"NANOSTRUCTURED HYDROXY APATITEFOR BIOMEDICAL APPLICATION"

A PROJECT WORK (BP812PW) SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

BY

PATEL NIRMAL M. (18BPH055)

SOLANKI NISARG R. (18BPH056)

PATEL SHUBHAM M. (18BPH089)

KHANDHARA VIHAR S. (18BPH100)

Semester VIII

UNDER THE GUIDANCE OF

DR. BHAGWATI SAXENA

INSTITUTE OF PHARMACY
NIRMA UNIVERSITY
SARKHEJ-GANDHINAGAR HIGHWAY
AHMEDABAD-382481
GUJARAT, INDIA

May 2022

CERTIFICATE

This is to certify that Project Work (BP812PW) entitled ***"NANOSTRUCTURED HYDROXY APATITE FOR BIOMEDICAL APPLICATION"*** *is the bonafide work carried out by* ***PATEL NIRMAL M. (18BPH055), SOLANKI NISARG R. (18BPH056), PATEL SHUBHAM M. (18BPH089), KHANDHARA VIHAR S. (18BPH100),*** *B.Pharm semester VIII under my guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2021-2022. This work is up to my satisfaction.*

Guide: Bhagwat

Dr. BHAGWATI SAXENA
M. Pharm., Ph.D.,
Assistant Professor,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Dr. TEJAL A. MEHTA
M. Pharm., Ph.D.,
Head, Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

Prof. Manjunath Ghate
M. Pharm., Ph.D.,
Director,
Institute of Pharmacy,
Nirma university

Date: 07 / 06 / 2022

CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work (BP812PW) entitled ***"NANOSTRUCTURED HYDROXY APATITE FOR BIOMEDICAL APPLICATION"*** *Submitted by* ***PATEL NIRMAL M. (18BPH055), SOLANKI NISARG R. (18BPH056), PATEL SHUBHAM M. (18BPH089), KHANDHARA VIHAR S. (18BPH100),*** *B.Pharm. Semester VIII is a bonafide review work carried out by us at the Institute of Pharmacy, Nirma University under the guidance of "Dr. Bhagwati Saxena". We are aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review work carried out by us is not reported anywhere as per best of our Knowledge.*

Patel Nirmal M. (18BPH055), H M Patel
Solanki Nisarg R. (18BPH056), N Solanki
Patel Shubham M. (18BPH089), S.K Patel
Khandhara Vihar S. (18BPH100)
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Guide: Bhagwat
Dr. BHAGWATI SAXENA
M. Pharm., Ph.D.,
Assistant Professor,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Date: 07 / 06 /2022

Date: 07 / 06 /2022

DECLARATION

We, ***PATEL NIRMAL M. (18BPH055), SOLANKI NISARG R. (18BPH056), PATEL SHUBHAM M. (18BPH089), KHANDHARA VIHAR S. (18BPH100),*** *students of VIIIth Semester of B.Pharm at Institute of Pharmacy, Nirma University, hereby declare that our project work (BP812PW) entitled* ***"NANOSTRUCTURED HYDROXY APATITE FOR BIOMEDICAL APPLICATION"*** *is a result of culmination of our sincere efforts. We declare that the submitted project is done solely by us and to the best of our knowledge, no such work is done by any other person for the award of degree or diploma or for any other means. We also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.*

Patel Nirmal (18BPH055), N.M. Patel
Solanki Nisarg (18BPH056), Solanki
Patel Shubham (18BPH089), S. Khate
Khandhara Vihar (18BPH100),
Institute of Pharmacy
Nirma University
Sarkhej - Gandhinagar Highway
Ahmedabad-382481
Gujarat, India

Date: 07 / 06 / 2022

ACKNOWLEDGEMENTS

We will take opportunity firstly thank almighty for his constant shower of blessings in all our endeavors. We would also like to take the opportunity to express our heartily thanks to all those who are related to our thesis in some or the other way and have been a part to frame it.

Secondly we would like to thank our parents for their timely supports and their absolute love for us.

In providing the fundamental picture of our thesis we would take this opportunity to express our heartily gratitude to our guide, Dr. Bhagwati Saxena, Associate Professor, Department of Pharmacology, Institute Of Pharmacy, Nirma University. Her timely guidance and support provided shape to this project because of which we are truly grateful.

Lastly we would like to thank Prof. Manjunath Ghate, Director, Institute of Pharmacy, Nirma University for providing platform to showcase our talent regarding this thesis.

ABSTRACT

Hydroxy Apatite has a wide range of applications in the pharmaceutical business. It has been used to treat calcium shortages including osteoporosis and rickets, among other things. Dietary supplements containing calcium phosphate are also available. Calcium phosphate is found in the teeth and bones of humans. The mineral hydroxyapatite(Ca10a(PO4)v6 (OH) 2, often known asaHA, is a naturally occurringcalcium phosphate.

The major mineral component of the human body's bones and teeth. The Nanostructured Hydroxyapatite nanoparticles have been synthesized by various methods. Hydroxyapatite can be utilized in medical devices since it is biodegradable and biocompatible, and it can be employed with nanotechnology. Different processes are used to synthesize nanostructured hydroxyapatite from both natural and manufactured sources. Nanostructured hydroxyapatite has a wide range of uses and current developments, including Magnetic Drug Delivery Systems, Gene Targeting Systems, Tissue Scaffolds, and as a carrier for a variety of issues.

Because they are prone to enzymatic degradation as well as oxidative reactions, Nanostructured Hydroxyapatite nanoparticles face numerous obstacles in terms of stability and use. Currently, research is underway to address this issue of nanostructured hydroxyapatite safety and stability.

I. INTRODUCTION

Bone is made up of approximately 8% water, 22% protein, and 70% mineral. The element of minerals Calcium phosphate (Ca P) is a kind of calcium that is found in bone. The chemical formulas for hydroxyapatite is Ca10 (PO4) six (OH) 2 the hexagonal crystalline structure, and so on. HAP makes up roughly 65 percent of bone mass and is essential for bone health. majority of its rigidity and strength Crystals of HAP in the bone are needle-like crystals that can be found in the a nanometre-sized range with a width of 5-20 nm and a length of 60 nm The synthesis of Nano-structured HAP is of interest. Due to its wide range of uses, it has sparked a lot of attention. Application in orthopaedics, dentistry, and drug delivery. Nano-sized HAP has a high surface energy, according to studies, which may improve mechanical characteristics and allow for faster implant surface turnover. HAP particles are connected with extracellular matrix proteins like collagen in bone tissues. HAP crystals and collagen fibres work together to improve bone mechanical characteristics and compensate for HAP's low formability and moderate breakdown rate. As a result, combining HAP with a polymer matrix would be perfect for maximising the benefits of each material while reducing the drawbacks.[1][3][5]

II. SYNTHESIS OF HYDROXY APATITE FOR BIOMEDICAL APPLICATIONS

3.1 Processing of plants to provide extracts for PMS Processing: -

One of the most important processes in the synthesis process is the creation of capping agents. Soxhlet extraction, distillation, and adsorption are examples of sample preparation processes. Some conventional basic separations include chromatography and HPLC. Techniques are used as well. The two methods are boiling and filtration. Conventional strategies that might be used to get basic information natural capping agents derived from raw or unprocessed plants form. Plant biochemical such as pectin and malic acid, tartaric acid, piperine, and sugar (sucrose) are all produced from the same source through separation operations from plant sources, with extraction being one of the most important processes.[44]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

Plant extracts are employed straight after treatment processes such as boiling and filtration in several synthesis procedures (methods). Because the plant extracts include particular phytochemicals that can initiate and control the morphology to obtain the required nanoparticles or nanostructures, they were used as the medium for preparing HAP nanoparticles.[6]

These procedures (methods) did not include chromatographic separation techniques (methods), such as HPLC, for obtaining specific extracts (such as alkaloids or plant organic acids) for use as capping agents.[38]

Gopi and colleagues prepared direct green templates for HAP synthesis using three types of fruit. Tamarind, grape, and banana extracts were used to make the extracts. Individual fruit peels and seeds were discarded, and 1 kg of pulp was mashed in 100 mL sterile double distilled water. The resulting mixture was filtered and dried at 40 degrees Celsius using what man No. 1 filter paper. The powder that resulted was immediately employed as a green template. Their goal was to use the tartaric acid in these three fruits to get an effective tempting effect.[44]

The well-known and commonly utilised extraction technique for obtaining High-performance liquid chromatography is used to find specific desirable capping or reducing agents for hydroxyapatite production (HPLC). HPLC is a type of liquid chromatography that uses instruments. A stationary phase made up of tiny particles is used achieving separations that are more efficient than those employed in liquid chromatography, as it is known.[44]

Green synthesis, particularly those involving plant extracts, is becoming increasingly popular, not just for Nano-HAP synthesis but for a wide spectrum of nanoparticle synthesis. Some of these nanoparticles have even been used in biological and pharmacological applications. As a result, crude green plant extracts should be investigated for synthesis. The use of less solvent volume, less time consuming,

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

less expensive, and may be improved for extracting phytochemicals from plants is a viable solution of incorporating extraction techniques.[44]

