*Quest Journals*
*Journal of Research in Environmental and Earth Science*
*Volume 2~ Issue 11 (2016) pp: 07-15*
*ISSN(Online) : 2348-2532*
www.questjournals.org

**Research Paper**

# Synthesis & Application of Hydroxyapatite Bioceramics from Different Marine Sources

Anchana devi.C[1] & Priya Perumal[2]
*PG & Research Department of Biotechnology, Women's Christian College, Chennai -600006, Tamil Nadu, India*

Received 14 September, 2016; Accepted 24 September, 2016 © The author(s) 2016. **Published** with open access at **www.questjournals.org**

***Abstract:*** *Waste shells of Crab (Portunus sanguinolentus), sea snail (Turritella attenuate and Babylonia umbilifusca) were used in the production of Hydroxyapatite bio ceramic compounds. Most of the marine structures are made up of pure calcium carbonate (calcite or argonite) with the addition of very small amount of an organic matrix, bioceramics are produced. Surface sterilization of the shell samples was done using distilled water & 4% Sodium hypochlorite and ground to powder form.Thermal analysis (DTA/TGA), Acid-Base back titration method and FTIR analysis of untreated samples was performed to estimate the calcium carbonate content and to calculate the stoichiometric molar ratio of Ca/P corresponding for Hydroxyapatite bioceramics (HA). This study focuses on a new and effective method of hot-plate heating and continuous stirring for 8 hours at 80°C, followed by treatment at 800°C for 4 hours in air in Muffle furnace was carried out for the effective synthesis of Hydroxyapatite bioceramics (HA). Structural characterization and elucidation was done using X-ray diffraction analysis, Scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The synthesized HA compounds was used to check its efficiency against normal microflora and micro-organisms responsible for causing oral cavities in the anti-bacterial activity using Agar- Disk diffusion method. Further, the synthesized HA compounds potential to safeguard against demineralization of teeth was checked.*

***Keywords:*** *Hydroxyapatite, Bioceramics, Sea shells, FT IR and DTA/TGA*

## I. INTRODUCTION

Bio ceramics are used for the repair, redesigning and reconstruction of diseased or damaged parts of the musculo-skeletal system, termed bioceramics, may be bio-inert (alumina, zirconia), resorbable (tricalcium phosphate), bioactive (Hydroxyapatite, bioactive glasses, and glass-ceramics), or porous for tissue ingrowth (Hydroxyapatite-coated metals, alumina). Applications include replacements for hips, knees, teeth, tendons, and ligaments and for repairing of periodontal disease, maxillofacial re- construction, augmentation and stabilization of the jaw bone, spinal fusion, and bone filling after tumor surgery. Carbon coatings are thrombo-resistant and can be used for prosthetic heart valves. The mechanisms of tissue bonding to bioactive ceramics are studied, which can result in the molecular designing of bio- ceramics for interfacial bonding with hard and soft tissues. Composites are being developed with high toughness and elastic modulus similar to bone. Therapeutic treatment of cancer has been achieved by localized delivery of radioactive isotopes using glass beads. Development of standard test methods for prediction of long-term (20-year) mechanical reliability under load is still needed.[1]

Bioceramics have been extensively researched for use in bone regeneration, and amongst them calcium phosphate ceramics are suggested due to their excellent biocompatibility and bioactive properties.[2]Hydroxyapatite is the most important bio ceramic materials for its unique bioactivity and stability. Naturally occurring and mostly available Hydroxyapatite is hexagonal in structure with the chemical formula of one unit cell, it is stable at physiological pH and exhibits bone bonding, forming strong chemical bonds with surrounding bone. This property has been recommended for rapid bone repair after major trauma or surgery. HA acts as an excellent implant material with its biological properties, extensively been used in medicine for implant fabrication of dense and porous bioceramics. Its general use includes biocompatible phase-reinforcement in composites, coatings on metal implants and granular filling for direct incorporation into human tissue.

A few methods were developed to prepare HA including hydrolysis hydrothermal or precipitation methods dry process, freezing method, hydroxylation of calcium phosphate, spray pyrolysis, and gel diffusion

*Corresponding Author: Anchana devi.C[1]

*Department Of Biotechnology, Women's Christian College, Chennai*

and sol-gel technique etc.[1] Their physical properties such as fractures toughness and fracture strength etc., depend on the crystal structure, compositions and sizes. The chemical species constituting HA crystals (Ca, P, O and H) are non-toxic. It is used as a promising material to reinforce filler for composites and to insulate agents for simple, rapid fraction of proteins and nucleic acids.

Synthetic Hydroxyapatite is similar in composition to the mineral component of bone and teeth this has led to the interest in the development of HA materials for biomedical applications.[3] Hydroxyapatite (HA) has the molecular structure of apatite with a stoichiometric atomic ratio Ca/P is 1.67. Its chemical formula is $Ca_{10}(PO_4)_6(OH)_2$, with 39% by weight of Ca, 18.5% P and 3.38% of OH. Hydroxyapatite crystallization occurs in a crystalline form.[4, 5]

Despite hydroxyapatites produced biologically are much more complicated, they have an atomic ratio Ca/P <1.67 and it contains ions and radicals of the HA and also traces of $CO_3$, Mg, Na, F and Cl. These amounts vary according at the specific type of tissue and closer to the value of Ca/P to 1.67, the greater the stability, inertness of the material inside the human body, better the bioactivity.

