

SYNTHESIS OF HYDROXYAPATITE COMPOSITE DOPED WITH SILVER OXIDE FOR ENHANCED ANTIBACTERIAL ACTIVITY

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

By

C. PRIYADHARSHINI

(41590014)



**DEPARTMENT OF PHYSICS
SCHOOL OF SCIENCE AND HUMANITIES**

SATHYABAMA

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

**Accredited with "A" grade by NAAC I 12B Status by
UGC Approved by AICTE**

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119.

May 2023



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BONAFIDE CERTIFICATE

This is to certify that this project report is the bonafide work of C.PRIYADHARSHINI (41590014) who carried out the project entitled "Synthesis of Hydroxyapatite composite doped with silver oxide for enhanced antibacterial activity" under our supervision from August 2022 to March 2023.

Internal Guide

(Dr. J. Anita Lett)

Head of the Department

(Dr. S. Ravichandran)

Submitted for Viva voce Examination held on 9.5.2023 2023

INTERNAL EXAMINER

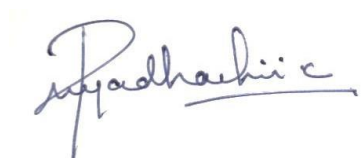
EXTERNAL EXAMINER

Dr. D. DINESH KUMAR

Associate Professor (Research)

DECLARATION

I C. PRIYADHARSHINI (41590014) hereby declare that the Project Report entitled “synthesis of hydroxyapatite composite doped with silver oxide for enhanced antibacterial activity” done by me under the guidance of Dr. ANITA LETT (Internal) is submitted in partial fulfilment of the requirements for the award of Masters of Degree in Physics.

A handwritten signature in blue ink, appearing to read 'Priyadharshini', with a horizontal line underneath the name.

DATE: 09-05-2023

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SIGNATURE OF THE CANDIDATE

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ABSTRACT

Recent years, the Nano-crystalline bio-ceramics are developing, due to the overcoming of post-implantation infection and osteo conduction, So that we consider it as a major challenge in medical application. In this project, we made an attempt to synthesize a Nano-crystalline bioactive hydroxyapatite of ceramic powder, using a simple precipitate and sol-gel technique, and studied its synthesis. In this synthesize calcium nitrate and ammonium dihydrogen phosphate were used as precursors for hydroxyapatite and AgNO_3 were used as precursors for silver oxide. The synthesize powder were analyzed by using **XRD, SEM /EDS, FTIR**, were performed to evaluate the mechanical properties. And also study the biocompatibility test of **hemolysis analysis** were performed to evaluate the absorption, and Bioactivity and biodegradation behavior of the sintered structures were assessed in simulated body fluid (SBF) and maintained in a dynamic state

Keywords: Hydroxyapatite, Biocompatibility, hemolysis analysis

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CHAPTER – 1

Introduction

Chapter – 1 INTRODUCTION

1.1 General introduction

Bioactive and bioresorbable phase of calcium phosphate bioceramics are materials of choice for bone – tissue engineering application because of their similarity of composition, ability to promote cellular functions and expressions, and osteoconductivity [1]. One of the most challenging fields of materials science is the one involving biomaterials. The scientific research takes a lot of effort in order to provide new and improved biomaterials with specific applications in medicine [4]. The prevention of implantation-associated bone infection is still a major challenge for both orthopedic and dental surgeries[2]. Currently most prosthetic implants are fabricated from titanium alloys due to their superior biocompatibility and enhanced corrosion resistance compared to stainless steels and cobalt-based alloys [3].And The most promising types of the implantable metallic biomaterials are titanium and its alloys, stainless steel and cobalt chromium alloys. Among these materials, titanium and its alloys attracted high importance due to its highest biocompatibility among other alloys, alongside with its combination of high mechanical properties, excellent corrosion resistance high strength and relatively low weight [7].However, the difference in mechanical properties between Ti and natural bone leads to negative effects, such as stress shielding. To mitigate these effects, many new Ti alloys have been developed for hard tissue implants, with a focus on controlling the alloy element and its content, phase, and other characteristics[5]. The concept of coating metal implant surfaces with HA combines the mechanical benefits of metal alloys with the biocompatibility of HA.Many coating techniques have been employed for the preparation of HA coatings, such as plasma spraying, dip coating, chemical solution deposition, sputter coating, biomimetic coating and electrophoresis[6]. The bond strength between the coating layer and the metal substrate is a very critical factor. Separation of the coating layer from the implant during service in the human body results in adverse effects on the implants and the surrounding tissue caused by detached particles [8]. The main reason of using HA coating on metallic substrates is to keep the mechanical properties of the metal such a load-bearing ability and, at the same time, to take advantage of the coating's chemical similarity and biocompatibility with the bone[8].

1.2 Bone Structure

Bone is not uniformly solid, but consists of a flexible matrix (about 30%) and bound minerals (about 70%) which are intricately woven and endlessly remodelled by a group of specialized bone cells. Their unique composition and design allows bones to be relatively hard and strong, while remaining lightweight.

Bone matrix is 90 to 95% composed of elastic collagen fibres, also known as ossein.

There are two types of bone tissue,

1. compact bone

2. spongy bone

1.2.1 Compact bone

Compact bone consists of tightly packed osteons or Haversian systems. The osteons consist of a central canal called osteonic canal or Haversian canal, which is surrounded by concentric rings (lamellae) of matrix [15]. Between the rings of matrix, the bone cells (osteocytes) are located in spaces called lacunae. Small channels (canaliculi) radiate from the lacunae to the osteonic (haversian) canal to provide passageways through the hard matrix. In compact bone, the haversian systems are packed tightly together to form what appears to be a solid mass. The osteonic canals contain blood vessels that are parallel to the long axis of the bone. These blood vessels interconnect, by way of perforating canals, with vessels on the surface of the bone.[15]

1.2.2 spongy (cancellous) bone

Spongy (cancellous) bone is lighter and less dense than compact bone. Spongy bone consists of plates (trabeculae) and bars of bone adjacent to small, irregular cavities that contain red bone marrow. The canaliculi connect to the adjacent cavities, instead of a central haversian canal, to receive their blood supply. It may appear that the trabeculae are arranged in a haphazard manner, but they are organized to provide maximum strength similar to braces that are used to support a building. The trabeculae

of spongy bone follow the lines of stress and can realign if the direction of stress changes [15]

Compact Bone & Spongy (Cancellous Bone)

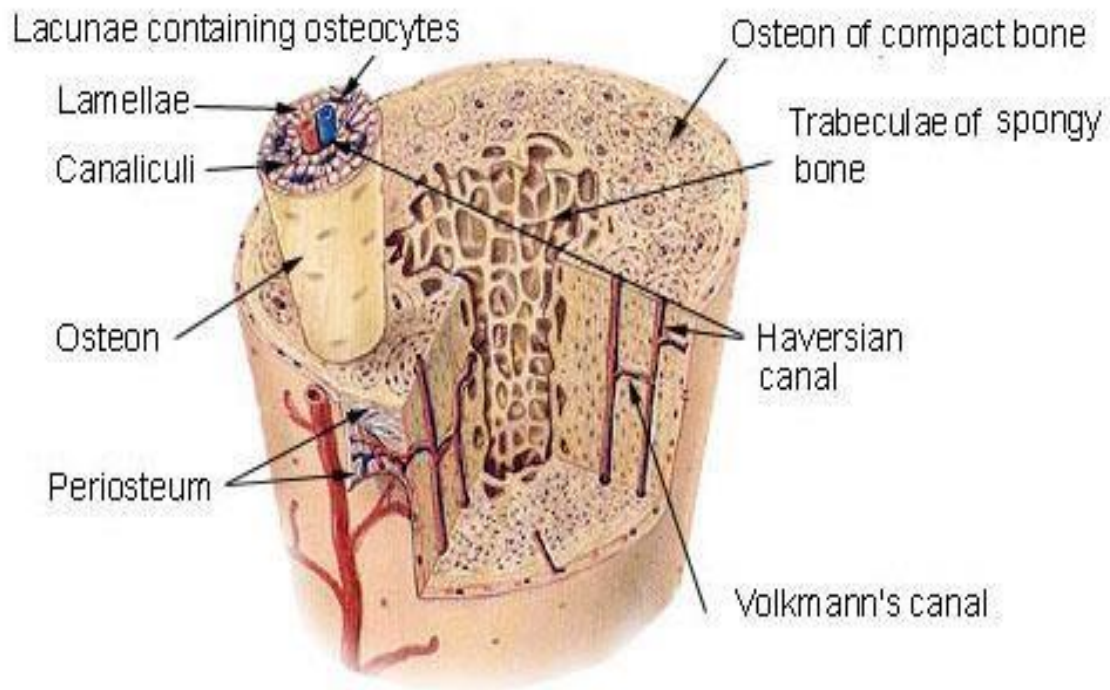


Figure1.1schematic diagram for compact bone and spongy bone[15]

1.3 BIOMEDICAL METERIAL

Biomedical materials are biocompatible metals, polymers, ceramics (including glasses and glass-ceramics), and composites. They are used in the biomedical field in various forms: particles, fibers, thin films, coatings, dense and non-porous, porous, etc[12]. Owing to the excellent combined properties of low elastic modulus, high specific strength, excellent corrosion resistance, complete inertness to body environment and superior biocompatibility, Ti and its alloys are a better choice for manufacturing biomedical implants [13]. All of these materials are required to have five prerequisites to be used as biomedical materials: sterility, biocompatibility, functionality, non-toxic and durability. Therefore composites which complement their ability each other have become common to be used [14].

1.3.1 TYPES OF BIOMATERIAL

1. Polymer-Based Biomaterial
2. Ceramic-Based Biomaterial
3. Natural Biomaterial
4. Inorganic Glass- Based Biomaterial
5. Regenerative Biomaterial
6. Hybrid Biomaterial Combination

1.3.2 ceramic based biomaterial

Biomaterial play a vital role in many application field in recent modern years. Ceramics are defined as oxides, nitrides, sulphides and carbides of metals and metalloids. These are important in the biomedical field due to their good and matchable physio-chemical properties with some of the human body parts. Initially, bioceramics porcelain was used in crown treatment during the 18th century. Then, in the 19th century, plaster of Paris was used in the dentistry application[16].Before recognising any material as an implant it is imperative to know that the said material is not causing any damaging effect, it should be biocompatible to the human body [17].The applications of ceramics increased many fold in the medical field in the 20th century due to processing technology advancements [5]. Bioceramic materialshaving excellent biocompatibility,poor degradability, high melting temperature, non-corrosive, bettermechanical properties with poor plasticity than metal-based biomaterials. Bioceramics are hard and fragile and low fracture toughness along with elastic modulus in comparison with bone[18,19,16].Bioceramics includes Alumina, Zirconia, Bioactive glass, Glass ceramics, Hydroxyapatite, resorbable Calcium phosphates and orthers they have been used in dentistry, orthopaedics, calcified tissues, implants, coatings, medical sensors, and many other applications [16,20]. It is also used for bony defects, root repair materials, apical fill materials, perforation sealing, soft tissue pair and regeneration [16,20,21]. Ceramic based biomaterials exhibit some advantages over metals and alloys like density, porosity, high elastic modulus, hardness and low cost. But they also have some demerits like poor sinterability, ductility and machinability[22]. Similarly, food wastes such as egg shell powder (ESP), egg shell membrane (ESM) contain calcium (Ca) and banana peel (BP) contain potassium (K), polyphenolic compounds, and essential amino acids which can be used for the

production of β -tricalcium phosphate (β -TCP), biphasic calcium phosphate (BCP), hydroxyapatite (HAp), mesoporous bioglasses, alternative to synthetic bioglasses used for prosthesis and medical therapies [16,23-25].

1.4 HYDROXYAPATITE

Among different classes of bioceramics, hydroxyapatite (HAp) is the emerging most bioceramic, which is widely used in various biomedical applications, mainly in orthopedics and dentistry [26]. Hydroxyapatite has been considered for use in the repair of osseous defects for the last 20 years. Recent developments have led to interest in the potential of porous hydroxyapatite as a synthetic bone graft [27]. Nanosized hydroxyapatite (HA) is the main component of mineral bone. Living bone constantly undergoes a coupled resorptive-formative process known as bone remodeling [9]. Any material upon implantation in the human body triggers some kind of response. This response is more pronounced at the tissue-implant interface since the interface is more reactive than the core of the material. Basically, the interface is considered as a two-dimensional imperfection where the atoms are not linked to the highest number of neighbours. As a result, they possess higher energy than core atoms of the materials [19]. The good biocompatibility nature of HAp makes it suitable for soft tissue regenerations like skin, muscle, and gums. The studies showed that the HAp can activate fibroblasts and accumulate vessel endothelial cells and thereby support the healing of skin wounds [35].

The four general types of tissue implant:

Table 1A comparison between different types of implant-tissue interactions and their subsequent consequences [19].

Sl.no	Implant-tissue response	consequences
1.	Toxic	Kills cells in the tissue surrounding the implant. Also can cause systematic damage to the patient
2.	Nearly inert	Non-adherent fibrous capsule develops to isolate the host from the implant
3.	Bioactive	Tissue- implant bond at the interface.
4.	Dissolution of implant	Implant dissolves and is superseded by the tissue

The greatest potential for bone substitution is shown by materials based on hydroxyapatite (HA), $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which can develop tight bonding with bone tissue, exhibits osteoconductive behavior, is stable toward bioresorption, and has no adverse effects on the human organism [10]. The use of low density HA, with highly interconnected porosity, has also been advocated as a viable alternative to bone grafts without the complications of sterilization, infection, rejection and inadequate supply [11]. This has opened the potential for using synthetic HAp to deliver pharmacological substances in many clinical applications with sustained release capacity for the treatment of osteomyelitis, osteoporosis, osseous cancers etc in which local delivery is effective with the need to fill defects in the skeleton. New economic and versatile methods for HAp synthesis are of interest due to the importance of this material for various biomedical applications. The current communication deals with various

methodologies for the preparation of synthetic HAp with optimum properties closer to those of living hard tissues like bone and teeth, aiming at better and more effective biomedical applications[26]. The preparation of HAp ceramics material have been carried out using different technique approaches like sol-gel, hydrothermal processing, microwave route, wet chemical route, emulsion system and sonochemical synthesis. Most of the researcher follows the wet chemical route due to the ease of handling, environment friendly and simple to optimise the process parameters.