Plant	Part	Extract/templating agent	Processing/extraction method	Type of HAp	Crystallite size (nm)	Morphology
Natural rubber latex (NRL) (*Hevea brasiliensis*)	—	Latex	Dilution with DI, preservation with ammonia solution	Nano-HAP	$X = 0$, (30.81 to 66.98) $X = 1$, (21.10 to 33.49)	Nanorods
Ginger	Roots	Aqueous filtrate	Distillation and filtration	HAp nanosheets	47 nm	Nanosheets
Tamarind (*Tamarindus indica*)	Fruit	Aqueous extract (tartaric acid)	Crushing, filtration, boiling, drying and crushing into powder	Nano-HAP	49	Uniform nanorods
Grape (*Vitis*)	Fruit	Aqueous extract (tartaric acid)	Crushing, filtration, boiling, drying, and crushing into powder	Nano-HAP	53	Nanorods (rod-like structure)
Banana (*Musa acuminata*)	Fruit	Aqueous extract (tartaric acid)	Crushing, filtration, boiling, drying and crushing into powder	Nano-HAP	57	Nanorods in the form of ice cubes
Banana (*Musa paradisiaca*)	Peel	Pectin	Fractional extraction	HAp, TCP	25.17–47.20	Discrete spherical HAP
Apple (*Malus domestica*)	Fruit (juice)	Malic acid	High-performance liquid chromatography (HPLC)	Pure nano-HAp	40	Ball-like structure
Prickly pear fruit (*Opentia ficus-indica*)	Peel	Pectin	Drying and grinding	HAp and beta tricalcium phosphate (TCP)	24	Cluster-like/ granular-like
Bitter orange (*Citrus aurantium*)	Peel	Pectin	Drying and grinding	HAp and tricalcium phosphate (TCP)	—	Bead-like structure
Pineapple	Fruit (juice)	Sucrose	Water extraction method by using HPLC	Nano-HAp	—	Cubic-like
Sugar cane	Stem	Sucrose	Water extraction method by using HPLC	Nano-HAp	—	Spherical shape
Carrot	Root	Sucrose	Water extraction method by using HPLC	Nano-HAp	—	Capsule-like
Moringa oleifera	Flower	Aqueous flower extract	Drying and boiling	Nano-HAp	41	Nanorods
Moringa oleifera	Flower	Flower extract	Soxhlet extraction	HAp, TCP	200	Crystalline plate like structure
Azadirachta indica	Leaves	—	Boiling and filtration	Nano-HAP	53	Hexagonal structure
Coccinia grandis	Leaves	—	Boiling and filtration	Nano-HAp	64	Hexagonal structure

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

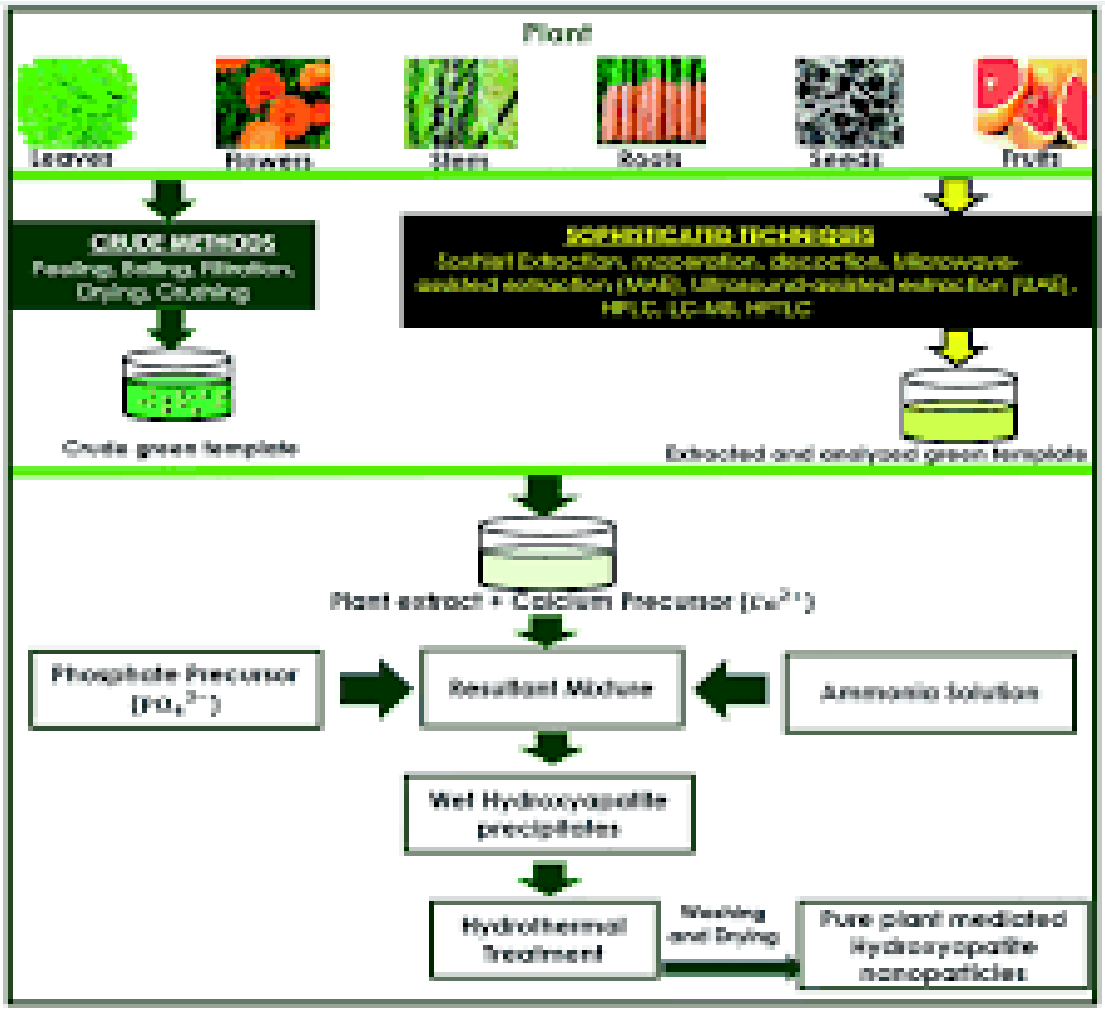

FIG I. Processing of plants to provide extracts for PMS Processing

3.2 Preparation of hydroxyapatites Nano ceramics using a wet chemical technique

The wet chemical process is used to make hydroxyapatite Nano ceramic. Tetra hydrate of calcium nitrate Di-ammonium hydrogen orthophosphate ($Ca(NO_3)_4H_2O$) and ($Ca(NO_3)_4H_2O$). The initial precursors are ($(NH_4)_2HPO_4$). Calcium nitrate tetra hydrate and

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

di-ammonium molar concentrations the concentrations of hydrogen orthophosphate are adjusted to achieve theoretical results. The calcium-to- phosphorus ratio is 1.66. Drop by drop, the precipitation process takes place. Di- ammonium hydrogen orthophosphate is added to calcium. Solution of nitrate tetra hydrate the ammonia is used to bring the pH level back to normal. The composition ofthe reaction mixture the process is carried out for 5 hours at 80degree Celsius with continuous stirring then aged for roughly 24 hours. The white precipitate is washed 3–4 times with double distilled water before being dried in an air oven at 100degree Celsius and sintered for 2 hours at 1000degree Celsius.[10]

The sodium and potassium ions are used to replace the calcium cation in the ion exchange mechanism. The ion exchange process is performed by dissolving produced HAP Nano ceramic powder in NaCl and KCl (0.1 M) solutions and shaking them at room temperature for 5 hours to achieve saturation. After that, the ion exchanged HAP is filtered and dried at 100degree Celsius. The samples for subsequent bioactivity and dielectric tests are pellets of pure and ion exchanged HAP produced using a hydraulic press at 5 tonne pressure.[45]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

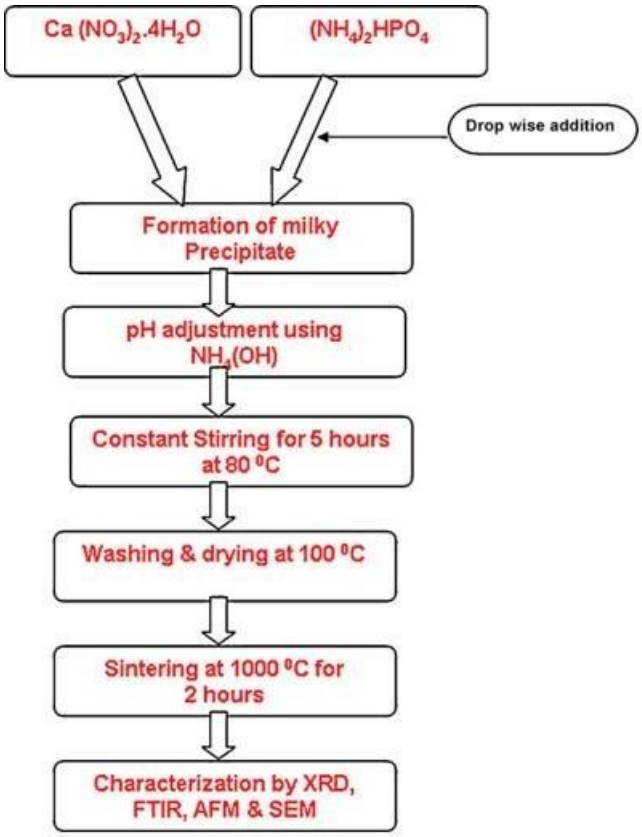

FIG II. SCHEME FOR SYNTHESIS OF HAP NANOCERAMIC BY WET CHEMICAL PROCESS

3.3 SOL – GEL METHOD

From an economic standpoint, the sol-gel process is seen as an efficient and appealing way for the creation of HAP that is more bioresorbable Similar to bone apatite in appearance. This procedure entails calcium, phosphorous precursor solutions (calcium and phosphorous source) are utilised for the process of preparing HAP Calcium, and magnesium ions deteriorate with age. Low-temperature phosphorous precursor solutions to get a sol, then at a greater temperature it is named after the process of creating a gel. As in the sol-gel approach Calcination of the gel obtained in this manner, HAP is produced when the temperature is raised. Calcium nitrate [$Ca(NO_3)_2$] and calcium diethoxide [$Ca(OEt)_2$] are the most commonly used Ca- precursors, while ammonium hydrogen phosphate [$(NH_4)_2HPO_4$] and triethyl phosphate are the most commonly used P-precursors.[5][34]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