Hydroxyapatite bio ceramics are effectively used in all areas of orthopaedics and orthodontics, to replace, partially or totally, parts of bone tissue. It is used as a filling material for bone. It creates physicochemical interaction, between ceramic and bone tissue, promotes binding and growth of new tissues.[6]

HA is also used for coating metallic prostheses, enhances the tissues a better suited and recognizable surface, given their characteristics and biocompatibility. Due to the stability and bioactivity of HA, a large number of applications have been developed in the field of maxillofacial surger.[7] Although due to their reduced mechanical properties it is primarily used in coatings for dental prostheses and metal plates, for the reconstruction of some cranial bones. It can also be used for fabrication of porous hydroxyapatite-gelatin/ chitosan/ polyurethane composite scaffolds for bone tissue engineering and proliferation of Hepatocellular Carcinoma Cells.[8]

Apart from the dental prostheses application, HA exhibits a greater potency to safeguard against the Dental Demineralizations. Demineralization of teeth is a process of decomposition of the crystal of $Ca_{10}(PO_4)_6(OH)_2$ due to the acidic conditions by releasing $Ca^{2+}$ and $PO_4^{3-}$ ions. Demineralization of tooth causing increased levels of $Ca^{2+}$ and $PO_4^{3-}$in saliva in direct contact with the tooth. Additionally, HA has been documented to possess antibacterial potentials against *Streptococcus mutans*, *Streptococcus anginosus (milleri)* and *Enterococcus faecalis* growth inhibition were increased with time. The antibacterial property should be used to good advantage as a bioactive biomaterial in dental and maxillofacial applications. [10]

## II. MATERIALS & METHODOLOGY

### 2. 1 Collection of samples

***Portunus sanguinolentus***(Sample A), Crab shells were collected from the local market, Sea snail shells ***Turritella attenuata*** (Sample B) and ***Babylonia umbilifusca***(Sample C),were collected from the Marina beach, Chennai, Tamil nadu, India.

### 2.1.2 Surface sterilization and Preparation of the sample

The shell samples (A, B & C), were first washed and cleaned with tap water several times to remove soil and surface contaminants and then rinsed with distilled water. Non- chemical shell processing methods such as breaking and crumbling were done using mortar and pestle. The powdered shell samples were sieved, to obtain (<100 µm) sized powdered particles and allowed to dry.

10 grams of fine powdered shell samples (A, B & C), were soaked in about 20ml of standard 4% solution of sodium hypochlorite (NaClO) in 3 different beakers and allowed to treat overnight at room temperature, to remove organic residues. After treatment, samples were washed with distilled water and carefully filtered using Whatmann filter paper grade 292. The NaClO treated fine powdered shell samples were allowed to dry overnight in an incubator at 100°C.

### 2.2 Preliminary characterization of the sample

### 2.2.1 Differential Thermal / Gravimetric analysis (DTA/TGA)

Thermogravimetric investigations of raw shell samples were carried out on dried samples using a Thermal Analysis SDT Q 600, to determine the content of $CaCO_3$. About 5 mg of powder was used in each measurement and the heating rates used were 10 °C $min^{-1}$.

### 2.2.2 Determination of calcium carbonate using back titration method

To measure the mass percent of calcium carbonate in the NaClO treated powdered shell samples (A, B & C), 0.25 g of NaClO treated powdered shell samples were weighed and added into each of three 250 ml conical flasks, labeled as "trial 1", "trial 2" and "trial 3". Few drops of ethanol were added to each flask, it acts as a wetting agent and helps the hydrochloric acid dissolve the $CaCO_3$.10 ml of 1.0 M HCl solution were added

to each of the labeled conical flasks, swirled well to wet all the solids. The solutions in the flasks are heated until it begins to boil and the shell samples dissolves completely.3-4 drops of phenolphthalein indicator were added to each flask. Titrate the shell samples against standardized 0.1M NaOH solution, until barely pink colour appears and persists for 30 seconds and fades slowly. Repeat the titration to obtain concordant values. The mass percent of calcium carbonate in the NaClO treated powdered shell samples (A, B & C) can be determined by calculating

$$\text{mass \%} = \frac{\text{mass of } CaCO_3, \text{g}}{\text{mass of eggshell, g}} \times 100$$

**2.2.3 Fourier Transform Infrared Spectroscopy (FTIR) analysis: (NaClO treated sample)**

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded within the range of 600 – 4000 $cm^{-1}$.

**2.3 Synthesis of Hydroxyapatite Bioceramics (HA)**

To obtain a stoichiometric molar ratio of Ca/P equal to 1.667 corresponding to Hydroxyapatite compound was calculated and batches of 10grams of NaClO treated fine powders (Sample A, B & C) were suspended in an aqueous solution of distilled water (wetting agent) in separate beakers. The samples were suspended in a hot-plate stirrer equipment and temperature was set to 80°C.After 15 minutes, equivalent amount of phosphoric acid (1M), was added drop by drop slowly ($CaCO_3$ in reaction with acid it raises, bubbles and leavening occurs to slower the reaction and avoid spillage $H_3PO_4$ is added drop wise) in order to obtain the Ca/P molar ratio as 1.667. The reaction was sustained in the hot-plate stirrer with continuous stirring for 8 hours at 80°C.After 8hours of stirring, the powders were removed from the liquid by filtration and dried overnight at 100°C in an incubator. The Phosphoric acid treated, dried powders were calcined at 800°C for 4hours in air in a Muffle furnace for complete substitution into Hydroxyapatite compounds.