1.5 Materials used in HAP doped Dental Implants:

1.5.1 Magnesium(Mg) nanoparticle:

Mg-doped HAp is very essential for the better bone development with higher antibacterial activity. Thus, due to this, several researchers tried to develop Mg-doped HAp using the different approaches such as wet chemical synthesis [37], hydrothermal [38], sol-gel [39], and microwave method [40]. However, till today the research on the production of mesoporous Mg-doped HAp synthesis is very limited. Hence, in this study we aimed to develop a simple and easy approach to synthesize eco-friendly mesoporous Mg-doped HApnanorods. The aim of this work to produce Mg-doped HAp for preventing microbial adhesion during implantation. In addition, the doping will be executed under the polyvinylpyrrolidone which is an organic modifier. Here, the HAp will be synthesized utilizing bio-waste blue mussel (*Mytilusedulis*) as a calcium source *via* the microwave-assisted synthesis method. Also, the antibacterial activity, cytotoxicity, and *in vivo* toxicity using zebra fish as a model are evaluated to confirm the biocompatibility and application of synthesized materials for implant applications[32]. Contrary to pure stoichiometric HA, biological apatites are nonstoichiometric, poorly crystalline, and often contain several ionic impurities such as Zn^{+2} , Mg^{+2} , Na^{+} , CO_3^{2-} , and F^{2-} . These elements are known to play a significant role in bone metabolism, growth, and nutriment absorption. For this reason, their incorporation in synthetic apatites is considered to lead to better biomaterials than pure HA. Among these substituting elements, magnesium is widely studied; it is one of the most important bivalent ions for maintaining bone health. It is the fourth most abundant cation in the human body; its presence inhibits crystallization, reduces crystal size, and decreases the proliferation and activity of osteoblast-like cells, 27-30

while its deficiency affects all stages of skeletal metabolism, causing cessation of bone growth, decrease of osteoblastic and osteoclastic activities, and bone fragility.³¹⁻³⁵

Numerous review papers are reported in the literature on the synthesis of hydroxyapatite containing Mg.³⁶⁻⁴⁰ The characters of the products are widely different depending on the parameters including type of process, physicochemical properties of the precursors, concentrations, temperature, aging, etc.⁴¹ In the present work, the primary objective was to study the incorporation of Mg ion in hydroxyapatite via a sol-gel method involving calcium nitrate, magnesium nitrate, and phosphoric pentoxide reactants in the sols preparation and to explore the impact of the Mg concentration on HA's crystal structure^[33]. Magnesium is one of the essential elements for all living organisms. Over 100 enzymes require the presence of magnesium ions for their catalytic action, including all enzymes utilizing or synthesizing adenosine-5'-triphosphate (ATP). Mg deficiency affects all skeletal metabolism stages causing cessation of bone growth, decrease of osteoblastic and osteoclastic activities, generation of osteopenia and bone fragility. Mg^{2+} ions are the most often described dopants for calcium in the HA structure. Doping HA with magnesium directly influences the mineral metabolism, modifies the dissolution rate of crystals and the biodegradation of the corresponding materials. It has been reported, that doping HA with 2 mol% Mg^{2+} and Zn^{2+} significantly enhanced osteoblast adhesion as compared to pure HA, however higher doses of Mg^{2+} caused the opposite effect. To obtain HA with improved mechanical properties and biological activity new dopant materials are researched. Several dopant elements are mentioned in the literature, including those, which normally cannot be found in the human body, like cations of La^{3+} , Y^{3+} , In^{3+} , Bi^{3+} . Of those, Y^{3+} and Bi^{3+} were the most effective at enhancing osteoblast response ^[34].

1.5.2 gold nanoparticle:

Gold nanoparticles (AuNPs) have been investigated for drug delivery, biosensors, thermotherapy in addition to its suggestion to be used for imaging^[36]. Gold doped HAp demonstrated enhanced antibacterial activity against the given bacterial species when

compared to HAp which was demonstrated in terms of inhibition zone with varying concentrations of gold doped HAp. With regards to biocompatibility study, gold doped HAp was exposed to MG-63 osteoblast. Gold doped HAp was prepared by the combination of bio-reduction method and hydrothermal method and its antibacterial activity was tested against *E. coli*, *S. aureus*, *K. pneumoniae*, and *S. pyogenes*[35]. The combination of HAP and AuNPs has been investigated for biomedical applications including bone regeneration.

Chai et al. have studied the efficiency of Au dopant into HAP to be used for albumin interactions. They found that the obtained composition displayed a good protein adsorption response that was 1.5 μM through the cell culture whereas it was 15.1 μM approximately in human blood. Whilst, Santos et al. found an interaction among mesenchymal stem cells and AuNPs on the surface of HAP and thus induced great osteoblastic differentiation in vitro. Due to the great influences of graft surface during the interaction process between the host environment and the implanted biomaterial, manipulating of the functional surface is a vital strategy to control fixation and improve the healing process[36]. Specifically, silver (Ag) and gold (Au) nanomaterials have involved scientists for their potential application in bone regeneration. The application of Au nanoparticles in cartilage and bone repair. Previous studies showed that Au nanoparticles promoted the osteogenic differentiation and mineralization of mesenchymal stem cells and MC3T3-E1 cells in-vitro through the extracellular signal-regulated kinases/mitogen-activated protein kinases signalling pathway.

Recently, Ribeiro et al, confirmed that silk fibroin/nano hydroxyapatite hydrogels with silver and gold nanoparticles simultaneously allowed the adhesion and spreading of osteoblastic cells. These analyses suggest that Ag and Au might play an important role in bone regeneration and formation. HA is one of the utmost common materials for numerous biomedical applications. Human bone matrix (mostly organic part) is designed by collagen and proteins. Moreover, HA has several properties such as: high porosity, good biocompatibility, suitable cell adhesiveness, high osteoconductivity and exceptional biodegradability[36].

1.5.3 copper nanoparticle

One of the dominant, reliable, inexpensive, and fast methods for the detection of bacteria in the field of sensing technology is luminescence-based detection. Metal nanoclusters comprising a few to hundred atoms are excellent fluorescent materials with high photoluminescence, excellent photostability, lower toxicity, and high water solubility. Among the metal nanoclusters, copper nanoclusters (Cu NCs) are explored with great attention in the area of bioimaging in vitro and in vivo. Copper nanoclusters have another advantage of being used in vivo as there are cellular and molecular mechanisms that regulate the uptake and efflux of copper unlike gold and silver metal ions. However, the HAP NPs doped with lanthanides have limited in vivo applications because of their toxicity, so as the case with organic dyes due to the drawbacks of photobleaching and poor stability. HAP owing to its high biocompatibility and adsorption property can be used for doping with metal nanoclusters such as copper. Herein, we report the synthesis of copper nanocluster-doped HAP NPs, which acted as nanocarriers for antibacterial drug delivery.

The as-synthesized doped HAP NPs were biocompatible, and they were also found to be useful for the bioimaging of bacterial cells[40]. Copper is also well known for its broad spectrum antimicrobial activity. Both Gram-negative and Gram-positive bacteria have been shown to be killed upon exposure to copper surfaces, copper ions in solution, and suspensions of copper nanoparticles. 12 The high surface area of Cu nanoparticles makes them susceptible to oxidation. However, the oxidized copper surface has also been shown to be antimicrobial upon contact and by releasing copper ions in solution.13,14 Copper has the advantage of being far less toxic to mammalian cells than silver.15 Further, Cu is an essential trace element in the human body.16 Cu^{2+} ions in small amounts facilitate the activity of several enzymes and helps in cross-linking of collagen and elastin of bones, while in higher concentration they inhibit osteoblast proliferation.17,18 Therefore, the ability to control copper loading is quite important for biomedical application in order to balance antibacterial activity against potential negative impacts on mammalian cells[41].

1.6 SYNTHESIS METHOD FOR NANO-PARTICLE

The various methods used for the synthesis of nano particles are

Precipitation method

Sol-gel method

Hydrothermal technique

Ball milling technique

Physical vapour deposition technique

Chemical vapour deposition technique

Sonochemical method

1.6.1 Precipitation method

The most popular and widely researched technique for synthesis of HAp is precipitation technique. This technique is also called as wet precipitation or chemical precipitation or aqueous precipitation. This technique is chosen widely to synthesize HAp in contrast to other techniques. Because, relatively large amount of HAp can be produced by precipitation technique in absence of organic solvents at a reasonable cost[29]. They employed calcium and phosphorus precursors in solutions. The experimental development involved the addition of 100 mL of a solution of 0.6 M of phosphoric acid (H₃PO₄) (Merck, 85%) to 100 mL of a suspension of 1.0 M calcium hydroxide (Ca(OH)₂) (J.T.Baker, 97%) at a speed of 7 mL/min. The chemical equation that describes the reaction is:



Figure 1 shows the first experimental precipitation process. A digital potentiometer Orion Mod. 1520 was used to measure the pH of the solution during mixing and aging. The mixture was warmed to 90°C for 1 hour to activate the chemical reaction and then stirred for another hour. During aging at room temperature, the HA was precipitated, filtered and washed with distilled water. The dried powder was calcined at up to 850°C for 4 to 6 hours; the obtained product was crystalline[30].

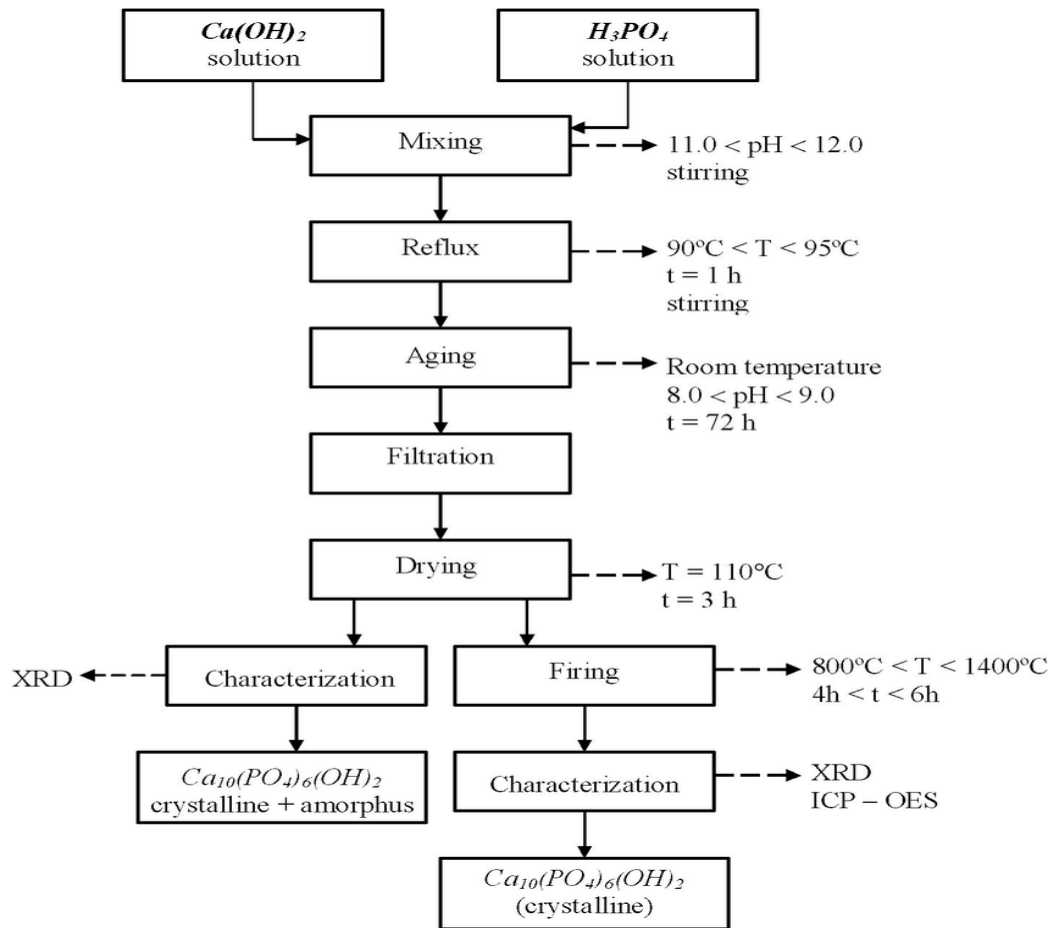
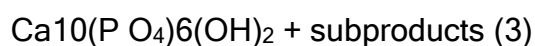
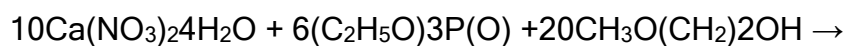


Fig 1.2 shows the synthesis of HAP by precipitate technique[30].

1.6.2. sol-gel method

Triethyl phosphate (Aldrich, 99.8%) and calcium nitrate tetrahydrated (Baker, 99.9%) were used, as well as 2-metoxiethanol ether (Aldrich, 99.3%) as an organic solvent. Esther phosphate was hydrolyzed with the release of water during the first stage of the reaction, generating HA (18). The equation of the chemical reaction is:

The equation of the chemical reaction is:



In order to obtain a Ca/P ratio of 1.667, 23.483g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 10.2 mL of $(\text{C}_2\text{H}_5\text{O})_3\text{P}(\text{O})$ and 15.6 mL of $\text{CH}_3\text{O}(\text{CH}_2)_2\text{OH}$ were used. The container was closed and constantly stirred until a homogenous mixture was obtained[30]. Figure 3 shows the procedure. Calcium nitrate tetrahydrated crystals and 2-metoxietanol were placed in a closed Erlenmeyer flask, stirring, and then triethyl phosphate was added. The mixture was aged for 4 days at 80°C with stirring. To start the gelation process the flask was opened and the temperature increased to $90^\circ\text{C} < T < 100^\circ\text{C}$. At the end of this stage there was a very viscous yellowish gel, which that was dried to obtain a grayish product and then washed. Finally it was calcined for 12h at 1200°C and HA was obtained[30].

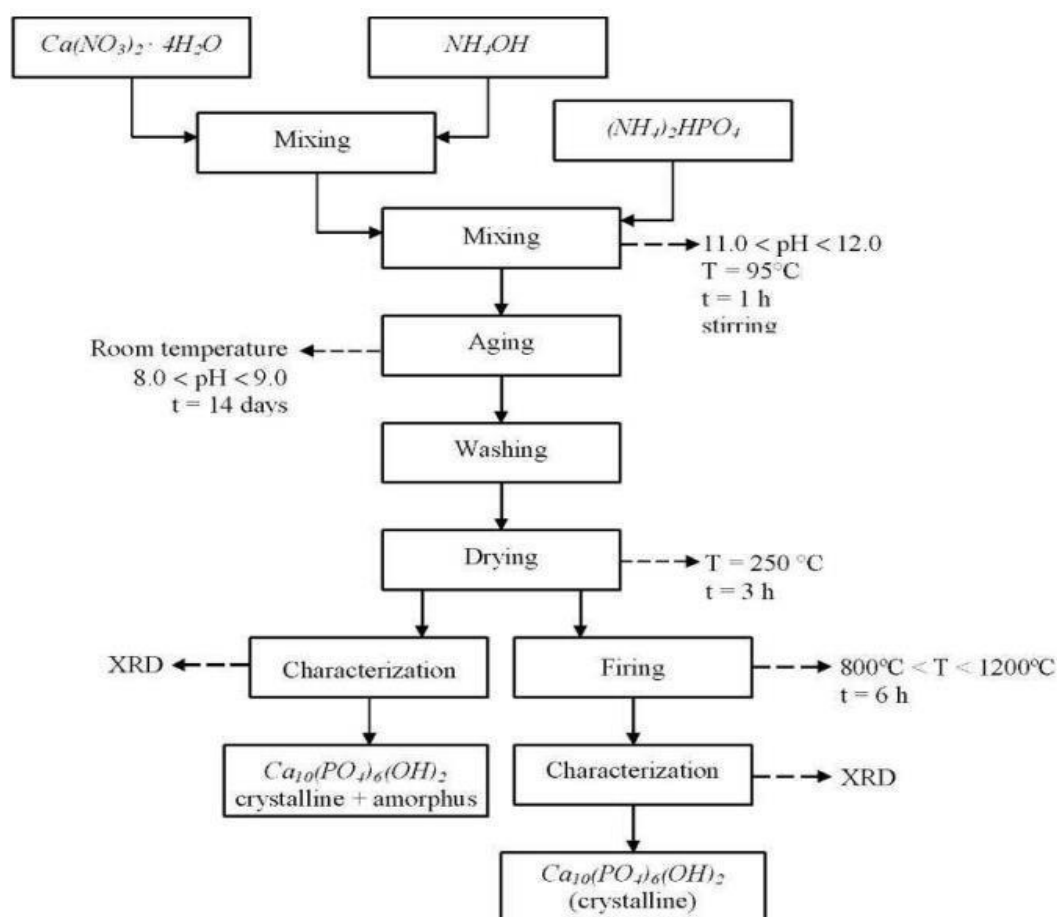


Fig 1.3 represent the synthesis of HAP by using sol-gel technique[30].