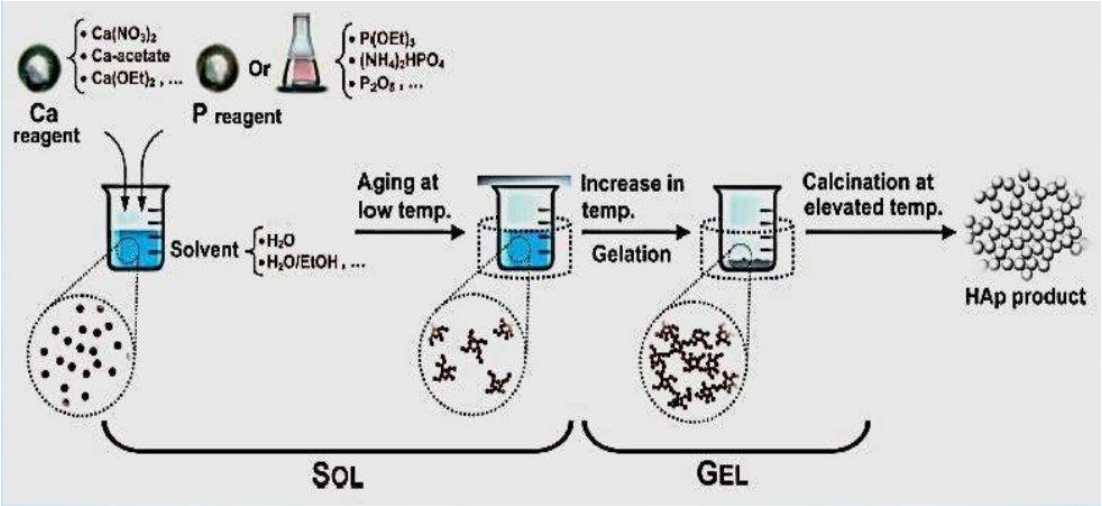

FIG III. SYNTHESIS OF HAP FROM SOL GEL METHOD

3.4 BIOGENIC ROUTES

Different mechanochemical processes can be used to extract crystalline Nano-HAP for biomedical and clinical applications from various biogenic sources and bio-wastes. Animal (bones, eggshells, etc.), plant (fruit peels, leaves, stems, roots, etc.), and aquatic (corals, seashells, fish scales/bones, etc.) sources are among them. Animaland aquatic sources have the most HAP, which when calcined produces Nano HAP. Similarly, eggshells are high in $CaCO_3$ and can be used to make CaO, which can then be turned into HAP as follows[11]

3.5 HAP SYNTHESIS WITH BIO WASTE EGG SHELLS:-

The production of hydroxyapatite may be done with easily accessible bio-waste. Due to its low cost, bio-waste is converted into profitable bioproducts.Because their physicochemical properties are identical to those of real human bone hydroxyapatite, HAP synthesised from natural resources such as eggshells has been shown to have good biocompatibility and biodegradability. India produces roughly 190,000 tonnes of eggshells each year, which contain about 91 percent CaCO3 and might be utilised to restore bone tissue as a biomaterial. Several scientists have established that hydroxyapatite generated from eggshells is a well-known osteoconductive material that may be used as a bone replacement. Eggshell has been used to make hydroxyapatite. Eggshells are used to make hydroxyapatites in two processes. The eggshell was heated

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

to 450degree Celsius for 2 hours at a rate of 5degree Celsius per minute in the first stage. The sample was then heated at 900 degree Celsius for 2 hours at a rate of 0.5 degree Celsius at the second phase, min1. Calcium oxide was converted to HAP in a phosphate solution. A 1.67 calcium/phosphate ratio is employed. Kattimani and colleagues investigated a bone implant made from eggshell hydroxyapatite alternative bone-building substance defect repair.[15][43]

3.6 Mechanochemical Method

It is referred to as the mechanical alloying procedure. This process is used to create nanocrystalline alloys and ceramics. The reagent molar ratio is preserved stoichiometric when pulverised on a planetary mill. The size of crystallites reduces as time passes. Calcium and phosphorus are combined in a certain ratio. When a powder combination is placed in a ball mill, it is exposed to high energy collisions from the balls, and mechanical force is employed to achieve chemical processing and transformation. Zirconia, aluminia, stainless steel, and other materials are used as milling medium. It is vital to maintain the milling ball ratio. The primary downside of this approach is the production of agglomerates and the lengthy processing time.

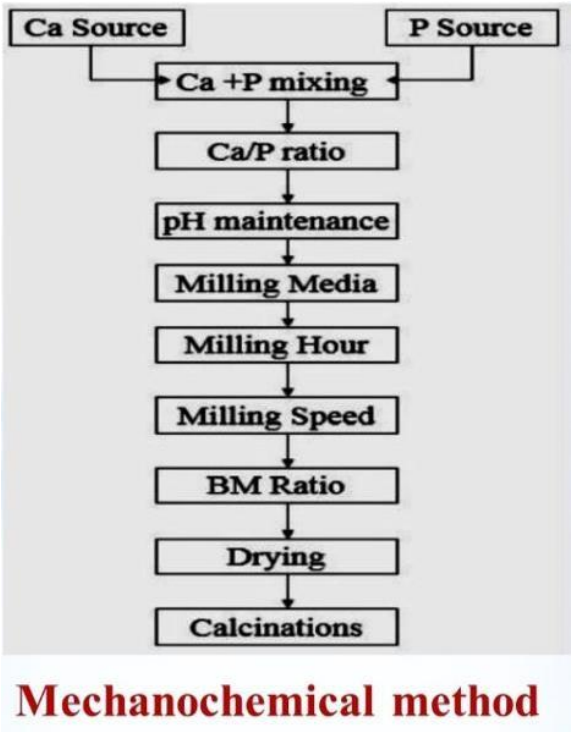

FIG IV. SYNTHESIS OF HAP FROM MECHANOCHEMICAL METHOD

3.7 Conventional Chemical Precipitation

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

It is the simplest way for synthesis and the most often utilised method for production of nanosized HaP. At pH 4.2, HaP is the least soluble, whereas CaP is often more stable in aqueous solution. Precipitation reactions are often carried out at pH levels greater than 4.2 and temperatures near to the boiling point of water. Calcium Hydroxide or Calcium Nitrate are Ca2+ sources, whereas orthophosphoric acid or dihydrogen phosphate are PO43- sources. The typical procedure comprises dropwise addition of one reagent to another with continuous and gentle stirring while the molar ratio of elements (Ca/P) is preserved at stoichiometry according to its ratio in HaP. The resulting suspension can be aged at atmospheric pressure or immediately cleansed, filtered, dried, and crushed into a powder. The benefits of this process are its low cost, easily available materials for synthesis, ease of operation, and low cost raw materials.

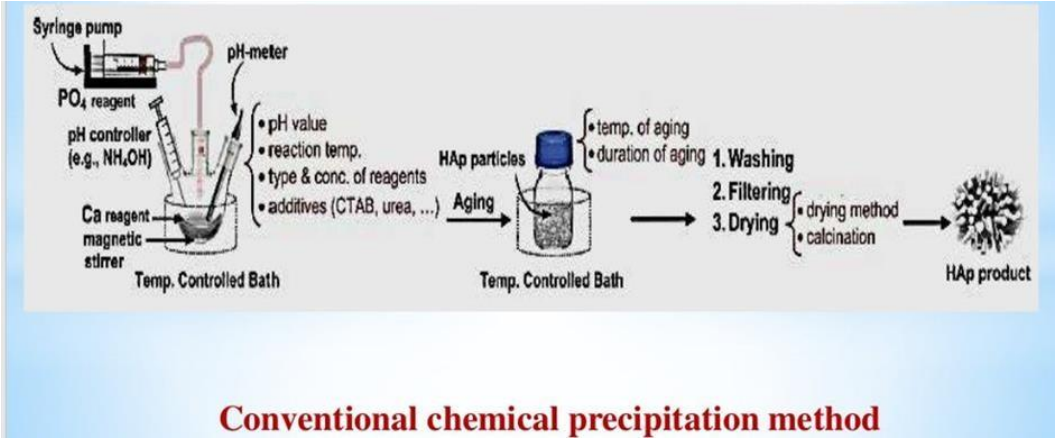

FIG V. SYNTHESIS OF HAP FROM CONVENTIONAL CHEMICAL PRECIPITATION METHOD

3.8 Hydrolysis method

The ingredients employed in this approach for the manufacture of HaP nanoparticles are Dicalcium phosphate dihydrate (DPCD) and Tricalcium phosphate (TCP). Because of the slow rate of OCP hydrolysis and the capacity of OCP to absorb contaminants, hydrolysis of Octa calcium Phosphate (OCP) has not been of considerable interest for the precipitation of HaP particles. The aqueous hydrolysis of HaP is divided into two stages, namely dissolution and precipitation. Under pH values greater than 6-7, the acidic phases of DPCA and DPCD are thermodynamically less stable and undergo transition into a more stable CaP. For example: HaP

3.9 Nonchemical Method

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

In this procedure, strong ultrasound radiation is used to initiate the reaction. The initial process is mixing, in which Ca and P Precursors are combined at a predetermined ratio and constant pH, followed by ultrasonic wave passage, drying, and calcination. The sonochemical approach has the advantage of taking less time and producing smaller, pure HaP crystals.