**2.4 Structure elucidation and Characterization of Hydroxyapatite Bioceramics (HA)**

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the FTIR were recorded within the range of 600 – 4000 $cm^{-1}$.

X-ray patterns, obtained on synthesized HA powders, were collected using an Analytical GE Seifert, equipped with detector powder diffract meter.

Scanning Electron microscopy (SEM) images were obtained using a Hitachi S-3400N instrument.

**2.5 Application of Hydroxyapatite Bioceramics (HA)**

**2.5.1 Anti-Bacterial Activity**

Bacterial inoculums were prepared by transferring a loop-full of bacterial culture from fresh culture plates to tubes containing 10 ml of Nutrient Broth (Hi-media) and incubated for 24 hours at37°C. The tubes were shaken occasionally to aerate and promote growth. The organisms responsible for oral cavity such as *Streptococcus* spp., *Lactobacillus* spp., and *Escherichia coli* and normal microflora such as *Staphylococcus aureus, Staphylococcus epidermidis* were used for the study. The antibacterial activity of the synthesized HA compound from Crab and Snail shell samples were evaluated by the agar disk- diffusion method. The petri-plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA), (Hi-media). The strains (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp., Lactobacillus spp., and Escherichia coli*) that had been incubated for 24 hours were used for the assay. A sterile cotton swab was dipped into the bacterial suspension and then evenly streaked over the entire surface of a sterile Mueller Hinton Agar plate to obtain uniform inoculums. The sterile paper discs were dipped in synthesized HA suspension (1g/1ml of saline) from samples (A, B & C), placed in the MHA plates and kept for incubation at 37°C for 24 hours. The inhibition of bacterial growth was determined by measuring the width of halo zone around the discs (mm).

**2.5.2 Teeth Demineralization Tested**

The Initial mass of the tooth samples were measured using a weighing machine. Each of 11 beakers were filled with 20 ml of Acetate buffer pH 5.0 solutions, with 0.1 M of acetic acid concentration and were prepared as Acetate buffer without the addition of NaF and $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C, Acetate buffer with only the addition 250 ppm of NaF, 500 ppm- Acetate buffer with addition of 250 ppm NaF and 500

ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C, 1000 ppm- Acetate buffer with addition 250 ppm of NaF and 1000 ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C, 1500 ppm- Acetate buffer with addition 250 ppm of NaF and 1500 ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C.(ppm was calculated based on 1ppm = 0.001grams).The cleaned tooth samples were immersed in each of 11beakers of solutions. The immersion time of tooth samples in solution are 3, 6, 9, 24 and 48 hours, respectively. The phosphate ion concentration in each solution was measured by using UV-Vis spectrophotometer with the wavelength of absorbance being 430 nm. The final mass of the tooth samples was measured after 48hours, and compared with the initial mass in order to estimate the teeth mass remaining on immersion in acetate buffer solution with a certain variation of the treatment.

## III. RESULTS &DISCUSSION

### 3. Preliminary characterization of the sample

### 3.1 Differential Thermal / Gravimetric analysis (DTA/TGA)

Preliminary characterization of the powdered shell samples without any treatment, except grinding was done using Thermal analysis (DTA/TGA) in order to estimate the amount of calcium carbonate. Results obtained after Thermo gravimetric analysis shows that the weight loss of raw shell below 500 °C, due to the burning of the organic matter is about 2-3%. The weight loss between 500 and 800 °C in all samples is attributed to the decomposition of aragonite according to the reaction: CaCO3 ↔ CaO + CO2

This value was estimated to be 84.8%, 83.6% and 81.3% and was used to calculate the stoichiometrically required volume of Phosphoric acid, to obtain a complete carbonate substitution are showed in fig 1.Studies have already shown similar results using thermal analysis (DTA/TGA) to estimate the amount of calcium carbonate. [11, 12]