1.6.3 hydrothermal technique:

Stock solutions, 1.0 M, were prepared by dissolving calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) (11.807 g) (Fisher Scientific ACS grade) and di-ammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) (6.603 g) (Fisher Scientific analytical reagent grade) in 50.0 ml distilled water. The stock solutions were diluted further with distilled water to give 0.10 M solutions, and subsequently mixed to yield suspensions with Ca/P molar ratios of 1.67 by the dropwise addition of 10.0 ml of 0.10 M diammonium hydrogen phosphate to 16.7 ml of 0.10 M calcium nitrate tetrahydrate. The pH of the reacting suspension was 5.1 (measured using a glass electrode). The solutions were transferred to a 125 ml Teflon-lined hydrothermal reactor (Model 4748, Parr Instrument). Reactions were performed by placing the unit in a laboratory oven for between 24 and 72 hours and at a temperature of 200°C , and then cooling to room temperature naturally. Following all experiments the reacted powders were washed by

suspending the sample in distilled water using an ultrasonic bath for 5 mins before sedimenting the particles by means of centrifugation. At the end of each spin cycle the liquid was decanted and replaced by an equal volume of fresh distilled water, this process was repeated 6 times, or more until the pH had returned to 7. In some cases a final wash was performed using methanol to limit agglomeration of HAp particles during drying. Samples for phase analysis were dried in a laboratory oven at $\sim 50^\circ\text{C}$ for ~ 4 hours. X-ray diffraction (XRD) of all prepared powders was performed, in the range $5 - 70^\circ (2\theta)$ in 0.025° steps at a scan speed of $0.05^\circ \text{ s}^{-1}$. HAp particle sizes were determined using a field emission scanning electron microscope (LEO 1530 FEGSEM) at 3 kV with a working distance of 3-4 mm. Dentine sections were prepared from human teeth and etched using 35 wt% phosphoric acid for 60s [7], to reveal the dentinal tubules.[31]

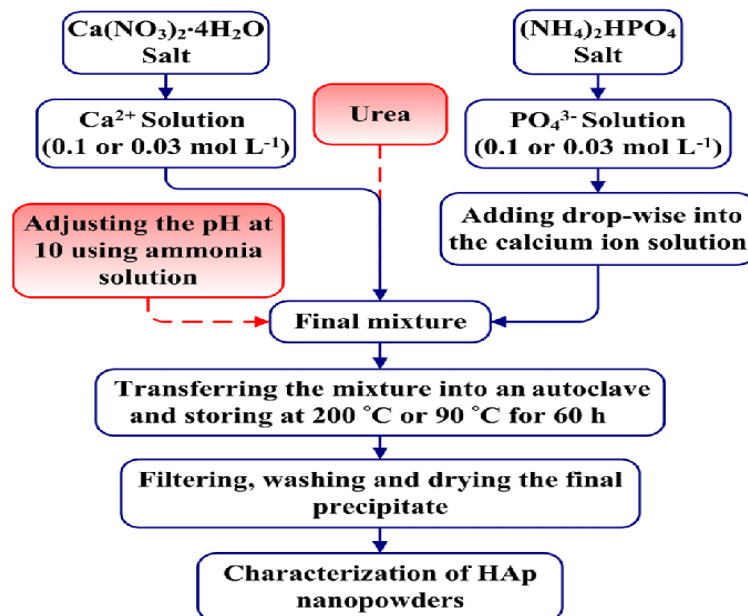


Fig 1.4 represent the synthesis of hap by using hydrothermal technique

1.6.4 Ball Milling through the Mechanical Method

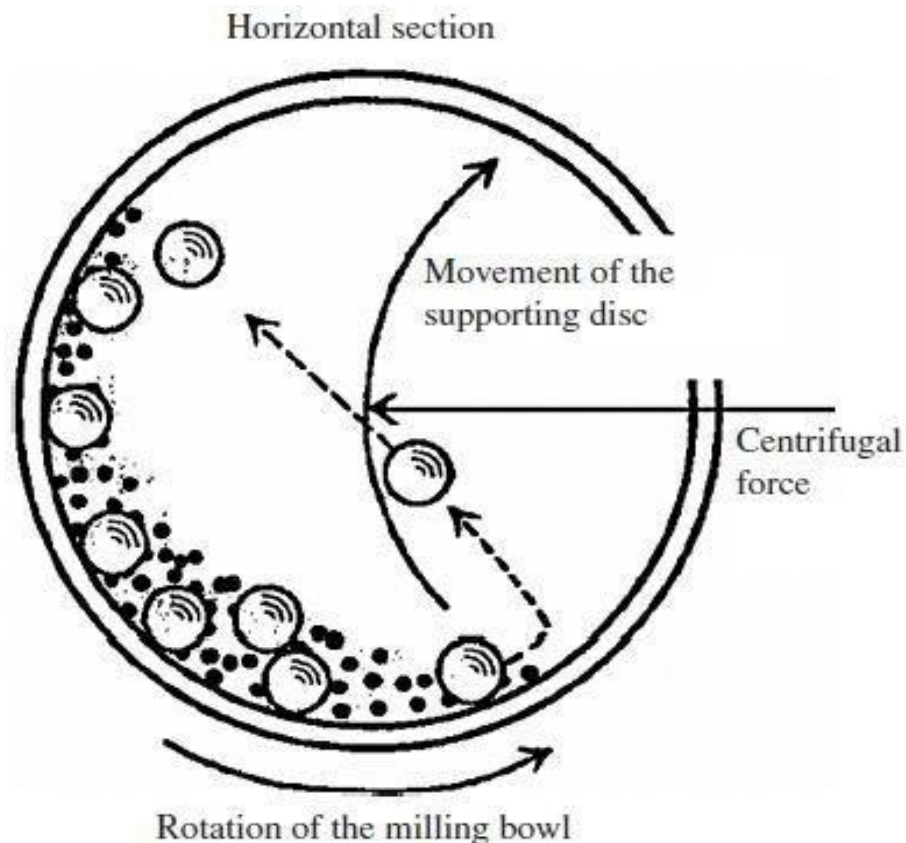


Fig 1.5 represent the ball milling technique

Ball milling is a mechanical method to fabricate nanomaterials. In this process, the materials are ground in a closed container. Small pebbles made of glass, ceramics, and stainless steel create shear force during grinding. Bulk materials are subjected to place in the closed container. By grinding process, the bulk materials are converted to fine-tuned nanomaterials. Using this method, we can fabricate metallic hydrides and nitrides. These nitrides possess essential properties that provide a variety of applications. Their hardness and stability are used to cut tools and tool coating in microelectronic applications (e.g., titanium nitride (TiN) alloy). It is synthesized using the reactive ball milling method. In this approach, the bulk metallic powder is placed in the closed container under the purging of the nitrogen gas atmosphere that is subjected to high-energy ball milling. The metallic powders are disintegrated to form tiny particles and oxygen-free active surfaces are created on the nanomaterials. In addition, nanotubes are ground to form very tiny powders. It is created by the collision of two or more pebbles in a closed container that creates very high pressure to get

fine nanotubes. The quality of the nanotubes is increased by adding functional moiety on the surface of the nanotube in the container. Some of the factors affecting the dispersion of nanotubes are the size of the pebbles, rotational speed, milling time, and amount of nanotube added.

As a result, nanotubes with less than 100 nm can be obtained by the grinding process. Using this process, the carbon nanotubes are transformed into fine carbon nanostructures and during this process, few damages occur on the surface of the nanostructures. The ball milling method is also used in the mechanical alloying method or attrition, a kind of top-down approach. The reduction of particles is done in the grinding process by the collision of pebbles that generates a frictional force, which gives rise in pressure, temperature, and internal energy. During this process, the materials are lying in the nanosized regime. The milling speed can be increased through surface functionalization of alkyl or aryl group on the surface of the single-walled carbon nanotubes (SWCNTs), which gives high solubilization in organic solvents. Metastable materials can be produced using high-energy ball milling method by maintaining thermal equilibrium.

This process slightly differs from the conventional ball milling methods because the conventional methods cannot produce sufficient energy to mill the materials. For example, nickel-niobium (Ni-Nb) alloy can be produced using high-energy ball milling methods. The advantage of the ball-milling method includes (i) making fine powder; (ii) being suitable to mill toxic materials; (iii) abrasive materials are milled using this technique. The disadvantage of the ball-milling method includes (i) the raise of contamination occurs from wear and tear in balls collision; (ii) increase in machine noise when the concealed cylinder is made of metal and it is reduced when the cylinder is made of rubber; and (iii) it is a time taking process[38].

1.6.6. Physical Vapor Deposition (PVD) Method

Physical vapor deposition (PVD) is a process applied to the synthesis of ultra-thin films and surface coatings. It is used to produce metal vapor that can be deposited on the conductive layer as ultra-thin films and alloy coatings. The whole process is carried in a vacuum held in a vacuum chamber about 10^{-6} torr from a cathodic-arc source. In a

clean atmosphere, vacuum deposition is held in the chamber and the metals are deposited as wider or sputtered in the localized area. Reactive PVD is designed in a method to deposit metal on the surface and reactive gas such as oxygen, nitrogen, or methane passed in the vacuum chamber. Plasma, the high energetic beam bombards the metal surfaces ensuring hard and dense coating. Using this method, we can synthesis nano-particles and allow to fabricate nanocomposites. Thin film formation is characterized by the metal ions in the vapor phase obtained from condense phase and return back to condense phase of thin films. The PVD includes evaporation and a sputtering process to fabricate thin films. The procedure for PVD includes sputtering process that is carryover in the vapor phase under supersaturation. In an inert atmosphere the metal vapors are promoted to condense phase, and it is subjected to thermal treatment to get nanocomposites. The advantage of the PVD techniques include (i) having improved properties as compared with the substrate material; (ii) inorganic and few organic materials are used, and it is an ecofriendly approach as compared to electroplating technique. This technique also faces few difficulties such as (i) coating with complex structures; (ii) it is not cost-effective and produces a low output; and (iii) it is a complex process [38].

1.6.7 Chemical Vapor Deposition Method (CVD)

Chemical vapor deposition (CVD) is a vacuum-based deposition process for producing better-quality, better-performance of solid materials. In this method, thin films deposits over the substrate and involves chemical reactions between species such as organometallic and other gases. The uniqueness of CVD is a multidirectional deposition method to coat over the substrate, while PVD uses a line-of-site impingement method. CVD is commonly used to deposit materials in various forms such as crystalline, amorphous, and epitaxial growth in the microfabrication process. In CVD, a mixture of gases interact chemically over the surface of bulk materials that leads to chemical decomposition forming a dense coating on the base of the material surface. For example, diamond crystals can be deposited over the silicon or molybdenum substrates using the CVD approach. Charged diamond nanoparticles can be produced using the hot filament chemical vapor deposition (HFCVD) process.

The diamond nanoparticles are captured using floating and grounded substrates. Some of the substrates are SiO, graphene membranes of copper TEM grid, and carbon. Different types of allotropes diamonds are identified as hexagonal and cubic diamond, i-carbon, and n-diamond particles. The diamond nanoparticles can characterize using Raman spectroscopy that has a sharp peak around 1332 cm^{-1} . The advantages of the CVD process are producing high purity thin films and fabricating abrupt junctions. Disadvantages of the CVD process are the product based on the properties of precursor and poor uniformity[38].

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).

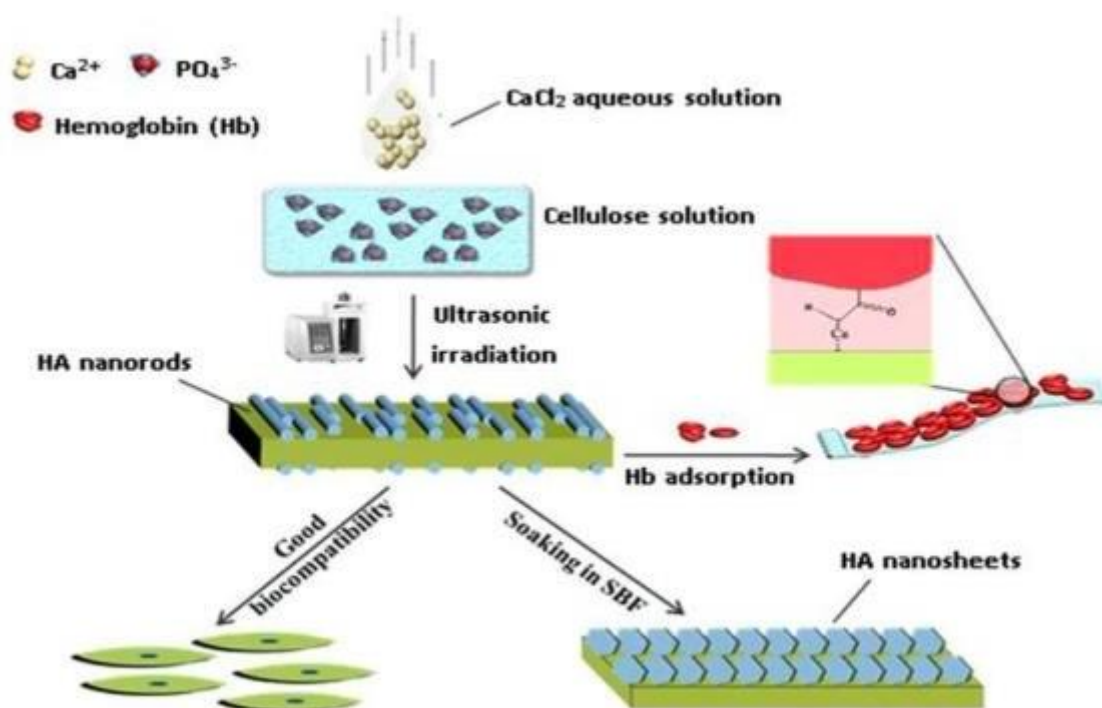


Figure 1.6 Diagrammatic representation of Sono chemical method.

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

1.7 BIOMATERIALS PROTEIN INTERACTION

Protein-biomaterials surface interaction has significance importance to develop a new class of biomaterials, specifically in the field of pharmaceuticals and tissue engineering. When a solid materials comes in contact with a fluid that contains soluble proteins, which rapidly adsorb onto the surface of the material[28]. The physicochemical properties are the main factor that influence the diffusion of a drug within the body. In particular, protein drugs show high hydrophilicity, large size and substantial physical and chemical lability; these features strongly influence the pharmacokinetic and pharmacodynamic behavior of the drug *in vivo*. They also limit the reactions, solvents and environmental conditions that can be used in the preparation and application of protein- or peptide-based pharmaceuticals. Most of the commonly employed protein drugs are administered systemically by intramuscular, intravenous, subcutaneous and intraperitoneal injections, and formulations often include excipients (e.g., buffers, preservatives, solubility enhancers), whose major role is that of improving the *in vivo* stability of the biomolecule [29]. As conformational alterations are usually induced in protein molecules upon adsorption on foreign surfaces, definite differences between the spectra of adsorbed and non-adsorbed proteins are generally apparent in the above-mentioned regions [30].

Recent advances in biotechnology demonstrate that peptides and proteins are the basis of new generation drugs [31]. A lack of protein causes the diseases like thrombosis, colon cancer, breast cancer, diabetes and osteoporosis. Hence, the proper sustained and targeted delivery of pharmaceutical offers several advantages in compare with classic administration through increment of drug amount towards the targeted area, improve the transportation mechanism, and protect the drug against inactivation, degradation and metabolization phenomena. In recent, HA nanoparticles has been elucidated to be an efficient carrier for bone growth factors or drug deliver [28]. The basic understanding of protein interaction with biomaterials, surface properties, adsorption kinetics and driving force for interactions. cells are not adhering without the presence of intermediate protein layer within foreign host biomaterial ,and cell molecules [28]. The protein properties like hydrophobicity and surface charge attribute the most effective contribution towards the adsorption of protein on

biomaterial [32]. The described features are needed to encounter to understand the protein adsorption phenomena (Table 1.1).

The most effective surface properties of the biomaterial on the protein adsorption are represented in (Table 1.2).

The relative bulk concentration of each protein and intrinsic surface activity has two driving forces of protein adsorption. Along with two major contribution, van der waals force of interaction and electrosatatic force of attraction plays an important role in protein interaction.

Table 1.1Effect protein properties on the adsorption phenomenon[28].

Properties	Effect
Size	Large molecules have more sites for surface contact
Charge	Molecules near their isoelectric point adsorb more readily
Hydrophobicity	More hydrophobic molecules adsorb more readily on hydrophobic surface
Stability	Less stable proteins form more surface contact points
Unfolding rate	Molecules that rapidly unfold can form surface contacts more quickly

Table 1.2 biomaterial surface that effect protein adsorption[28].

