

SYNTHESIS AND CHARACTERIZATION OF SILVER DOPED NANO HYDROXYAPATITE

**Submitted in partial fulfillment of the requirements for the award
of Master of Science degree in Physics**

By

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DEPARTMENT OF PHYSICS

SCHOOL OF SCIENCE AND HUMANITIES

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **M.PRIYADHARSHINI (Reg.No.40590013)** who carried out the project entitled **"SYNTHESIS AND CHARACTERIZATION OF SILVER DOPED NANO HYDROXYAPATITE"** under my supervision from **NOVEMBER 2021 to MARCH 2022**

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I **M.PRIYADHARSHINI (Reg.No.40590013)** hereby declare that the project report entitle “**SYNTHESIS AND CHARACTERIZATION OF SILVER DOPED NANO HYDROXYAPATITE**” done under the external guidance of **Dr. S. Manisha Vidyavathy**, Associate professor, Department of Ceramic Technology and internal guidance of **Dr. J. Anita lett** is submitted in partial fulfillment of the requirements for the award of Master of Science degree in Physics.

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(M.PRIYADHARSHINI)

ABSTRACT:

The manufacturing of hydroxyapatite biomaterial is gaining popularity because hydroxyapatite is an important component in the recovery of bone and tooth tissues. The calcium carbonate-rich white cockle shell can be employed as an alternate source of material for hydroxyapatite synthesis. The utilization of white cockleshell waste in hydroxyapatite manufacturing helps save money while also protecting the environment.

The goal of this work is to investigate how a conventional precipitation method can be used to produce hydroxyapatite from white cockleshells and dope it with green synthesized silver, as well as to characterize the material's physical, chemical FTIR, XRD, TEM, and SEM with EDX and biological properties such as antibacterial assay for medical applications.

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LIST OF ABBREVIATIONS

- SEM –SCANNING ELECTRON MICROSCOPE
- XRD –X-RAY DIFFRACTION
- TEM - TRANSMISSION ELECTRON MICROSCOPE
- AFM- ATOMIC FORCE MICROSCOPE
- EDX – ENERGY DISPERSIVE X-RAY
- SAED - SELECTIVE AREA ELECTRON DIFFRACTION
- ZOI – ZONE OF INCUBATION

1 INTRODUCTION:

1.1 BIOLOGICAL IMPLANTS:

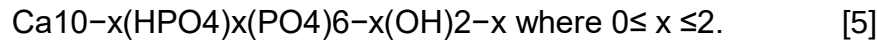
Implants are designed to replace a missing biological component. Sustain a damaged biological structure. Are to improve the functionality of an existing biological system. Depending on the functionality of the material, the surface of the implant that contacts the body may be made of titanium, silicone, or appetite. The use of metallic biomaterials to replace biomedical implants dates back to the 19th-century Clinical success has been declared for these metallic biomaterials due to their mechanical qualities That meet the requirement of human bones However Important concerns with the material when it is used as an implant exist, such as the release of toxicity and dangerous metal ions through wear and corrosion process after extended implantation Aside from that, due to different components of human bones and metal implants, the bonding strength between bones and implants are regarded weak. Result bioceramicmic materials like Alumina, zirconia, and hydroxyapatite are used as a coating material for metallic biomaterials with the crucial intention to solve the issue, that Metallic biomaterials have.

Bioceramics based on calcium hydroxyapatite, in general, offer great biocompatibility, corrosion resistance, and compressive strength, making them a promising candidate for implants. [1]. Bioceramic materials are not only used for coating implants but are also used to make scaffolds. To date, many commercial approaches for developing the HAP coating on metallic biomaterials have been introduced. Plasma spraying, Sol-gel dip-coating electrochemical deposition, high-velocity suspension plasma spray, or some of the techniques used.[2]

1.2 HYDROXYAPATITE:

Hydroxyapatite is a calcium phosphate that resembles human heart tissues and bones it has a hexagonal shape and has the same stoichiometric Ca/P ratio as bone appetite. Hydroxyapatite is a chemically comparable material to the actual bone that can be used as a bone substitute. Human bone is made up of 65 to 75% hydroxyapatite calcium phosphate mineral as hard tissue, with the remaining 25% made up of collagen, an organic molecule, and water to let the body operate. In HA the calcium to phosphate molar ratio, CIP ranges from 1.2 to nearly two.[3] [4]. The

stoichiometric molar ratio of HA is 1.67; however, because of the minor amounts of. Other components such as carbon, nitrogen, iron, and other elements or integrated. This is not the actual value that is found in the organisms. So, the general formula is as given below



1.2.1 CRYSTAL STRUCTURE OF HYDROXYAPATITE $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$:

Hydroxyapatite has a hexagonal shape and the same stoichiometric ratio as the bone appetite. Hydroxyapatite Calcium ions react with negatively charged phosphate and/or carboxyl groups on the biomolecule to form a stable complex, as well as ionic interactions between positively charged groups on a biomolecule (typically a protein) and the phosphate groups in hydroxyapatite.

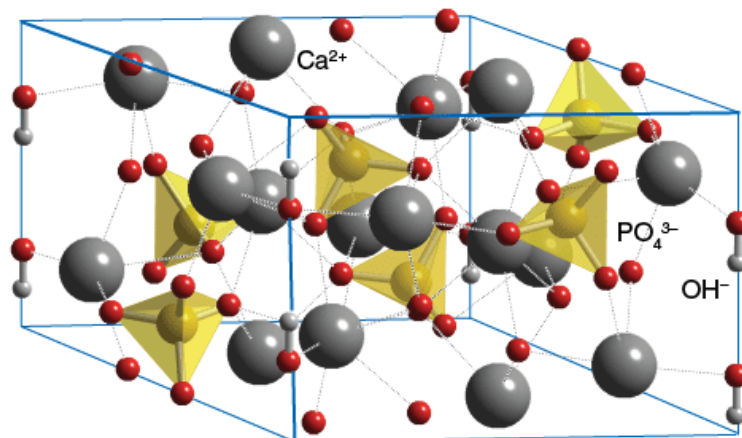


Figure 1.1 Crystal Structure of Hap.

The OH^- ions represent the individual oxygen atoms, with H outside the cell. Phosphate and hydroxide ions help the calcium ions coordinate. Its chemical formula is usually written as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ because the crystal unit cell of hydroxyapatite has ten Ca ions (10 Ca^{2+}).

1.2.2 PHYSICAL PROPERTIES:

- Hydroxyapatite is well-known for its brittleness.
- The color of appetite varies depending on its types; however, it is typically green, grey, red, brown, blue, Violet, or colorless.



Figure 1.2 Apatites of different colors

- It can be transparent or opaque.
- The majority of hunger is fluorescent when exposed to UV radiation.
- The density and porosity of a material determine its compressive strength.
- In a pH. 2.0 solution, the apatite degrades.
- HA is found to be the same as human tooth enamel.

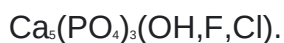
Table 3 [9] lists the typical features of dense hydroxyapatite. As a result, improving hydroxyapatite's mechanical characteristics would broaden its range of applications.

TABLE 1.1: Dense hydroxyapatite's typical qualities.

PROPERTIES	AMOUNT.
Theoretical density	3.156 g/cm ³
Bending strength	20-80MPa
Hardness	500-800 Vickers,2000-3500 Knoop
Tensile strength	40-100 MPa
Compressive strength	100-900 MPa
Fracture toughness	1MPa ^{1/2}
Young's modulus	70-120 GPa

1.2.3 CHEMICAL PROPERTIES:

- Many foreign ions find a home in appetite. As a result, a decent of other additional minerals can also be expected.
- The empirical formula for hydroxyapatite, fluorapatite, and chlorapatite is shown below.



- The hydroxyl groups of fluorine and chlorine can be mutually replaced [6].

TABLE 1.2: Stoichiometry of Hap:

COMPONENTS	STOICHIOMETRY (wt%)
Ca	39.90
P	18.50
OH	3.38

As indicated in Table 2, synthetic hydroxyapatite has a similar composition to the mineral component of bone and teeth.[8]

TABLE 1.3 :

Comparison of the composition of hydroxyapatite present in different areas of the human body (HA)[7].

COMPOSITION (wt%)	BONE	DENTINE	ENAMEL
Ca/P ratio	1.71	1.61	1.63
Calcium	34.8	35.1	36.5
Phosphorous	15.2	16.9	17.1
Organics (%)	25	20	1.5
Inorganics (%)	65	70	97
Water (%)	1.5	10	16

1.2.4 BIOLOGICAL PROPERTIES:

All living species rely on the bone for basic structure and support. They protect the sensitive organs as well as the principal structural backup. The skull covers our brain, and the vertebral column protects the delicate spinal cord. Traumatic or physiological bone regeneration abnormalities pose a significant challenge and are a global health concern. Bone regeneration is a natural process that occurs during normal fracture healing; nevertheless, there are times when enormous amounts of bone regeneration are required in a short amount of time. To compensate for the poor bone regeneration process, numerous techniques are now being explored. As a result, investigations in this area are being conducted to develop artificial bone regeneration procedures.

Hydroxyapatite is well known for its explicit biological properties

- Biocompatibility
- Bioactivity
- Osteoconductive
- Nontoxicity and noninflammatory nature

which makes it the most suitable material for coating bone replacements and for making biological scaffolds.

1.2.5 NATURAL SOURCES AND METHODS FOR PREPARING HYDROXYAPATITE:

Natural hydroxyapatite is typically derived from biological sources or wastes such as mammalian bone (e.g., bovine, camel, and horse), marine or aquatic sources (e.g., fishbone and fish scale), shell sources (e.g., cockle, clam, eggshell, and seashell), plants and algae, and mineral sources (e.g., limestone). Figure 1 depicts the sources and techniques used to synthesize natural HAp. Stoichiometric HAp is primarily composed of calcium and phosphorus, with a Ca/P molar ratio of 1.67. This is the most effective ratio for promoting bone regeneration [18]. Natural HAp is non-stoichiometric and deficient in either calcium or phosphorus [19]. Calcium positions are the most common vacancy in HAp, where cations such as Na⁺, Mg²⁺, and Al³⁺ are substituted, while carbonate ions can substitute for either phosphate or hydroxyl ions and fluoride ions substitute for hydroxyl ions [19].

Some natural HAp contains trace elements that mimic the apatite produced from human bone [20]. Trace elements are necessary for bone regeneration and speed up the process of bone formation [18]. Balamurugan et al. discovered that adding 3–5 mol percent silicon to synthetic hydroxyapatite (Si-HAp) increased cell growth density, which boosted osteoblast growth [21]. Capuccini et al. discovered that the presence of 1–10% strontium ions in synthetic HAp increased osteoblast activity and differentiation while inhibiting osteoclast proliferation and production [22]. Carbonate-substituted hydroxyapatite has also been shown to improve osteogenesis by increasing resorption [23]. Because HAp extracted from natural sources is available in large quantities, it can be considered an environmentally friendly,

sustainable, and cost-effective process for fabricating these materials. This can have a positive impact on the economy, the environment, and overall health.

Table 1.4: Sources of Hydroxyapatite:

SOURCE	METHODS
MINERALS • Lime stone	• Calcination + precipitation. • Calcination +hydrothermal • Calcination+ ultrasonic irradiation.
PLANTS AND ALGAE • Stalk (leaves and flowers), • Fruit peels • Algae • Wood	• Microwave irradiation, • Pyrolysis. • Hydrothermal • Chemical precipitation. • Alkaline hydrolysis. • Calcination. • Combination of the methods.
MAMMALIAN • Bovine • Camel • Cow • Pig	• Calcination • hydrothermal • combination of methods. • Alkaline hydrolysis.
AQUATIC • Fishboneses • Fish scales	• Calcination • Alkaline hydrolysis, • Hydrothermal • Combination of methods.

SHELLS • Cockleshell • Clams • Mussel shell • Eggshell	• Calcination • Hydrothermal • Chemical precipitation. • Sol-gel precipitation • Mechanochemical method.
--	--

1.3 APPLICATION:

Plaster of Paris (calcium sulfate) was historically the first widely tested artificial bioceramic, but it now has ex vivo applications. Surgeons were already using plaster of Paris as a bone-filling substitute by the end of the nineteenth century to provide information on the recent history of CaPO_4 , bioceramics, and biomaterials. In 1920 Fred Houdlette Albee (1876-1945), the inventor of bone grafting [24], attempted for the first time to implant a laboratory-produced CaPO_4 as an artificial material to repair surgically created defects in rabbit bones. He also invented some other orthopedic surgical advances [26].

Currently, hydroxyapatite is gaining popularity as an implant material with applications in dentistry and orthopedics.[26]. Blends of polycaprolactone (PCL)/HAP, PCL/collagen (Col)/HAP, PCL/gelatine (Gel)/HAP, poly-L-lactic acid (PLLA)/Col/HAP, and poly3-hydroxybutyrate-3-hydroxy valerate (PHBV)/HAP were studied by various research groups as a substitute for bone tissue engineering [63]-[68]. Scaffolds with HAP polymeric composites improved the new bone tissue development with increased osteointegration, osteoblast adhesion, and calcium mineral deposition on its surface [27].

- Engineering of bone tissue.
- Orthopaedic, traumatology, spine, maxillofacial, and dental void fillers.
- Coating for orthopedic and dental implants
- Periodontal defects are repaired.
- augmentation of the edentulous ridge
- Endodontic procedures such as pulp capping
- Mechanical furcation perforation repair and apical barrier formation

- Restorative glass ionomer cement (GIC) and restorative composite resin fillers.
- Desensitizing agent in post-bleaching teeth
- toothpastes with remineralizing agents
- Early treatment of carious lesions
- Gene and drug delivery

1.3.1 BIOCERAMIC COATING:

The human body accepts hydroxyapatite very well since it has a similar chemical composition to the human bone. Without HAP coating, the body may work to isolate an implant from surrounding tissues. Plasma spraying is currently the only commercially acceptable method of applying hydroxyapatite coatings to metallic implants. Titanium and stainless-steel implants are frequently coated with hydroxyapatite to fool the body and reduce implant rejection. In cases where there are bone voids or defects, hydroxyapatite can be used as well. This procedure involves the placement of powders, blocks, or beads of the material in the affected areas of bone. It encourages bone growth and repairs the defect due to its bioactivity. This method could be used instead of allogenic or xenogeneic bone grafts. Healing times are typically shorter when hydroxyapatite is used than when it is not.

1.3.2 BONE FILLING:

Hydroxyapatite can be used to fill bone defects or voids in powders, porous blocks, or beads. These can occur when large sections of bone must be removed (for example, in the case of bone cancer) or when bone augmentations are required (e.g., maxillofacial reconstructions or dental applications).

The bone filler will act as a scaffold, promoting rapid filling of the void by naturally forming bone, and will serve as an alternative to bone grafts. It will also become a part of the bone structure, reducing healing times when compared to the case where no bone filler was used.

1.3.3 DERMA FILLING:

The use of hydroxyapatite has evolved in tandem with advances in aesthetic medicine, from a surface-oriented two-dimensional approach focusing on the treatment of facial wrinkles and folds to a three-dimensional strategy addressing both soft and hard tissue volume loss in the face and hands.[30]

A FILLER MADE OF CALCIUM HYDROXYAPATITE IS USED TO SOLVE THE FOLLOWING ISSUES:

- To replenish the amount of collagen lost in the cheeks and chin;
- To make the nasolabial folds disappear;
- Getting rid of marionette lines;
- To get rid of wrinkles and blemishes on the skin;
- To improve the firmness and elasticity of the skin;
- To correct the oval shape of the face;
- Hands' dermis should be smoothed and replenished.
- The tip of the nose is lifted.

