in the image. Their diameter is nearly 1/10 micrometers.

3. SEM Analysis for the Solid State Sintered HAp Materials of (HERP and Ca(OH)₂) Mixtures

The presence of many microcrystalline structures/particles/spherulites and micro pores are found.

The presence of particles less than 1 micrometer can be found. Also, the overall image for 5KX gives agglomerated particles which may lead to good correlation among particles.

E. TGA Analysis

A sample of 10.115 mg human bone was subjected to TGA. As mentioned in the literature; the first significant weight loss occurs nearly at 200°C (0.9112 mg) representing 9.008%, which may be associated with the dehydration of the sample. Following that interval, the sample reduced its weight nearly 5.6358 mg at 650°C; it has occurred due to the bone structure collagen elimination. This reaction continues up to 936.88°C, with a lowered rate. Above that temperature, a fine TGA curve descending slope is observed up to maximum analyzed temperature of 1432.97°C with the total weight loss of 54.79%, this being associated with the collagen remains removal and the incipient transformation of HAp in β – TCP [25].

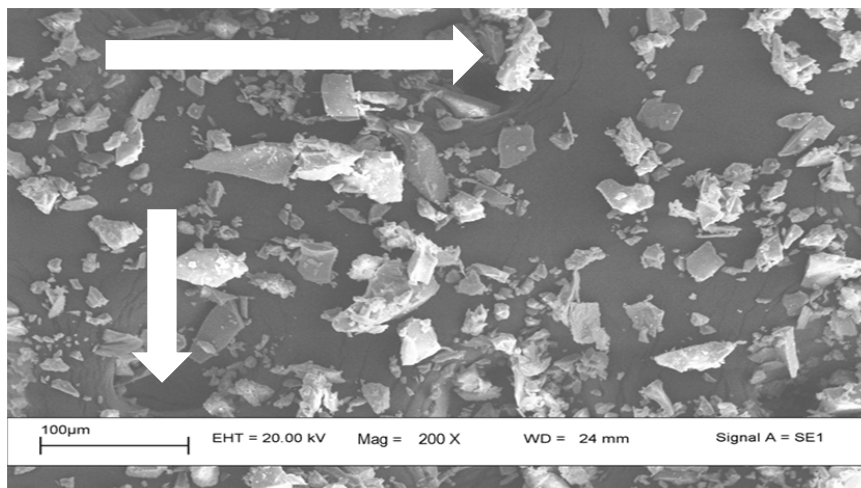


Fig. 8 SEM image for the synthesized HAp material of (HERP + Ethanol) mixture, 200 X

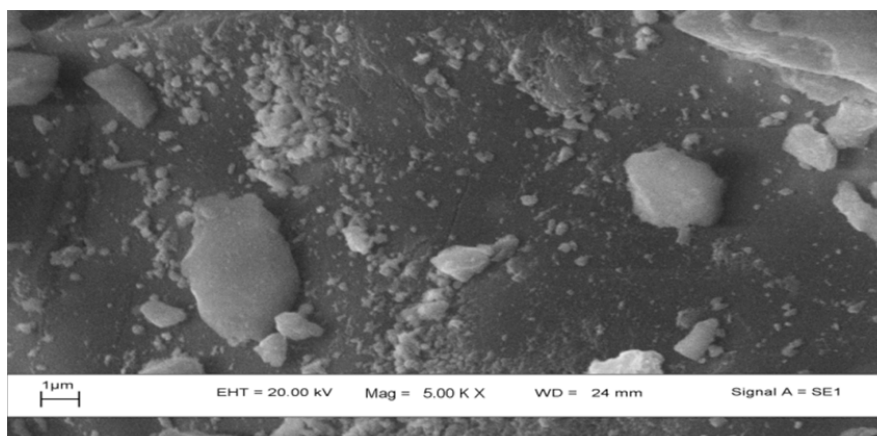


Fig. 9 SEM image for the synthesized HAp material (HERP + Ethanol) mixture, 5KX

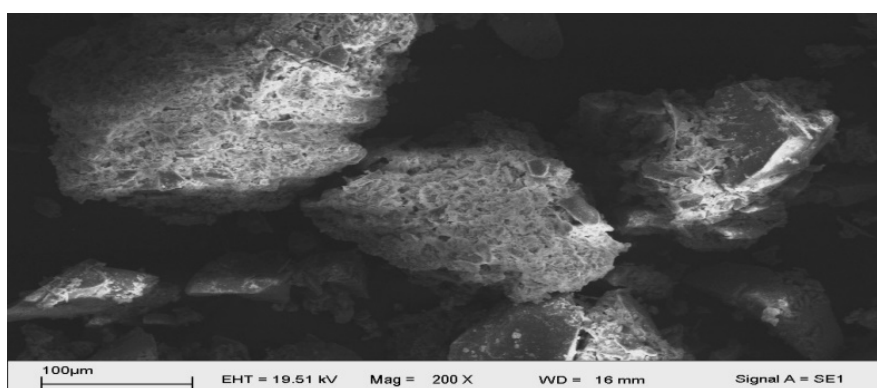


Fig. 10 SEM image for the synthesized HAp material of (HERP +dil.Nitric +Ethanol) mixture, 200X