Feature	Effect
Topography	Greater texture exposes more surface area for interaction with protein
Composition	Chemical makeup of a surface will determine the type of intermolecular forces governing interaction with protein
Hydrophobicity	Hydrophobic surfaces tend to bind more proteins
Heterogeneity	Non- uniformity of surface characteristic result in domains that can interact differently with proteins
Potential	Surface potential will influence the distribution of ions in solution and interaction with proteins

the host defence mechanism and circulate to its intended site of action[28].

Chapter - 2

Literature survey

1. Nano-crystalline hydroxyapatite doped with magnesium and zinc: synthesis and characterization.

Author: Samar J. Kalita, Himesh A. Bhatt

In 2007, Samar J. Kalita, Himesh A. Bhatt investigated a Nano-crystalline hydroxyapatite doped with magnesium and zinc synthesis and characterization. In this research, an attempt to synthesize nanocrystalline bioactive hydroxyapatite ceramic powder in the lower-end of Nano-range (2-10nm) and study its sintered structures. Here the methodology is used to synthesize the Nano-crystalline by Simple low temperature sol-gel technique. The obtained characterization of **XRD, SEM, VICKERS HARDNESS TESTING, COMPRESSION TESTING**. and also involved the studies of the Nano-crystalline.

Citation: Samar J. Kalita, Himesh A. Bhatt, Nanocrystalline hydroxyapatite doped with magnesium and zinc: Synthesis and characterization, Materials Science and Engineering: C, Volume 27, Issue 4, 2007, Pages 837-848, ISSN 0928-4931, <https://doi.org/10.1016/j.msec.2006.09.036>

2. Composition-Tailoring of ZnO-Hydroxyapatite Nano-composite as Bioactive and Antibacterial Coating ACS Applied Nano Materials.

Author: Geuli, Ori and Lewinstein, Israel and Mandler, Daniel

In 2019, Geuli, Ori and Lewinstein, Israel and Mandler, Daniel investigated a Composition-Tailoring of ZnO-Hydroxyapatite Nanocomposite as Bioactive and Antibacterial Coating ACS Applied Nano Materials. In this article The synergic combination of hydroxyapatite and ZnO holds beneficial properties. Here highly phase tunable pure ZnO-Hap nanocomposite coatings were fabricated. ZnO-Hap nanocomposite coatings were fabricated via electrophoretic deposition. HAp synthesized by precipitate method and ZnO nanoparticles were synthesized by solvothermal synthesis. The obtained characterization of STEM, HR-TEM, SEM, FTIR, XRD. In vitro studies, bioactivity, cytotoxicity, and antibacterial test.

Citation: Geuli, Ori and Lewinstein, Israel and Mandler, Daniel Composition-Tailoring of ZnO-Hydroxyapatite Nanocomposite as Bioactive and Antibacterial Coating ACS

3. "Synthesis and Antimicrobial Activity of Silver-Doped Hydroxyapatite Nanoparticles".

Author: Carmen StelutaCiobanu, Simona Liliana Iconaru, Mariana Carmen Chifiriuc, Adrian Costescu, Philippe Le Coustumer, Daniela Prodo.

In 2013, Carmen StelutaCiobanu, Simona Liliana Iconaru, Mariana Carmen Chifiriuc, Adrian Costescu, Philippe Le Coustumer, Daniela Prodo investigated a "Synthesis and Antimicrobial Activity of Silver-Doped Hydroxyapatite Nanoparticles" The aim of this study was the evaluation of $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ Nanoparticle (Ag:HAP-NPs) for their antibacterial and antifungal activity. Here the methodology used The $\text{Ca}_{10-x}\text{Ag}_x(\text{PO}_4)_6(\text{OH})_2$ ceramic powder, was prepared (Ca/P molar ratio:1.67) using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ by coprecipitation method. The obtained characterization of, HR-TEM, SEM, FTIR, XRD. In vitro Antibacterial and Antifungal activity.

Citation: Carmen StelutaCiobanu, Simona Liliana Iconaru, Mariana Carmen Chifiriuc, Adrian Costescu, Philippe Le Coustumer, Daniela Prodo, "Synthesis and Antimicrobial Activity of Silver-Doped Hydroxyapatite Nanoparticles", *BioMed Research International*, vol. 2013, Article ID 916218, 10 pages, 2013.
<https://doi.org/10.1155/2013/916218>

4. Hydroxyapatite coating on titanium substrate by electrophoretic deposition method: Effects of titanium dioxide inner layer on adhesion strength and hydroxyapatite decomposition, Surface and Coatings Technology.

Author: OnderAlbayrak, Osman El-Atwani, SabriAltintas

In 2008, OnderAlbayrak, Osman El-Atwani, SabriAltintas investigated Hydroxyapatite coating on titanium substrate by electrophoretic deposition method: Effects of titanium dioxide inner layer on adhesion strength and hydroxyapatite decomposition, Surface and Coatings Technology. In this paper As an inner layer between Ti6Al4V substrate

and HA coating, nano-sized titanium dioxide (TiO₂) powders were deposited, using different coating voltages, in order to connect substrate and HA tightly. Nano-sized hydroxyapatite (HA) powders were prepared by a chemical precipitation method and electrophoretically deposited on Ti6Al4V substrates. After the sintering stage, adhesion strengths of coatings were measured by shear testing, phase changes were studied by X-ray diffraction, and coating morphology was analyzed through scanning electron microscopy observations.

Citation: Onder Albayrak, Osman El-Atwani, Sabri Altintas, Hydroxyapatite coating on titanium substrate by electrophoretic deposition method: Effects of titanium dioxide inner layer on adhesion strength and hydroxyapatite decomposition, Surface and Coatings Technology, Volume 202, Issue 11, 2008, Pages 2482-2487, ISSN 0257-8972, <https://doi.org/10.1016/j.surfcoat.2007.09.031>.

5. Comparative investigation on the adhesion of hydroxyapatite coating on Ti-6Al-4V implant.

Author: E. Mohseni, E. Zalnezhad, A.R. Bushroa

In 2014, E. Mohseni, E. Zalnezhad, A.R. Bushroa investigated Comparative investigation on the adhesion of hydroxyapatite coating on Ti-6Al-4V implant. This

review comprehensively analyzes nine techniques mostly used for deposition of HA onto Ti-6Al-4V alloys. The techniques reviewed are Plasma sprayed deposition, Thermal spray, Dip coating, pulsed laser deposition, sol-gel. The advantages and disadvantages of each method over other techniques are discussed. The adhesion strength and the factors affecting the adhesion of HA coating on Ti-6Al-4V implants are also compared.

Citation: E. Mohseni, E. Zalnezhad, A.R. Bushroa, Comparative investigation on the adhesion of hydroxyapatite coating on Ti-6Al-4V implant: A review paper, International Journal of Adhesion and Adhesives, Volume 48, 2014, Pages 238-257, ISSN 0143-7496, <https://doi.org/10.1016/j.ijadhadh.2013.09.030>

6. Synthesis of antimicrobial silver/hydroxyapatite nanocomposite by gamma irradiation, Radiation Physics and Chemistry

Author:A. Akhavan, N. Sheikh, F. Khoylou, F. Naimian, E. Ataeivarjovi

In 2014, A. Akhavan, N. Sheikh, F. Khoylou, F. Naimian, E. Ataeivarjovi investigated Synthesis of antimicrobial silver/hydroxyapatite nanocomposite by gamma irradiation, Radiation Physics and Chemistry. The formation of AgNPs incorporated In the hydroxyapatite composite was studied as a function of γ -irradiation doses. Silver nanoparticles (AgNPs) were synthesized through γ -irradiation reduction of silver ions into hydroxyapatite as a solid support. The XRD pattern of nanocomposites was recorded. The FT-IR spectra of the samples were obtained, TEM, and Antibacterial Test.

Citation: A. Akhavan, N. Sheikh, F. Khoylou, F. Naimian, E. Ataeivarjovi, Synthesis of antimicrobial silver/hydroxyapatite nanocomposite by gamma irradiation, Radiation Physics and Chemistry, Volume 98, 2014, Pages 46-50, ISSN 0969-806X, <https://doi.org/10.1016/j.radphyschem.2014.01.004>.

7. Characterization of Magnesium-Doped Hydroxyapatite Prepared by Sol-Gel Process

Author:Ziani, S., Meski, S., &Khiredine, H

In 2013, Ziani, S., Meski, S., &Khiredine, H investigated Characterization of Magnesium-Doped Hydroxyapatite Prepared by Sol-Gel Process. In this paper explained about the Pure and doped hydroxyapatite (HA) nanocrystalline powders ($\text{Ca}_{10-x}\text{Mg}_x(\text{PO}_4)_6\text{OH}_2$) were synthesized using sol-gel process Pure and doped hydroxyapatite (HA) nanocrystalline powders ($\text{Ca}_{10-x}\text{Mg}_x(\text{PO}_4)_6\text{OH}_2$) were synthesized using sol-gel process and characterized by thermogravimetry (TG), (FTIR), (XRD), and inductively coupled plasma (ICP), (SEM), and (TEM).

Citation: Ziani, S., Meski, S., &Khiredine, H. (2013). *Characterization of Magnesium-Doped Hydroxyapatite Prepared by Sol-Gel Process. International Journal of Applied Ceramic Technology*, 11(1), 83–91. doi:10.1111/ijac.12093.

8. Silver and gold doped hydroxyapatite nanocomposites for enhanced bone regeneration

Author:Kumar, V. B., Khajuria, D. K., Karasik, D., &Gedanken, A

In 2019, Kumar, V. B., Khajuria, D. K., Karasik, D., &Gedanken, A investigated Silver and gold doped hydroxyapatite nanocomposites for enhanced bone regeneration. In this paper We report the osteogenic potential of silver(Ag), gold (Au), or silver-gold doped hydroxyapatite nanoparticles(Ag-Au-HA)in zebrafish (ZF) jawbone regeneration (JBR) model. The hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), Ag-HA, Au-HA, and Ag-Au-HA nanomaterials were synthesized by the coprecipitation procedure. The surface structure were analysed y SEM, TEM, FTIR, UV, XRD, EDS

Citation: Kumar, V. B., Khajuria, D. K., Karasik, D., &Gedanken, A. (2019). *Silver and gold doped hydroxyapatite nanocomposites for enhanced bone regeneration. Biomedical Materials*. [http//doi:10.1088/1748-605x/ab28e4](http://doi:10.1088/1748-605x/ab28e4).

9. Structural studies of magnesium doped hydroxyapatite coatings after osteoblast culture

Author:W. Mróz, A. Bombalska, S. Burdyńska, M. Jedyński, A. Prokopiuk, B. Budner, A. Ślósarczyk, A. Zima, E. Menaszek, A. Ścisłowska-Czarnecka, K. Niedzielski

In 2010, W. Mróz, A. Bombalska, S. Burdyńska, M. Jedyński, A. Prokopiuk, B. Budner, A. Ślósarczyk, A. Zima, E. Menaszek, A. Ścisłowska-Czarnecka, K. Niedzielski investigated Structural studies of magnesium doped hydroxyapatite coatings after osteoblast culture. In this paper, three target materials consisting of non-modified and magnesium modified hydroxyapatite with Mg content of 0.6 wt.% and 1.8 wt.% were ablated using an ArF excimer laser. Hydroxyapatite and magnesium modified hydroxyapatite were deposited on nitrited Ti6Al4V substrates by use of pulsed laser deposition technique. The obtained coatings were analyzed using X-ray diffraction, FTIR and AFM methods in order to determine the influence of magnesium on their

phase and chemical composition, crystallinity, surface morphology and biological properties.

Citation: W. Mróz, A. Bombalska, S. Burdyńska, M. Jedyński, A. Prokopiuk, B. Budner, A. Ślósarczyk, A. Zima, E. Menaszek, A. Ścisłowska-Czarnecka, K. Niedzielski, Structural studies of magnesium doped hydroxyapatite coatings after osteoblast culture, Journal of Molecular Structure, Volume 977, Issues 1-3, 2010, Pages 145-152,ISSN 0022-2860, <https://doi.org/10.1016/j.molstruc.2010.05.025>.

10. Antibacterial Copper–Hydroxyapatite Composite Coatings via Electrochemical Synthesis.

Author: Rashmi Ghosh, Oliver Swart, Sabrina Westgate, Benjamin L. Miller, and Matthew Z. Yates*,`

In 2019 Rashmi Ghosh, Oliver Swart, Sabrina Westgate, Benjamin L. Miller, and Matthew Z. Yates*,` investigated Antibacterial Copper-Hydroxyapatite Composite Coatings via Electrochemical Synthesis. The observation of antibacterial activity from a relatively low loading of copper on the bioactive HA support suggests that multifunctional implant coatings can be developed to supplement or supplant prophylactic antibiotics used in implant surgery that are responsible for creating resistant bacteria strains. In the first stage, HA Nanocrystals were grown on titanium using the cathodic electrolytic synthesis. Antibacterial activity was measured by culturing *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) in the presence of coatings having varying copper contents.

Citation: Antibacterial copper-hydroxyapatite composite coatings via electrochemical synthesis Rashmi Ghosh, Oliver Swart, Sabrina Westgate, Benjamin L. Miller, and Matthew Z. Yates*,

CHAPTER 3:
Aim and Objective

3.1 AIM AND OBJECTIVES OF THE CURRENT INVESTIGATION:

3.1.1 AIM OF THIS STUDY:

The goal of the work is to synthesize Nano-hydroxyapatite using diammonium hydrogen phosphate precursor and prepare Silver Oxide and hence prepare doped silver- hydroxyapatite by precipitation technique.

- Synthesis hydroxyapatite via diammonium hydrogen phosphate precursor
- Synthesis of Nano-silveroxide particle by sol-gel method
- Doping process to prepare silver doped - hydroxyapatite
- Characterization of the material synthesized:
- X-ray diffraction (XRD)
- Fourier transform infrared spectroscopy (FTIR)
- Scanning electron microscopy (SEM)
- Energy-dispersive X-ray spectroscopy (EDAX)
- Biological Properties:
- Hemolysis assay

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 when coated with it.
- Conducting a comparison of the final product made from different precursors and concentrations of metal dopants.

Chapter- 4

Material and Methodology

4.1 EXPERIMENTAL PROCEDURE

4.1.1 Synthesis of pure hydroxyapatite(HAP):

In this thesis, the pure HAPNano-particle was synthesised by Precipitation method. In this method, we split process into steps.

STEP 1: In step 1, the precursors of calcium nitrate(164.088g/mole) was dissolved with 50ml of distilled water in a 200ml beaker through under vigorous stirring of 30 minutes, we found a mix solution.

STEP 2: In step 2, the precursors of ammonium dihydrogen phosphate($\text{NH}_4\text{H}_2\text{PO}_4$) taken as amount of 94.9714g/mole, was dissolved with 50ml of distilled water in a 200ml beaker through under a vigorous stirring of 30minutes, we found a mix solution

STEP 3: In step 3, the mix solution of $\text{NH}_4\text{H}_2\text{PO}_4$ was taken in the burette and added a dropwise into the beaker of mix solution of calcium nitrate through under the vigorous stirring of 2-4 hrs, then we have a mix sol.