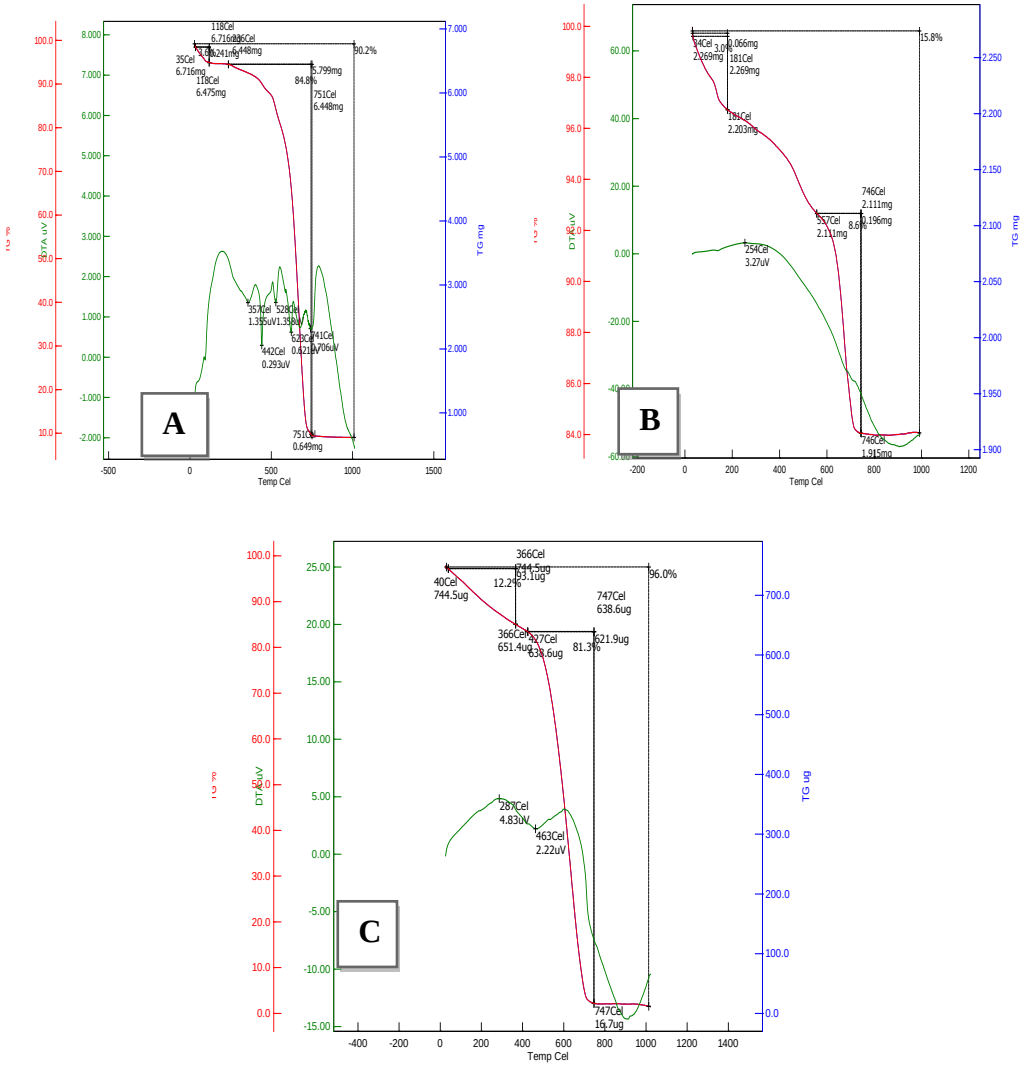


**Fig 1.**Thermal analysis of untreated samples- Sample **A**, Sample **B**, Sample **C**.

### 3.2 Determination of calcium carbonate using back titration method

**Table 1:** Titration of $CaCO_3$ in shell samples:

| | **Sample A** | **Sample B** | **Sample C** |
|---|---|---|---|
| Mass of ground shell sample | 0.25 g | 0.25 g | 0.25 g |
| Volume of HCl used | 5ml of 0.1M HCl | 5ml of 0.1M HCl | 5ml of 0.1M HCl |
| Volume of NaOH used in titration | 0.17 ml | 0.2 ml | 0.22 ml |
| Mass of $CaCO_3$ in sample | 0.17g | 0.21g | 0.21g |
| Average Percentage of $CaCO_3$ in sample | 68% | 84% | 84% |

Based on the back titration method, the amount of calcium carbonate in the NaClO treated samples are used to calculate the stoichiometric ratio corresponding for the synthesis of Bioceramic compound.

### 3.3 Fourier Transform Infrared Spectroscopy (FTIR) analysis: (NaClO treated samples)

The characterization of the NaClO treated samples are done using FTIR in order to check the absorbance bands assigned to the $CO_3^{2-}$ group was observed at 873.6, 1481(Sample A); 814, 896 and 1470 (Sample B & C). Studies have already shown similar results using FTIR analysis to confirm the absorbance bands assigned to $CO_3^{2-}$ group.[11, 12]

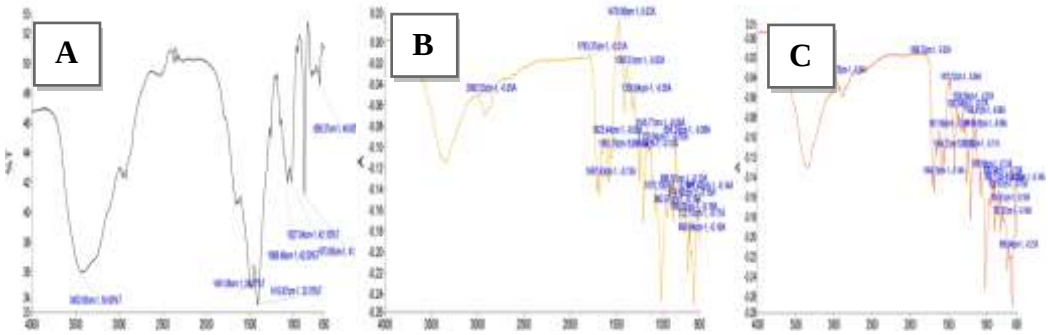


**Fig 2.** FTIR analysis of NaClO treated samples Sample A, Sample B, Sample C.

### 3.4 Synthesis of Hydroxyapatite Bioceramics (HA)

This work introduces a new and effective approach for producing fine powders of Hydroxyapatite bioceramic by using hot plate heating and stirring. Based on the preliminary characterization of the samples, stoichiometric molar ratios based on mass corresponding to each sample was calculated to maintain Ca/P-1.67 ratio specific for Hydroxyapatite. Corresponding to the Calcium levels in each sample, equal volume of Phosphoric acid was added in order to maintain the Ca/P ratio. The samples are allowed to react and transform under hot-plate stirrer @ 80°C in room temperature for 8 hours continuous stirring, the powdered shell samples were removed by filtration and dried at 100°C overnight in incubator. The dried powder samples are smooth, orangish in colour (Sample A), soft, white in colour (Sample B) and rough, greyish in colour (Sample C). The Dry shell powders were calcined using Muffle furnace for 4 hours in air at 800°C. The thermally treated powders were converted into ash- white in colour with similar texture for all the samples (Fig 3).