1.3.4 SCAFFOLD:

A biological scaffold in three dimensions is made up of at least three sets of polymer waveguides that extend in at least three different directions. A self-supporting structure is formed by at least three sets of polymer waveguides that interpenetrate each other at several nodes. The polymer waveguides may be biodegradable in some embodiments. In some embodiments, the three-dimensional biological scaffold may also include one or more coating layers for covering the polymer waveguide's surfaces. They are necessary for tissue engineering because they direct cell growth and facilitate tissue or organ formation. Polymer scaffolds are primarily used in the construction of scaffolds. There are some basic requirements for scaffold design. First, they should be porous, i.e., have high porosity. Following that, they should have a proper and convenient biodegradation rate. Third, it should be biocompatible and have sufficient mechanical strength. Finally, it should have a large surface area and promote cell proliferation.[29]

1.3.5 DENTAL APPLICATION:

Nano-HA is used to treat dentin hypersensitivity due to its bioactive properties and ability to stimulate the mineralization process. When the dentinal tubules are exposed, it is thought that the fluid circulation within the tubules may cause excitation of the receptor cells in the pulp, resulting in hypersensitivity. This usually appears in response to cold, hot, sweet, or tactile stimuli. Nano-HA can easily penetrate dental tubules and act as a mineralizing agent, occluding the tubule and stopping the circulation of fluid within. In addition, chemical reactions with the tooth structure ensure an effective and long-lasting therapeutic effect with resistance to mechanical or chemical factors. Based on its surface remineralization property and the addition of an appetite coating on the tooth's surface, nano-HA is preferred over desensitizing agents in the treatment of hypersensitivity.[31]

1.4 CONVENTIONAL METHODS FOR PRODUCING NANOHYDROXYAPATITE:

In general, HAp nanoparticles are synthesized using a variety of techniques, depending on the desired application, such as hydrothermal, sol-gel, mechanochemical, precipitation, hydrolysis, emulsion, combustion, Sonochemical, and pyrolysis. Furthermore, several parameters, such as reaction method, temperature, stirring time, pH, sintering process, starting materials, and solvents, can be changed to obtain HAp with specific size and morphology.

1.4.1 PRECIPITATION METHOD:

Wet precipitation, among other conventional methods, is a low-cost, simple, and environmentally friendly method for producing nano-HAp. The purity of the resulting powder is very high due to the close control of processing parameters such as pH, time, stirring speed, and reaction temperature. According to recent reports, precipitation is a viable method for producing HAp nanoparticles for biomedical applications (Boudemagh et al., 2019). Peng et al. (2015) investigated the effect of rotational speed on the precipitation of HAp nanoparticles. The findings suggested that the rotational speed be increased to increase particle size. A rotational speed of 1600 rpm (rotation per minute) was optimized for the production of rod-like HAp (Peng et al., 2015). However, in vitro biological studies revealed that carbonated HAp increased proliferation rate when compared to silicon-substituted HAp (Bang et

al., 2015). Zhang et al. (2016) investigated the thermal stability and morphological changes in precipitation method mediated HAp fabrication, and the results revealed that increasing the temperature for sintering as well as reaction increased the crystallinity of the HAp synthesized. Even though the HAp synthesized was thermally stable up to 1000 C, decomposition of HAp was observed above 1000 C, and crystallinity studies revealed the presence of a secondary phase, -TCP. Furthermore, when nano-HAp was calcined at 1000 C, electron microscopic images revealed nano-particle aggregates (Zhang et al., 2016). Furthermore, by optimizing the addition rate, reaction temperature, and calcination temperature, Yelten-Yilmaz and Yilmaz (2018) created spherical HAp nanoparticles with an average particle diameter of 200–300 nm. The results show that an additional rate of 5.5 ml/min C was optimal for producing uniformly spherical nanoparticles. The mechanical strength of the powder calcined at 1200 C was greater than that of the powder calcined at 900 C. (Yelten-Yilmaz and Yilmaz, 2018).

Similarly, Othman et al. (2016) stated that wet chemical precipitation is a highly flexible technique because the parameters can be controlled to produce nano-HAp with the desired morphology, size, and surface chemistry. Furthermore, Yelten-Yilmaz and Yilmaz (2018) created spherical HAp nanoparticles with an average particle diameter of 200–300 nm by optimizing the addition rate, reaction temperature, and calcination temperature. The results show that an additional rate of 5.5 ml/min C was optimal for producing uniformly spherical nanoparticles. The mechanical strength of the powder calcined at 1200 C was greater than that of the powder calcined at 900 C. (Yelten-Yilmaz and Yilmaz, 2018) Similarly, Othman et al. (2016) stated that wet chemical precipitation is a highly flexible technique because the parameters can be controlled to produce nano-HAp with the desired morphology, size, and surface chemistry.

In a nutshell, the precipitation method can be thought of as a simple method for producing HAp nanoparticles with specific properties. Furthermore, the method of preparation affects the crystallinity, size, and shape distribution of the HAp particles. The ability to optimize the synthesis parameters is a critical advantage of this method for large-scale HAp nanoparticle production (Ramesh et al., 2015).

1.4.2 HYDROTHERMAL METHOD:

The hydrothermal technique is a simple procedure for producing specific morphologies of nanoparticles, such as rods, flowers, and other unusual shapes, due to its ability to induce the growth of one-dimensional nanostructures and the ability to optimize the parameters by changing the reaction parameters [1]. When performed in an autoclave, the reaction occurs at a specific pressure and temperature of $T > 100^\circ\text{C}$, yielding highly crystalline nanostructures.

The hydrothermal method was used to fabricate HAp nanorods and HAp/magnesium nanocomposites, and the results show that increasing the magnesium concentration can reduce HAp crystallinity and crystal size. Similarly, it was discovered that the cooling rate after hydrothermal synthesis determines the size and shape of HAp nanoparticles and that rapid cooling could result in smaller-sized (60nm) nanoparticles [2]. Furthermore, the solvents hexamethylenetetramine (HMTA) and cetyltrimethylammonium ammonium bromide (CTAB) was used to produce uniformly distributed HAp nanoparticles. Furthermore, the solvents hexamethylenetetramine (HMTA) and cetyltrimethylammonium ammonium bromide (CTAB) was used to produce uniformly distributed HAp nanoparticles. Similarly, hexagonal HAp nanoparticles substituted with Neodymium (III) (Nd^{3+}) were hydrothermally prepared, and their optical properties for bioimaging applications were studied [3]. Furthermore, the authors described the simple hydrothermal preparation of porous hollow hydroxyapatite microspheres, as well as their drug loading, release, anticancer, and cytocompatibility properties [4]. However, HAp microspheres were mildly toxic to normal cells. Furthermore, HAp nanobelts were prepared using additive-free hydrothermal methods, and the results demonstrated the effect of sodium hydroxide on nanobelt growth (An et al., 2016). Furthermore, Kang et al. (2011) developed a novel method for depositing super-hydrophobic HAp on the surface of magnesium alloys using simple hydrothermal techniques to reduce the corrosion rate in implants (Kang et al., 2011).

Furthermore, Zoran et al. (2016) used two-stage hydrothermal processes to describe the growth mechanism of one-dimensional HAp nanostructures (nanowires) and analyzed their morphological changes by varying the reaction parameters (Stojanovic c et al., 2016). The hydrothermal approach has several advantages, the

most important of which are its simplicity and low energy consumption. Furthermore, it is environmentally friendly and can be scaled up to produce bulk nano-materials (Stojanovic c et al., 2009). Unlike other synthesis methods, the hydrothermal approach can produce nano-sized HAp without the use of an additional phase, and the closed environment provides an ideal environment for rapid chemical reactions (Zhu and Chen, 2014). Furthermore, a low-cost instrumentation setup results in an excellent dispersion in solution during the reaction, resulting in a low production cost of nano HAp (Kusnieruk et al., 2016).

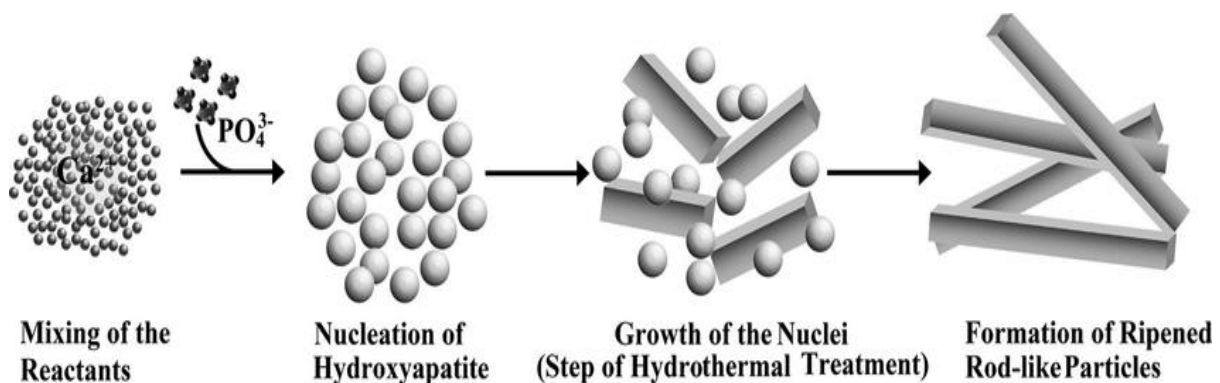


Figure 1.3 Diagrammatic representation of Hydrothermal method.

1.4.3 MECHANOCHEMICAL METHOD:

Mechanochemical is a simple and low-cost method for producing ceramic nanocomposites. The morphology of the resulting powder is influenced by process variables such as milling jar, reagents, milling media, milling time, ball-to-powder ratio, and rotation speed in this technique. Mechanical milling is advantageous for bulk material production due to its reproducibility, simplicity, and solvent-free environment. Harilal et al. (2018) demonstrated that increasing the milling time increased the calcium-to-phosphate ratio of the synthesized HAp, whereas electron micrographs revealed the presence of highly agglomerated powder particles with an average particle diameter of 26 nm. Harilalet et al. (2018). Rasha et al. (2017) also prepared carbonated hydroxyapatite nanoparticles by varying the milling time, resulting in HAp with increased hardness and compressive strength. Increased milling time, on the other hand, reduced the amount of porosity, which is necessary for osteoconductive (Youness et al., 2017). Adsila et al. (2016) used a mechanochemical approach to create HAp nanoparticles doped with magnesium

ions, and the results suggested that doping magnesium with HAp could induce the formation of secondary phases such as β -tricalcium phosphate (TCP) and brushite. However, magnesium ion doping significantly improved the hardness of HAp nanoparticles (Adzila et al., 2016).

Furthermore, Fakharzadeh et al. (2017) investigated the morphological and crystal structure changes of pure HAp and silver-doped HAp. The findings revealed the presence of aggregates of spheroids with dimensions of 25–33 nm and 30–32 nm of pure and silver-doped HAp nanoparticles, respectively (Fakharzadeh et al., 2017). Furthermore, Nandi et al. (2019) synthesized novel HAp with dolomite as a calcium source. The thermogravimetry curve confirmed the thermal stability of the HAp synthesized up to 1050 °C. Furthermore, the XRD results revealed the presence of secondary phases such as monocytite and β -TCP at 1200 °C. (Amirthalingam et al., 2019). Similarly, Bulina et al. (2016) used 0.6 M of silicon-substituted HAp synthesized by mechanochemical method and found no secondary phases up to 1200 °C when compared to undoped HAp. At 1000–1100 °C, however, increasing the silica content results in the formation of silicocarnotite (Bulina et al., 2016). Similarly, Javadinejad et al. (2017) used the mechanochemical method to synthesize HAp nanoparticles from calcium hydroxide and phosphorus pentoxide as raw materials, and the results indicated that increasing the heating rate can induce nanoparticle agglomeration (Javadinejad et al., 2017). Interestingly, Hannora (2019) used the mechanochemical method to synthesize manganese-substituted HAp nanoparticles, which resulted in decreased crystallite size at 1200 °C, agglomeration of nanoparticles, and decreased hardness due to an increase in manganese concentration (Hannora, 2019).

Ebrahimi et al. (2019) used a mechanochemical method to create a bioresorbable scaffold for tissue engineering applications using carbonated HAp/ β -TCP composites. The results showed that carbon substitution improved bioresorbability, and secondary phase β -TCP formation was also observed during thermal treatment (Ebrahimi et al., 2019). This method has several advantages, including the ability to produce nano-HAp in a short period with or without solvents, a high yield at room temperature, and distinct morphology by optimizing process parameters such as milling speed and reaction time (Kosachan et al., 2017).

1.4.4 SONOCHEMICAL METHOD:

High-frequency sound waves were employed in the Sonochemical process to speed up reactions involving nanomaterial production. Furthermore, when compared to other approaches, this method has the advantages of shorter reaction times, lower temperatures, and higher purity of the synthesized nanomaterials, making it more efficient. Türk et al. (2017) used a Sonochemical approach to construct hexagonal and rod-like HAp nanostructures utilizing various calcium and phosphate sources, including CaCl_2 , $\text{Ca}(\text{CO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Ca}(\text{OH})_2$, $(\text{NH}_4)_2\text{HPO}_4$, and K_2HPO_4 (Türk et al., 2017). Similarly, Ekka et al. (2018) developed zirconium oxide decorated HAp nanorods for photocatalytic applications, claiming that up to 95 percent degradation of phenols and their derivatives was accomplished with good stability (Ekka et al., 2018).

Deng et al. (2017), for example, used a single and dual ultrasonic reactor to explore the morphological alterations of nano-HAp caused by the sonication process. A dual ultrasonic reactor was used to successfully generate a uniform distribution of pure HAp nanorods with a stoichiometry of 1.66, whereas single and non-sonicated samples resulted in aggregated nanorods. As a result, a dual ultrasonic reactor was proposed as a useful medium for producing homogenous HAp nanoparticles by S. Balu et al (Deng et al., 2017; Mehta et al., 2017). Furthermore, natural rubber was used as a template for HAp production via sonication. The results showed that as the ultrasonic irradiation time increased, the size of the particle diameter decreased. In addition, thermogravimetry results revealed that non-stoichiometric HAp was more stable than stoichiometric HAp (Utara and Klinkaewnarong, 2015). Deng et al. (2019) also investigated the biological properties of HAp nanoparticles synthesized using a dual-frequency ultrasonic method. The study emphasized that nano-HAp is less toxic to normal human bronchial epithelial (16 HBE) cells up to 25–400 g/ml, and its hemolysis percentage was also less than 2% at 25–200 g/ml (Deng et al., 2019).

The ultrasonic irradiation method was used to create needle-like HAp nanoparticles from limestone and orthophosphoric acid (H_3PO_4) as precursor materials. The results of this study show that ultrasonic irradiation can hasten the growth of needle-like morphologies with minor secondary phases. It was discovered that a 20 kHz irradiation power and a 3 h irradiation time were optimal for producing

needle-like nano-HAp (Klinkaewnarong and Utara, 2018). Thus, ultrasonication is used to investigate the crystal structure and shape of HAp particles. It also aids in the production of fine nanosized HAp by promoting chemical reactions and physical effects. Furthermore, this method is highly recommended for creating core-shell HAp nanoparticles (Ingole et al., 2017).

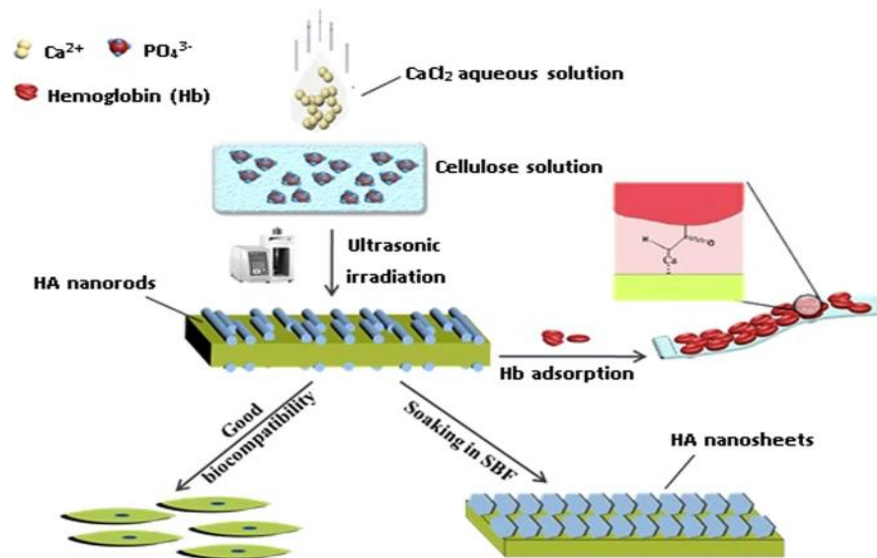


Figure 1.4 Diagrammatic representation of Sono chemical method.