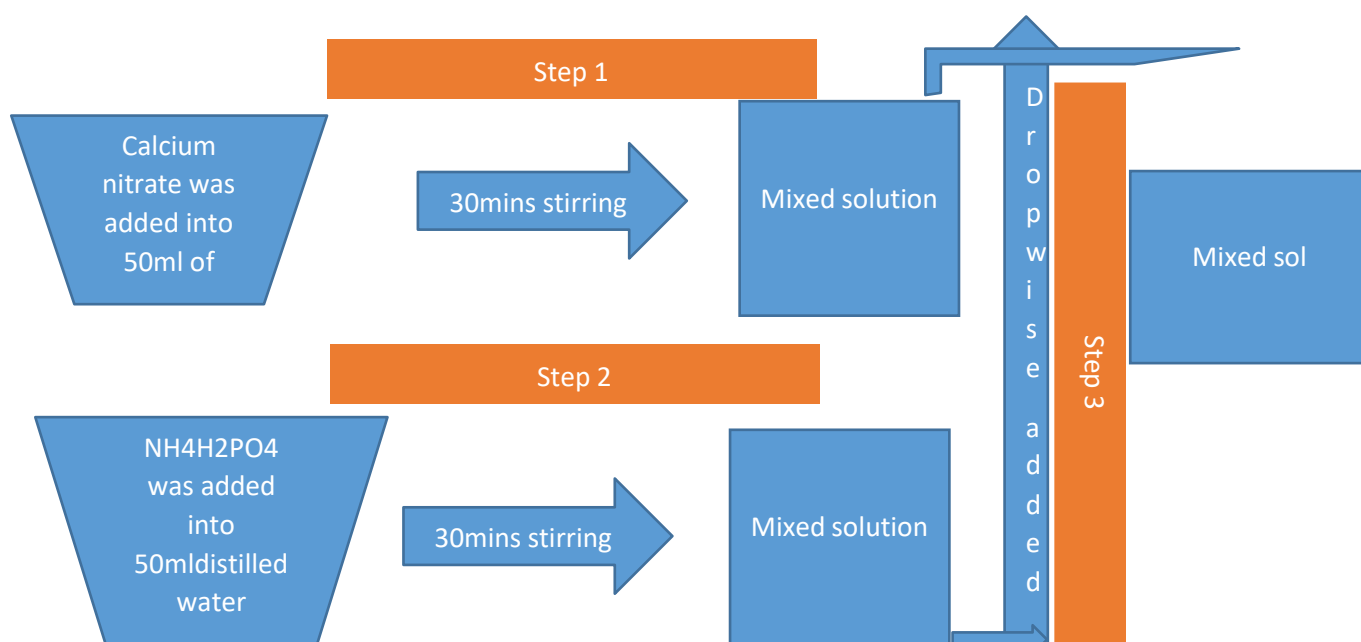


Fig 4.4 Experimental synthesis of HAP by precipitation method.



Fig 4.5 1 and 2 represent the steps process of mixed sol.

STEP 4: The mixed sol was adjusted by PH9 by adding ammonium hydroxide(NH_4OH) and then stirring for 1 day which mean 24hrs. then the sol was filtered by using watmann no.1 filter paper, then the filtered sol was placed a hot oven at 80deg C for 8hrs. the found dired powder was taken into the mortar pestle and grained it well until the powder Pure HAP fromed. Then the grained powder was again placed into the hot furnance under 800 degC for 2hrs until the deep dry powder was formed. Then the formed dried powder was graining by using mortar pestle then the pure HAP was formed.



Fig 4.6 Image 3 represent the dry HAP Powder

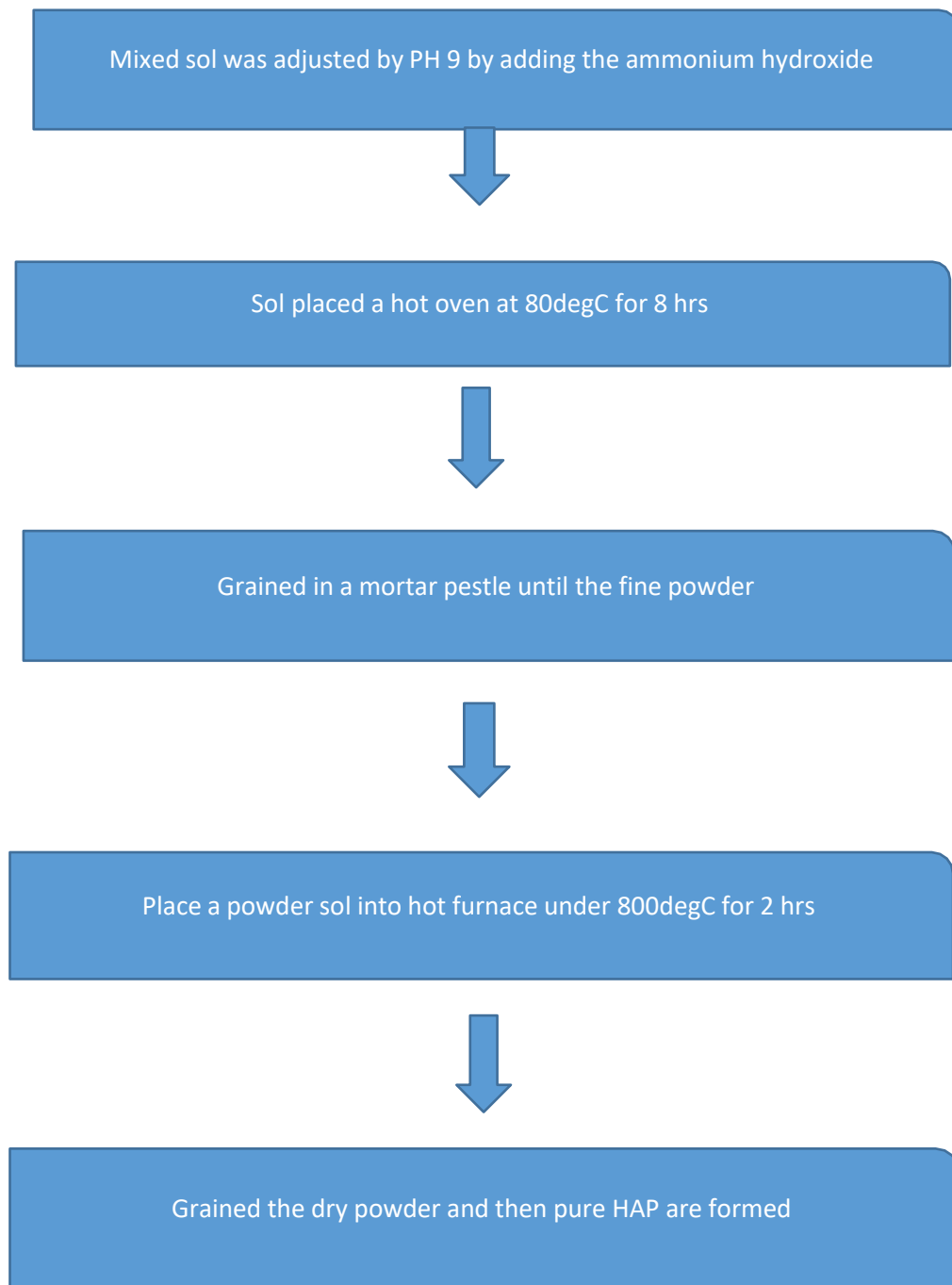


Fig 4.4 represent the step 4 process of synthesis HAP by precipitation method.

4.1.2 Synthesis of silver-oxide by sol-gel process:

In this thesis the silver oxide nanoparticle was synthesis by sol-gel methods.

STEP 1: In step 1, the precursors of AgNO_3 (3.398g) was dissolved in 20ml of deionized water in a 200ml beaker through under vigorous stirring of 30 minutes, we found a mix solution.

STEP 2: In step 2, the citric acid(1.92g) solution was prepared by 10ml of deionized water in a 200ml beaker under stirring of 30 minutes.

STEP 3: In this step, the two separately prepared solution were mixed continuously stirred for 30mins.

STEP 4: the ethylene glycol was then added dropwise with continuous stirring for 3-4 hrs with ethylene glycol acting as a sol stabilizer.

STEP 5: the obtained resultant precipitate was washed with distilled water and then dried at 80degC in an oven for 3hrs. the dried powder was grained by mortar pestle and then, finally the nanoparticles were put into the muffle furnace, here maintained at a constant temperature of 200degC for 3hrs. and then again the dried powder were grained finely by mortar pestle, then the silver nanoparticle were obtained

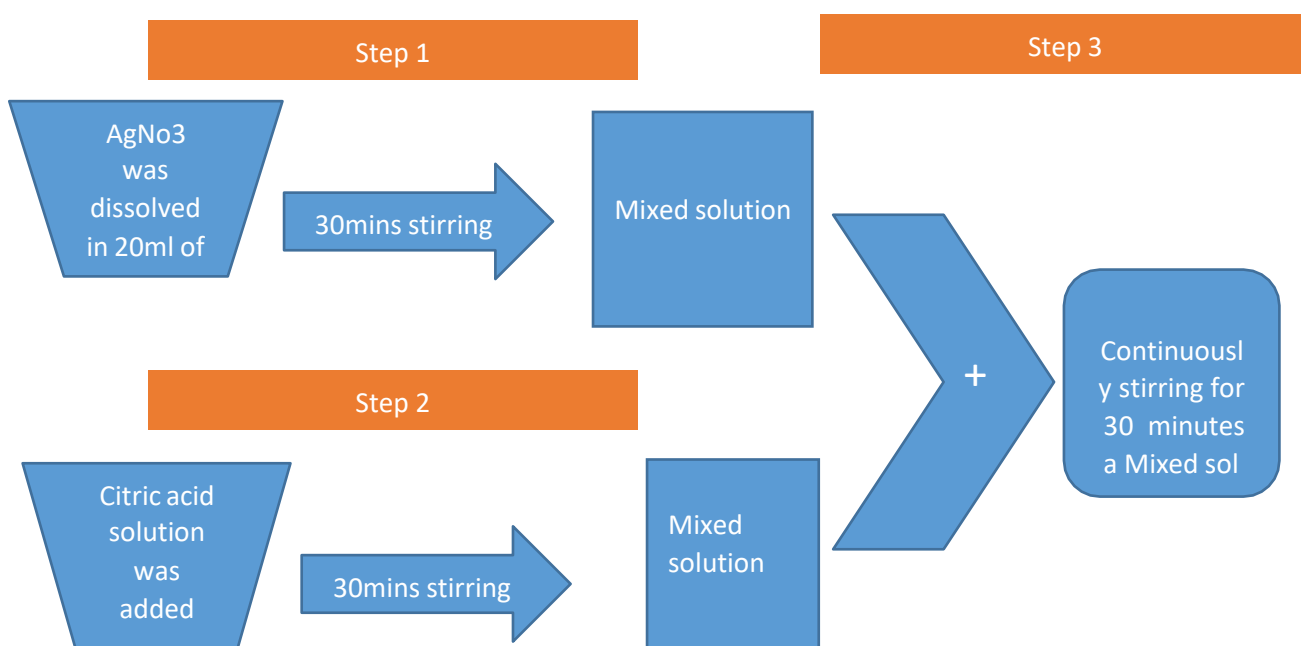


Fig 4.4 synthesis of pure silver oxide by sol-gel technique

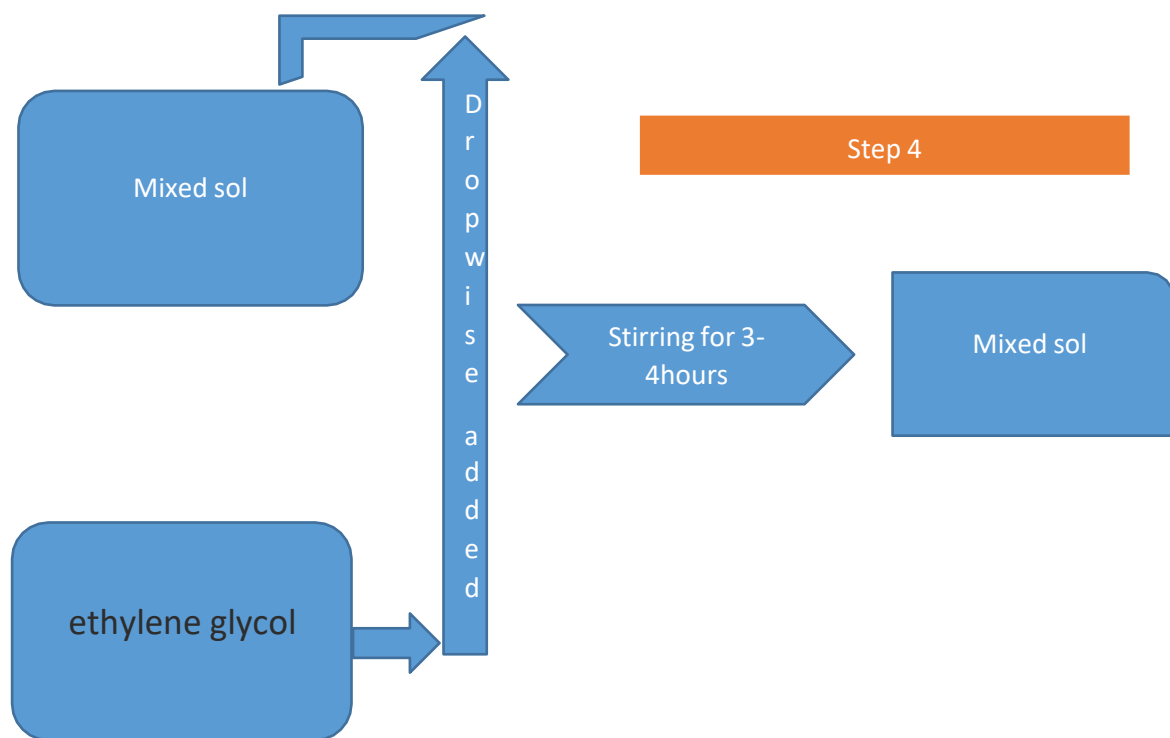


Fig 4.5 Step 4 process synthesis of pure silver oxide by sol-gel process

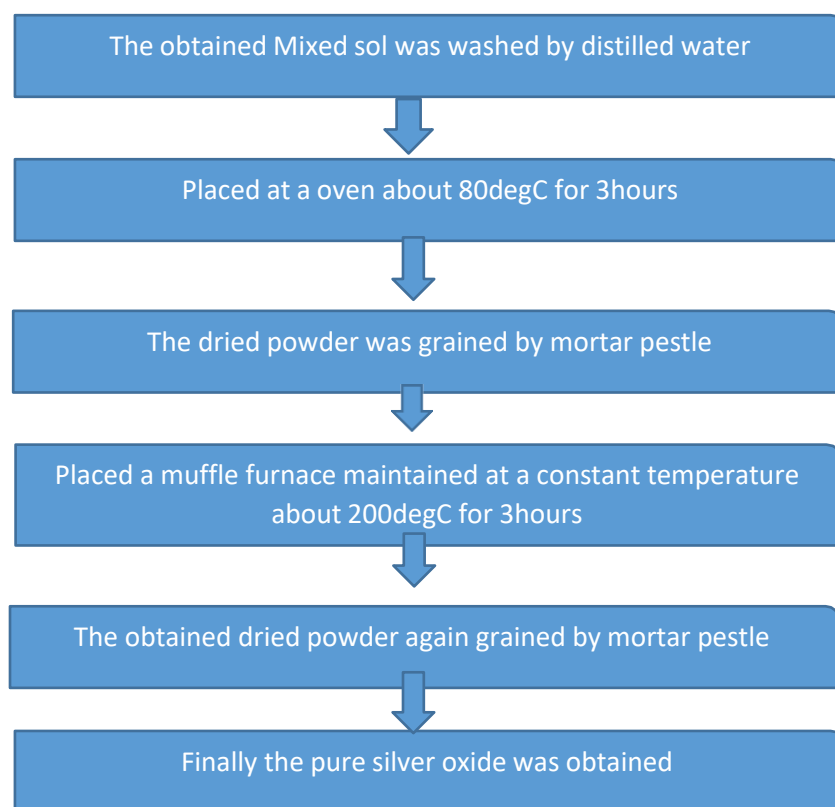


Fig 4.5 represent the synthesis of pure silver oxide of the step 5 process

4.1.3 Synthesis of HAP doped with Silver Oxide Nanoparticle:

The synthesized nanoparticle of pure HAP and silver oxide doped by the ratio of 5:1 which means 5 parts of pure HAP doped with 1 part of silver oxide. The measured two synthesized pure HAP and pure silver oxide was taken, through the mortar pestle and graining well until it mixed for 1-2 hours, finally a doped HAP with AgO₂ nano powder were obtained. Now the obtained HAP doped AgO₂ given to the characterisation like XRD, SEM, FTIR, BIOCOMPATIBILITY studies.