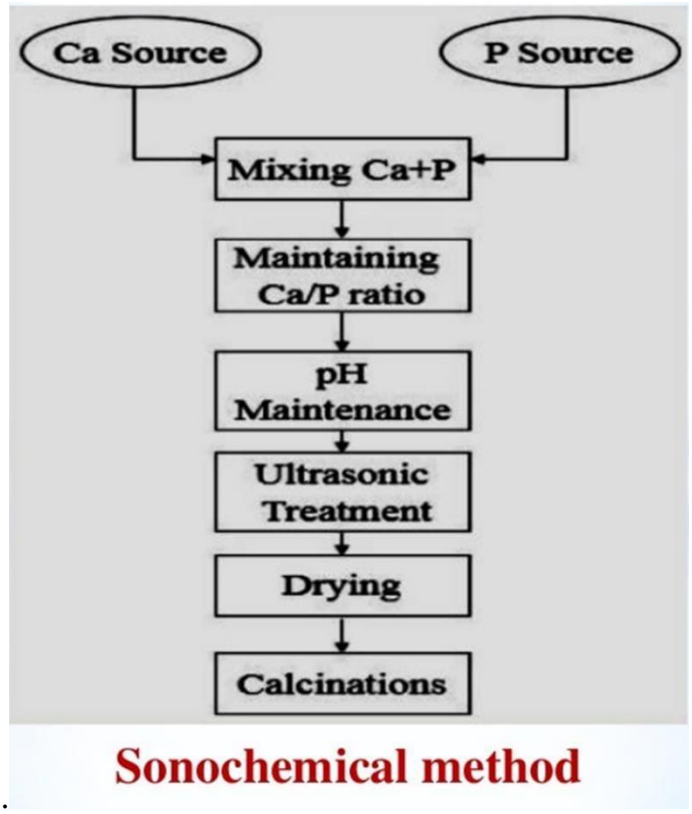

FIG VI. SYNTHESIS OF HAP FROM SONOCHEMICAL METHOD

3.10 Combustion Method

Combustion process of HaP involves rapid exothermic reaction. Firstly solutions of Ca (NO3)2 and (NH4)2HPO4 are firstly mixed followed by adding concentrated HNO3 which results in formation of white precipitates. A Single or mixture of two or more fuels is subsequently incorporated into resulting solution. The final step is fast cooling to induce maximum nucleation and to prevent further particle growth. Vigorous exothermic reaction occur between fuel and oxidizer which gives rise to a very high local temperature that cause formation of a solid Calcium Phosphate powder. Depending on the type of fuel, used product formed may be either crystalline or amorphous. Both require a calcination step to remove organic residues and crystalize the phase formed respectively. Advantage of Combustion method is that is completed in less time, good chemical homogeneity.

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

3.11 Spray Pyrolysis

To get a high crystalline product, various post-treatments or long-term ageing at extreme temperatures may be necessary during HaP synthesis. Spray pyrolysis yields stoichiometric, homogeneous, crystalline HaP. The Aerosol technique or gas phase method is another name for the Pyrolysis process. Spray Pyrolysis is the process of spraying precursor solutions into a flame or hot zone of an electric furnace using an ultrasonic generator, followed by a high-temperature reaction of the produced vapours and gases to create end powder, often in an agglomerated form. High temperatures cause the evaporation of precursor droplets, which is followed by the nucleation and development of nanoparticles in gas phase. The drawbacks of Spray Pyrolysis include inadequate control of processing variables and secondary aggregate formation.

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

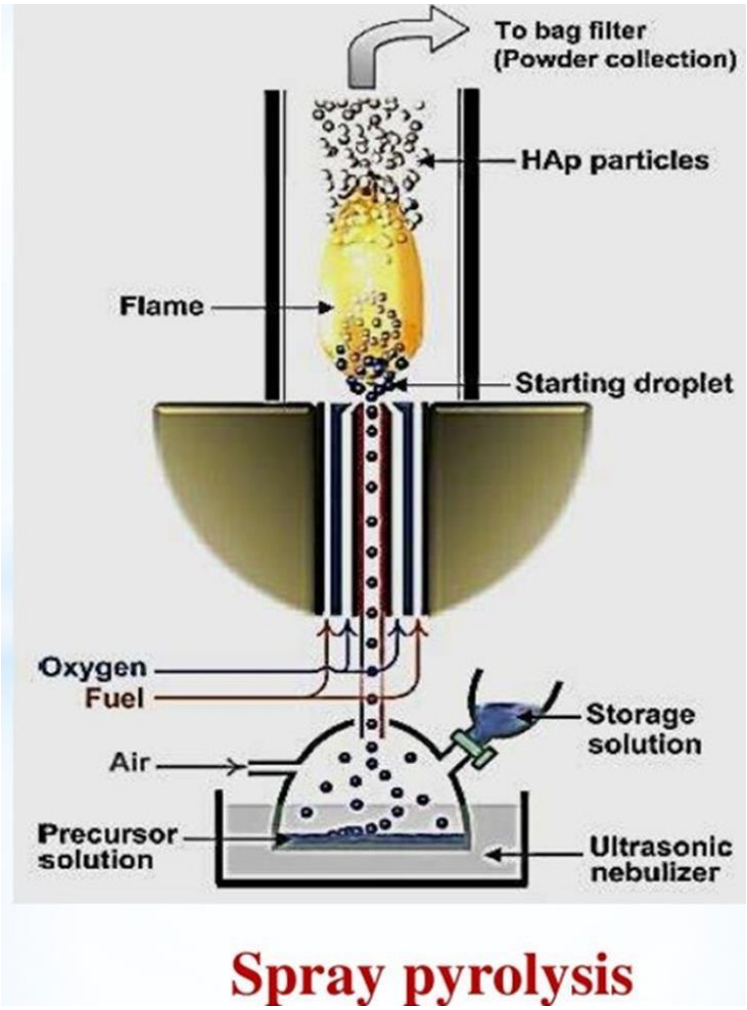

FIG VII. SYNTHESIS OF HAP FROM SPRAY PYROLYSIS

III. The following are biological sources for synthesising HAP from bio-waste:-

4.1 SCALES OF FISH

Rocha and colleagues described the synthesis and identification of biogenic compounds HAP made from bio-waste from fish scales. The gathered fish scale debris soaked in distilled water and cleaned unwanted salts, bloodstains, and greasy substances were all eliminated. Deproteinization of the moist fish scales after being Overnight drying using 0.1 M HCl peripheral washing, followed by a thorough cleaning numerous times a number of times using only distilled water Protein-rich The scales of fish are cleaned. sodium hydroxide (NaOH) at 5% (w/v) in a anxious yet not too so solution at 70 degrees Celsius for 5 hours The result was a fine white precipitate, following which it was

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

filtered, then dried in a dehydrator after being rinsed with double distilled water. Preheat the oven to 60degree Celsius. The alkaline solution was used to absorb the remaining fish scale residues, and 50 percent NaOH was added. The scales of processed fish are calcined for 4 hours at 1000degree Celsius. HAP was thoroughly washed with deionized water till the washing solution was obtained to achieve neutral pH before being dried at 60 degree Celsius. FTIR, XRD, and scanning electron microscopy are examples of different analytical methods (SEM), were used to analyse the biosynthesized HAP. Recently, alkaline heat treatment was used to synthesise HAP from fish scales (Tilapia Nilotic a).[43]

4.2 BOVINE BONES

The bovine bones were prepared using a combination of spray drying and a mechanical ultrasonic procedure to produce HAP. Surface flesh-containing bovine bones were removed and cleansed. Bone marrows were removed by boiling with deionized water for 5 hours. In addition to tendon for a total of three times, the boiling processes were carried out. The bones were then sundried after defatting and deproteinizing. until the tint changed to a yellowish hue The bone fragments that had been treated calcined for 3 hours in a muffle furnace at 800degree Celsius, then 24 hours of ball milling Approximately eight grammes of the sample were taken, with 12 percent of the total weight. In a glass beaker, sonication media was introduced and sonicated with an electric mixer. 100 Hz amplitude range was tested. The product was sonicated, then spray-dried to create HAP powder, which was then examined using FTIR and XRD. Studies have shown that using mesenchymal stem cells from bone marrow allograft to construct a 3D scaffold of calcined bovine bone covering is viable.This study demonstrated the efficacy of calcined bovine bone mixed with mesenchymal stem cells from bone marrow in the treatment of osteoporosis-related bone abnormalities.[42][38]

4.3 MARINE SUPPLIES

Nowadays, Corals are a type of marine organism that lives in the ocean employed to generate Phosphorus and HAP minerals in the ocean. The existence of 3D skeletal

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

structure, morphology, excellent porosity structure, and increased degrees of interconnectivity with the host site are all positive indicators are all advantages of marine species for biomedical applications. Calcium carbonate from snail shells (Pomacea Lineata) has been transformed to HAP powder in an in vitro procedure including immersion in phosphate solution at room temperature. The XRD approach demonstrated after 7 days in phosphate buffer, nucleation occurred on the implant coated HAP, but the FTIR examination confirmed After 30 days of immersion, the aragonite is completely converted to HAP. SEM analysis revealed a surface morphology of flowers caused by a sponge-like arrangement of connected platelets that promoted bone marrow cell adhesion. Hydrothermal and thermal calcinationprocedures were used to synthesise HAP from the native coral exoskeleton, respectively. The production of pure HAP phase was confirmed by XRD; there were no subsequent stages of tricalcium phosphate (TCP), calcium oxide (CaO), or calciumcarbonate ($CaCO_3$). The peaks, on the other hand, are compared to HAP's conventionallattice parameters. The creation of the HAP phase was confirmed by XRD tests, and EDX indicated the inclusion of Si and Mg in addition to Ca, P, and O.[34]