The Sonochemical method of preparing cellulose/HAp nanocomposites is depicted schematically.

1.4.5 SOL-GEL PRECIPITATION METHOD:

The first wet chemical method developed for the fabrication of HAp was sol-gel, which includes several protocols such as hydrolysis, polycondensation, gelation, aging, and drying (Owens et al., 2016). The main advantage of the sol-gel technique is its ability to produce a homogeneous particle mixture by adjusting the parameters, as well as a reduction in evaporation loss because the process is carried out at low temperatures. The first step is to combine the Ca and P precursors. Ca/P ratio and pH as a constant value, followed by ultrasonic wave passage, drying, and calcination. Environmental parameters can be used to prevent sample contamination (Ma, 2019). Based on the solvent used, sol-gel processes are broadly classified into two types: those based on water (aqueous) and those based on organic solvents (non-aqueous) (Rao et al., 2017). Ben-Arfa et al. (2017) recently stated that due to

their logarithmic aging period, sol-gel is one of the time-consuming methods for obtaining nano-HAp.

As a result, they developed a novel technique that did not require aging, and the final products were found to be similar to conventional HAp, with low bioactivity (Ben-Arfa et al., 2017). Similarly, Türk et al. investigated the effect of solvents and calcination time on the synthesis of HAp nanoparticles using a sol-gel approach (2019). When water and ethanol were used as solvents, the results revealed that calcium hydroxide and calcium oxide impurities were present in the nano-HAp, which increased the crystallinity after different calcination times (Türk et al., 2019). Similarly, Shalini et al. (2017) used the sol-gel method to prepare HAp nanorods with gelatine composites and studied their drug-loading and release capabilities. Because of the weak physical interaction between HAp and the drug, the results emphasized that standalone HAp nanoparticles were not suitable for sustained drug-release applications. As a result, when compared to HAp alone, the drug-loaded HAp with gelatine nanoparticles demonstrated a significant, sustained and enhanced drug-release rate of up to 30%. (Shalini et al., 2017). Bolino et al. also investigated the effect of thermal treatment during the sol-gel synthesis of HAp/zirconia composite (2017). According to the reports, optimizing the thermal treatment can improve the bioactivity and biocompatibility of the composite.

Thermal treatment at 1000 C increases the crystallinity of HAp, as well as its biocompatibility and protein adsorption. Protein adsorption is the first step in the adhesion and multiplication of cells on the surface of HAp (Bolino et al., 2017). However, the unpredictable aging conditions and low yield are significant drawbacks of the sol-gel approach to nano-HAp particle fabrication (Kottegoda et al., 2011; Taheriana et al.).

1.4.6 MICROEMULSION METHOD:

Microemulsions were first used to prevent nanoparticles from aggregating. This method, however, is now a widespread synthesis technique, particularly for producing aggregation-free ceramic nanoparticles with specified morphologies. Using a one-step microemulsion approach, Foroughi et al. (2015) made magnetic HAp nanoparticles. The results of the vibrating sample magnetometer (VSM)

revealed the presence of nano-HAp magnetic properties up to 700 degrees Celsius, which gradually declined as temperature increased due to nanoparticle aggregation (Foroughi et al., 2015). Foroughi et al. (2016) used the microemulsion process to create HAp/magnesium ferrite nanoparticles with a magnetic property of up to 700 degrees Celsius (Foroughi et al., 2016). Gao et al. (2016) used the emulsion approach to create HAp nanoparticles with spherical and rod-like morphologies for heavy metal ion removal.

During the early phases of the synthesis process, electron microscopic pictures revealed the presence of rod-shaped HAp, which later converted into spherical nanoparticles with an average diameter of 60 nm as the concentration of glycine was increased. Furthermore, the mass spectroscopy results demonstrated that the spherical nanoparticles can absorb Cd^{3+} ions in large amounts. When compared to the various morphologies of HAp nanoparticles, it is argued that the spherical morphologies are the best contender for water purification applications (Gao et al., 2016). Using cyclohexane, n-octyl alcohol, and the CTAB microemulsion system at pH 11, Huang et al. (2019) created mesoporous HAp nanoparticles with an average pore size of 19.56 nm. The presence of agglomerated particles without surfactants was observed using electron microscopy. Surfactants, on the other hand, resulted in morphologies that were consistent (Hajimirzaee et al., 2019; Huang et al., 2019). The high yield of nano-HAp with a narrow particle size distribution, low aggregation, easy operating method, and homogenous topography are the main advantages of this technology (Fan et al., 2013)

1.4.7 COMBUSTION METHOD:

Among the temperature-mediated methods for preparing nanomaterials, the combustion method is a simple exothermic reaction method for producing nanoparticles with desired properties, particularly for the synthesis of composite materials, ceramic nanomaterials, and alloys (Aruna and Mukasyan, 2008). Furthermore, there are three types of combustion methods based on the reactants used in the fabrication, namely solid-state combustion, aqueous combustion, and gas-phase combustion (Virji and Stefaniak, 2014). Among these methods, the microwave-assisted combustion method was used to create HAp nanoparticles with antibacterial properties to prevent biofilm formation on the surface of implants. An

antioxidant assay using (2,2-diphenyl-1-picrylhydrazyl) DPPH assay, electron spin resonance (ESR) signals, and intelligent ink testing results show that radicals from nano-HAp are responsible for the bactericidal effect (Lamkhao et al., 2019). Similarly, Kaygili et al. (2018) prepared a magnesium-substituted stacked plate and rod-like HAp at 600 C in various ratios.

According to the findings, increasing the magnesium content can reduce the lattice parameters of the crystals as well as the crystallinity of HAp by increasing the lattice strain (Kaygili et al., 2018). In contrast, the same effect was observed when incorporating chromium ions due to the replacement of calcium ions. The MTT assay results showed that nano-HAp was more biocompatible with HeLa (cervical cancer) cells and L929 (mouse fibroblast) cells up to 800 g/ml of dosage (Bandgar et al., 2017). In addition, 100–200nm HAp nanoparticles were synthesized at 300 C using glycine as a fuel. With particle agglomeration, the samples demonstrated adequate porosity and mechanical strength (Rao et al., 2016). Thus, the advantages of this method include low production costs, large production volumes, and energy efficiency (Xiu et al., 2005). However, reports on the methods of synthesis of nano-HAp using the combustion method are scarce, owing to their main disadvantage of particle agglomeration (Varma et al., 2016).

1.4.8 PYROLYSIS METHOD:

Carbon nanomaterials such as carbon nanotubes, graphene oxide, and carbon quantum dots are synthesized using the pyrolysis method (Wang et al., 2019a). A carbon source is evaporated at high temperatures in this process, and the material is deposited as nanoparticles over the substrate, which is placed inside the inert chamber (Zahid et al., 2018). Yokota et al. (2017a, b) used sintering at 1250 C to create sodium-substituted HAp with improved thermal stability. The biomineralization analysis revealed that sodium substitution improves apatite bonding ability. However, when compared to pure HAp, sodium substitution increased the grain size and density of nanosized-HAp (Yokota et al., 2017b).

Similarly, HAp nanoparticles were synthesized at 900 C using the ultrasonic-assisted spray pyrolysis method, and their size and morphological modifications were studied as the raw material concentration was increased. The findings revealed

that increasing the precursor concentration increased particle diameter. Raising the sintering temperature resulted in increased HAp density (Cho et al., 2016). Furthermore, spray pyrolysis was used to investigate the substitution of potassium for the HAp structure. The addition of potassium resulted in the formation of hydroxide vacancies, which induced grain growth by altering the molar ratio of calcium phosphate (YOKOTA et al., 2017a). There are various types of pyrolysis, and each has a distinct advantage over others in the synthesis of nanosized-HAp, which is generally beneficial in yielding HAp via the straightforward approach of using complex precursors (Zhang et al., 2019b).

1.4.9 HYBRID METHOD:

To reduce reaction time, a hybrid (combined) method, such as a microwave-assisted hydrothermal (MAH) approach, is usually used. It also has the capability of altering the final product under certain conditions (Yang and Park, 2019). Kumar et al. (2018) created HAp nanoparticles with spherical (20–30 nm), rod (20–40 nm width, 50–100 nm length), needle (10 nm width, 90–100 nm length), and Fiber-like (10 nm diameter) morphologies using the microwave-assisted hydrothermal technique and organic modifiers such as ascorbic acid, cetyltrimethylammonium bromide (CTAB), and poly (Kumar et al., 2018). Similarly, Yu et al. (2018) used the microwave-assisted hydrothermal method to create ultralong HAp nanowires for the fabrication of flexible fire-resistant paper. Only 8.5 percent of weight loss was observed up to 1000 C (Yu).

Furthermore, conventional precipitation methods and ultrasonic-assisted precipitation methods have recently been used to synthesize HAp nanorods for fluoride removal applications. It is worth noting that ultrasonication increases the yield of uniform HAp nanorod bundles from 83 percent to 90 percent, and aggregated nanorods were observed in the conventional precipitation method (Mehta et al., 2017). Furthermore, microwave-assisted solid-state synthesis was used to create fluorinated HAp with fluorine substitution for improved mechanical properties. The results revealed that the addition of water (10–20%) influenced grain size, as well as biological and mechanical properties (Peng et al., 2019). Furthermore, for the mesoporous HAp synthesis, a microwave-assisted hydrothermal technique was used in conjunction with organic modifiers such as

sodium dodecyl sulfate (SDS) and oxalic acid. The results demonstrated that organic modifiers effectively modify the shape, crystallinity, and pore size. It should also be noted that oxalic acid produced rod-like HAp with calcium oxalate particles measuring 10 nm in width and 20–40 nm in length, whereas SDS produced pure HAp measuring 20–45 nm in width and 55–100 nm in length (Karunakaran et al., 2019b).

The euphorbium-doped, heparin-coated HAp nanoparticles were synthesized using an ultrasound-assisted co-precipitation technique, and the resulting nanoparticles demonstrated significant luminescent properties and stability while using ultrasonication followed by sterilization. Furthermore, morphological changes were observed as the ultrasonication process produced rod-shaped HAp and the precipitation method produced fibrous HAp nanoparticles (Xing et al., 2019). The main advantage of the hybrid method is that it can produce nanoparticles with unique properties that are not possible with other stand-alone synthesis approaches.

CHAPTER 2

2. LITERATURE SURVEY:

The abstracts of the literature publications that were extremely important and beneficial in the completion of the research are listed below.

Satheesh Kumar et.al [34] successfully replace damaged or diseased bones, implant materials must mimic natural human bones in terms of biocompatibility, osteoconductive, and mechanical stability. To replace conventional implant materials that have caused toxicity issues, synthetic hydroxyapatite was combined with bioglass to mimic natural bones. This study provided an overview of the conventional approaches for fabricating nano-HAp, their limitations, and the significance of using marine organisms and marine food wastes as a precursor for biogenic nano-HAp production. Furthermore, in vivo and in vitro analyses of nano-HAp derived from marine sources, as well as their potential biomedical applications, were discussed.

Gehan T et. al [36] used the wet precipitation method was to create hydroxyapatite (HA) nanoparticles from mussel shells, which were then tested on human mesenchymal and epithelial cells. This study sheds light on the significance of combining stem cells and nanomaterials, which could lead to advancements in tissue engineering to create novel treatments for a variety of diseases

Sri Devi et. al. [37] used oyster shell, which is mostly calcium carbonate, was used as a starting material for the synthesis of hydroxyapatite powder. The pure calcium oxide was obtained by calcining the oyster shell powder at 1200°C. The HA powder was obtained at pH 10 based on XRD results, as confirmed by the standard JCPDS file No. 076-0694. Furthermore, based on the XRD results, the crystallize size of HA was determined to be 89.5 nm.

C.S. Ciobanu et. al [38] looked into the possibility of increasing hydroxyapatite (HAp) bioactivity by substituting silver oxide. Wet precipitation with exposure to DC glow discharge non-thermal plasma was used to create Ag₂O-HAp nanoparticles. for the MCF-7 breast cancer cell line was performed, and the cytotoxicity effect was investigated. The biocompatibility of the synthesized silver oxide doped HAp demonstrates its potential for future biomedical applications.

Soo-Ling Bee et. al [39] obtained Monophase silver-doped hydroxyapatite ($\text{Ag}_x\text{Ca}_{10-x}(\text{PO}_4)_6(\text{OH})_2$; 0.002×0.04) nanoparticles were prepared using a neutralization method and investigated for potential medical applications. The method consists of dissolving Ag_2O in a solution of H_3PO_4 , and slow addition to the suspension of $\text{Ca}(\text{OH})_2$ was used for the homogeneous distribution of silver ions. The hydroxyapatite sample with the highest silver content demonstrated the greatest antimicrobial activity, killing all *E. coli* cells and reducing viable counts of *S. aureus* and *Candida albicans* by more than 99 percent. AFM studies show that a silver-doped hydroxyapatite sample causes significant morphological changes in microorganism cells, which may be the cause of cell death. Hemolysis ratios of silver-doped hydroxyapatite samples were less than 3%, indicating good blood compatibility and potential as biomaterials.

Bishnupriya Nayak et al [40] in this research was to develop new hydroxyapatite-based material with high biocompatibility. X-ray diffraction (XRD), scanning electron microscopy (SEM), and X-ray Photoelectron Spectroscopy was used to characterize the structural properties of the samples (XPS). Photoelectron Spectroscopy analyses confirm the presence of calcium (Ca), phosphor (P), oxygen (O), and silver (Ag) in the Ag: HAp. Murine macrophage viability and activation were increased by nanocrystalline silver doped HAp.

ThivaharanVaradavenkatesan et. al [41] due to their high calcium content, food industry wastes such as eggshells, bones, and seafood shells that are typically piled up in landfills can indeed serve as an excellent precursor to the formation of bioceramic materials such as hydroxyapatite. this review investigates the potential applications of various biowaste-derived hydroxyapatite in multidisciplinary fields such as biomedical (bone tissue engineering, metallic implant coating, drug delivery) and environmental pollution remediation.

N.I. Agbeboh et.al [41] synthesized nanocrystalline hydroxyapatite (HAp) bioceramics using dead *Pila globosa* shells are described in this paper. The snail shell's high calcium carbonate content (94.76 percent) confirmed its suitability as a precursor for HAp synthesis using inductively coupled plasma atomic emission spectroscopy. The investigation of the dielectric properties and AC conductivity supported the material to have a low dielectric loss (1.73) and a high dielectric

constant (296.6). The frequency dependence of AC conductivity validated the power law and impedance analyses demonstrated the material to be grain type. Overall studies indicate that the synthesized HAp could be a potent material for versatile applications

N.I. Agbeboh et al.[43] also mimicked the natural properties while also exhibiting unique properties such as high porosity, surface area, crystallinity, stability, mechanical properties, and biocompatibility. These unique properties make them ideal biomaterial candidates for use in a wide range of biomedical applications, including surgical implants, bone grafts, drug delivery agents, and environmental remediation to remove toxic dyes and heavy metals from wastewater. The review enlightens readers on the various synthesis methods for HAP and HAP-containing composites. It also discusses the most recent advancements in environmental and biomedical applications.

CHAPTER 3:

3.1 AIM AND OBJECTIVES OF THE CURRENT INVESTIGATION:

3.1.1 AIM OF THIS STUDY:

The goal is to synthesize Nano-hydroxyapatite from white cockle shells using two different precursors, diammonium hydrogen phosphate and orthophosphoric acid, and to dope them with green synthesized Nano-silver particles in three different concentrations before going to the comparative studies on their mechanical and biological properties for future biological applications.