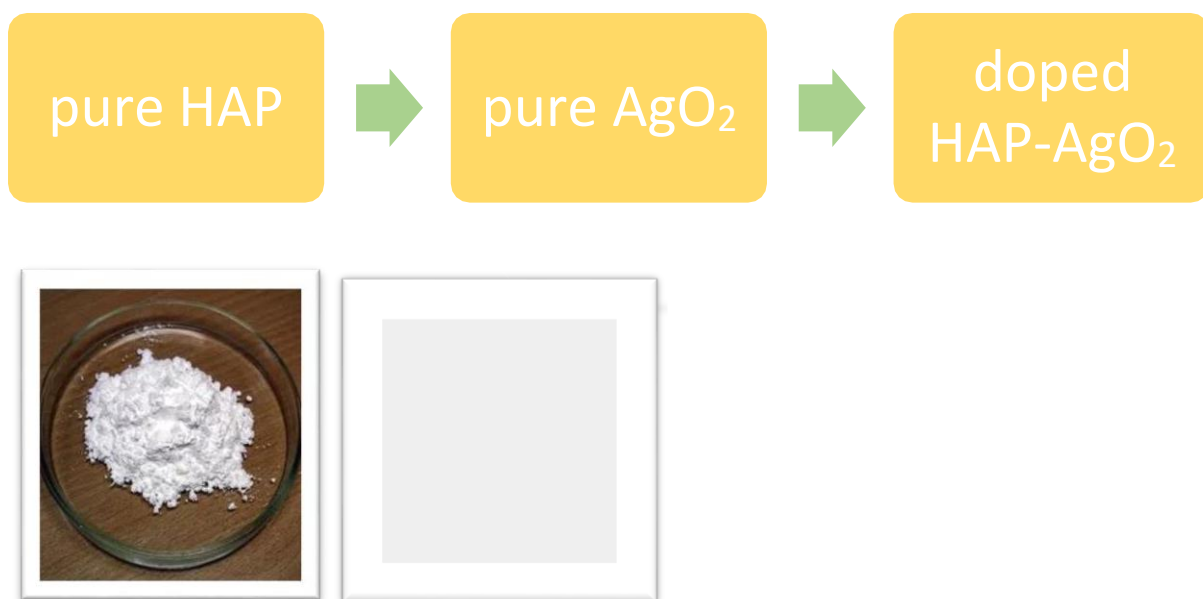


Fig 4.6 schrematic diagram for synthesis of HAP doped silver oxide

4.2 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 Xrays 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).

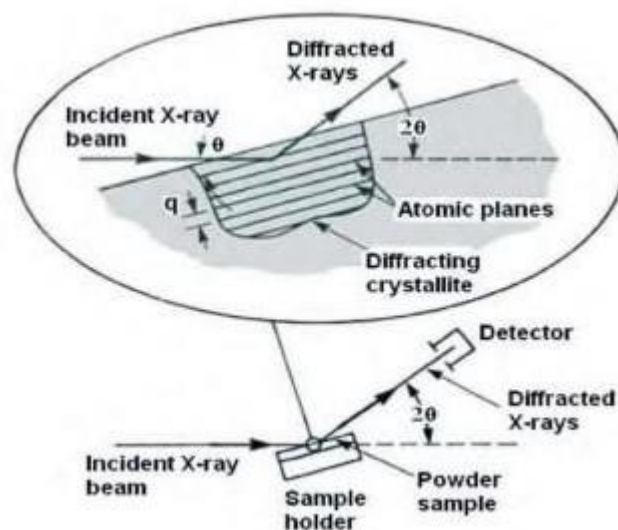


Figure 4.2 X-Ray diffractometer

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 2 and a scan step time of 32 s. The generator was set to 40kV and 25mA. The Lynx Eye detector was used.

4.3 FTIR (Fourier Transform Infrared):

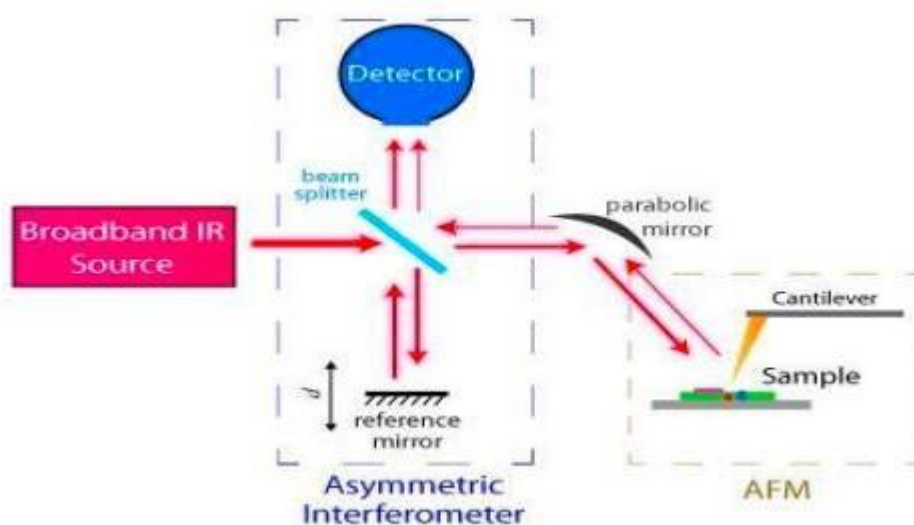


Figure 4.3 FTIR Spectrometer.

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 ($4000\text{--}400\text{ cm}^{-1}$) wavelength. The Fourier Transform Spectrometer converts a timedomain plot into a frequency domain spectrum.

4.4 SCANNING ELECTRON MICROSCOPE (SEM):

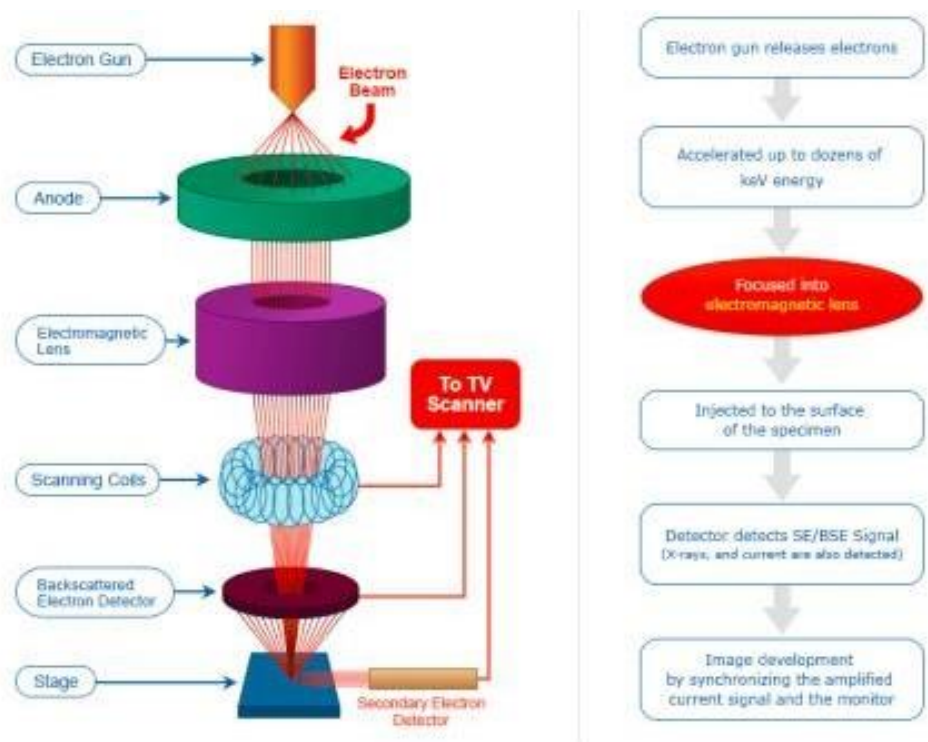


Figure 4.4 Scanning Electron microscope.

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 working 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.

4.5 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.

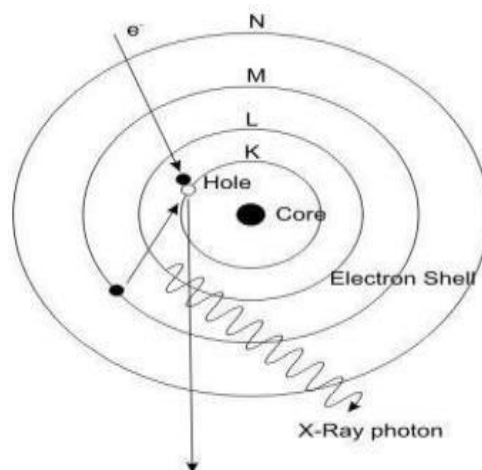


Figure 4.5 Working Principle of EDX

4.6 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].

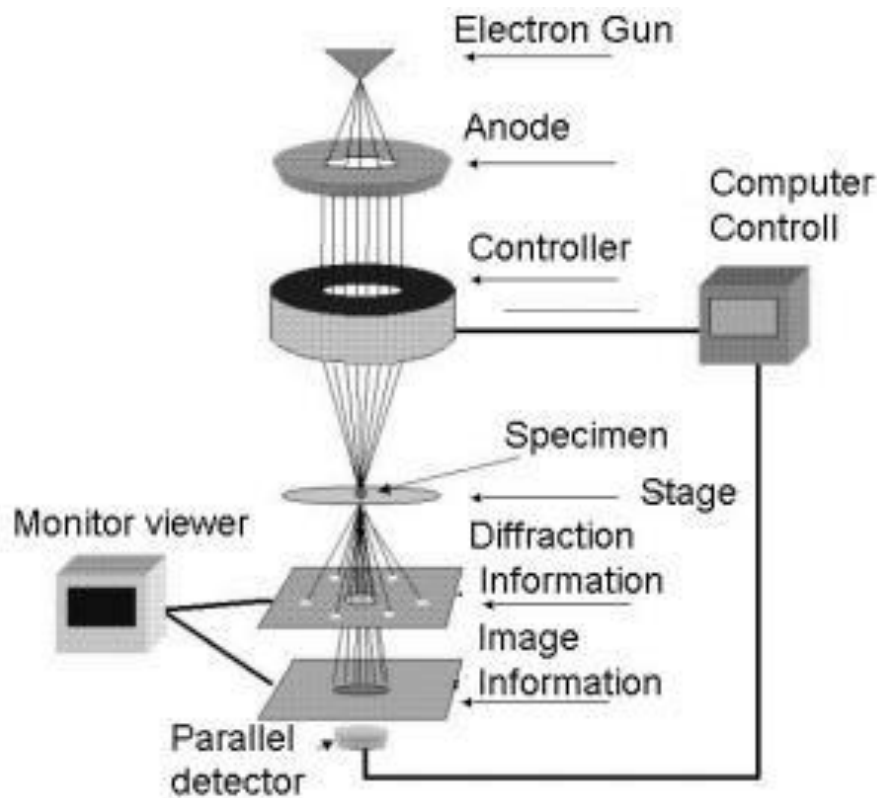


Figure 4.6 Transmission electron microscope.

4.7 Biocompatibility studies:

Hemolysis analysis:

Hemolysis is damage to red blood cells (RBCs), which results in the release of the iron-containing protein hemoglobin into plasma. In this case, the rupture of the RBCs is due to improper blood collection or sample handling. This kind of hemolysis may be due to phlebotomist using too thin needle size, inappropriate tubes mixing, or a too prolonged tourniquet time, as well as due to causes related to the transport or storage of the samples. In addition, this type of hemolysis is the one on which the clinical laboratory plays a more important role trying to reduce its incidence. To do this, it must first be estimated and managed properly by including in the report the degree of interference, e.g. through comments, as well as by managing indicators to know their impact on the blood collection centers, strengthening the training where sample hemolysis rates are higher than the quality objectives set[42]. Here we describe an in

vitro assay specifically developed for the analysis of nanoparticle hemolytic properties. for standard hemolysis test, a fresh human blood was collected in anti-coagulant heparin- containing tubes. The samples were immersed in 6ml extraction medium of sterilized physiological saline and incubated at 37degC for 30minutes under static conditions. Here, the negative control was sterilized physiological saline and the positive control was distilled water. The anti-coagulated human blood was diluted by sterilized physiological saline. The 0.2ml extract of each sample was incubated for 60mins at 37degC in water bath, therefore each tube was centrifuged and the absorbance of the supernatant was measured at 545nm[42].

The hemolysis ratio (Z) was calculated by following formula;

$$Z = (AS - AN / AP - AN) * 100$$

Where,

Z - Hemolysis ratio.

AS - Absorption value of the sample.

AN - Absorption value of the negative control.

AP - Absorption value of the positive control.

When Z is >5, the hemolytic grade is hemolytic

Z is 2-5, the hemolytic grade is slightly hemolytic

Z is 0-2, the hemolytic grade is non-hemolytic

The spectral absorption value of AN, AP, AS was recorded on schimadzu UV-1601 at 545nm.

CHAPTER -5

Result and Discussion

Chapter – 5 Result and Discussion

5.1 Result and Discussion

5.1.1 XRD

HAP NPs were synthesized by a precipitation technique for calcium and phosphate precursors and characterized by various techniques. We conducted an XRD analysis to further clarify the phase content. Figure 5.1a shows a XRD image of pure HAP synthesized NPs. It can be seen that the NPs have a two intense peaks at 31.13 and 31.87 of the pure HAP NPs which is corresponding to the (211) of pure hap formed crystal. It is evident that no other peaks than HAP are detected.

AgO₂ NPs were prepared by sol-gel synthesis, where figure 5.1b show the XRD pattern of typical peaks of highly crystalline well define AgO₂. The strong orientation towards the (110) index supports the elongated morphology of AgO₂. The index (111) of AgO₂ show the crystalline in nature. no other impurities are detected.