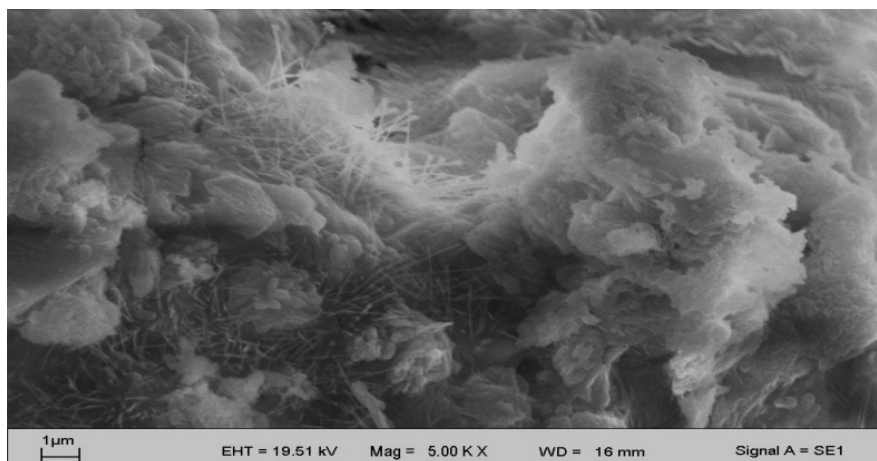


Fig. 11 SEM image for the synthesized HAp material of (HERP+ dil.Nitric + Ethanol) mixture, 5KX

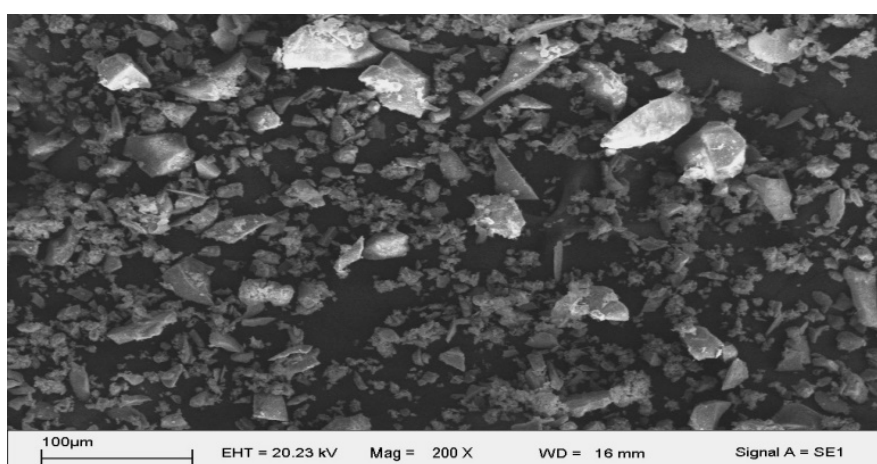


Fig. 12 SEM image for the synthesized HAp material of (HERP + Ca(OH)₂) mixture, 200X

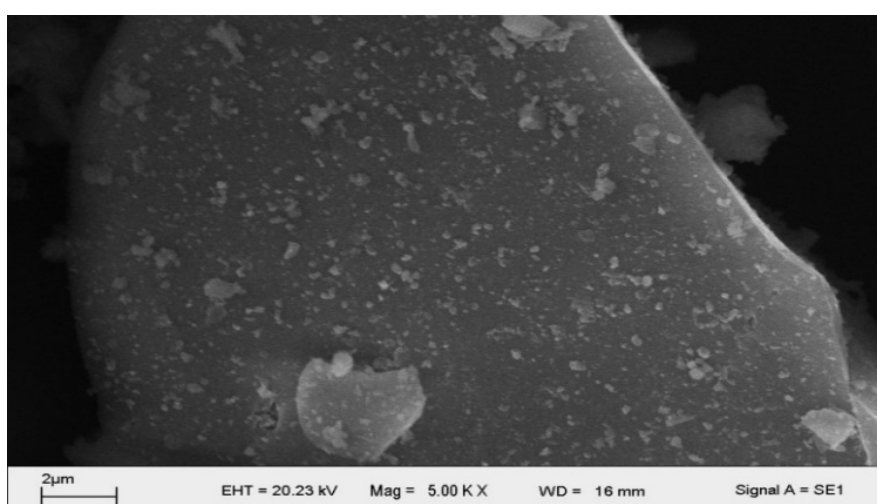


Fig. 13 SEM image for the synthesized HAp material of (HERP + Ca(OH)₂) mixture, 5KX