- Synthesis hydroxyapatite via sea shells
- Green synthesis of Nano-silver particle via rose extract
- Doping process
- Characterization of the material synthesized:
 - X-ray diffraction (XRD)
 - Fourier transform infrared spectroscopy (FTIR)
 - Scanning electron microscopy (SEM)
 - Energy-dispersive X-ray spectroscopy (EDAX)
 - Transmission Electron Microscope (TEM)
- Biological Properties:
 - Anti Bacterial Assay
- Mechanical properties:
 - Vickers hardness

3.1.2 PROSPECTS FOR FUTURE RESEARCH:

- Novel materials can be tested by adding a metal dopant, which improves the biocompatibility and mechanical properties of the implant material itself or when coated with it.
- Conducting a comparison of the final product made from different precursors and concentrations of metal dopants.

CHAPTER – 4

4.1 METHODS AND METHODOLOGY

4.1.1 PREPARATION OF CALCIUM OXIDE FROM WHITE COCKLE SHELL:

Typical raw seashells are composed primarily of calcium carbonate (95 - 97%). (CaCO_3). To obtain calcium oxide the following steps were carried out.

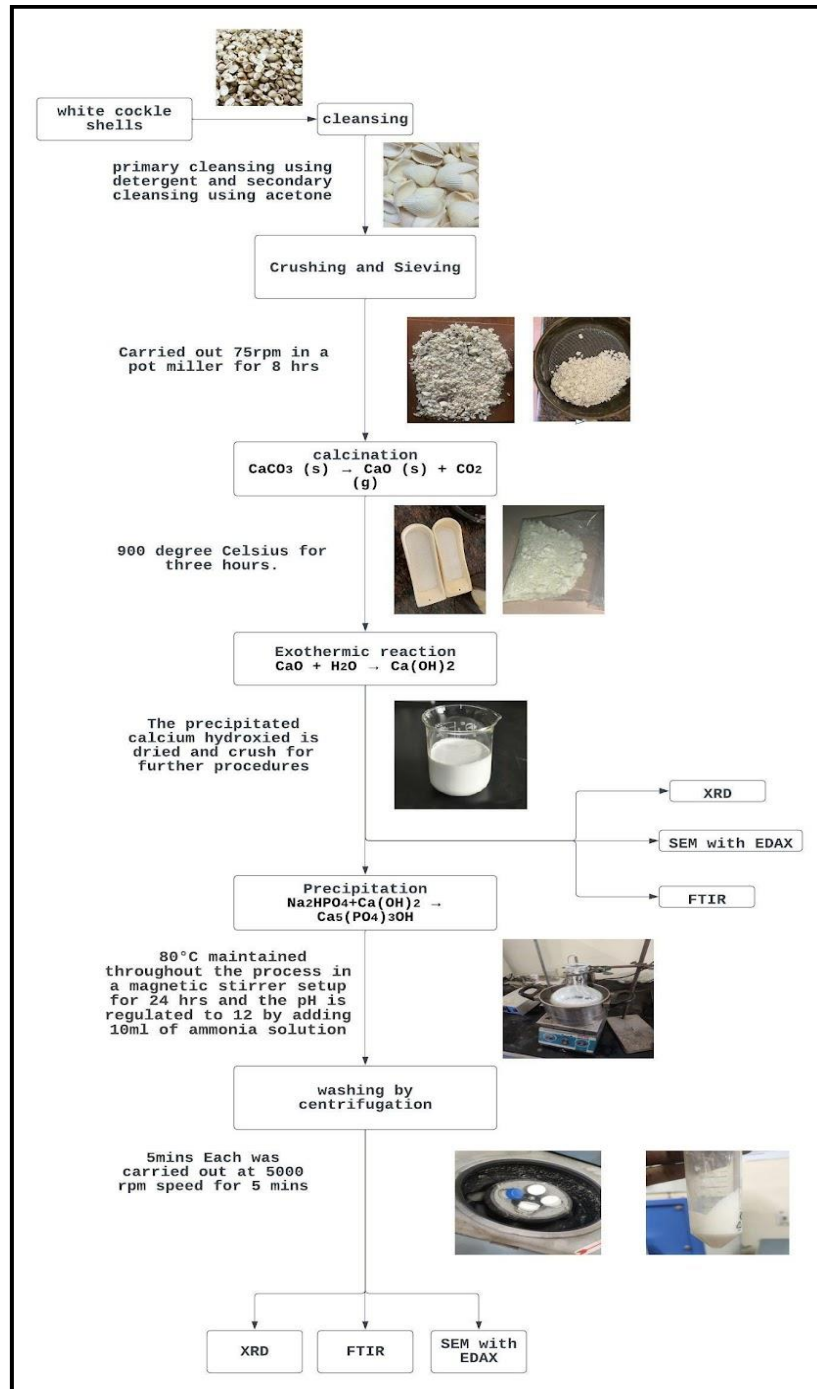


Figure 4.1 process of the synthesis of Hydroxyapatite from seashells

4.1.2 CLEANSING:

- The collected shells were first cleaned with detergent water to remove sand.
- The shells were then cleansed with acetone to remove algae and other organic material from the shell for 30 minutes.

4.1.3 CALCINATION:

Calcination is a balanced reaction. CaCO_3 decomposes to lime if the ambient partial pressure of CO_2 is less than the equilibrium partial pressure at a given temperature. When the temperature rises above the dissociation temperature of the carbonates in the limestone, the reaction begins. This is usually between 780 and 1340 degrees Celsius. Thus, the rate of decomposition is determined by the partial pressure of CO_2 , the reaction temperature, and the particle size. The process is dependent on an adequate firing temperature of at least 800 degrees C to ensure decomposition and a good residence time, i.e., holding the lime/limestone for a sufficiently long period at temperatures ranging from 1,000 degrees C to 1,200 degrees C to control its reactivity. During this process, the CO_2 passes out.

- The shells were crushed using a pot miller and sieved using a 1mm mesh before the calcination process.
- The shell powder was collected in an alumina crucible and kept at a high temperature of 900 degrees in a furnace for 3 hr.

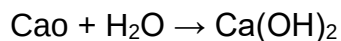
REACTION: $\text{CaCO}_3 (\text{s}) \rightarrow \text{CaO} (\text{s}) + \text{CO}_2 (\text{g})$.

4.1.4 PREPARATION OF CALCIUM HYDROXIDE:

Calcium oxide reacts with water to form slaked lime $\text{Ca}(\text{OH})_2$, which means that calcium oxides combine with free molecules of O^- and H^+ . This reaction generates a lot of heat and makes a hissing sound, so it is classified as exothermic.

- 25 grams of CaO is added to 100 ml of water and kept in a magnetic stirrer.
- The precipitated $\text{Ca}(\text{OH})_2$ is collected and dried in an oven at 80° .

REACTION:



4.1.6 HIGH ENERGY BALL MILLING:

Coarse-grained structures dissociate during the high-energy ball milling process as a result of severe cyclic deformation caused by milling with stiff balls in a high-energy shaker mill.

- 15 g of the collected shell-derived calcium hydroxide is taken in the zirconium-coated jar.
- 25 zirconium balls were added for fining the calcium hydroxide.
- The powder is milled at 5000 rpm speed for 6 hr.
- After 6 hr the finely milled powder is collected.

4.2 HYDROXYAPATITE VIA WET PRECIPITATION METHOD:

Chemical precipitation is the process to convert the calcium hydroxide to Hydroxyapatite using Diammonium hydrogen phosphate as a phosphate source and a precursor.

- Then one mole of finely milled calcium hydroxide is added to 100 ml of water.
- Then. Diammonium hydrogen phosphate is used as the phosphate source in this process. 0.6 moles of diammonium hydrogen phosphate are mixed in 100 ml of distilled water.
- In a boiling flash, the calcium hydroxide solution is taken and placed in an oil bath arranged at 80 degrees Celsius, unstirred for 5 minutes, and the pH is checked to be 8.
- Then 10 ml of ammonia solution is added to the calcium hydroxide solution and stirred for 10 minutes and the pH is checked to be 12.
- Diammonium hydrogen phosphate solution is added. Drop by drop allowed to mix for 24 hours.
- The solution is collected and centrifuged to obtain the precipitated material.
- The material is dried in an oven at 60 degrees Celsius for 24 hours. The material is then collected.

4.3 PREPARATION OF SILVER NANOPARTICLES FROM ROSE EXTRACT:

Silver nanoparticles were made using the biogenic technique, and anthocyanin was extracted from rose petals treated with 0.1M AgNO₃.

4.3.1 PREPARATION OF ROSE EXTRACT:

- Fresh rose petals (*Rosa indica*) were collected from the koyambedu flower market rose petals were washed multiple times with distilled water to eliminate minor contaminants and dust particles, then placed in a 500 ml clean and dry beaker with 250 ml 98 % ethanol and left to infuse for two hours at room temperature.
- The mixture was filtered using Whatman filter paper 1 to get clear and fine extract after the aqueous extraction by infusing rose in ethanol.
- First, 0.1M silver nitrate solution was made in a conical flask with 100ml of de-ionized water, and then 10 ml of rose petal extract (anthocyanin) was dropped into the silver nitrate solution and stirred for 24 hours.
- The flask was covered with aluminum foil during the operation to avoid photodegradation by preventing sunlight from interacting with the solution.
- The color of the fluid changed, suggesting the formation of silver nanoparticles.

4.4 DOPING PROCESS USING COPRECIPITATION METHOD:

- In this method, one mole of finely milled calcium hydroxide is added to 100 ml of water, followed by preparing diammonium hydrogen phosphate solution by adding diammonium hydrogen phosphate in 100ml of water in a separate beaker.
- In a boiling flash, the calcium hydroxide solution is taken and placed in an oil bath set at 80 degrees Celsius, stirring occasionally for 5 minutes, and the pH is tested to be 8.
- The pH is then tested by adding 10 mL of ammonia solution to the calcium hydroxide solution and stirring for 10 minutes.
- 10ml of previously prepared silver solution with silver Nanoparticles were added drop by drop and allowed to mix for 20 minutes.
- 100ml of diammonium hydrogen phosphate solution is added as a phosphate source drop by drop and allowed to mix for 24 hours.
- To acquire the precipitated material, the solution is collected and centrifuged.

- The material is dried for 24 hours at 80 degrees Celsius in a hot air oven. After that, the material is then grained using a pestle and mortar and collected for characterization.

4.4 X-RAY DIFFRACTION:

The use of X-ray diffraction (XRD) to characterize crystalline materials is a powerful non-destructive technique. It describes structures, phases, preferred crystal orientations (texture), and other structural parameters such as average grain size, crystallinity, strain, and crystal defects. Constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample produces X-ray diffraction peaks. The distribution of atoms within the lattice determines the peak intensities. Atoms in crystalline material are arranged in periodic patterns of three-dimensional barriers. The atoms in amorphous are arranged chaotically and randomly. X-ray diffraction data can be used to calculate the interlunar distance between two successive planes of atoms. When a monochromatic X-ray beam interacts with a target material, the scattering of such X-rays from atoms within the target material is the most important effect. In materials with a normal structure, dispersed X-rays interact both constructively and destructively (i.e. crystalline).

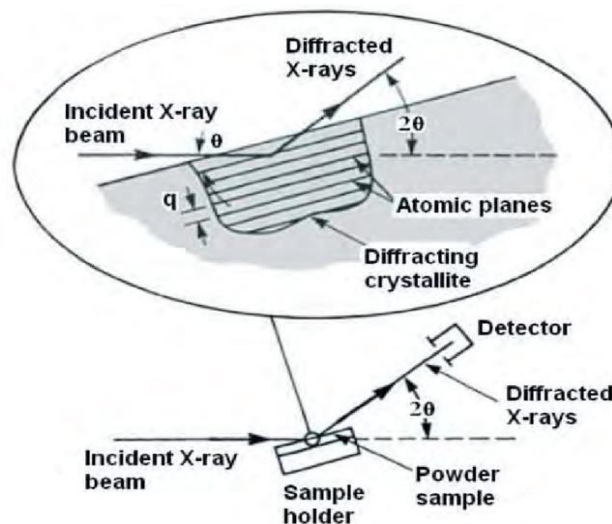


Figure 4.2 X-Ray diffractometer

The diffraction method is used in this example. X-ray diffraction by crystals is described by Bragg's Law, $n = 2d \sin$. Potential diffraction directions are determined by the size and shape of the material's unit cell. The intensity of diffracted waves is

determined by the atoms' type and arrangement in the crystal structure. An X-ray beam focused on a powder of arbitrarily oriented crystallites can see all possible interatomic planes. If the experimental angle is systematically changed, all possible diffraction peaks from the powder will be detected. As a result, x-ray diffraction was used to examine the sample's molecular and atomic structure. Samples were scanned from 10° to 100° (2), at a scan rate of $0.02^\circ/\text{s}$ and a scan step time of 32 s. The generator was set to 40kV and 25mA. The Lynx Eye detector was used.

4.5 FTIR (Fourier Transform Infrared):

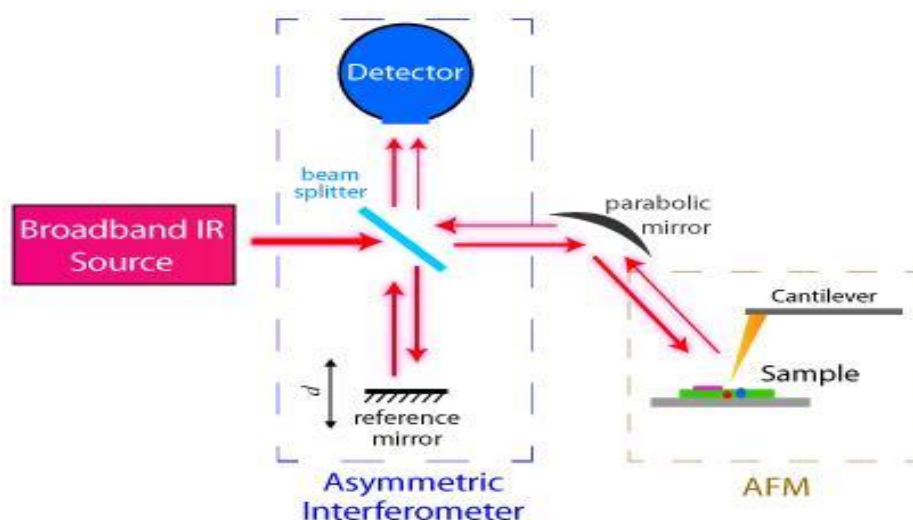


Figure 4.3 FTIR Spectrometer.

FTIR (Fourier Transform Infrared) Spectroscopy is also known as FTIR Analysis or FTIR Spectroscopy. This method of infrared spectroscopy is used to identify organic, polymeric, and, in some cases, inorganic materials. Infrared light is used in the FTIR test to scan samples and observe bond properties. When there are unknowns, FTIR analysis services can identify compounds and the general type of material being analyzed. This technique is used to determine the purity of some inorganic samples and is extremely accurate for determining polymer composition. The Michelson interferometer is at the heart of all modern FTIR spectrometers. It is made up of a beam splitter, a fixed mirror, and a moving mirror.

Fourier Transform Spectroscopy is a fundamental mathematical technique for separating the frequency components of a complex wave. Traditional IR spectrometers are ineffective in far IR because the sources are weak and the detectors are insensitive. Because of FTIR, this energy-constrained area has

become more accessible. It has the interference pattern is generated by a two-beam interferometer, and the spectrum is generated by Fourier transforming the output as the path difference between the two beams changes. Solid samples are spread in KBr or polyethylene pellets, depending on the area of interest. FTIR analysis determines a sample's molecular composition and structure by measuring its absorbance of infrared light at various wavelengths. However, it is simpler to consider transmittance rather than absorbance, so the FTIR graph is a transmittance (percentage) vs. wavenumber plot. Also increased the utility of the mid-infrared (4000–400 cm^{-1}) wavelength. The Fourier Transform Spectrometer converts a time-domain plot into a frequency domain spectrum.