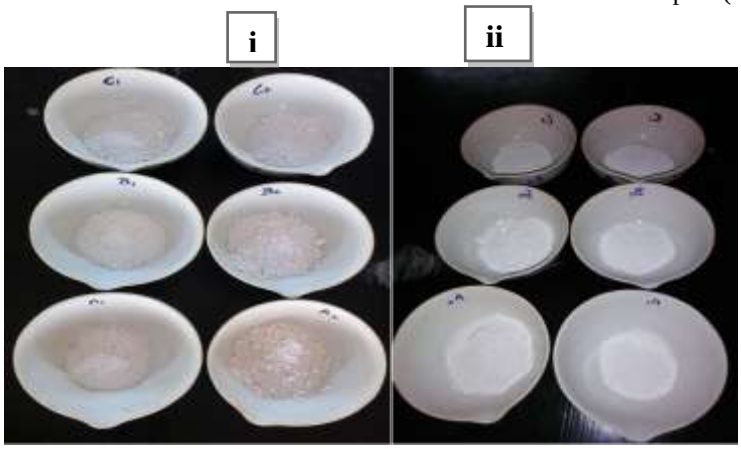


**Fig 3.** Samples treated in Muffle furnace (i) before treatment, (ii) after treatment.

**3.5 Structure elucidation and Characterization of Hydroxyapatite Bioceramics (HA)**

**3.5.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis: (Synthesized HA)**

The FT-IR spectra of shell samples (A, B &C) after sintering at a temperature of 800°C shows that Hydroxyapatite is the dominant compound formed. The most characteristic chemical groups in the FTIR spectrum of synthesized HA are $PO_4^{3-}$, OH-, $CO_3^{2-}$. $PO_4^{3-}$ group forms intensive IR absorption bands at 673.5 cm-1 and 1036, 1083 cm-1(Sample A); 613 cm-1 and 1029, 1068 cm-1(Sample B) and 613.76cm-1 and 1028, 1100cm-1(Sample C). Adsorbed water band is relatively wide, from 3600 to 3000 cm-1, with an explicit peak at 3537 cm-1 (Sample A); 3641 cm-1 (Sample B) and 3434 cm-1 (Sample C). $CO_3^{2-}$ group forms weak peaks at 878.07 cm-1(Sample A); 875 cm-1(Sample B) and 879cm-1(Sample C), with more intensive peaks at 1475.01 cm-1(Sample A); 1472 cm-1(Sample B) and 1563cm-1(Sample C) indicating a shift in peak range corresponding to the formation of HA. (Fig 4). The corresponding absorbance bands confirm the presence of the synthesized bio ceramic compound- HA. Earlier studies have already shown similar results using FTIR analysis to confirm the absorbance bands assigned to Synthesized HA corresponding to bands at chemical groups $PO_4^{3-}$, OH-, $CO_3^{2-}$. [9,11,12,13]