IV. BASIC PROPERTIES AND CHEMICAL IDENTITY

HAP is an appetite mineral with the general formula $M_5(ZO_4)_3X$, where M is a rare-earth metal such as Ca^{2+}, Cd^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, or Mg^{2+}, and ZO4 could be Ca^{2+}, Cd^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, or Mg^{2+}, and ZO_4 PO_4 CO_3 SO_4, with OH-, F-, Cl-, or CO_3 as X^{2-}. The formula for Ca5 [(PO4)3OH] is Ca5 [(PO4)3OH] HAP, Ca10 [(PO4)6(OH)2] is the unit cell's formula, and its pKsp (Ksp = solubility) is at 37 degrees Celsius, product) equals 58.65. As a result, The calcium orthophosphate phaseHAP is the most stable with a pH between 4.2 and 12.4.[34]

The following formula can be used to express the unit cell of stoichiometric HAP: $M_{14}M_{26}(PO_4)_6(OH)_2$, where M1 and M2 are two crystallographic locations for 10 calcium atoms, respectively. Four Ca atoms occupy M1 position in the HAP unit cell, 9 oxygen atoms from PO4 tetrahedra surround them.[11]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

One of the two OH- groups and six O atoms from the PO4 tetrahedra coordinate the remaining six Ca atoms in the M2 site. HAP is subject to substantial ion substitution in biological settings, therefore human bone is best represented as $(Ca\ Z)_{10}(PO_4,Y)_6(OH,X)_2$, where $Z = Na^+$, Mg^{2+}, K^+, Sr^{2+}, etc., $Y = CO_3^{2-}$, HPO_4^{2-}, and $X = Cl^-$, F^-.

Regardless of whether it is found in enamel, dentin, cementum, or bone, biogenic apatite is always impure and non-stoichiometric. The most popular is CO3. contaminant.2 (3–8% by weight), whereby Na+ (0.5–1 weight percent), Mg2+ (0.4–1.2 weight percent), K+ (0.03–0.08 weight percent), and Cl- are negligible contaminants. (0.01 – 0.3 weight %), and F- (0.01 – 0.06 weight %), respectively. OH-Cl-, for example, is one of these substitutions. PO_4CO_3 is the third element. The apatite structure is known to be weakened and fractured by Ca2+, Mg2+, and Sr2+ Others, such as OH- F-, are known to strengthen it while also increasing its solubility properties decrease a substance's solubility.[11][34]

In fact, with the exception of fluoride, Most of these contaminants improve thesolubility of the resultant apatite stoichiometry, which is why fluorine is included in toothpaste formulas, despite health concerns regarding its negative effects on gut flora and indications that it might potentially be a neurotoxic, it is widely used. Furthermore, large quantities of fluoride in the apatite structure have been demonstrated to cause increased porosity and material weakness.

CO_3^{2-} is the most prevalent dopant in biological apatite40, and carbonated HAP has been demonstrated to have better bioactivity than pure HAP, which has been linked to the carbonated phase's increased solubility. CO32- ions have also been demonstratedto impede down crystal formation in the HAP crystal structure. This replacement is so complicated that it touches on the most fundamental concerns in crystallography and crystal formation processes, in addition to bio mineralization. When apatite is formed by precipitation in the presence of air, the B-type of carbonated HAP is formed, in which CO_3^{2-} ions replace PO_4^{3-} ions. When HAP is made via an annealing procedure, it is usually Due to the high mobility of OH- groups, A-type forms occur when CO_3^{2-} ions replace OH-. However, A-type substitution was also seen in HAP produced by precipitation at ambient temperature, indicating that this is not always the case. A mixture of A and B types is common in biological apatite. In both cases, the fact that divalent carbonate replaces trivalent phosphates or monovalent hydroxyls

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

necessitates adjusting the stoichiometry of the molecule or promoting the introduction of other impurities into the crystal lattice to compensate for the imbalanced charges. Carbonated HAP has the chemical formula Ca10-x/2. [(PO4)6-x (CO3) x] [(OH)2-2y(CO3)y], where x and y are the numbers of CO_3^{2-} ions substituting for PO_4^{3-} and OH-, respectively, based on charge neutrality.[10]

The most prevalent HAP structure is the hexagonal system with the P63/m space group. The P21/b space group defines the less frequent symmetry of the HAP lattice in the monoclinic crystal system. Pure hexagonal HAP has unit cell dimensions of a = b = 0.94 nm and c = 0.69 nm. However, because each of the substitutions affects the lattice properties, biological HAP deviates from the ideal crystal structure of ions depending on the additions and their positions in the lattice. When HAP is carbonated, for example, A-type substitution results in bigger CO_3^{2-} ions replacing smaller OH- ions, extending the an axis and shrinking the c axis, whereas B-type substitution results in the opposite effect. Crystallinity, thermal stability, shape, solubility, and other physicochemical and biological characteristics ofthe material are commonly impacted by changes in crystal lattice parameters.[7]

HAP is a ceramic substance having ionic and covalent chemical bonding, it explains the intricate interplay of latent characteristics that HAP and ceramic materials in general are prone to CAP, as expected, can take on a variety of crystal forms depending on stoichiometry and formation circumstances. Table 1 lists some of the major CAP phases, with the crystal structure of HAP depicted in Fig.

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

Table 1. The main CAP phases obtainable upon precipitation from the solution.

Phase	Chemical formula	Space group	pK_{sp} at 37 °C	Ca/P molar ratio
Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	Triclinic $P^{\bar{1}}$	1.14	0.5
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$	Triclinic $P^{\bar{1}}$	1.14	0.5
Dicalcium Phosphate (DCPA, Monetite)	$CaHPO_4$	Triclinic $P^{\bar{1}}$	7.0	1
Dicalcium Phosphate Dihydrate (DCPD, Brushite)	$CaHPO_4 \cdot 2H_2O$	Monoclinic I_a	6.6	1
α-Tricalcium Phosphate (α-TCP)	$Ca_3(PO_4)_2$	Monoclinic $P2_1/a$	25.5	1.5
β-Tricalcium Phosphate (β-TCP, Whitlockite)	$Ca_3(PO_4)_2$	Rhombohedral R3cH	29.5	1.5
Tetracalcium Phosphate (TTCP)	$Ca_4(PO_4)_2O$	Monoclinic $P2_1$	37.5	2
Octacalcium Phosphate (OCP)	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	Triclinic $P^{\bar{1}}$	97.4	1.33
Hydroxyapatite (HAP)	$Ca_{10}(PO_4)_6(OH)_2$	Pseudo-Hexagonal $P6_3/m$	117.3	1.67

TABLE II. THE MAIN CAP PHASES OBTAINABLEUPON PRECIPITATION FROM THE SOLUTION

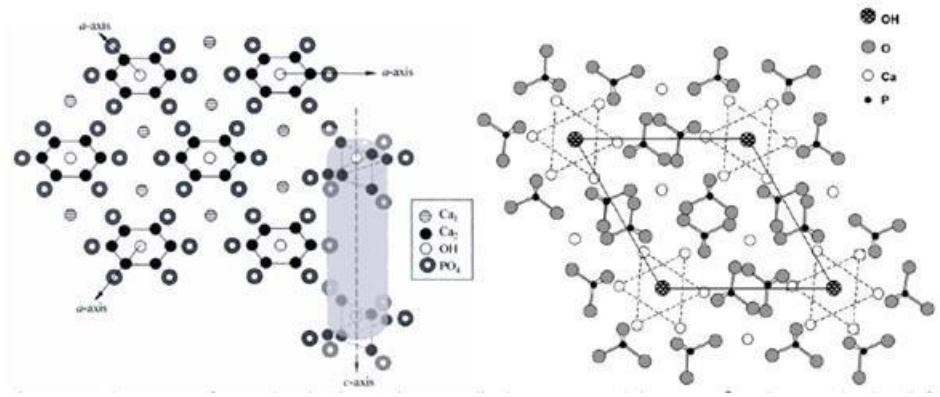

FIG VIII. CRYSTAL STRUCTURE OF HAP

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

V. APPLICATIONS OF NANOSTRUCTURED HYDROXYAPATITE

Because of its mechanical behaviour, similarity to bone, and teeth minerals, HAP has been widely employed to repair bone and periodontal abnormalities, alveolar ridge, as dental materials, middle ear implants, a tissue engineering systems, and bioactive coatings on metallic osseous implantsd. HAP particles are considered to hinder the growth of many kinds of cancer cells, according to recent research. HAP and its derivatives have a wide range of industrial and technical non-medical uses, including chromatography adsorbents for the purification and separation of proteins and nucleic acids catalysts, laser host materials, fluorescence materials, ion conductors, and gas sensors. Furthermore, HAP has excellent properties for water treatment and the remediation of heavy metal contaminated soils.[30]

6.1 HYDROXYAPATITE AS A DRUG CARRIER

Amino acids, steroids, hormones, proteins, vaccines, phenolics, acetylsalicylic acid, genes, antigens, enzymes, antibiotics, and anti-cancer medications have all been delivered using a variety of ceramic drug delivery systems. In physiological conditions, HAP has a low solubility and could be employed as a carrier for local drug administration via surgical implantation or injection. (1) Pharmaceuticals conjugated/loaded with implanted HAP scaffolds, (2) porous HAP/nHAP granular particles, and (3) polymer coated HAP/- nHAP particles are three major types of drug delivery via HAP. There are various advantages of using HAP as a drug carrier agent:[15]

Biodegradation periods for HAP nanoparticles are typically longer, which is important for diffusion-controlled drug release kinetics. Ceramic matrices that degradeslowly or are nearly no degradable can hold medications for longer periods after administration. By reducing the medication concentration in the circulation, HAP's regulated localised drug delivery reduces toxicity to other organs. The drug concentration might be managed so that it never exceeds the toxic threshold or falls

below the minimum effective level, as well as avoiding the need for recurrent drug administration.[11][18]

The loaded drug's concentration over the porous HAP surface is determined by the drug molecules' penetration behaviour through the micro pores, the velocity of penetration, and the drug's pharmacokinetic profile. Engineered HAP can assure gradual drug release due to its microporous structure and superior biological responsiveness to physiological conditions. • By simple adsorption, HAP can be functionalized/bonded with both positively and negatively charged molecules.