4.6 SCANNING ELECTRON MICROSCOPE (SEM):

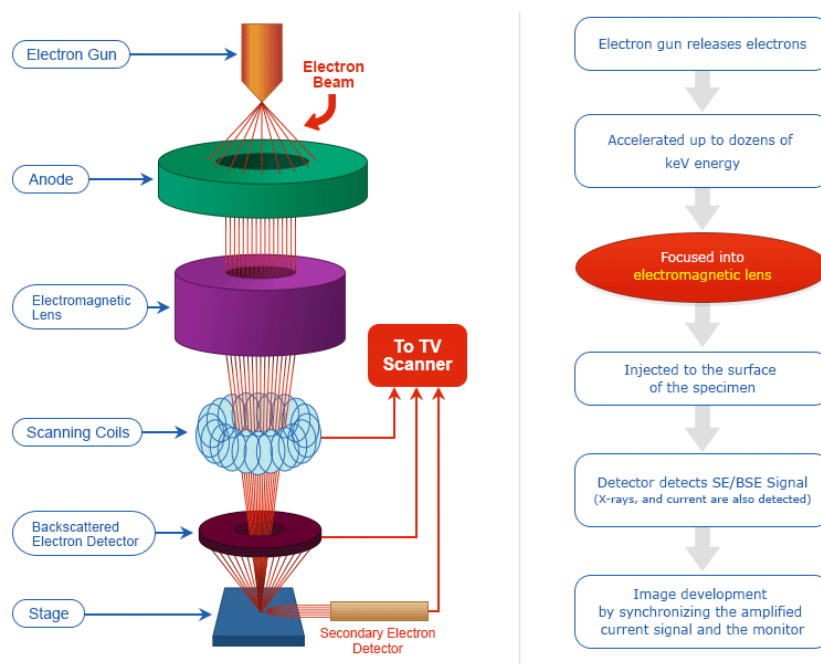


Figure 4.4 Scanning Electron microscope.

The scanning electron microscope, which is more recent than the TEM, is used to create images of the surface of specimens. SEM is a type of electron microscope that scans the surface of materials by focusing and scanning specimens with a beam of electrons moving at low energy. The walking principle. Unlike the transmission electron microscope. Users of the scanning electron microscope emitted electrons. The primary electron beam is produced by a scanning electron microscope and an electron gun. These beams are directed over the surface of the

specimen after passing through electromagnetic lenses. The primary electron beam knocks electrons out of the surface of the specimen and the secondary electrons thus produced are transmitted to an electron collector, amplified and used to produce an image on a viewing screen or photographic plate. The image is called a scanning electron micrograph. The electron column is where the electron beam is generated under vacuum, focused to a small diameter and scanned across the surface of a specimen by electromagnetic deflection coils. The lower portion of the column is called the specimen chamber.

The electron beam is thermos-ionically emitted from an electron gun fitted with a tungsten filament cathode. These thermos-electrons are gathered as an electron beam flowing into the metal plate anode. By applying a positive voltage (1-30 Kv) to the anode, if the hole is made at the center of the anode, the electron beam flows through this hole. If the Wehnelt electrode is placed between the cathode and anode and applies a negative voltage to it, we can adjust the current of the electron beam. At this time, the electron beam is finely focused by the action of the Wehnelt electrode. By applying a positive voltage (1-30 Kv) to the anode, if the hole is made in the center of the anode, the electron beam will pass through it. We can control the current of the electron beam by connecting the cathode and anode with the Wehnelt electrode and applying a negative voltage to it. The action of the Wehnelt electrode finely focuses the electron beam at this point. After the beam passes through the anode, it is influenced by two condenser lenses, which cause the beam to converge and pass through the focal point. The aperture's function is to reduce and exclude extraneous electrons from the lenses. The final lens aperture located below the scanning foils determines the diameter or spot size. The resolution and depth of the field will be determined in part by the size of the spots on the specimen. The reduced spot size will also result in increased resolution and depth of field at the expense of brightness.

4.7 ENERGY-DISPERSIVE X-RAY SPECTROSCOPY (EDAX):

In the x-ray method, the elemental composition of materials is determined using Energy Dispersive X-Ray Analysis (EDX), also known as EDS or EDAX. This method is suitable for the material and product testing as well as troubleshooting. This is a technique for determining the chemical or elemental composition of a

sample. It is based on the interaction of a sample with an X-ray source. Its ability to characterize elements is largely due to the basic concept that each element has a unique atomic structure that allows for a unique collection of peaks on its electromagnetic emission spectrum. [19]. It's a powerful but straightforward method for determining which elements and, by extension, chemical compounds are present in a sample. EDAX consists of detecting the characteristic X-rays emitted by each element after bombarding a sample with high-energy electrons in an electron microscope. Using a technique known as X-ray mapping, details about a sample's elemental composition can then be overlaid on top of a magnified image of the sample. The amount of X-rays produced by each element present in a sample is proportional to the concentration of that element, making EDS particularly useful (mass or atomic fraction). This is how X-ray measurements can be converted into a final X-ray spectrum and the amounts of various chemicals found in a sample determined. Material science is without a doubt the discipline that has benefited the most from EDAX.

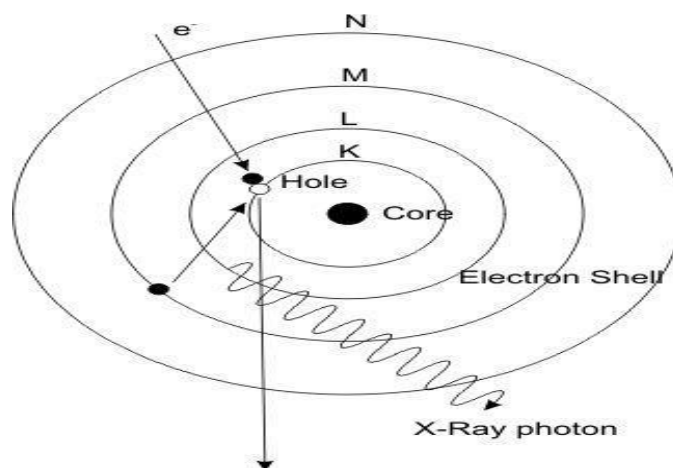


Figure 4.5 Working Principle of EDX

4.8 TRANSMISSION ELECTRON MICROSCOPE (TEM):

Transmission electron microscopy (TEM) is a high-resolution technique used to reveal structural details, size distribution, and morphology of lipid and protein nanoparticles, as well as hard nanoparticles made of metallic particles, carbon, or plastics. The interaction of a high-energy electron beam with a thin sample is the foundation of TEM. Images of the nanoparticle are produced by amplitude and phase

variations in the transmitted beam. Negative-stain, freeze-fractured, and cryogenic TEM can be classified based on sample preparation. Nanoparticles are adsorbed onto the support of an electron microscope and a heavy-metal stain is applied in negative-stain TEM. Although this type of TEM is simple to use, its use in nanoparticle research is hampered by artifacts such as aggregation, particle collapse, and nonuniform deposition during sample preparation. Another limitation of this technique is the difficulty in controlling the adsorption of nanoparticles to the electron microscope support. TEM and SEM both show the size, degree of aggregation, and dispersion, as well as the heterogeneity of nonmaterial. When compared to SEM, TEM has more advantages in terms of providing spatial resolution in high-quality and analytical measurements. The TEM provides precise particle size of bright field images as well as darkfield images, and it provides details about nanoparticles by utilizing energetic electrons to provide information about morphologic, compositional, and crystallographic information. The three main techniques used in TEM are imaging, spectroscopy, and diffraction. TEM works based on optical microscopy. Electrons are replaced by photons, and glass lenses with electromagnetic lenses and images are viewed on a screen rather than through an eyepiece. The benefits of TEM include high magnification and the ability to provide information about compound and element structures. TEM requires specialized maintenance, and only black-and-white images are acceptable as output [34].

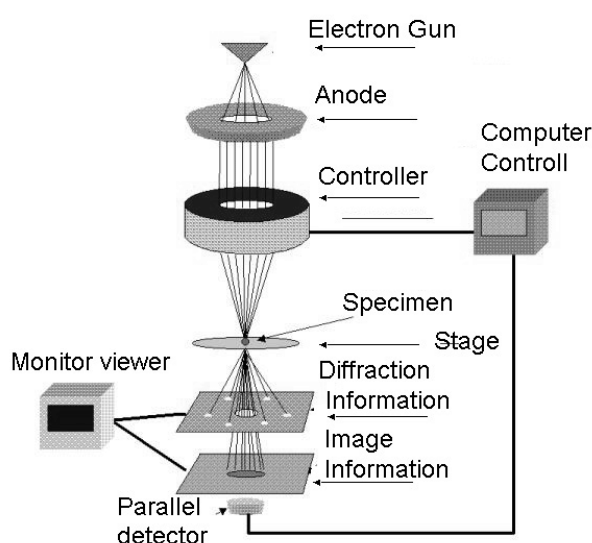


Figure 4.6 Transmission electron microscope.

4.9 MTT ASSAY:

To investigate the antitumor activity of synthesized silver doped HAp nanoparticles, it is necessary to first determine the cytotoxicity concentration of the synthesized silver doped HAp. The cytotoxicity test determines the highest concentration of extract that is not toxic to cell lines. Cell death and viability were estimated after the addition of the synthesized silver nanoparticles. Additional metabolic intervention experiments, such as the MTT assay, confirm the result. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) A cytotoxic drug is administered to cells that are in the exponential phase of growth. The duration of exposure is usually determined by the time required for maximum damage to occur, but it is also influenced by the drug's stability. After the drug is removed, the cells are allowed to proliferate for two to three populations doubling times (PDTs) to distinguish between cells that are viable and capable of proliferation and those that are viable but cannot proliferate. The number of surviving cells is then determined indirectly through MTT dye reduction. After the MTT–formazan has been dissolved in a suitable solvent, the amount of MTT–formazan produced can be determined spectrophotometrically.

4.9.1 PROCEDURE:

T-25 flask was trypsinized and 5 ml of complete media was added to the trypsinized cells. 15 ml of sterile falcon tube was centrifuged at 500 rpm in the swinging bucket rotor (~400 x g) for 5 min. the media was removed and the cells were resuspended to 1.0 ml with complete media. The Cells were removed aseptically. They were then counted and recorded per ml. the cells (CV=CV) were diluted to 75,000 cells per ml. complete media was used to dilute the cells. 100µl of cells (7500 total cells) was added into each well and incubated overnight. The Vero cells and the Hep 2 cells were grown in six-well plates and the media was removed using the micropipette. The cells were washed with phosphate buffer saline. The cells were then fixed with 1ml of cold ethanol and were incubated for about 5 mins. After fixation, the cells were then washed with distilled water. The fixed cells were then viewed under a normal light microscope of radical 40x magnification T-25 flask was trypsinized and 5 ml of complete media was added to the trypsinized cells. 15 ml of sterile falcon tube was centrifuged at 500 rpm in the swinging bucket rotor (~400 x g) for 5 min. the media was removed and the cells were resuspended to 1.0

ml with complete media. The Cells were removed aseptically. They were then counted and recorded per ml. the cells (CV=CV) were diluted to 75,000 cells per ml. complete media was used to dilute the cells.100µl of cells (7500 total cells) was added into each well and incubated overnight. The spent media was removed and fresh media and synthesized silver nanoparticles of varying concentrations were added. The final volume was 200µl per well. The MTT plate was incubated in a CO₂ incubator for 4 hrs at 37°C. After 4 hrs, the media and the synthesized silver nanoparticles were removed into the new MTT 96 well plate. 20µl of 5 mg/ml MTT and 80µl of media was added to each well. One set of wells was included with MTT but no cells were added to the control under aseptic conditions. The plates were then incubated for 3.5 hours at 37°C in a culture hood. The media was removed. The cells were not disturbed and it was not rinsed with PBS.150µl of DMSO (MTT solvent) was then added. The plates were then covered with aluminum foil and the cells were mixed with DMSO. The absorbance was read at 570 nm with a reference filter at 630 nm.

$$(\%) \text{ Percentage of cytotoxicity} = \frac{(\text{control} - \text{sample}) \times 100}{\text{Control}}$$

The Vero cells and the Hep 2 cells were grown in six-well plates and the media was removed using the micropipette. The cells were washed with phosphate buffer saline. The cells were then fixed with 1m.

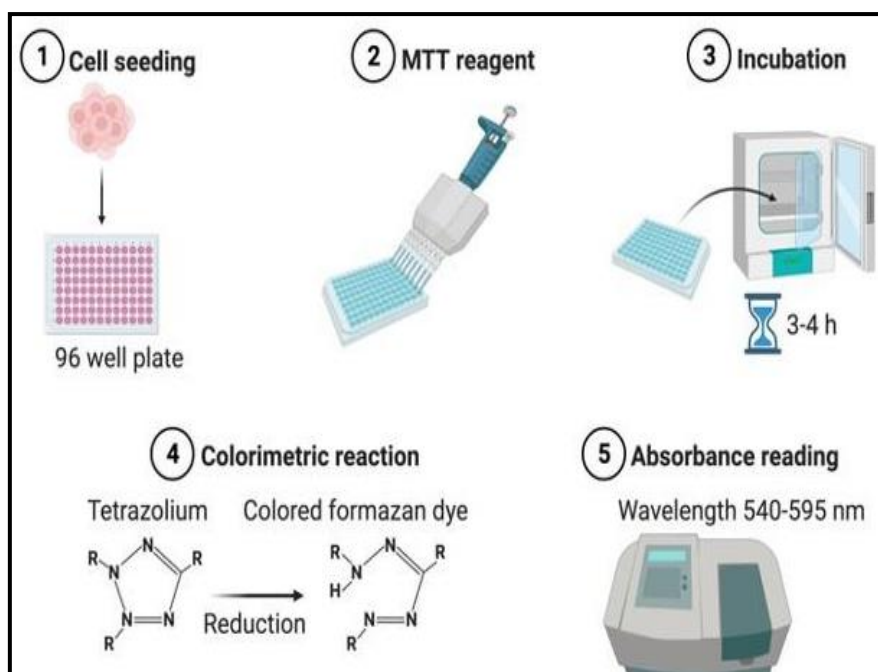


Figure 4.7 Procedure of MTT Assay.

4.10 ANTIMICROBIAL ACTIVITY:

The antibacterial assays were performed on *Escherichia coli* and *Staphylococcus aureus*, *B. subtilis*, *Listeria monocytogenes*, and *Salmonella Typhi* using the diffusion method. To summarise, bacteria were grown on Nutrient broth/agar medium. Fresh overnight cultures of inoculum (100 l) from each culture were spread on nutrient agar plates. The cork borer was used to drill five wells: one in the center and four around it. In the center well, a 15g standard erythromycin antibiotic disc was placed, and the surrounding wells were loaded with 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl of the synthesized nanoparticle solutions from *Ananas comosus*. The plates were then incubated in an incubator at 370°C for 24 hours before being visually examined for the formation of ZOI (zone of inhibition).

CHAPTER 5

5 RESULT AND DISCUSSION:

5.1 SEM IMAGES AND EDAX

SEM images can help us understand the morphology of the particles and learn more about their sizes. It is a surface test that only provides an overview of the surface.