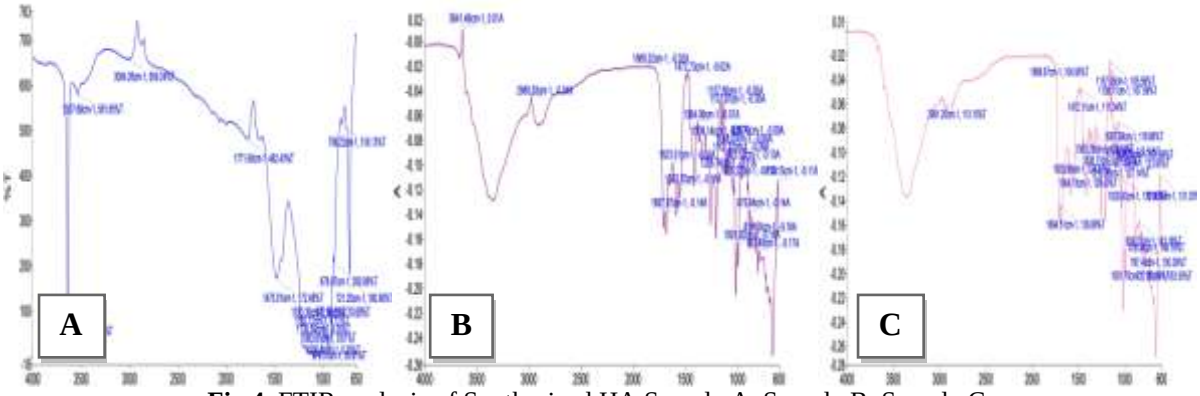


**Fig 4.** FTIR analysis of Synthesized HA Sample A, Sample B, Sample C.

**3.5.2 X-ray diffraction analysis**

XRD analysis was performed on the Synthesized HA from shell samples (A, B & C) where similar to the HA peaks corresponding to the ICDD card 01-089-6438 are indicated. Since the XRD pattern of the samples obtained by thermal transformation at 800◦C exhibits the diffraction pattern belonging to Hydroxyapatite, highest intensity peak at 31.2572◦ corresponding to crystalline has ,the second highest peak intensity is 31.7783◦, and the third highest peak intensity is 28.0565◦ crystals suitable for $Ca_{10}(PO_4)_6(OH)_2$ with increase in intensity due to planes around (2 1 1), (0 0 2), (3 0 1), (2 2 2) and (2 1 3) for Sample A, B & C. XRD patterns of Sample A exhibits multiple peaks which confirms presence of HA as well as many other compounds. Sample B & C confirms the complete transformation of calcium carbonate templates into HA as well as few other compounds (Fig 5). Further treatment with increase in temperature of calcination can help in determining pure HA phases and elimination of other compounds.

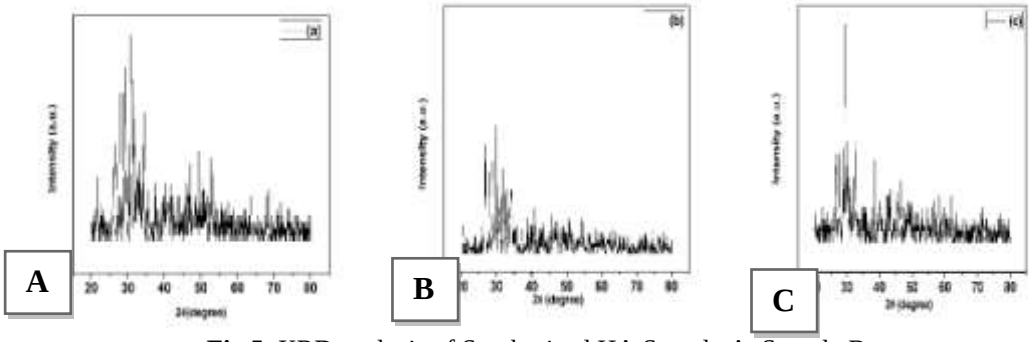


**Fig 5.** XRD analysis of Synthesized HA Sample A, Sample B,

**3.5.3 Morphological characterization- Scanning Electron microscopy analysis**

The morphologies of synthesized HA powders, are observed small in size and only a few particles are large in size, few exhibit nano- structures, surface is smooth and non- porous (Sample A). The treated HA samples are observed as regular and round in shape and partially agglomerated and fibrillar nano- structures

(Sample B). The SEM images exhibits morphology of small, round particles, clump with each other to form bigger agglomerates with few nano- fibrillar structures (Sample C) (Fig 6)

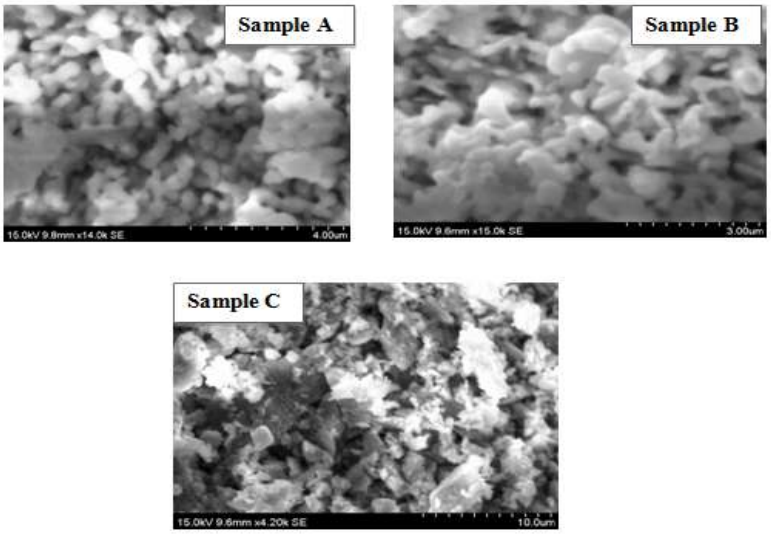


**Fig 6.** SEM pictures of Synthesized HA Sample A, Sample B,

### 3.6 Application of Hydroxyapatite Bioceramics (HA)

#### 3.6.1 Anti-Bacterial Activity

**Table 2 :** Anti-bacterial activity using Synthesized HA.

| Organisms | Zone of Inhibition (in mm) | | |
|---|---|---|---|
| | **Sample A** | **Sample B** | **Sample C** |
| ***Staphylococcus aureus*** | 3mm | 0mm | 2mm |
| ***Staphylococcus epidermidis*** | 0mm | 0mm | 0mm |
| ***Streptococcus species*** | 8mm | 10mm | 0mm |
| ***Lactobacillus species*** | 10mm | 14mm | 15mm |
| ***Escherichia coli*** | 23mm | 20mm | 20mm |

**The Inhibition of bacterial growth was determined by measuring the width of halo zone around the discs:**

- Synthesized HA from Sample A inhibits the growth of *Staphylococcus aureus, Streptococcus spp., Lactobacillus spp., and Escherichia coli.* No effect is seen against *Staphylococcus epidermidis.*
- Synthesized HA from Sample B inhibits the growth of *Staphylococcus epidermidis, Streptococcus spp., Lactobacillus spp., and Escherichia coli.* No effect is seen against *Staphylococcus aureus*.
- Synthesized HA from Sample C inhibits the growth of *Staphylococcus aureus, Lactobacillus spp., and Escherichia coli.* No effect is seen against *Staphylococcus epidermidis* and *Streptococcus spp*.