HAP does not swell or change porosity, and it remains generally stable when the pH and temperature of the fluid change. Ceramics' low swelling ratios limit the release of a burst of pharmaceuticals, which is a common problem with hydrogels such the poly (2-hydroxyethyl methacrylate) (pHEMA) drug-delivery system. Synthetic HAP nanoparticles can have the same chemistry, crystalline structure, and size as elements of targeted tissues (for example, different kinds of CaP in bone or teeth).[20][25]

HAP could be tailored as nanoparticles with favourable electrical (e.g., ferroelectric and dielectric), mechanical (e.g., piezoelectric, ultrahigh hardness, etc.), magnetic (e.g., superparamagnetic), and optical (e.g., photo thermal effects, electroluminescence, etc.) properties, which are rarely seen in polymeric nanoparticles, through doping.[28][35]

6.2 Examples of Effective Conjugation of Therapeutic Drugs with Hydroxyapatite (HAP) through Different Approaches

Type of HAp	Plant used for preparing extract/template	Biomedical application of nanostructured HAp
HAp nanosheets	Ginger (*Zingiber officinale* Roscoe)	Bone tissue regenerative medicine
Nano-HAp	*Azadirachta indica, Coccinia grandis*	Antibacterial activity
Nano-HAP	*Parkia biglobosa*	Antibacterial activity
Nano-HAp	Tamarind seed, guar gum	Drug loading and *in vitro* drug release
Nano-HAp	Basil, lavender	Antimicrobial activity
Nano-HAp	Peppermint plant (*M. piperita*)	Antimicrobial activity
Honeycomb-like poly(lactic acid) nano-HAp	Bitter gourd fruits	Bone tissue regeneration
Cocoon shaped nano AgHAp	*Azadirachta indica* gum	Antibacterial activity
Strontium-hydroxyapatite (SrHA)	*Hippocampus kuda* Bleeler	Drug delivery system for bone tissue repair
Reduced graphene oxide/hydroxyapatite composite (RGO–HA)	*Melissa officinalis*	Cell viability

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

Drug Conjugation Approach	Conjugated Drug	Performance
Cleavable covalent linkage	Doxorubicin (DOX)	Mesoporous HAp acts as an excellent carrier for DOX molecules with a loading efficiency of ≈ 93%, which is much higher than that of the conventional HAp particles. Variation of pH of the release medium (PBS) from 7.4 to 5.5 increases the drug release from 10% to about 70%.
	Raloxifene	Raloxifene used in osteoporosis therapy, inhibits osteoclast. Forms a covalent bond with nHAp-based biomaterial by interfacing with (3-aminopropyl)-Triethoxysilane (nHAp coating material). No drug loading or releasing kinetics were studied. By employing FTIR, the functional groups were studied and possible mechanism of raloxifene conjugation on HAp surface has been discussed. HAp contains hydroxyl groups which may react with the 3-aminopropyl-triethoxy silane and subsequently condenses the amino group of the silane with the Ketone group of raloxifene.
	Ofloxacin	β-cyclodextrin (β-CD) has covalent interaction with HAp. Coupling agents are used to improve the drug (ofloxacin) loading capacity and for sustained release. The adsorption capacity of ofloxacin on β-CD-grafted HAp (β-CD-g-HAp) composite was found to be about 30 mg/g at 37 °C in 24 h. The release of ofloxacin from β-CD -gHAp slows down to 28% and 21% in pH 2.0 and 7.4, respectively, within 2 h.
Attachment through physical interaction	Ibuprofen	Ibuprofen exists in dimeric form both in solid and liquid state. Ibuprofen—nHAp complex formation occurs most likely through the dissociation of Ibuprofen dimer into monomeric species. Hydrogen bonding of the hydroxyl group of Ibuprofen to the hydroxyl group of the apatite, together with the interaction of the ibuprofen carbonyl group to HAp Ca center.
	Sodium ampicillin	HAp contains negatively charged hydroxyl groups (OH^-), which are the potential bridging agents to sodium ampicillin, which has positively charged groups such as amine group (NH_3^+), sodium ions (Na^+) and some hydrogen ions (H^+). Three stage release kinetics, independent of loading concentration (1, 5 and 10 mg of ampicillin/ml) has been observed. The first stage, characterized by a fast release (first 2 min), followed by the slower second stage, which lasts approximately 12 min. In the final stage, a residual volume of sodium ampicillin is released, which lasts until the finish of sodium ampicillin in the samples (~30 min.).
	Vancomycin	Vancomycin was loaded with HAp and PCL coated HAp scaffold via immersion-adsorption method. The drug release studied for short (1 h) and prolonged (72 h) time durations. Compared to the abrupt initial burst (as high as ~70–80% release) in uncoated HAp samples, the PCL coated HAp showed much lower initial burst (~44%). The study revealed ~90% release of the drug within 24 h for the pristine HAp sample.

FIG IX. CONJUGATION OF OTHER DRUGS WITH HAP

6.3 Drug Delivery through Magnetic HAP

Since Freeman and colleagues first proposed the idea of employing magnetism in medicine in the 1960s, a huge amount of research has been done in this field, leading to the development of various magnetic particles and vectors. The goal behind targeted drug delivery and targeted drug therapy is to send a drug directlyto the damaged areas of the body under various conditions, treating them effectively and without causing any adverse effects. Iron oxides make up the majority of magnetic HAP nanoparticles. There are eight different types of magnetic HAP

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

nanoparticles: (1) Fe -doped, (2) Fe and Pt co -doped, (3) iron oxide doped, (4) Cobalt -ferrite doped, (5) Mn and Fe doped, (6) ND and Gd co-doped, (7) 153Sm and Gd integrated, and (8) iron oxide, Fe, and Cu doped HAP nanoparticles. Hou and colleagues used the co precipitation approach to make magnetic HAP (mHAP) nanoparticles, which they used in a mouse model to treat tumours using magnetic hyperthermia. In a 15-day investigation, mice that had only been injected with mHAP and had been treated inside a magnetic field showed a significant reduction in tumour volume. Important information about the biocompatibility of the HAP and mHAP nanoparticles can be found in the blood test results of the experimental mice's liver and kidney functions. The experimental mice's normal blood urea nitrogen (BUN) and creatinine levels show normal kidney activities; however, the raised alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels suggest aberrant liver functions due to HAP and mHAP metabolism through the liver. Because all of the mice survived the experimental investigation, the raised ALT and AST levels are not lethal. All of the animals in the study had normal alkaline phosphatase (ALP) levels, indicating that bone metabolism was normal even after the nanomaterials were introduced into the body system.[33][39][36][15]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

Drugs with HAp	Application	Nature of HAp
Paclitaxel, Cis-diamminedichloroplatinum (II), Di(ethylenediamineplatinum) medronate, Methotrexate	Anticancer drug delivery	Nanoparticles, Scaffold or block of nanoparticles of HAp
Eu^{3+} and Gd^{3+} doped	Tri-modal contrast for MRI, X-ray and NIR fluorescence	Nanoparticles of 30 nm average size
Fe^{2+} and Pt^{2+} doped	Lung cancer and hyperthermia	Pt and Fe doped nanoparticles
Arbekacin sulfate, Vancomycin, Ceftriaxone, Gentamicin, Cefoperazone sodium, Flomoxef sodium, Hydrocortisone,	Antibiotic drug delivery	Nanoparticles, Scaffold or block of HAp
Iron doped Poly lactic acid (PLA)	MRI contrast agent, scaffold	HAp nanoparticles
Iron (Fe^{3+} and Fe^{2+}) doped	Targeted drug delivery, Imaging	Needle shape, superparamagnetic
Fe_3O_4, Fe and Cu doped composite	Targeted drug delivery and MRI	Porous nanopartides
Iron (Fe^{3+}) doped	Nano-probes for drug releasing	Rod shaped nanoparticles of 75 nm average size
Tb^{3+}/Gd^{3+} dual-doped	Bimodal imaging applications	Spherical (40–100 nm)
Functionalized with luminescent and magnetic $Na(Y/Gd)F4:Yb^{3+}$, Er^{3+}	Drug delivery and MRI bimodal contrast agents	HAp nanoparticles
Dual doped with Ho(III) ion and Fluorescein isothiocyanate labeled	*in vitro* cell imaging and as T2 MRI contrast agent	Magnetic and luminescent
Dye (FITC) functionalized	Cellular imaging, drug delivery	Needle shape nanoparticles (50–100 nm)
Luminescent mesoporous Eu-doped	Luminescent drug carrier and bioimaging probes	Rod shaped, 20–40 nm wide and 100–200 nm length
Ln (Eu^{3+}, Tb^{3+}) doped	Luminescent drug carriers and bioimaging probes	Rod shaped nanoparticles
$CePO_4$:Tb doped	Redox luminescent switch and bioimaging probes	Needle shaped, 50–100 nm length, 5–10 nm wide
Cr^{3+} doped	Bioimaging/biosensing	Nanoparticle, 40–100 nm