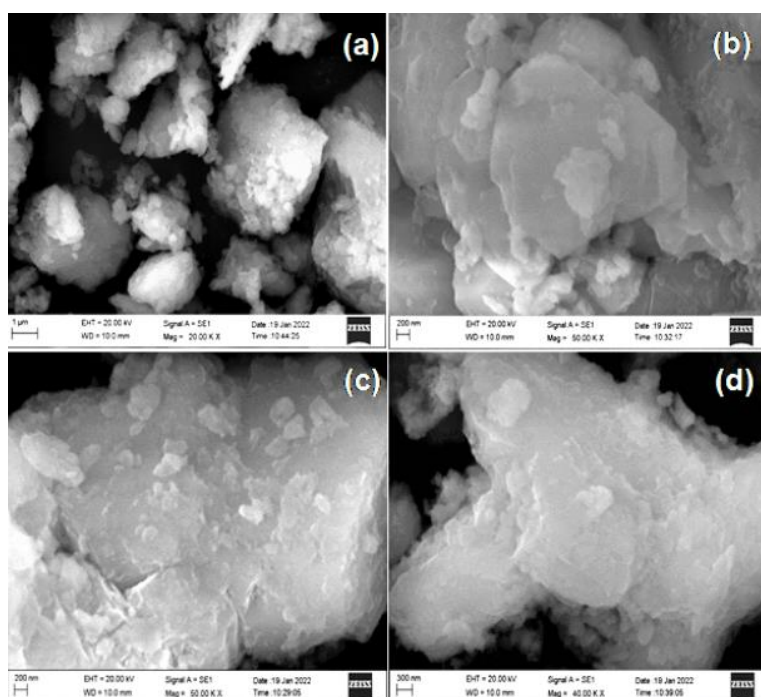


Figure 5.1 SEM images of Calcium Hydroxide.

The surface morphology of calcium hydroxide was investigated by scanning electron microscope the result of characterization shows that calcium hydroxide, where having irregular shapes with slight agglomeration. The size distribution ranges from 200nm-1 μ m.

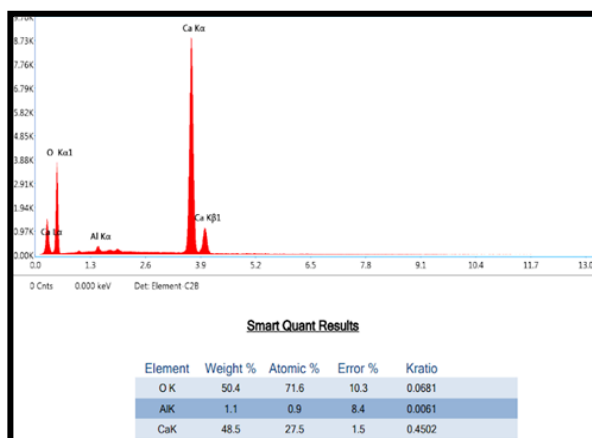


Figure 5.2 EDAX Spectra of Calcium Hydroxide

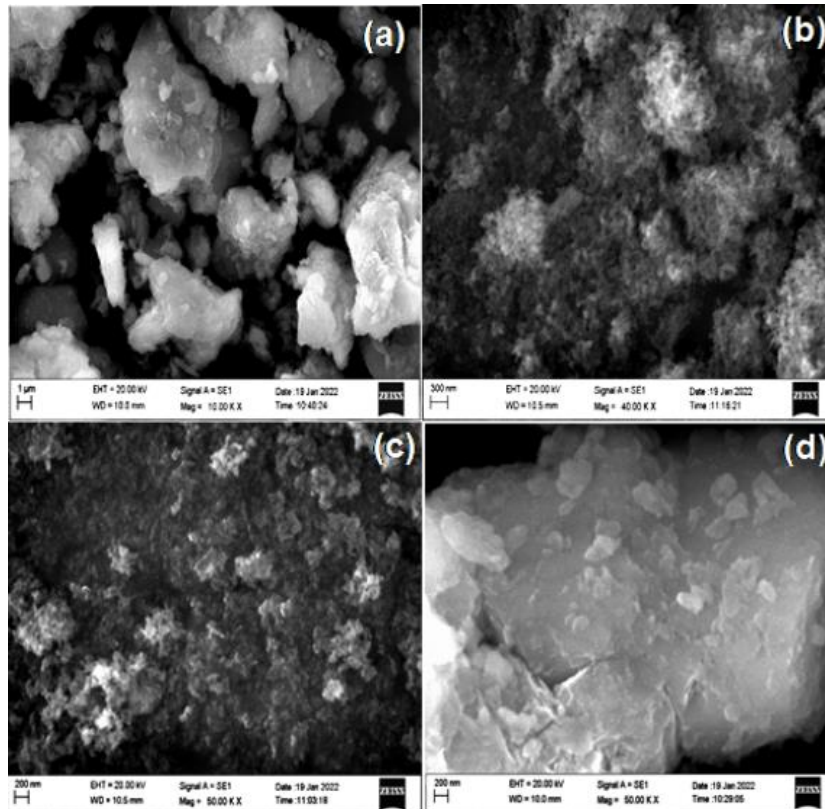
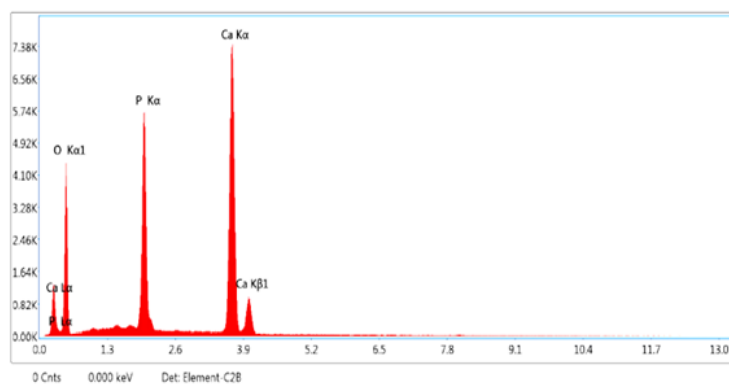


Figure 5.3 SEM images of Hydroxyapatite.

The result and characterization show that the hydroxyapatite synthesized is slake-shaped With a size distribution ranging from 200nm-1 μ m.



Smart Quant Results

Element	Weight %	Atomic %	Error %	Kratio
O K	45.9	66.1	10.2	0.0687
P K	16.0	12.0	3.1	0.1286
CaK	38.1	21.9	1.8	0.3431

Figure 5.4 EDAX Spectra of Hydroxyapatite

5.1.3 SEM IMAGES AND EDAX SILVER DOPED HYDROXYAPATITE:

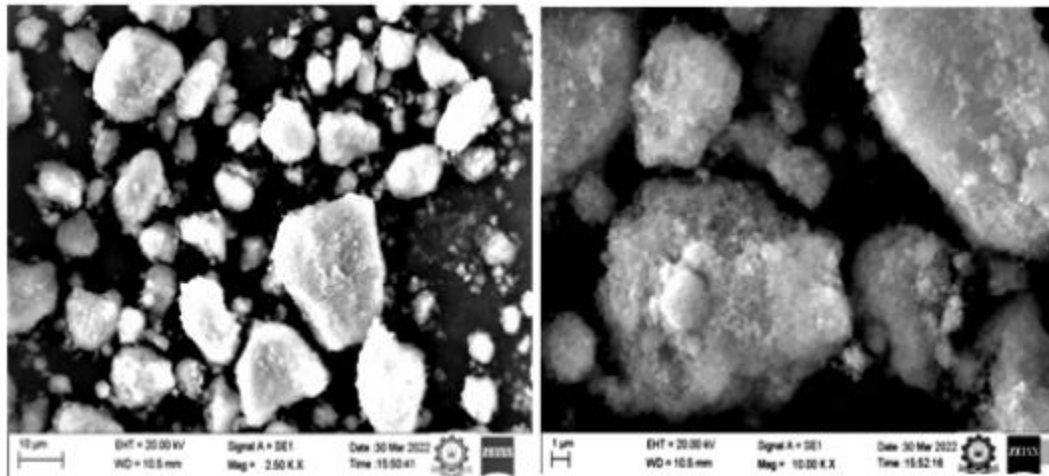
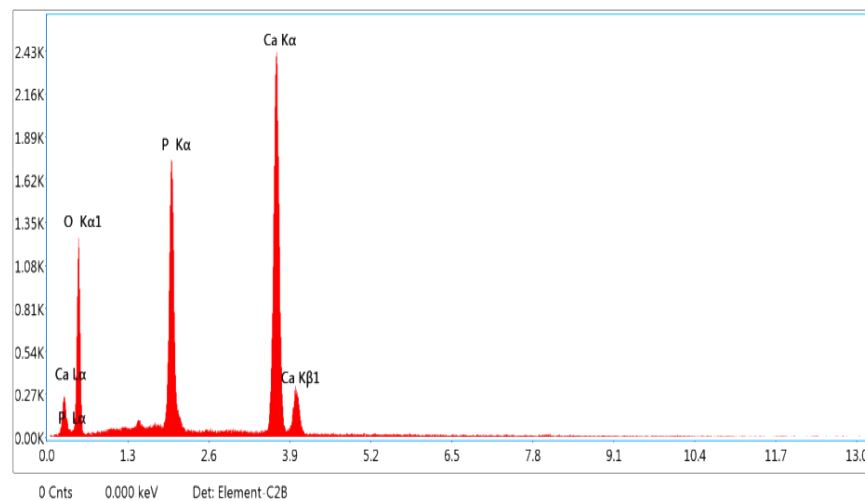


Figure 5.5 SEM images of Silver doped Hydroxyapatite.

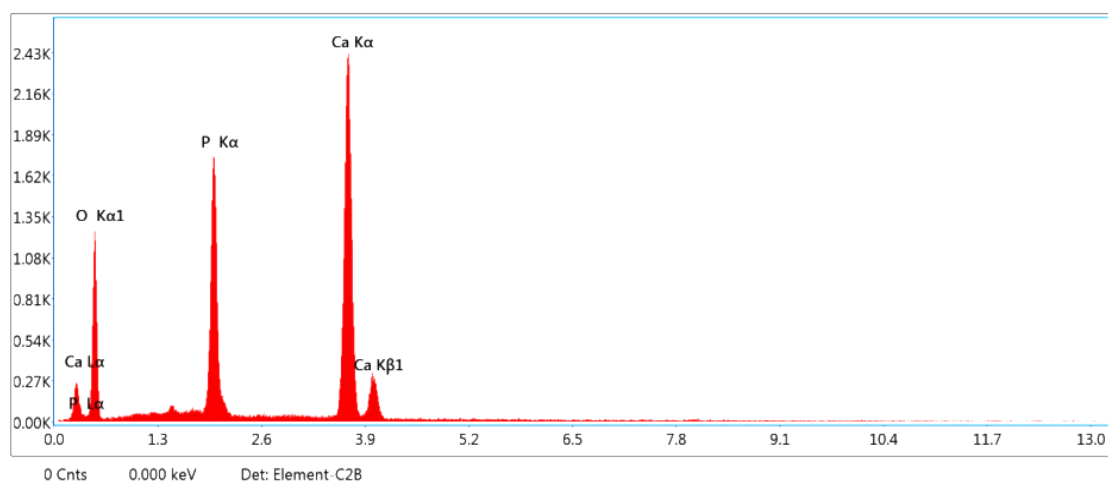
The surface morphology of silver doped hydroxyapatite showed the distribution size measured for this material was 1µm.



Smart Quant Results

Element	Weight %	Atomic %	Error %	Kratio
O K	44.7	65.1	10.8	0.0658
P K	16.1	12.1	3.5	0.1291
CaK	39.3	22.8	2.1	0.3544

Figure 5.6 EDAX Spectra of Silver Doped Hydroxyapatite



Smart Quant Results

Element	Weight %	Atomic %	Error %	Kratio
O K	44.7	65.1	10.8	0.0658
P K	16.1	12.1	3.5	0.1291
CaK	39.3	22.8	2.1	0.3544

Table 5.3 EDAX Spectra of Silver doped Hydroxyapatite

5.2 TRANSMISSION ELECTRON MICROSCOPE:

5.2.1 TEM image of hydroxyapatite:

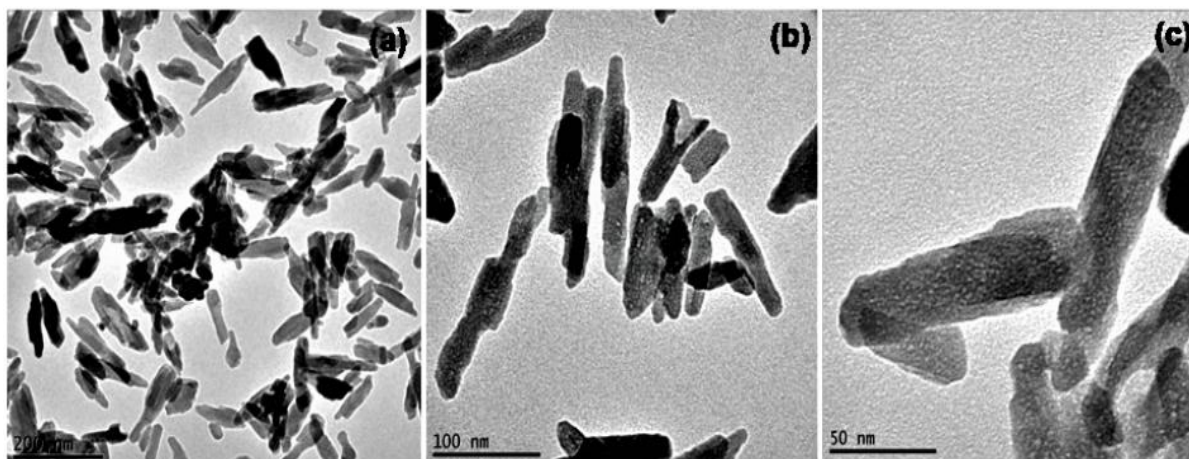


Figure 5.7 TEM image of hydroxyapatite:

The structure and morphology of the samples were further confirmed by the TEM and TEM images of the prepared nano-hydroxyapatite, as shown in Figure. The transmission electron microscopic analysis confirms the presence of the rod shape morphology of the prepared hydroxyapatite nanoparticle with a particle size of

around 100nm-50nm. The d-spacing values can now be determined by measuring the distance between two symmetric diffraction points in the FFT image.

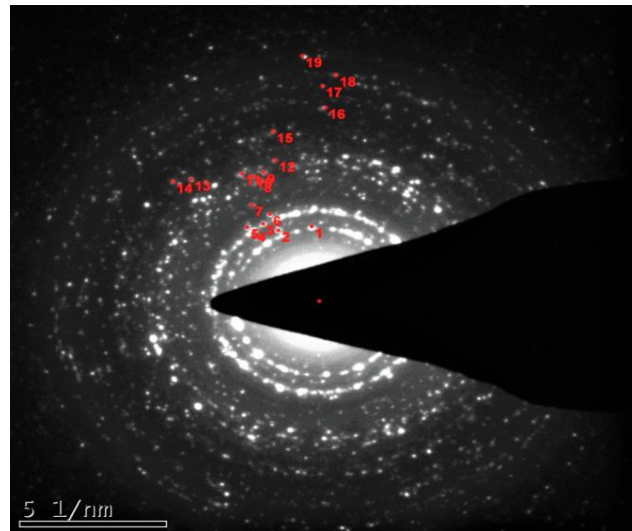


Figure 5.8 SAED (Selective Area Electron Diffraction) pattern for HAp

Table 5.4 D-Spacing (HAp)

Spot	d-spacing
1	0.3127
2	0.3274
3	0.2766
4	0.2350
5	0.2368
6	0.2023
7	0.1952
8	0.1992
9	0.1592
10	0.1459
11	0.1331
12	0.1229
13	0.1241
14	0.11030
16	0.09473
17	0.09270

Table 5.4 shows the d-spacing values which represent **the distance between two symmetric diffraction points**. Figure 5.8 shows the d-spacings of the planes with 2theta values 9,10,11 which nearly overlapped with each other. The figure shows the SAED (Selective Area Electron Diffraction) pattern.

