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CHEMICAL SYNTHESIS OF BONE-LIKE HYDROXYAPATITE FROM CUTTLE
FISH BONES AND ITS CHARACTERIZATION

Abin Abraham Sebastian¹ D.Ajith Kumar² S.S.Divya³

¹PG Scholar, Udaya School of Engineering

²Assistant Professor, Udaya School of Engineering

³Assistant Professor, Udaya School of Engineering

¹abinabrahamseban@gmail.com,

²ajith.biomedical@gmail.com,

³divyassbme@gmail.com

ABSTRACT

Hydroxyapatite (Hap) was synthesized from cuttle fish bone by using a wet chemical method at room temperature. In this method powdered cuttle fish bones were reacted with phosphoric acid (H₃PO₄). The synthesized Hap was characterized by Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Fine and Crystallized Hap was obtained. The particular cuttlefish bones are biocompatible and used in implants for osseointegration.

Keywords: Carbonate Hydroxyapatite, cuttlefish bones, Calcium Phosphate, Chemical synthesis.

INTRODUCTION

Apatite are a group of phosphate minerals. They are usually referred as Hydroxyapatite, fluoroapatite and chloroapatite in the crystal form with high concentration of OH⁻, F⁻ and Cl⁻ ions. Hard tissues contain from 70 to 90wt% of inorganic phase in human and animal known as "biological apatite". The chemical composition corresponds to the general formula (Ca,M)₁₀(PO₄,CO₃,Y)₆(OH,F,Cl)₂, where M represents minor elements such as Sr, Pb and Ba, while Y represents acid phosphate, HPO₄²⁻, sulfates, borates, vanadates, etc.(1). Biological apatite is commonly calcium deficient and it always carbonate substituted. For this reason, it is more appropriate to refer to it as "carbonate apatite" (CA) (1). The carbonate ions substitute primarily for the phosphate groups of biological apatite, which is designated as Type B substitution. However, the hydroxyl (OH) groups can also be substituted, which is in turn designated as Type A substitution (1). The carbonate

content present in human bones, dentine and enamel, fluctuates from 3 to 8wt% (1, 2).

The stoichiometric chemical composition of Hydroxyapatite (Hap) is Ca₁₀(PO₄)₆OH₂. Hap is a synthetic material which is attractive for biomedical applications. Hard tissues can be replaced by using Hydroxyapatite (Hap) (3). A modified form of Hydroxyapatite known as bone mineral is present up to 50% of bone by weight (4). In many modern implants like hip replacement, dental implants and bone conduction implants coatings are formed using Hydroxyapatite. The Hydroxyapatite will help to promote osseointegration (5). Local delivery of drugs in bone can be done using porous Hydroxyapatite (6). It can be also used in repairing of early lesions in tooth enamels (7).

Cuttlefish bones are hard and brittle internal structure. It is primarily composed of aragonite and is

chambered, gas filled shell (8). It is used commonly as calcium –rich dietary supplement for caged birds, reptiles, crabs, snails etc (9).

MATERIALS AND METHODS

Synthesis and Processing

To remove the organic matter present in cuttlefish bone it is washed with distilled water and with H_2O_2 . Take H_2O_2 in a beaker along with the cuttlefish bone and heat it in a heating mantle at a temperature of $90^\circ C$ for about 5 to 10 minutes. Foam will be formed. Again wash the sample with distilled water and heat with H_2O_2 . Continue the step 4 to 5 times and keep the sample for drying in hot air oven for 2 to 5 minutes. The dried sample is grinded for 60 minutes to micro sized particles using mortar and pistol. The powder sample now obtained is Calcium Carbonate ($CaCO_3$). (10)

A 2gm of cuttlefish bone powder is taken and 20ml phosphoric acid is added. Phosphoric acid is added drop by drop and stirred well using a magnetic stirrer. Presence of carbonate can be identified; bubbles will be formed when the phosphoric acid drops get mixed with cuttlefish bone powder. Fine chap particles and a residual aqueous solution were obtained. The entire sample is filtered and washed several times using deionized water. The washing continued until the ph becomes neutral. Then the sample is dried at $100^\circ C$ for 6 hours. (10)

Characterization

Fourier Transform Infrared Spectroscopy (FTIR) characterization is used to get an infrared spectrum of absorption, emission and photoconductivity. It collects spectral data in a wide range. It is used for determining functional groups in the synthesized Hydroxyapatite (HAp) (11, 12). The morphology and size of the synthesized Hydroxyapatite crystals were studied and analysed by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM). Scanning Electron Microscopy (SEM) characterization a focused beam of electrons produces an image of a sample by scanning it. SEM produces black and white images with single value per pixel (13). Atomic Force Microscopy (AFM) characterization is used to get images in nanometers

range resolution. AFM provides 2 dimensional images of a particular sample (14).