A sample product of 14.6270 mg was used to analyze under Thermal analyzer. There is a small drop in weight nearly 100°C that may occur due to the elimination of moisture in the sample. Then nearly 625°C, 0.955% (0.1397 mg) weight loss was observed. That may occur due to the elimination of some

gas from the sample. Then again, from 625°C to 1450°C there is a weight loss indicating 1.974% (0.2887 mg) which has occurred due to the incipient transformation of produced HAp in β – TCP. Therefore, it indicates the formation of HAp in products. Final weight remained at 1434.21°C that was

97.07% of the started weight of 14.6270 mg.

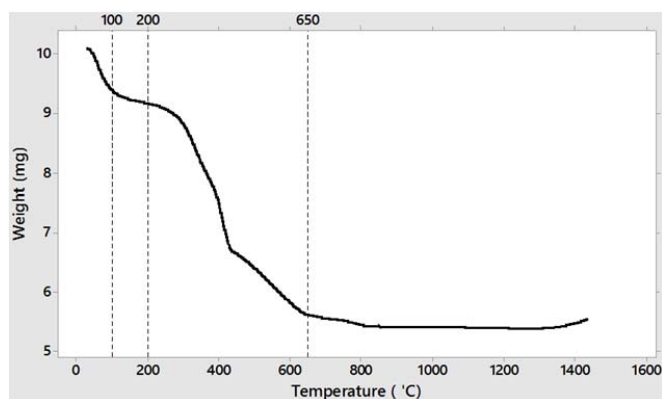


Fig. 14 TGA curve for Human bone

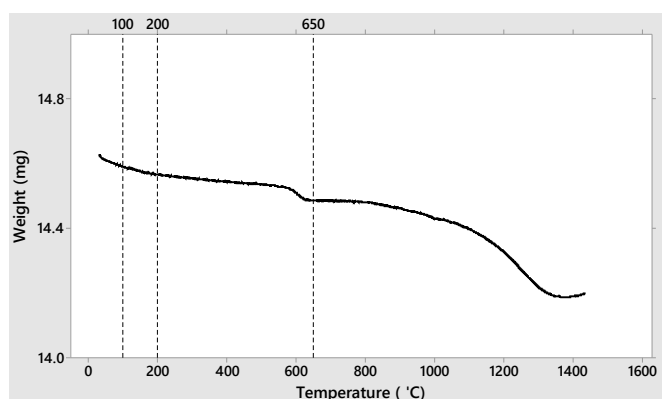


Fig. 15 TGA curve for synthesized HAp products

When comparing human bone and HAp products, there is the least amount of weight loss in the synthesized HAp product sample than bone. As a result, it can be concluded that the synthesized products perform high thermal stability and good material stability in nature and application.

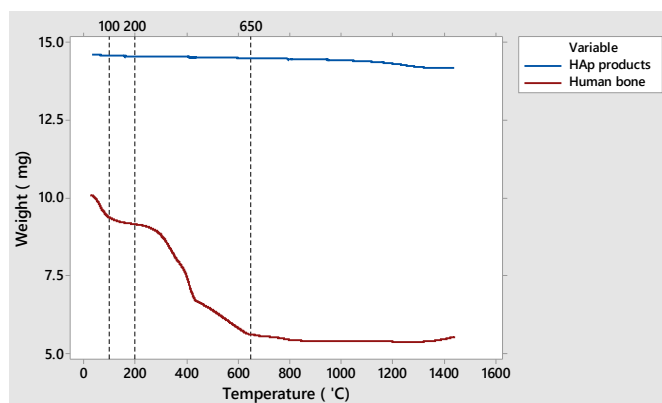


Fig. 16 Comparison between TGA curves of human bone and synthesized HAp products

IV. CONCLUSION

Finally, it can be concluded that, there is a possibility of hexagonal hydroxyapatite formation from Eppawala high

grade rock phosphate available in Sri Lanka. Solid state sintering gives higher yield than sol-gel method. Sol-gel synthesized products show more microstructural properties, osteoconductive properties and good correlation via SEM images. When considering sol-gel synthesized product cost, using HERP and ethanol will more cheaper than using HERP, nitric and ethanol. But, there may also be different properties between HERP and ethanol products and HERP, nitric and ethanol products when considering the SEM images of both products. Synthetic hydroxyapatite is usually white in color, but considering the physical properties of the products, they are brown in color. This may occur due to the impurities present in the HERP raw material. For better results, impurity removed raw material can be used.

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