5.2.2 TEM IMAGE OF SILVER DOPED HAP:

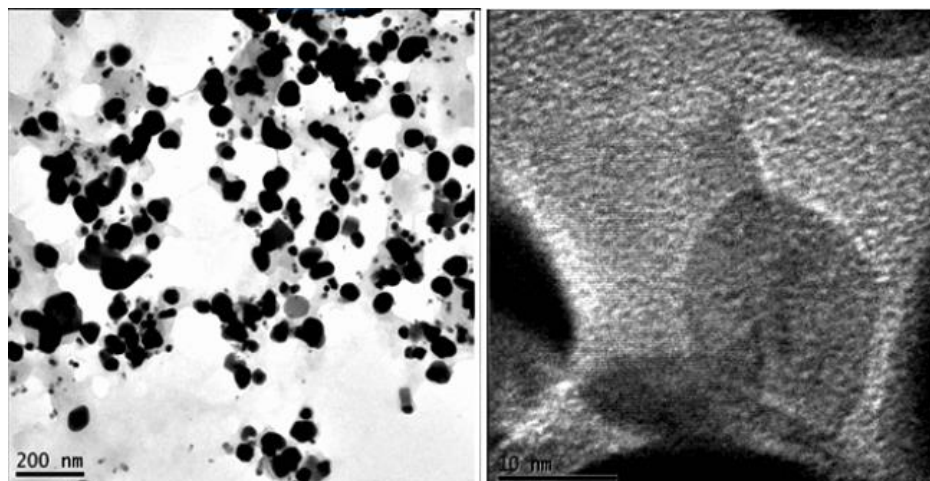


Figure 5.9 TEM image of Silver doped Hydroxyapatite.

The transmission electron microscopic analysis confirms the presence of the rod shape morphology of the prepared hydroxyapatite nanoparticle with a particle size of around 200nm from the about figure

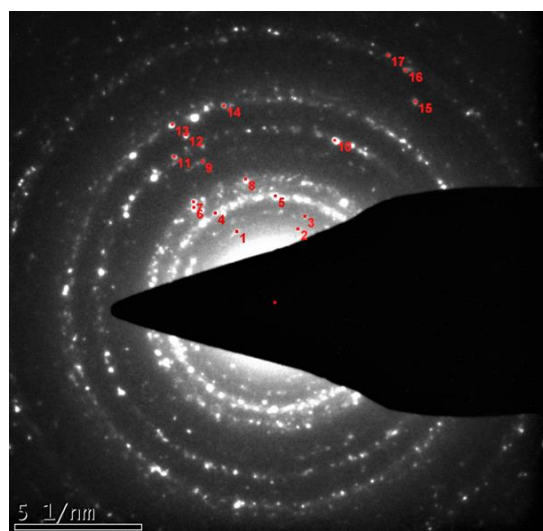


Figure 5.10 SAED (Selective Area Electron Diffraction) pattern for Ag/Hap.

Figure 5.9 shows the SAED (Selective **Area Electron Diffraction**) pattern for the silver doped hydroxyapatite form by the coprecipitation method.

Table 5.5 D-Spacing (*SILVER DOPED HAP*)

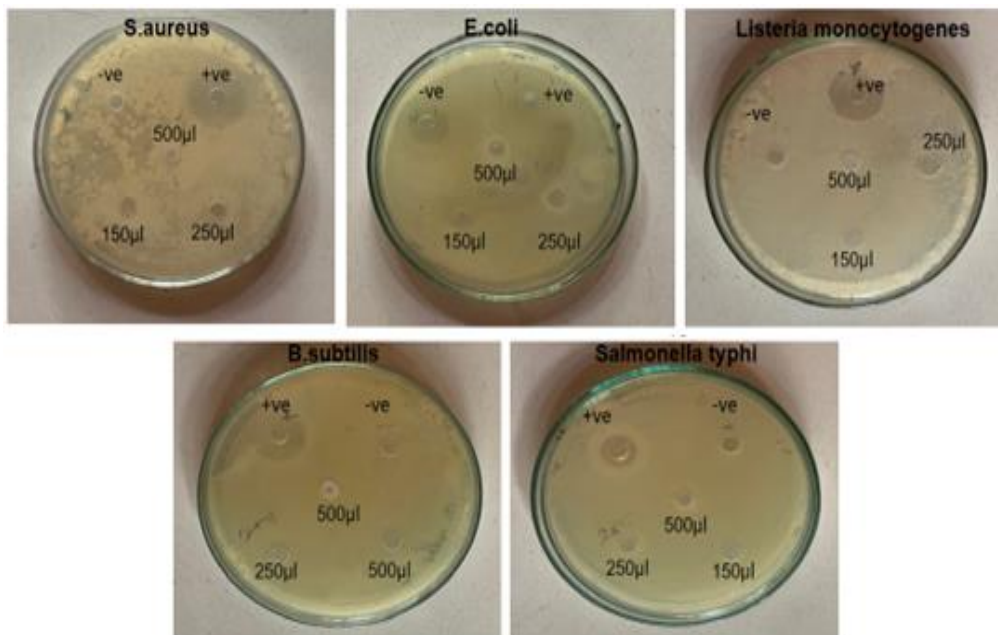
Spot	d-spacing
1	0.3427
2	0.3234
3	0.2800
4	0.2826
5	0.2645
6	0.2274
7	0.1959
8	0.1860
9	0.1858
10	0.1778
11	0.1757
12	0.1547
13	0.1462
14	0.1465
15	0.1319
16	0.1187
17	0.1128
18	0.1040

The d-spacing values, which represent the distance between two symmetric diffraction spots, are shown in the table.

5.3 ANTIMICROBIAL ACTIVITY:

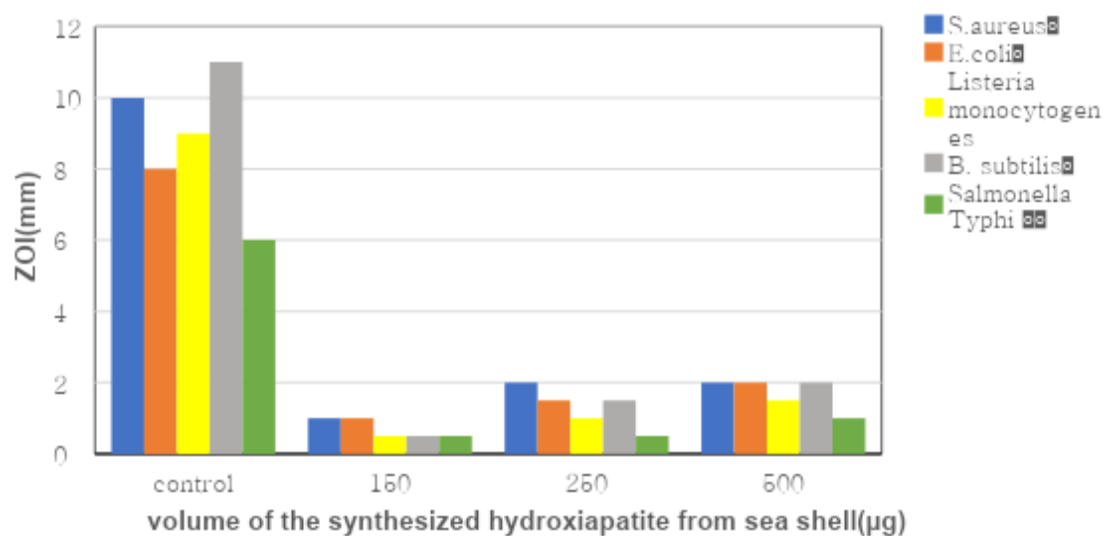
Furthermore, nanoparticles showed a very small amount of antibacterial activity against *S.aureus*, *E.coli*, *B. subtilis*, *Listeria monocytogenes*, and *Salmonella Typhi* due to the presence of natural sea urchins

5.3.1 ANTIMICROBIAL ACTIVITY OF HYDROXYAPATITE :



Figures 5.11 Streak plating for Hydroxyapatite

Figures 5.10 show that the antimicrobial Kirby-Bauer test is depicted in this diagram. There was a 1-2mm inhibition zone visible. As a result, this chemical has only a minor ability to inhibit microbial activity. For each plate



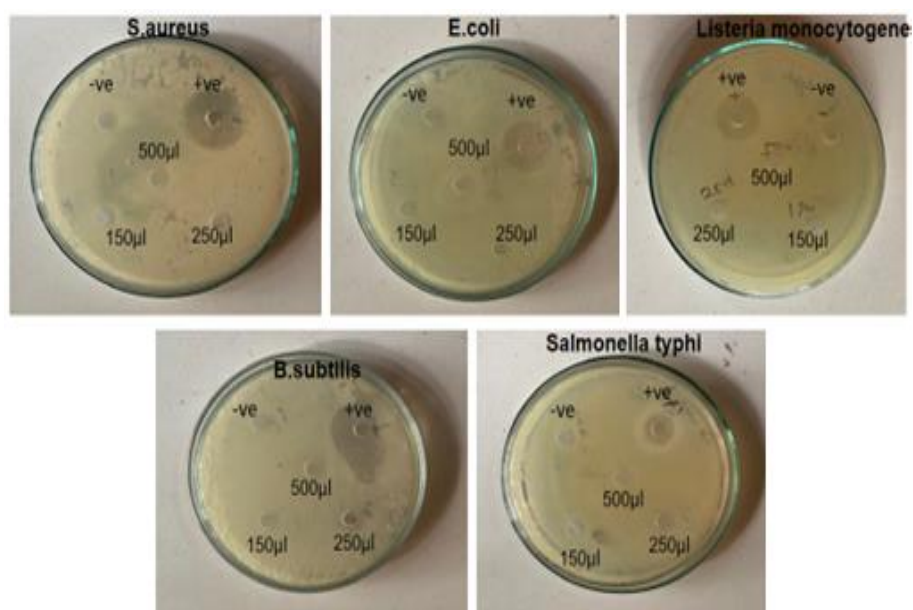
Figures 5.12 ZOI formed by Hydroxyapatite

Table 5.6 ZOI formed by Hydroxyapatite

The table shows the ZOI (mm) formed by synthesized Hydroxyapatite from seashells

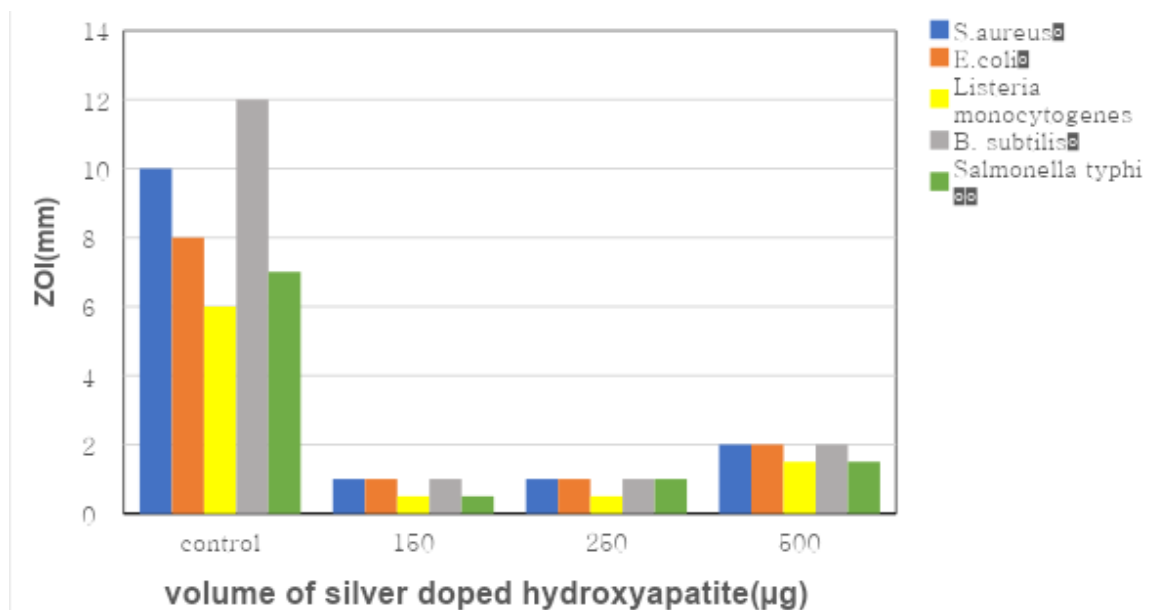
	S.aureus	E.coil	B. subtilis	Listeria monocytogenes	Salmonella Typhi
Control (15 µg)	10	8	9	11	6
150 µl	1	1	0.5	0.5	0.5
250 µl	2	1.5	1.5	1.5	0.5
500 µl	2	2	2	2	1

5.3.1 ANTIMICROBIAL ACTIVITY OF SILVER DOPED HYDROXYAPATITE :



Figures 5.13 Streak plating for silver doped Hydroxyapatite

It revealed a 1-2mm inhibition zone. As a result, this substance has very little potential to restrict microbial activity.



Figures 5.14 ZOI formed by silver doped Hydroxyapatite

Table 5.7 ZOI formed by Silver - Hydroxyapatite

The table represents ZOI (mm) formed by synthesized Silver doped Hydroxyapatite

	S.aureus (mm)	E.coil (mm)	B. subtilis (mm)	Listeria monocytogenes (mm)	Salmonella Typhi (mm)
Control (15 µg)	10	8	6	12	7
150 µl	1	1	0.5	1	0.5
250µl	1	1	0.5	1	1
500µl	2	2	1.5	2	1.5

5.4 X-RAY DIFFRACTION (XRD) PATTERN :

Powder XRD was used to characterize the products produced. The (002), (102), (211), (300), (212), (311), (113), (620), (222), (312), (213) diffraction peaks were detected at 25°, 28°, 31°, 32°, 33°, 39°, 42°, 43°, 45°, 46°, 48°, 49° for Hap readings. A tiny impurity peak may also be found at 30°, 35°, 43°, and 57°, which has an impact on purity. The given hkl values and the 2θ values have been cross-checked with the previous data available and comply with the previous results. The (111), (002), (102), (211), (300), (202), (301), (113), (222), (320), (213) diffraction peaks were detected at 22°, 25°, 29°, 31°, 32°, 33°, 35°, 43°, 46°, 48° and 49° for Ag/Hap. The crystallite size and the average crystallite size were also calculated from the obtained data using the Debye Scherrer equation.

$$\text{The Scherrer equation is: } D = 0.9\lambda / \beta \cos \theta$$

Where, D – crystallite size in nm

K – Scherrer constant = 0.9

λ – Wavelength of the x-ray used = 0.154058 nm

β – FWHM in radians θ – peak positions in radians

FWHM and peak positions were calculated from the plot using the Origin application, after which the size was calculated by applying the above formula. The Hap's average crystallite size is 20.86 nm (calculated using the above equation) which is equivalent to natural bone crystallite size, and the lattice strain is 0.0055 nm.

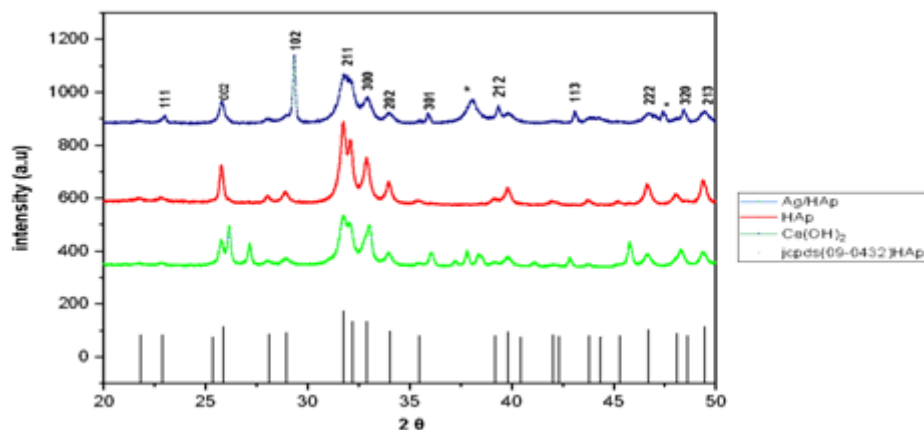


Figure 5.15 XRD Pattern for Calcium Hydroxide, Hap, Ag/Hap
* represents impurities.