The Inhibition of Bacterial growth around the discs is due to the release of HA compounds into the surrounding medium. Synthesized HA compounds from samples A, B & C are more effective against the causative agents of oral cavities (*Streptococcus spp., Lactobacillus spp., and Escherichia coli.*) and variations in effect in seen towards normal microflora (*Staphylococcus aureus, Staphylococcus epidermidis*). (Table 2)

#### 3.6.2 Demineralization of tooth

**Table 3:** Teeth mass remaining on immersion in acetate buffer solution with a certain variation of the treatment for 48 hours.

| Code | Sample | Initial mass (gram) 0 hour | Final mass (gram) 48 hours |
|---|---|---|---|
| **1** | Acetate buffer | 2.04 | 2.02 |
| **2** | NaF | 1.73 | 1.72 |
| **500 ppm** | A | 1.73 | 1.73 |
| | B | 1.39 | 1.39 |
| | C | 1.26 | 1.27 |

| | | | |
|---|---|---|---|
| **1000 ppm** | A | 1.57 | 1.59 |
| | B | 1.37 | 1.39 |
| | C | 1.09 | 1.12 |
| **1500ppm** | A | 1.42 | 1.44 |
| | B | 1.28 | 1.30 |
| | C | 1.08 | 1.15 |

1- Acetate buffer without the addition of NaF and $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C.
2- Acetate buffer with only the addition of NaF.
500 ppm- Acetate buffer with addition of NaF and 500 ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C.
1000 ppm- Acetate buffer with addition of NaF and 1000 ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C.
1500 ppm- Acetate buffer with addition of NaF and 1500 ppm of $Ca_{10}(PO_4)_6(OH)_2$ from Sample A, B and C.

**3.6.3 Relationship between tooth mass and the addition of Hydroxyapatite:**

The decrease in the rate of tooth demineralization with the addition of $Ca_{10}(PO_4)_6(OH)_2$can also be observed through analyzing the tooth mass reduction in the higher concentration fifth variation of acetate buffer solution. The greater concentrations of $Ca_{10}(PO_4)_6(OH)_2$ in the acetate buffer solution were teeth immersed exhibit the increase in mass of teeth in the solution.

The increase of mass of teeth after soaking in Samples A, B & C at 1000 ppm and 1500 ppm concentration can be due to deposition or coating of synthesized HA on the teeth and decreasing the rate of demineralization. Sample C were observed to be very efficient in deposition at all three variations.
The tooth samples exhibit a brightening or whitening effect after treatment for 48 hours in acetate buffer solution with variations in concentration of synthesized HA.

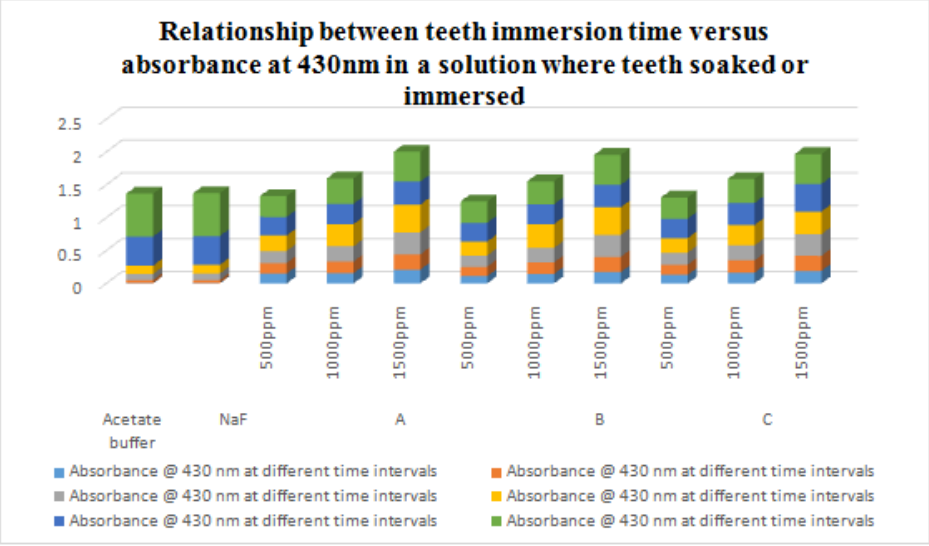


**Fig.8.**Relationship between teeth immersion time versus absorbance at 430nm in a solution where teeth soaked or immersed.

**Inhibition of Tooth demineralization:**

The relationship between the soaking time of teeth is responsible for theincreaseofthe$PO_4^{3-}$ion levels in solution where the tooth was soaked; it appears that with the increase in addition of $Ca_{10}(PO_4)_6(OH)_2$ into the acetate buffer equals to the decrease in rate of demineralization(Fig.29). It can be altered by the amount of $PO_4^{3-}$ ions in solutions; the additionof$Ca_{10}(PO_4)_6(OH)_2$ions showed lower amount of $PO_4^{3-}$ions compared to solutions without the addition of $Ca_{10}(PO_4)_6(OH)_2$ (Table.7). This proves that the $Ca_{10}(PO_4)_6(OH)_2$ synthesized from the shell samples were effective for protection against tooth demineralization.