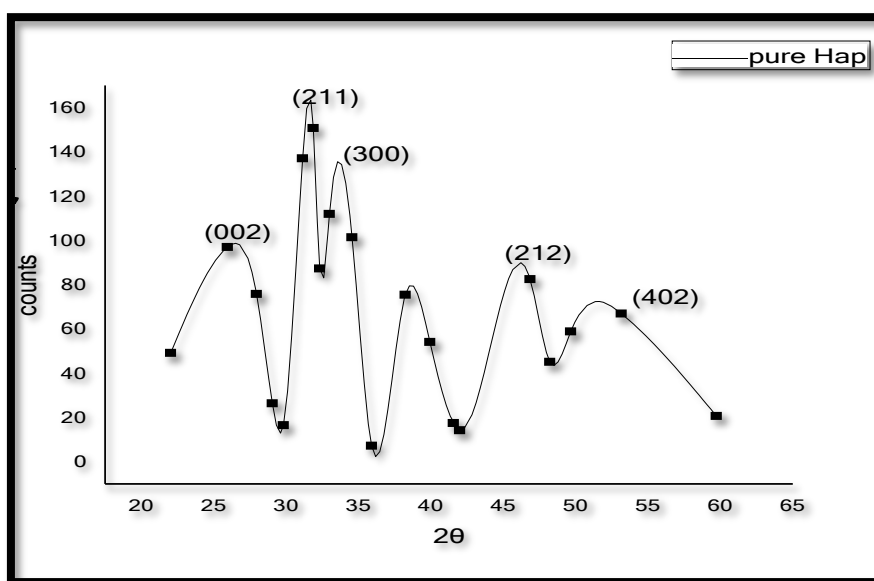


Fig 5.1a show the XRD image of Synthesized pure HAP NPs

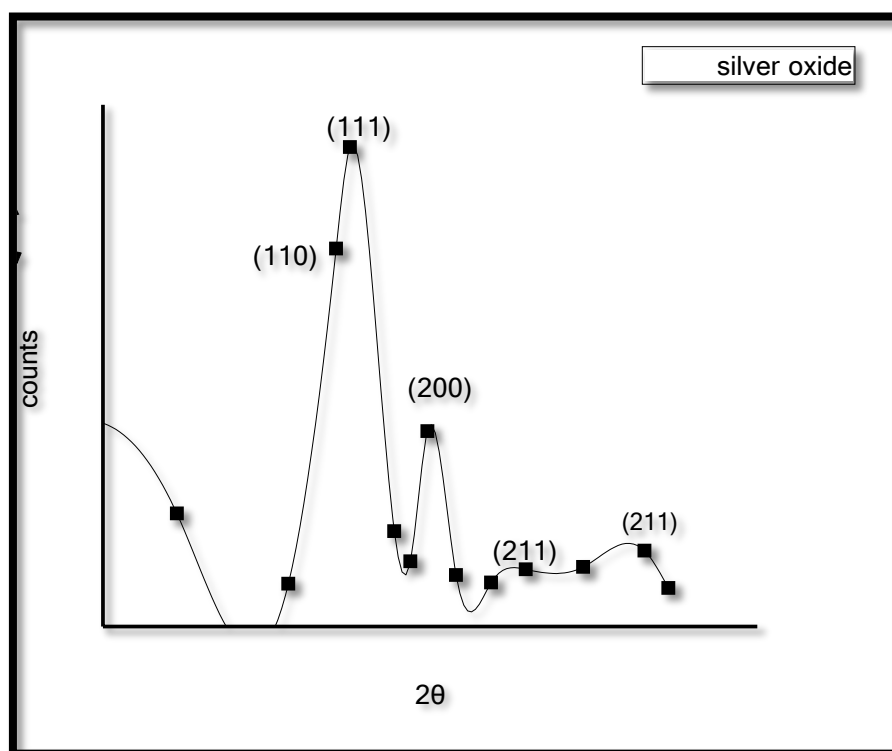


Figure 5.1b show the XRD peaks of pure silver oxide

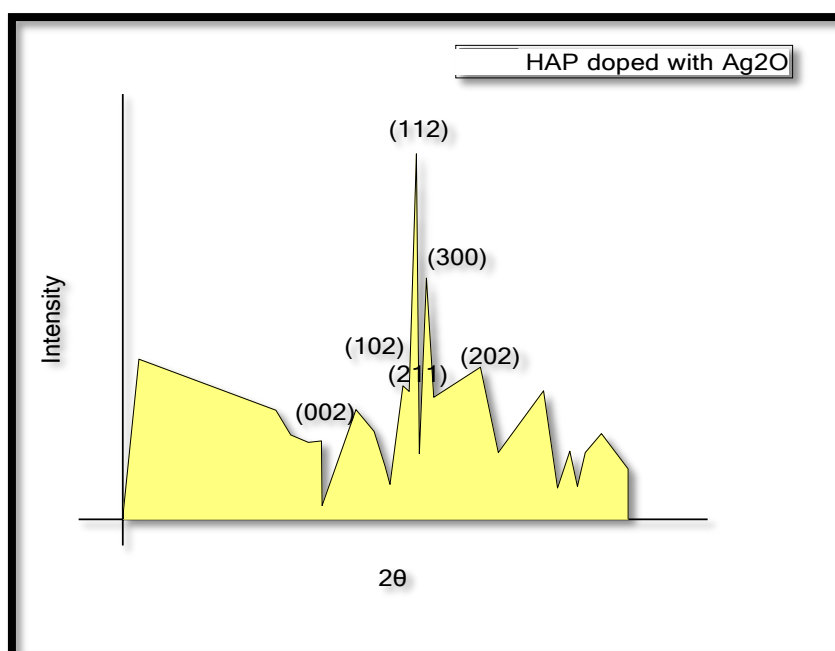


Figure 5.1c show the xrd peaks of AgO₂-HAP

For stable AgO₂ – HAP sample were prepared by adding a solvent ratio of 1:4. And the doped AgO₂, figure 5.1c show the XRD pattern of intensive peaks of crystalline, and from the fig of 5.1c also shows the two separate peaks of silver oxide and HAP were present in the crystalline peaks.

5.1.2 SEM AND EDAX

HAP NPs were synthesized by a precipitation technique for calcium and phosphate precursors and characterized by various techniques. Figure 5.2a shows a SEM image of pure HAP synthesized NPs. It can be seen that the NPs have an agglomerated shape. The average particle size was 200nm ± 2000nm based on SEM. Fig 5.2b shows the selected area electron diffraction pattern of the HAP NPs. Table 5.1 shows the EDS element analysis of the HAP NPs. The obtained powder is composed of Ca, P, and O, where the latter is associated with carbon impurities. The atomic Ca/p ratio is 1.73, which is slightly higher than the expected stoichiometric Ca/P ratio (1.67) in HAP.

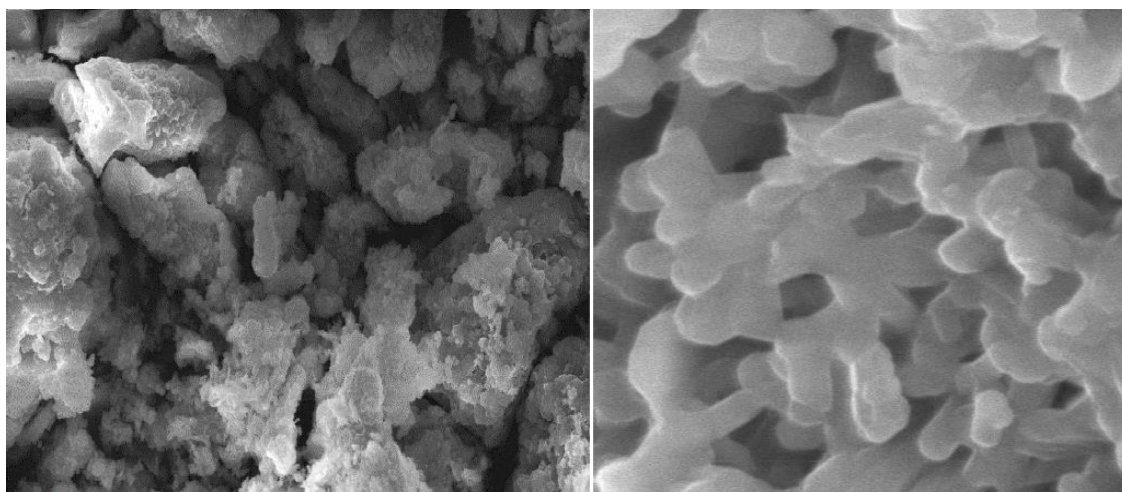


Fig 5.2a shows the SEM image of 200nm-2000nm

AgO₂ NPs were prepared by sol-gel technique, figure 5.2c shows a SEM images that reveals that AgO₂ NPs have an agglomerated structure with elongated shape. here the average particle size was 200nm – 2000nm based on SEM . Figure 5.2d shows the selected area electron diffraction pattern of the HAP NPs. Table 5.2 shows the EDS

element analysis of the pure AgO₂ NPs. The obtained powder is composed of Ca, P, O, and Ag. Where the latter is associated with carbon impurities.

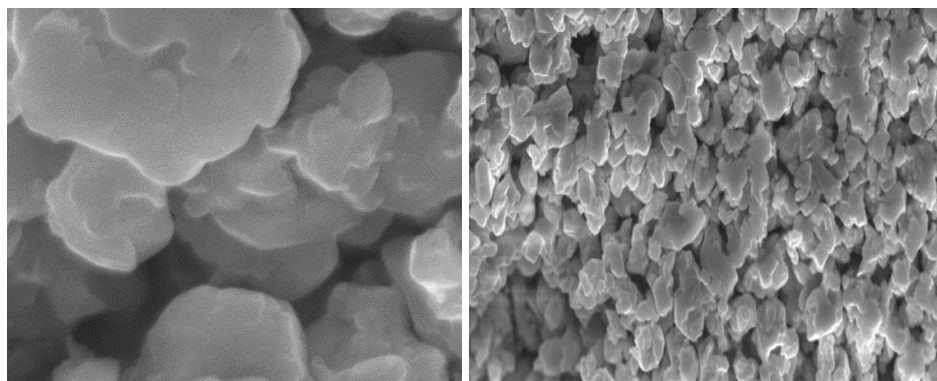


Fig 5.2c shows the SEM image of 200nm-2000nm

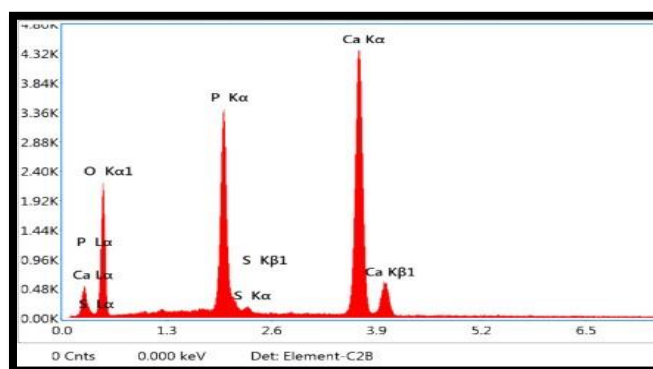


Fig 5.2b show that the image of pure HAP EDS analysis

Table 5.1 shows the EDS element analysis of the HAP NPs

ELEMENT	WEIGHT %	ATOMIC%	ERROR%	K RATIO
OK	43.4	63.8	10.6	0.0629
PK	16.6	12.6	3.2	0.1340
SK	0.6	0.4	16.6	0.0047
CaK	39.4	23.1	2.0	0.3548

Table 5.2 shows the EDS element analysis of the AgO₂ NPs

ELEMENT	WEIGHT %	ATOMIC%	ERROR%	K RATIO
O k	23.1	67.0	11.7	0.0342
AgL	76.9	33.0	2.0	0.7209

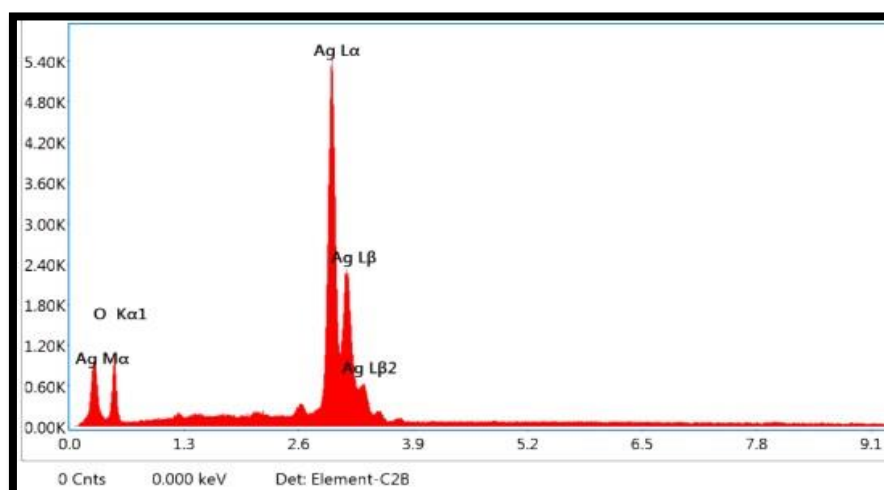
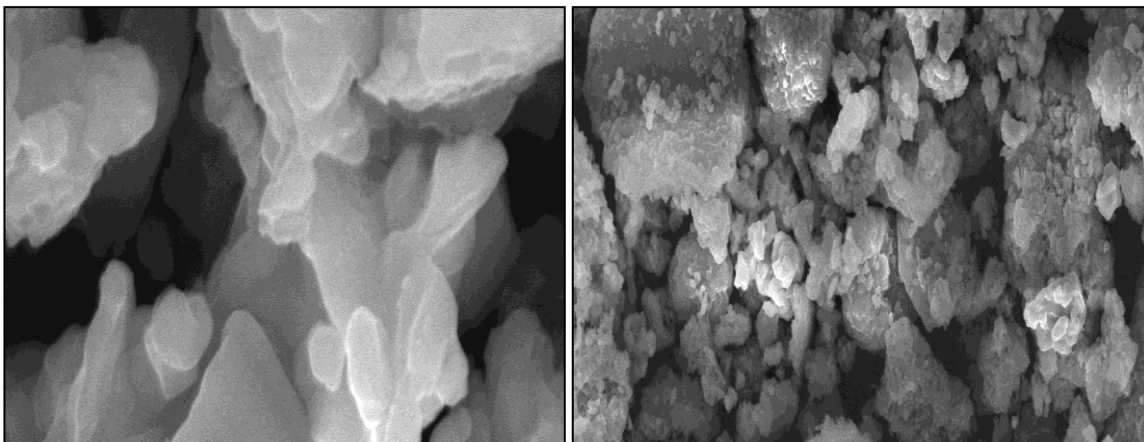


Fig 5.2d show that the image of pure AgO₂ EDS analysis

HAP doped silver oxide NPs were prepared by taking a certain ratio of individual sample and grinding well, figure 5.2e shows a SEM images that reveals that HAP-AgO₂ NPs have an agglomerated structure with elongated shape. here the average particle size was 200nm – 2000nm based on SEM . Figure 5.2f shows the selected area electron diffraction pattern of the HAP-AgO₂ NPs. Table 5.2 shows the EDS element analysis of the pure AgO₂ NPs. Here, The obtained powder is composed of Ca, P, O, and Ag. Where the latter is associated with carbon impurities.



5.2e shows a SEM images of HAP-AgO₂ NPs.

Table 5.3 shows the EDS element analysis of the HAP-AgO₂ NPs

ELEMENT	WEIGHT %	ATOMIC%	ERROR%	K RATIO
O K	47.4	69.7	10.5	0.0769
P K	14.0	10.6	3.6	0.1112
S K	0.4	0.3	26.6	0.0029
AgL	8.1	1.8	6.5	0.0652
CaK	30.2	17.7	2.6	0.2641

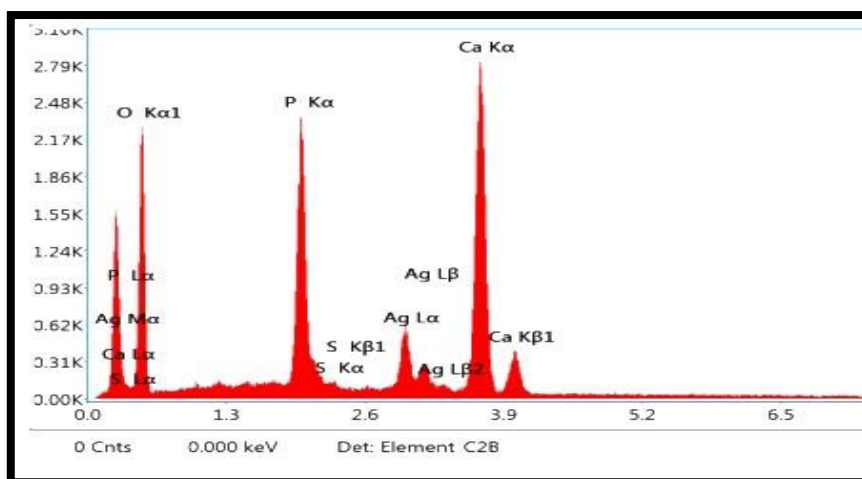


fig 5.2f show that the image of HAP- AgO2 EDS analysis

5.2.3 Fourier Transform Infrared Spectroscopy

FTIR spectra of HAP, AgO₂, and HAP-AgO₂ are show in the figure. The characteristic absorbance peaks of HAP obtained in the region of 1000 – 1400 cm⁻¹, where the intense peaks at 522, 600 cm⁻¹ are associated with metal-oxide [M-O] stretching, are shown in the figure 5.4a,