5.5 The Fourier Transform Infra-red analysis:

The Fourier Transform Infra-red analysis is used to identify the functional group present in the crystal. The FTIR spectrum of the AgHAp has been recorded on a spectrophotometer within the wavenumber range 500 cm^{-1} to 4000 cm^{-1} . The peak observed at 3566.4 cm^{-1} corresponds to O-H stretching bond. Whereas, the peak at 1643.35 cm^{-1} shows the presence of —C=C— stretch. The band at 1458.18 cm^{-1} is for the C—H bond. The peak observed at 565 cm^{-1} corresponds to the C—Cl stretch. Most peaks appeared to be medium.

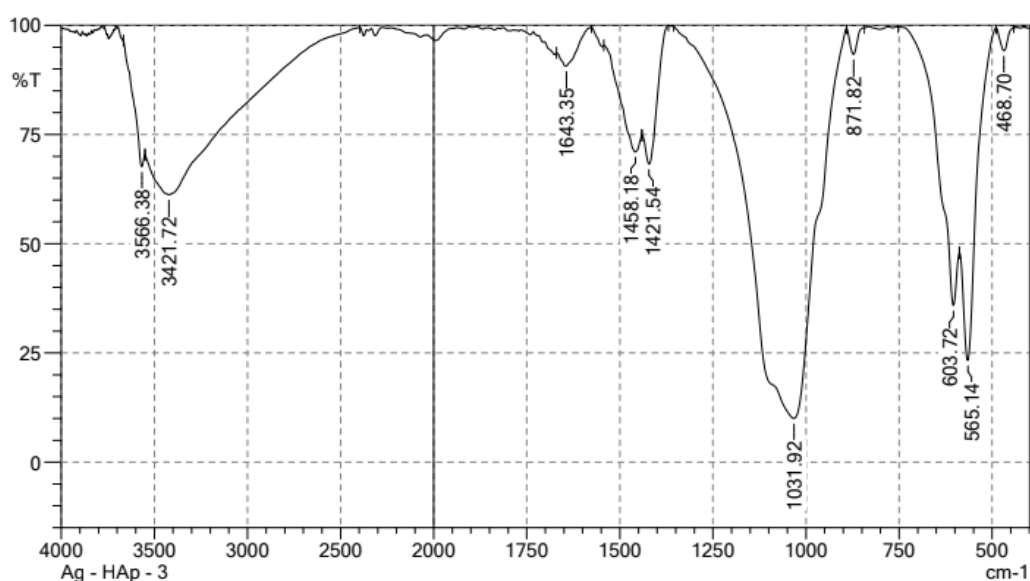


Figure 5.16 FTIR spectrum of Ag/Hap.

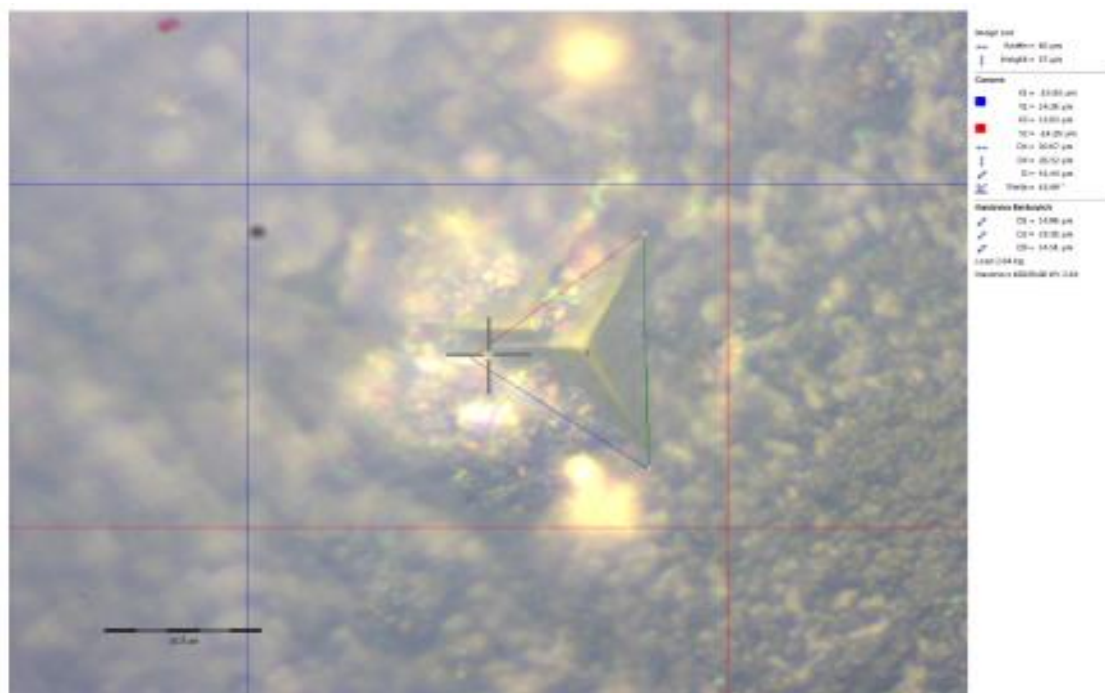
Table 5.8 : Functional group corresponding to the spectral peak

3566.38	OH stretch	Alcohols, water	Strong
3421.72	O—H stretch, H—bonded	alcohols, phenols	Strong, broad
1643.35	—C=C— stretch	alkenes	Medium
1458.18	C—H bend	alkanes	Medium
1421.54	C—C stretch (in—ring)	aromatic	Medium
1031.92	C—N stretch	Aliphatic amines	Medium
871.82	C—H “oop”	aromatics	Strong
603.72	C—Br stretch	alkyl halides	Medium
565.14	C—Cl stretch	Alkyl halide	Medium
468.70	(C—I) stretch	Halogen compound	Strong

5.6 HARDNESS TEST:

The hardness test was performed on an automated hardness tester with a Vickers diamond indenter (Model No-LV-700AT, LECO Co, MI) with a maximum load of 100.00mN applied to two samples of each composition type during the hardness test, and they were assessed for hardness at three distinct sites with the applied loads. It was calculated, reported, and compared the average of these data. For the applied load the obtained Vickers Hardness is 92,878 (Vickers) for the shell derived Hap and 91.26 (Vickers) for the silver doped Hap.

5.6.1 INDENTATION IMAGE OF Hap:



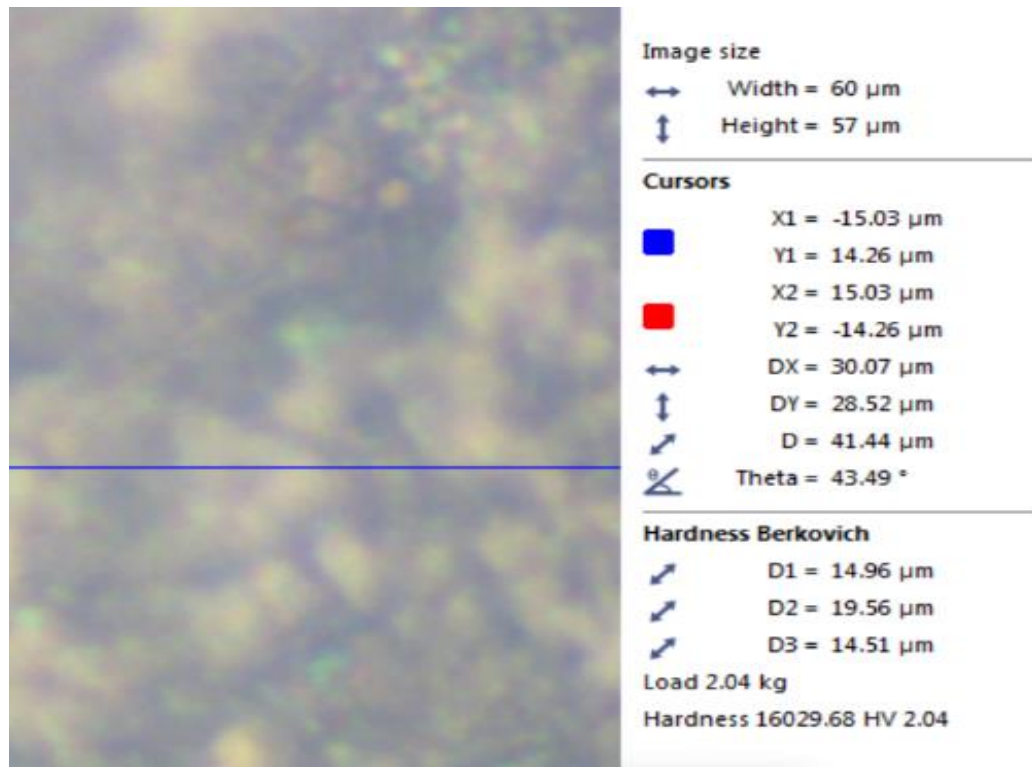


Figure 5.17 Indentation image

Table 5.9 Hardness test

Material	Vickers hardness HV_{IT} (Vickers)	Young's Modulus E_r (GPa)	Indentation Modulus E_{IT} (GPa)	Indentation Hardness, HIT (MPa)
HAp	92.878	18.397	19.850	1002.881
Ag/HAp	91.26	985.16	27.08	985.16

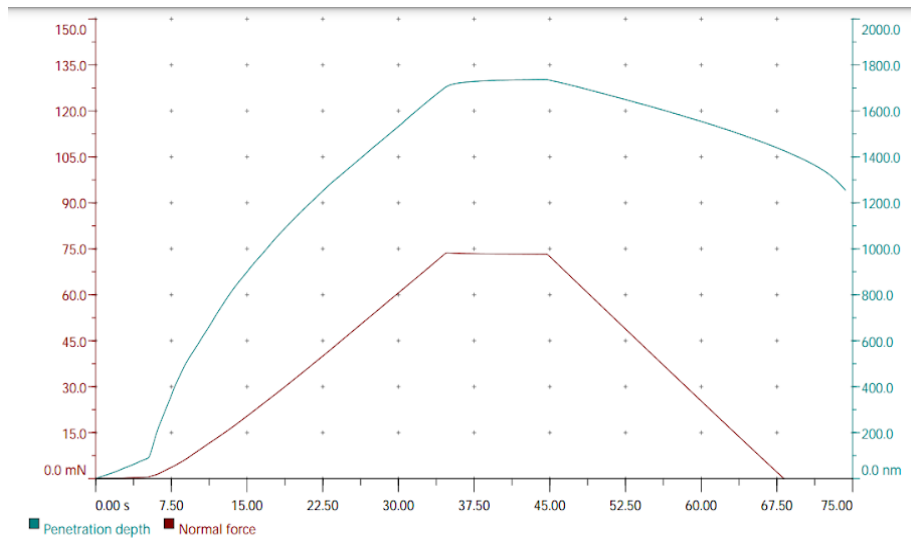


Figure 5.18 Indentation curve (Hap)

The graph shows the indentation curve obtained for the applied loads for the Ag/doped Hap.

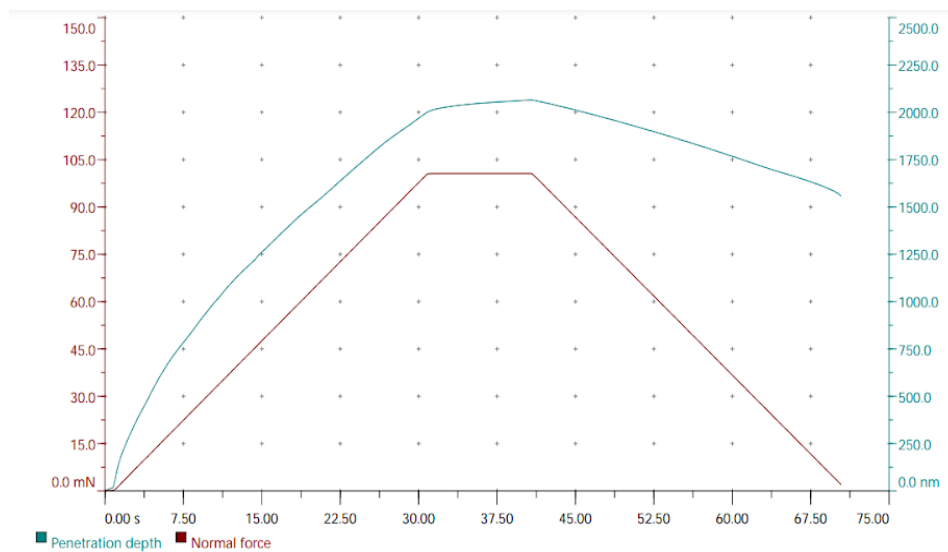


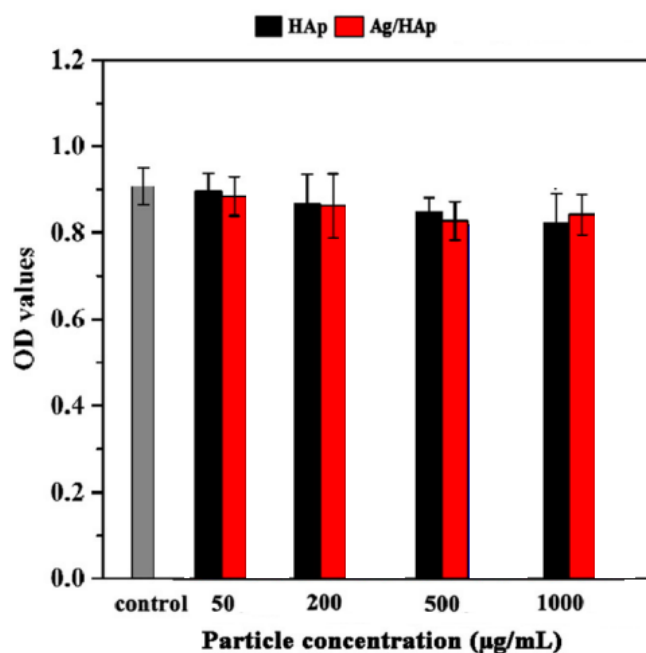
Figure 5.19 Indentation curve (Ag/Hap)

The graph shows the indentation curve obtain for the applied loads for the Ad/doped Hap.

5.7 MTT ASSAY

Cytotoxicity studies are robust studies and, in recent years, were considered as one of the most important factors for the development of new bone substitutes. Interestingly, some materials which were known to be inert, are in fact toxic in large

quantities at the nanoscale. Employing such types of nanoparticles, which are toxic in nature over a longer period of time, could significantly diminish the therapeutic efficiency of cell-based therapy. The structural properties, dosage, and chemical composition of the particles lead to the toxicity of the nanoparticles on the biological entities.



. Figure 5.20 In vitro cytocompatibility assay of Ag/HAp against VERO cells after incubation for 24 h.

The OD values directly proportional to cell viability was determined with regard to the concentration of nanoparticles (50–1000 µg/mL). All the data exhibited a downward trend as the increasing concentration of either un-doped or Ag doped HAp nanoparticles. No significant differences were founded in lower concentration compared with blank control group. However, at higher concentration Ag/HAp (1000 µg/mL) group exhibited certain cytotoxicity with only 62% cell viability when compared with blank control group.

CHAPTER 6

6 CONCLUSION

Due to their structural and compositional closeness to real bones, material strength, biocompatibility, and osteoconductive properties, nanosized hydroxyapatite particles are frequently suggested in the field of implants. Furthermore, essential materials can be incorporated into nano-HAp to enhance and incorporate unique features. Synthetic production of nano-HAp, on the other hand, can have negative toxic side effects and is not recommended for biomedical use. As a result, biogenic technologies are used to create nano-HAp with improved biological features that are less hazardous. Marine wastes, such as fish bones and shells, are gaining appeal in nano-HAp fabrication since it is an innovative way to recycle trash into a useful material.

It's worth noting that using waste from marine sources as a precursor material for nano-HAp synthesis is a novel way to turn trash into very valuable material. However, until now, the stability of the produced nano-HAp in biological fluids has been a significant restriction. In the future, biomolecules could be used as capping agents to solve this problem. As a result, marine waste-assisted nano-HAp synthesis could be a viable alternative to traditional synthetic methods for producing non-toxic nano-HAp particles for implant manufacturing and other biomedical applications.

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