RESULT AND DISCUSSION

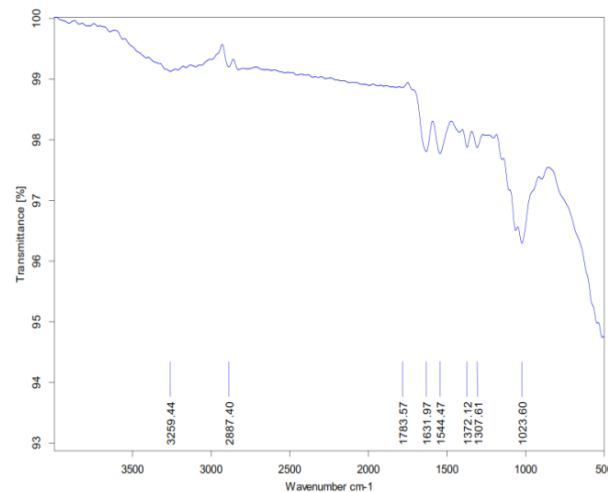


Fig. FTIR for Hydroxyapatite Synthesized

The carbonate ions are present in the range of 1500-2000 and the phosphate ions are present in the range of 1000-1500.

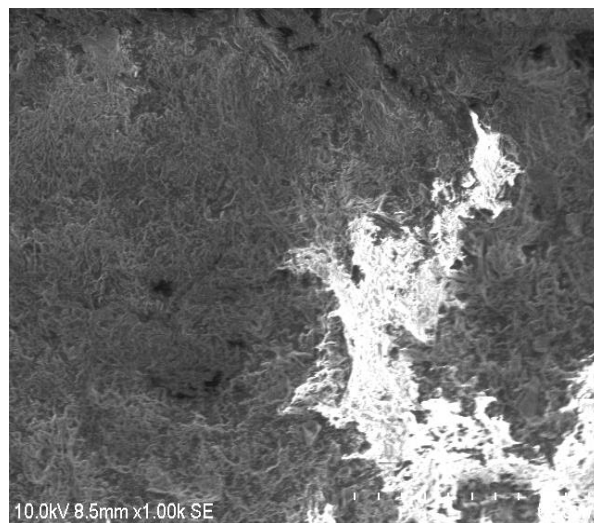


Fig. Scanning Electron Microscopy Image for Hydroxyapatite Synthesized

SEM is used for viewing the sample at a high resolution. The SEM image of Hap is given in above figure.

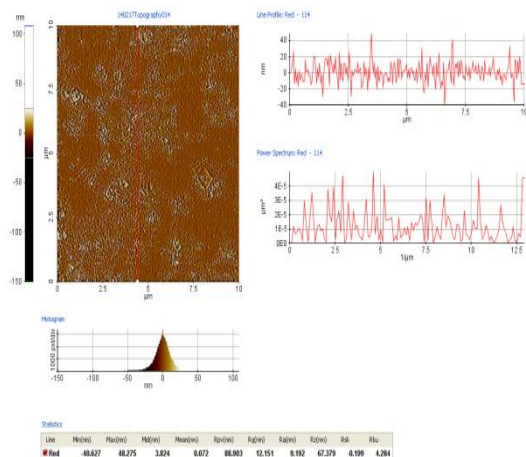


Fig. Two-dimensional image of Atomic Force Microscopy Characterization of Hydroxyapatite synthesized.

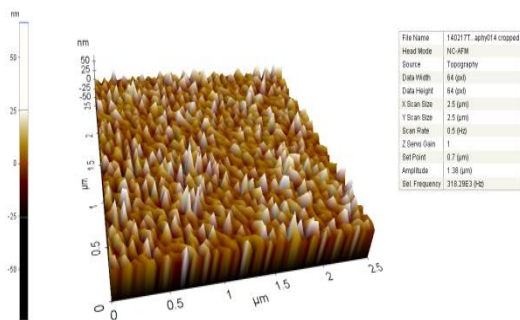


Fig. Three-dimensional image of Atomic Force Microscopy Characterization of Hydroxyapatite synthesized.

Atomic Force Microscopy (AFM) is used for viewing the sample in the resolution range of nanometers and the sample reaches the range of nanometer size.

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