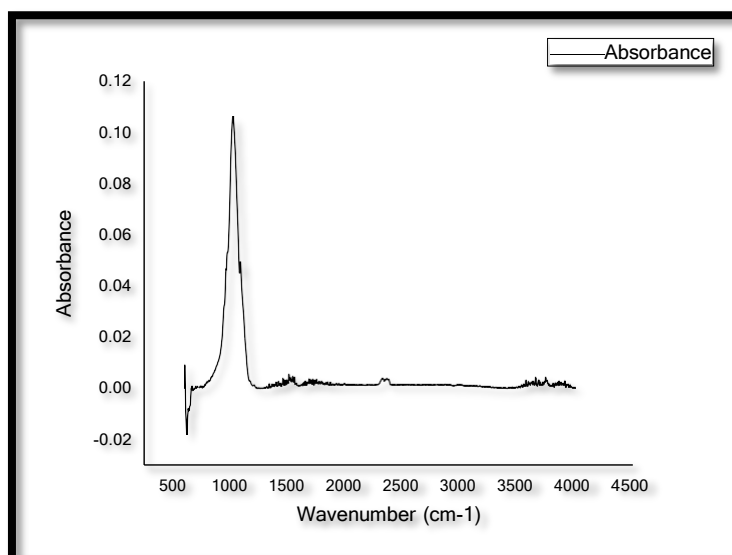


Figure 5.4a shows that the absorbance of PURE HAP

The characteristic Transmittance peaks of HAP obtained in the region of 1000 – 1400 cm^{-1} , where the intense peaks at 522, 600 cm^{-1} are associated with metal-oxide [M-O] stretching, are shown in the figure 5.4b

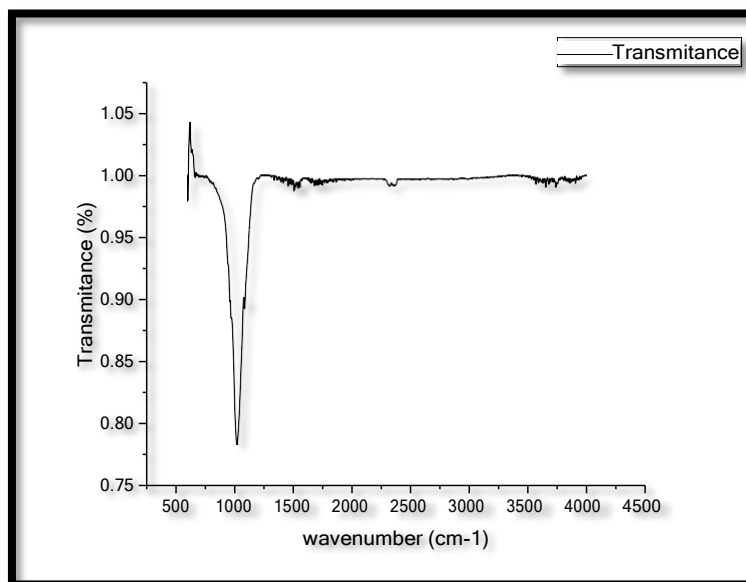


Figure 5.4b show that the Transmittance peaks of pure oxide

FTIR spectra of AgO_2 , are show in the figure 5.4b. The characteristic absorbance peaks of HAP obtained in the region of 1000 – 1400 cm^{-1} , where the intense peaks at 522, 600 cm^{-1} are associated with metal-oxide [M-O] stretching, the 3500 – 4000 cm^{-1} are attributed to the OH group, are shown in the figure 5.4c,

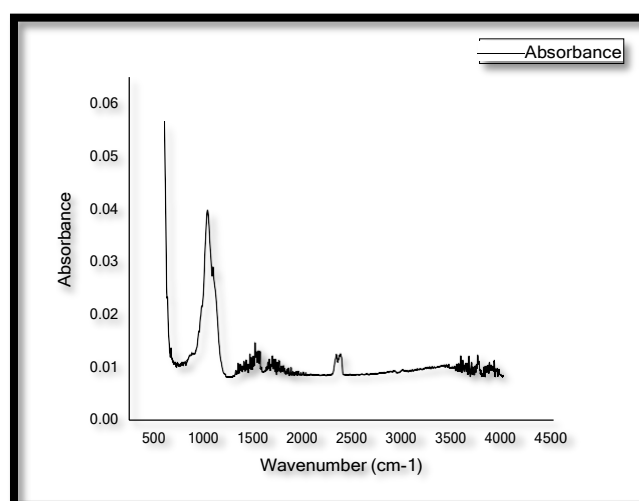
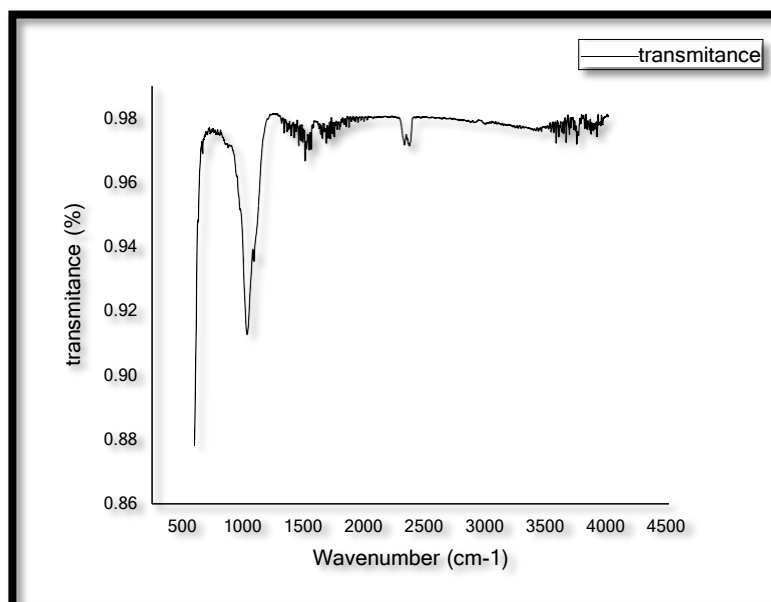


Figure 5.4c show that the absorbance peaks of silver oxide

The characteristic Transmittances peaks of silver oxide obtained in the region of 1000 – 1400 cm^{-1} , where the intense peaks at 522, 600 cm^{-1} are associated with metal-oxide [M-O] stretching, are shown in the figure 5.4d



f

Figure 5.4d show that the transmittance peaks of silver oxide

FTIR spectra of HAP-AgO₂, are show in the figure 5.4e. The characteristic absorbance peaks of HAP obtained in the region of 1000 – 1400 cm^{-1} , where the intense peaks at 522, 600 cm^{-1} are associated with metal-oxide [M-O] stretching, The two intense peaks at 1500 - 2000 cm^{-1} are associated with CO₃²⁻ impurities. the 3500 – 4000 cm^{-1} are attributed to the OH group, are shown in the figure 5.4c. It can be seen that the peaks of hap doped AgO₂ are intensified, the HAP content in the dispersion increases, where the strong peak of AgO₂ at 500 - 600 cm^{-1} obtained.

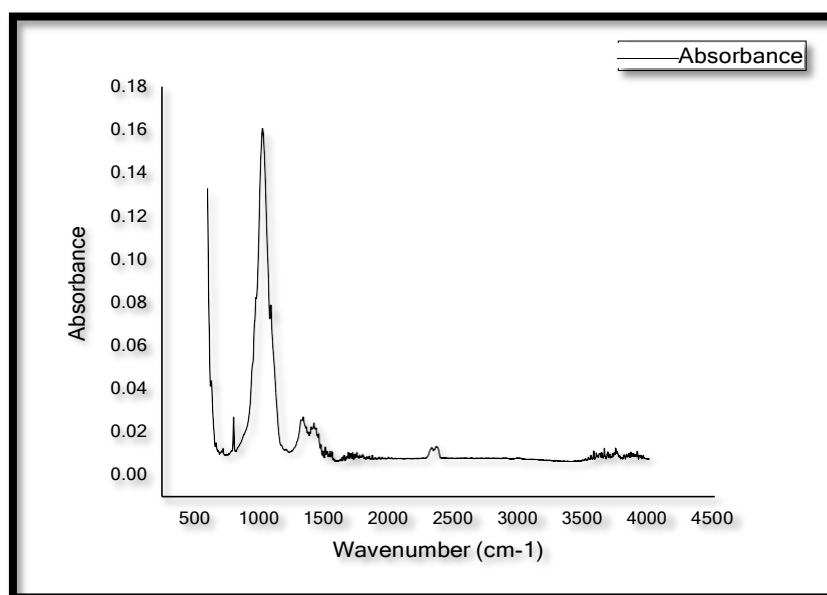


Figure 5.4e show that the absorbance peaks of HAP-AgO₂

The characteristic Transmittances peaks of HAP- AgO₂ obtained in the region of 1000 – 1400 cm⁻¹, where the intense peaks at 522, 600 cm⁻¹ are associated with metal-oxide [M-O] stretching, are shown in figure 5.4f

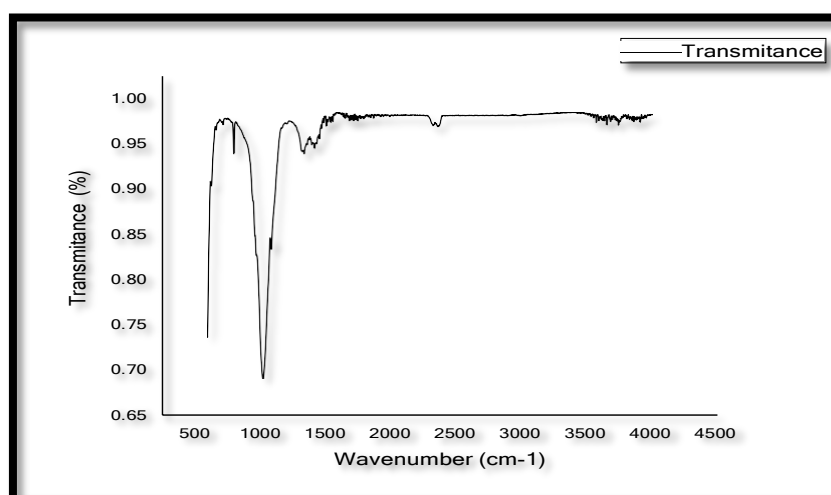


Figure 5.4f show that the transmittance peaks of HAP-AgO₂

5.2.4 Biocompatibility studies

5.2.4.1 Hemolysis analysis:

Hemolysis is damage to red blood cells (RBCs), which results in the release of the iron-containing protein hemoglobin into plasma. Here we describe an in vitro assay specifically developed for the analysis of nanoparticle hemolytic properties. For standard hemolysis test, a fresh human blood was collected in anti-coagulant heparin-containing tubes. The samples were immersed in 6ml extraction medium of sterilized physiological saline and incubated at 37degC for 30minutes under static conditions. Here, the negative control was sterilized physiological saline and the positive control was distilled water.

The anti-coagulated human blood was diluted by sterilized physiological saline. The 0.2ml extract of each sample was incubated for 60mins at 37degC in water bath, therefore each tube was centrifuged and the absorbance of the supernatant was measured at 545nm.

The hemolysis ratio (Z) was calculated by following formula;

$$Z = (AS - AN \div AP - AN) \times 100$$

The spectral absorption value of AN, AP, AS was recorded on schimadzu UV-1601 at 545nm.

So, the obtained absorption spectrum values of AN, AP, AS are noted, the noted value are applied in the formula and calculate the Z value.

The absorption value for the sample of synthesized pure HAP,

$$AS = 0.0134 \quad AN = 0.0081 \quad AP = 0.896$$

$$Z = (0.0134 - 0.0081) \div (0.896 - 0.0081) \times 100$$

$$Z = 0.59 \%$$

The absorption value for the sample of synthesized pure AgO₂,

$$AS = 0.0349 \quad AN = 0.0096 \quad AP = 0.896$$

$$Z = (0.0349 - 0.0096) \div (0.896 - 0.0096) \times 100$$

$$Z = 2.85 \%$$

The absorption value for the sample of synthesized pure HAP,

$$AS = 0.0239 \quad AN = 0.0094 \quad AP = 0.896$$

$$Z = (0.0239 - 0.0094) \div (0.896 - 0.0094) \times 100$$

$$Z = 1.6354 \%$$

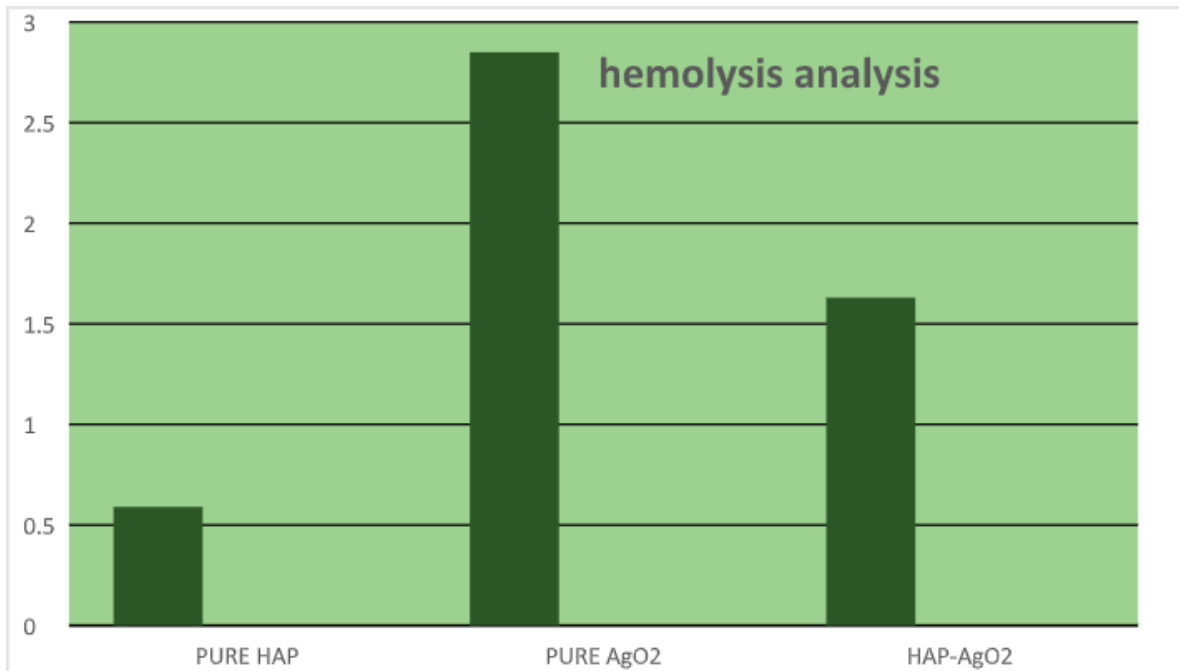


Fig 5.3 show that the range value of hemolysis analysis

Conclusion and References

6. Conclusion

Nanocrystal line hydroxyapatite bio ceramic powder can be synthesis by the simple precipitate & sol-gel processing technique. Powder particle-size of nano-HAP can be refined by doping with silver oxide nanoparticles. X-ray diffraction analysis showed that the apatite phase started to appearing at temperature as low as 250 °C and a complete crystallization was achieved at 500 °C. The reflection intensity of the major peaks in the calcined powder improved with increasing temperature indicating an increase in hydroxyapatite phase content, which is attributed to both the removal of the organic residuals and the improved crystallinity of the apatite. Pure nano-HAp showed a sintered density of 3.07 g/cm³ when sintered at 1250 °C. Sintering at 1300 °C resulted in sintered density values of 3.12 g/cm³ in samples of pure nano-HAp. The maximum sintered density of 3.29 g/cm³ was achieved in composition A1.0 (nano-HAp doped with 1.0 wt.% of MgO). The surface hardness of nanocrystal line Hap ceramics is influenced by the presence of magnesium and zinc as dopants, during powder synthesis. 20% increase in hardness was recorded in samples of composition A1.0, compared to pure nano-HAp. Nano crystalline powder improved compression strength of HAp ceramics. Presence of dopants further improved compressive strength. 34% improvement in compression strength was recorded for composition A1.0. Biodegradation studies conducted in dynamic SBF brought out the fact that presence of these dopants in nanocrystalline hydroxyapatite ceramics reduces the rate of resorption and surface hardness degradation, compared to pure nanocrystalline hydroxyapatite.

7. References

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