## IV . CONCLUSION

The samples of Crab shells (***Portunus sanguinolentus***), sea snail shells (***Turritella attenuata***and ***Babylonia umbilifusca***) were collected. Most of the marine structures are made up of pure calcium carbonate (calcite or argonite) with the addition of very small amount of an organic matrix, bioceramics are synthesized. Surface sterilization of the samples were done using Distilled water & 4% Sodium hypochlorite and ground to powder form. Thermal analysis (DTA/TGA), Acid-Base back titration method and FTIR analysis of untreated samples were performed to estimate the calcium carbonate content and to calculate the stoichiometric molar ratio of Ca/P corresponding for Hydroxyapatite bioceramics (HA). A new and effective pretreatment method using hot-plate heating under continuous stirring for 8 hours at 80°C, followed by treatment at 800°C for 4 hours in air in Muffle furnace were carried out for the effective synthesis of Hydroxyapatite bioceramics (HA). Structural characterization and elucidation were done using X-ray diffraction analysis, Scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The synthesized HA compounds were used to check its efficiency against normal microflora and organisms causing oral cavities and it is proved to be effective by inhibiting the organisms growth with clear halo zones of inhibition in the anti-bacterial activity. Further, the synthesized HA compounds potential to safeguard against demineralization of teeth were checked and proved to efficient with the increase in addition of $Ca_{10}(PO_4)_6(OH)_2$ into the acetate buffer corresponds to the decrease in rate of demineralization by reducing the amount $PO_4^{3-}$ ions in solutions and in higher concentration levels, an increase in mass of teeth after 48 hours treatment exhibits the deposition or coating of synthesized HA on the teeth by decreasing the rate of demineralization, this study also observed a brightening or whitening effect after treatment for 48hours in acetate buffer solution with variations in concentration of Synthesized HA.

## REFERENCES


[1]. Hench L.L, American Ceramic Society, 74, 1991, 1487.
[2]. Hench L.L, , American Ceramic Society, 81,, 1998, 1705.
[3]. Hench L.L, Ducheyne P, Kahan A, Martens M, Bursens A, Mulier J.C, Biomedical Materials Research, 14, 1980, 225.
[4]. Elliot, J.C., Mackie, P.E. and Young, R.A. . 'Monoclinic hydroxyapatite', Science, 180, 1983, 1055-1057.
[5]. Kay, M.I., Young, R.A. and Posner, A.S. , 'Crystal structure of hydroxyapatite', Nature 204, 1964, 1050.
[6]. Oonishi H, , 'Orthopaedic applications of hydroxyapatite', Biomaterials, 12, 1991, 171178.
[7]. Oonishi H, "Development and application of bioceramics in orthopaedic surgery" Hard Tissue Repair and Replacement, 3, 1992.
[8]. Block, M.S. & Kent, J.N. , "Prospective review of integral implants", In: The dental clinics of North America, Hydroxyapatite-coated implants, 36( 1), 1992.
[9]. Cao Xianying, Du Jingjing, Wen Feng, Cao Yang, and Pang Sujuan, , 'Effect of Nano-HAP on Proliferation of Hepatocellular Carcinoma Cells', Bioceramics Development and Applications,4, 2010, 110136,
[10]. Indah Raya, Erna Mayasari, Afdaliah Yahya, Muhammad Syahrul, Andi Ilham Ltunra, 'Shynthesis and Characterization of Calcium Hydroxyapatite Derived from Crab Shells (Portunus pelagicus) and Its Potency in Safeguard against to Dental Demineralizations', International Journal of Biomaterials, 2015, 469176.
[11]. Tin-Oo MM, Gopalakrishnan V, Samsuddin AR, Al Salihi K A, Shamsuria O, 'Antibacterial property of locally produced hydroxyapatite', Archives of Orofacial Sciences 2, 2007, 41-44.
[12]. Iulian Antoniac, Iaidoro Giorgio Lesci, Ana-lulia Blajan, Greta Vitioanu, Aurora Antoniac, 'Bioceramics and biocomposites from marine sources', Key Engineering Materials 672.276-292, 2015.
[13]. Innocent J. Macha, Lutfiye Sevgi Ozyegin, Joshua Chou, Ramazan Samur, Faik Nuzhet Oktar and Besim Ben-Nissan,'An Alternative Synthesis Method for Di Calcium Phosphate (Monetite) Powders from Mediterranean Mussel (Mytilus galloprovincialis) Shells', Journal of The Australian Ceramic Society, 49, 2013, 122-128.
[14]. Ratner B.D, Hoffman A.S, Schoen F.J, Lemons J.E, 'Biomaterials Science', Elsevier Academic Press,, 2004, 851.
[15]. Dasgupta Adak, Purohit, 'Synthesis of Nano-crystalline Hydroxyapatite from Dead Snail Shells for Biological Implantation', Trends Biomater. Artif. Organs, 25, 2010, 101-106.
[16]. Gunduz O, Sahin Y. M., Agathopoulos S, Ben-Nissan B, and Oktar F.N, Journal of Nanomaterials" 2014, 382861- 6.