TABLE III. APPLICATION OF DRUGS WITH HAP WITH HAP'S NATURE

6.4 APPLICATION OF MAGNETIC HYDROXYAPATITE NANOPARTICLES

FIG X. APPLICATION OF MEGNATIC HAP PARTICLES

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

6.5 Tissue engineering scaffolds and bone fillings:-

Bone grafting surgery is frequently required to treat major bone deficiencies caused by tumour resection, trauma, or infection. When using auto grafts and allografts for reconstructive procedures, issues such as compatibility of donors, immunological rejection, and pathogen transfer can develop. Organic/inorganic composite materials can now be made to look like real bones. The porous materials that form canoperate as a temporary extracellular matrix, stimulating tissue regeneration and growth in the natural way. The increased qualities of such materials are due to a combination of the inorganic ceramic phase's compressive strength and the polymer's toughness and flexibility. When compared to the other CaP phases, crystalline HA is the calcium phosphate with the slowest breakdown rate. Furthermore, as one of the most important components of bone, HA is both biocompatible and osteoconductive, promoting human osteoblast cell adhesion, development, and proliferation. As a result, hydroxyapatite incorporation into a polymeric matrix is of tremendous interest.[30][15][18]

Hap inclusion into polymer matrix, i.e. nanoparticle aggregation and adherence to the polymer matrix, is the most important element influencing the characteristics of such composite materials. Mineral precipitation in the polymer solution is one method of dealing with these problems.[25][26]

Natural polymers have a high cellular affinity, while synthetic polymers have a higher mechanical strength and a variable breakdown rate. Because of their resemblance to extracellular fluids, hydrogels have recently received a lot of attention as biopolymers.[18][19]

Chitosan, gelatine, alginate, collagen, cellulose, and silk fibroin are the most often utilised natural polymers. Poly (vinyl) alcohol, poly-2-hydroxyethylmethacrylate, polycaprolactone, polylactic-co-glycolic acid (PLGA), polylactic acid (PLA), and other synthetic polymers are used. The majority of natural polymers have high bioactivity and biocompatibility; nevertheless, during scaffold production, care must be made to avoid denaturation. When it comes to synthetic polymers, it is a different story. In the case of synthetic polymers, however, this is a small concern, biocompatibility must be taken into consideration. Three -dimensional (3D) printing

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

techniques are gaining a lot of attention as a potential solution for making on-demand bone scaffolds for regenerative medicine. This technology allows for the creation of a wide range of structures employing computer -aided designs based on medical tests that enable imaging of damaged tissue.[30][8]

Personalized implants or grafts might be possible thanks to 3D medical printing technologies. Composite materials with polymer and ceramic elements are known to exhibit properties that combine the bioactivity and mechanical strength of the ceramic phase with the biodegradability, toughness, and flexibility of the polymer phase. As a result, numerous studies have been conduct to develop methods for 3D printing polymer-ceramic composites, with polymer-hydroxyapatite composites being of particular interest. 3D-printed bone tissue engineering scaffolds, such as those based on polycaprolactone (PCL)/HA, polylactide (PLA)/HA, or polypropylene fumarate) (PPF) /HA, were found to promote bone repair in vitro and/or in vivo studies.[6][5]

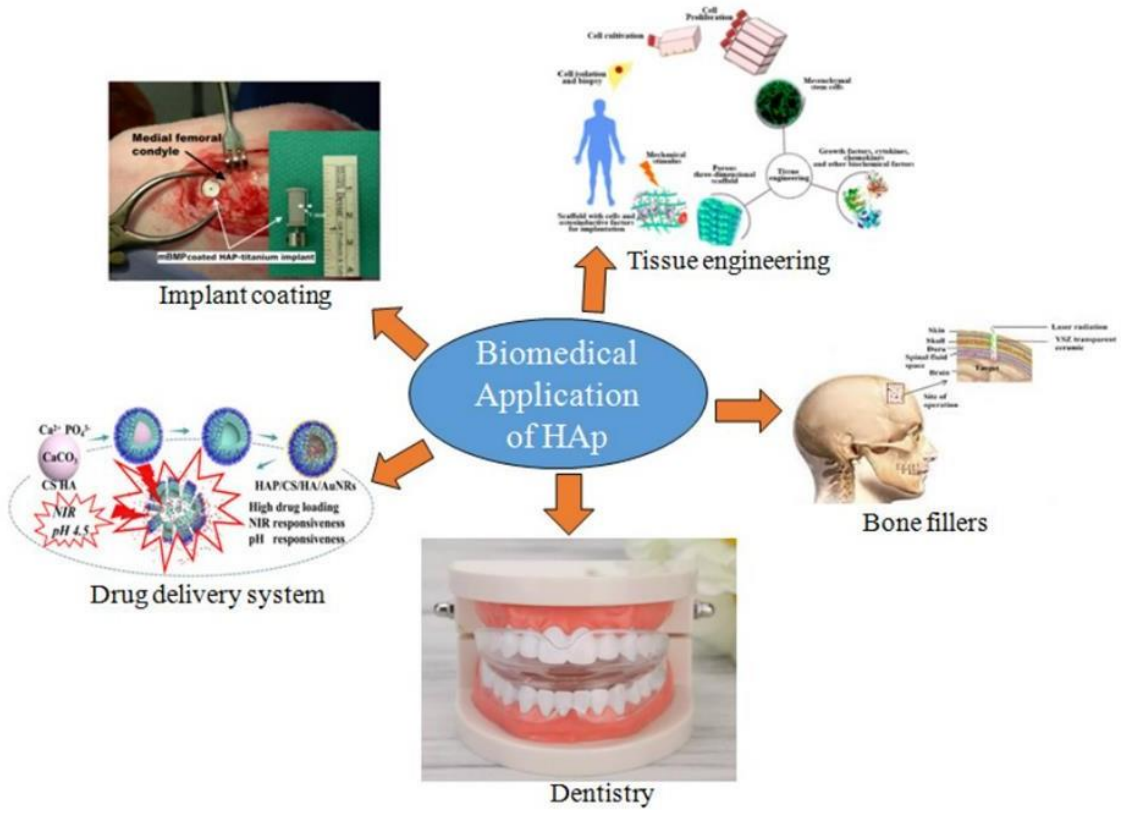

FIG XI. BIOMEDICAL APPLICATION OF HAP

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

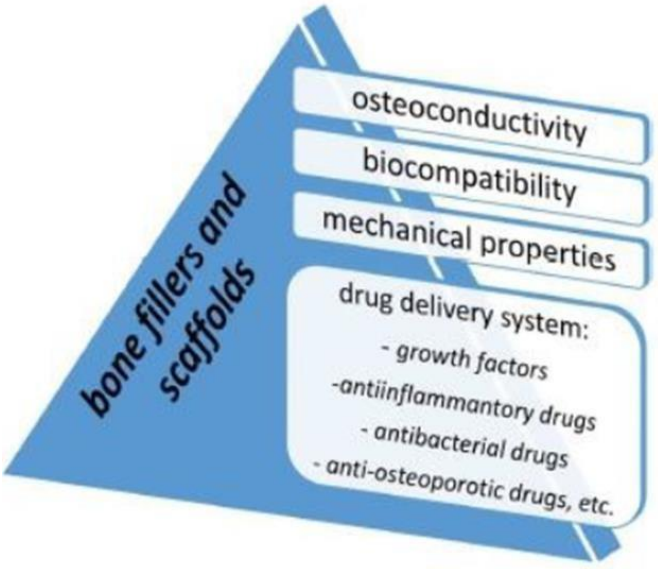

FIG XII. BONE FILLERS AND SCAFFOLDS

Osteoconductive materials should be use in the remodelling of bones. As a result, numerous attempts to synthesise bone fillers and scaffolds that act as carriers for the regulated release of growth factors have been make. Healing large bone abnormalities is still a difficult task. New methodologies inspired by natural bone healing mechanisms are need to develop new materials for bone replacement and regeneration. Not only should bone fillers and scaffolds be biocompatible, but they should also provide osteoinductive support for bone healing. Furthermore, they should serve as a delivery mechanism for various growth factors, as well as anti - inflammatory and antibacterial medications, preventing their rejection after use. Composite bone-like scaffolds made of Nano-sized HA and biodegradable polymers are promising bone replacement and regeneration materials.

6.6 Preparation of implant coating

Because of their fine mechanical qualities, endurance, low density, and chemical stability in human fluids, the majority of implants are comprised of titanium or its alloys. Metallic implants have a high degree of hardness and mechanical strength, but they have poor biocompatibility and Osseo integration. Because of its high biocompatibility, chemical stability, and osteoconductivity, hydroxyapatite has

been extensively teste as a coating for metal implants to improve their properties. Such coatings have been shown to aid Osseo integration of implants with surrounding tissues. Carbon nanotubes were included into the HA coating, resulting in homogeneous, crack-free composite coatings with improved crystallinity, biocompatibility, and bonding strength. Bacterial cells can cling to the implant's surface after it is place in a living creature, resulting in biofilm formation and infection. As a result, implants with not only good biocompatibility but also antimicrobial qualities are increasingly being sought. Antibiotics can be deliver locally via the antibiotic -loaded HA implant coating, preventing infection or inflammatory reactions after surgical implantation.[6][19]

VI. CHALLENGES OF NANOSTRUCTURED HYDROXYAPETITE PARTICLES

The biosafety of hydroxyapatite nanoparticles is still a subject of debate.

There is little evidence that the combination is bio toxic as a drug delivery system or a protein-coupling agent.

As a result, the use of hydroxyapatite in commercial medicinal applications is still debatable. Regulatory approval is currently restricted nanoparticles as fillers in the bone matrix.

The possibility of particle wear production and the accompanying these nanoparticles have osteocytes capability, are now regard to be the downsides of nanoparticles. Nanoparticles are easily releasable due to their susceptibility to hydrolysis and oxidation. Wear particles can damage your car in two ways. To begin with, hydroxyapatite wear particles might be stuck in the joint, degrading the remaining components and causing cellular inflammation, especially when utilised in joint replacement solutions. Because of the inflammatory reaction, following net bone loss, implant loosening will happen. Second, wear particles might escape the insertion site, such as through the synovial fluid. When hydroxyapatite debris from body fluid is transport up into the circulation via the intercellular gap between endothelial cells, migration can be particularly harmful.[30][25][24]

NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

Lahiri and colleagues investigated if the size of wear particles has any effect on cytotoxicity.

The size of the wear debris ranged from 300 nanometres to 9.5 millimetres, owing to the natural aggregation of hydroxyapatite nanoparticles (average 3 mm). When carbon nanotubes and Nano-hydroxyapatite were combine, the particle size was decreased (100 nm to 3 mm, average 600 nm). The effects of Nano-hydroxyapatite wear particles on osteoblast function have been demonstrate, in the investigation. This was thought to be because to problems with phagocytosis of bigger particles.[15][18]

Shi and colleagues, who employed to treat osteosarcoma, researchers used two different sizes of Nano-hydroxyapatite (20 and 80 nm), backed up this finding. Apoptotic cell death was seen in both types of hydroxyapatite nanoparticles, while thelarger particles caused more apoptosis (80 nm). Other researchers speculated that the reported enhanced cytotoxicity against macrophages could be attributable particle sizeis less important than intracellular calcium levels, because in lysosomes, hydroxyapatiteparticles of various sizes might be progressively degraded.[28][30]

Nano-hydroxyapatite has been immerse; bio stability was determined by testing in very acidic solutions for Ca ion depletion. Lim and colleagues, for example, submerged discs and teeth coated with hydroxyapatite, for up to three months in an extremely acidic solution (pH 3.26).[17][19]

Despite the fact that the calcium concentration in both samples increased with time, both substrates were thought to have exceptional chemical resistance and stability.

Hydroxyapatite nanoparticles are now use in commercial goods and have acquired regulatory approval. Ostium (Osiris, Orenburg, Germany) is a nanoparticle-sized precipitated hydroxyapatite paste containing about 40% water that is used to treat bone deficiencies as an injectable bone filling material. Preclinical research that is comprehensive, includes tests for biocompatibility and Geno toxicity, has established that the product is marketable in Europe and the US.

Another concern about Nano-hydroxyapatite is the possibility of particle agglomeration inside blood vessels. Calcium hydroxyapatite is well known to cause arterial calcification as part of atherosclerosis. Although the exact mechanism is unknown,

Nano-hydroxyapatite may build in blood vessels similarly to cholesterol. Because hydroxyapatite and cholesterol have comparable crystal lattices, it is possible that long-term hydroxyapatite nanoparticle use will result in atherosclerosis issues.

VII. CONCLUSION

Nano-hydroxyapatite looks to offer various benefits over micrometre-sized particulate hydroxyapatite. Deposition of hydroxyapatite using traditional techniques, wet chemistry, sol–gel chemistry, and electrophoresis are examples of these techniques., appear to be well adapted to producing hydroxyapatite particles with diameters considerably below 1 mm; however, repeatability of The presence of nanoparticles looks to be an issue.

The capacity of Nano-hydroxyapatite to cross cellular membranes makes it appealing. A Nano-hydroxyapatite scaffold is use to encapsulate pharmaceuticals or targeted delivery might be possible if coupled to a Nano-hydroxyapatite particle, particularly in drug-delivery applications. The known dissolving rate of apatite materials, particularly hydroxyapatite, could be crucial in addressing the problem of burst drug release and absorption of hydrophobic compounds into cells. The health advantages of employing Nano hydroxyapatite, however, are not restricted to bone problems. Rather, there is emerging evidence that Nano-hydroxyapatite is beneficial either alone or in combination with other agents, is particularly effective in the treatment of tumours.

While there are some worries about nanoparticle biosafety, there is no conclusive proof that consumers are being harm by Nano-hydroxyapatite, and this will continue in the future. When compared to micrometre-sized particles, in vitro and in vivo tests revealed no harmful effects . Nano-hydroxyapatite has been demonstrate to be biocompatible in studies.

Its advantages stem from synthetic hydroxyapatite’s benefits as a bone filler, scaffold, and coating material, it is widely recognise. When particles are create at the nanoscale, they have a larger surface area they are therefore suited for cellular processing. Because Nano-hydroxyapatite as a targeted drug delivery vehicle is still in its infancy, within the next decade, the full breadth of Nano-long-term hydroxyapatite's benefits will almost certainly reveal.

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NISARG SOLANKI [18BPH056], NIRMAL PATEL [18BPH055], SHUBHAM PATEL [18BPH089], VIHAR KHANDHARA [18BPH100]
INSTITUTE OF PHARMACY, NIRMA UNIVERSITY, AHMEDABAD

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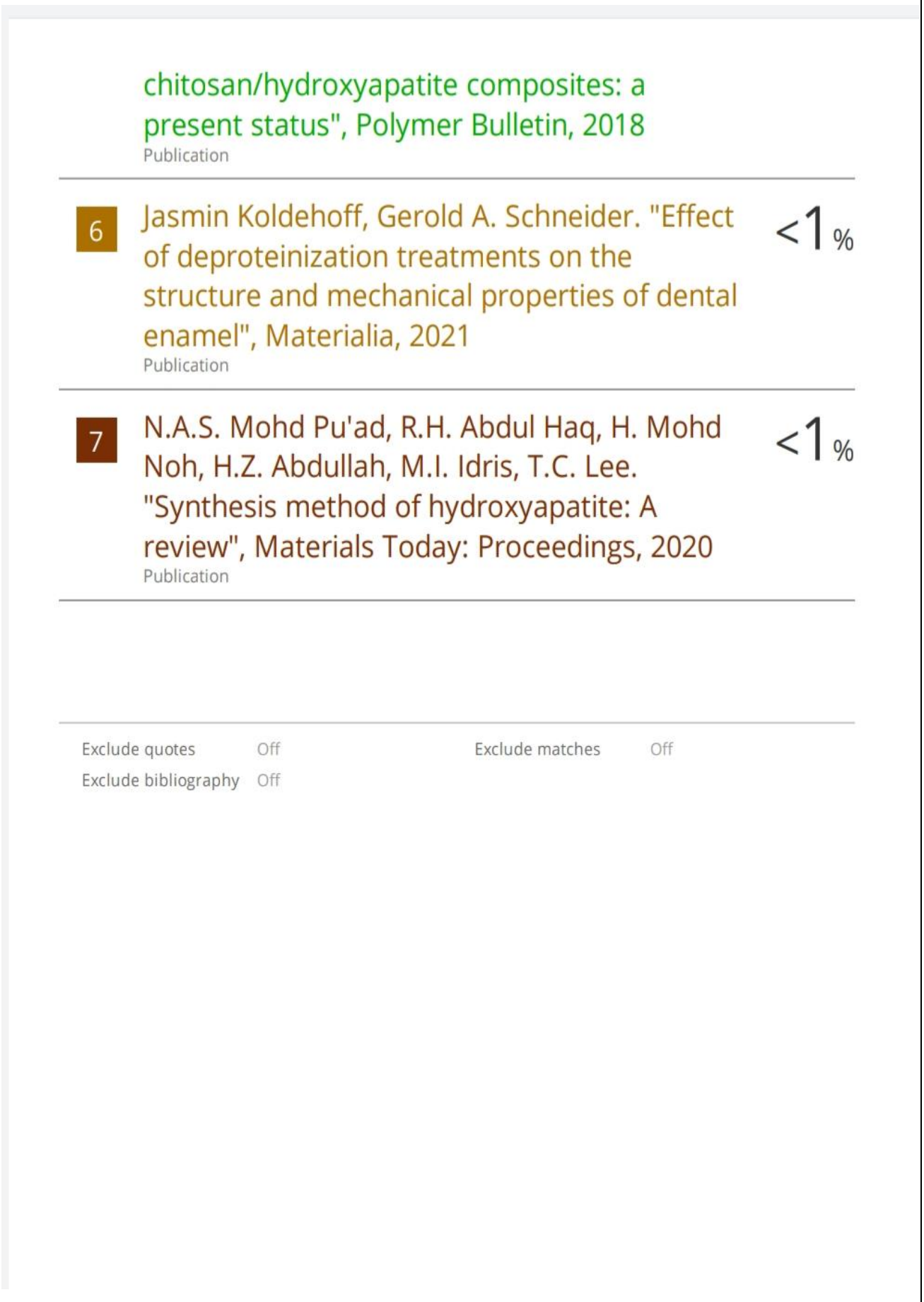

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