

CHAPTER 2 LITERATURE REVIEW

The primary objective of conducting a literature review was to generate a thorough and comprehensive summary of the available research pertaining to the present subject matter and to identify the lacuna in present studies. This task involves simplifying essential findings, theories, approaches, and discussions in the area of study. The present chapter supported the identification of gaps and trends by highlighting areas that require additional investigation or where contradictory findings are present. The study includes evaluating the merits and deficiencies of various research endeavours, scrutinizing the dependability of the evidence put forth, and determining any partialities or limitations in the body of literature. Avoiding duplication of existing studies and ensuring that the present research makes a relevant contribution to the subject is also beneficial. **This chapter provides evaluations of research articles that are currently available on the following subjects:**

- ❖ Synthesis of hydroxyapatite
- ❖ Metallic doping
- ❖ Fabrication of biocompatible composite
- ❖ Tribological behavior
- ❖ Coating of hydroxyapatite

2.1 Synthesis of Hydroxyapatite

2.1.1 Bibliometric Analysis on “Synthesis of Hydroxyapatite”

Bibliometric analysis is a quantitative method for evaluating scientific literature, academic publications, and research outputs based on bibliographic data. This process entails the methodical analysis of bibliographic data in order to detect recurring patterns, trends, connections, and impact metrics that are specific to a particular field of study or

transcend multiple disciplines [37]. The basic statistical analysis can be performed using the data/ graphs provided by Scopus, Web of Science, Dimensions, Lens, PubMed, etc. However, effective bibliometric analysis can be performed using VOSviewer software, which provides various analysis like co-authorship, co-occurrence, citation, bibliographic coupling, and co-citation analysis. The subset includes data units such as authors, countries, organizations, keywords, documents, and references. The obtained graphs have three different visuals namely network visualization, overlay visualization and density visualization.

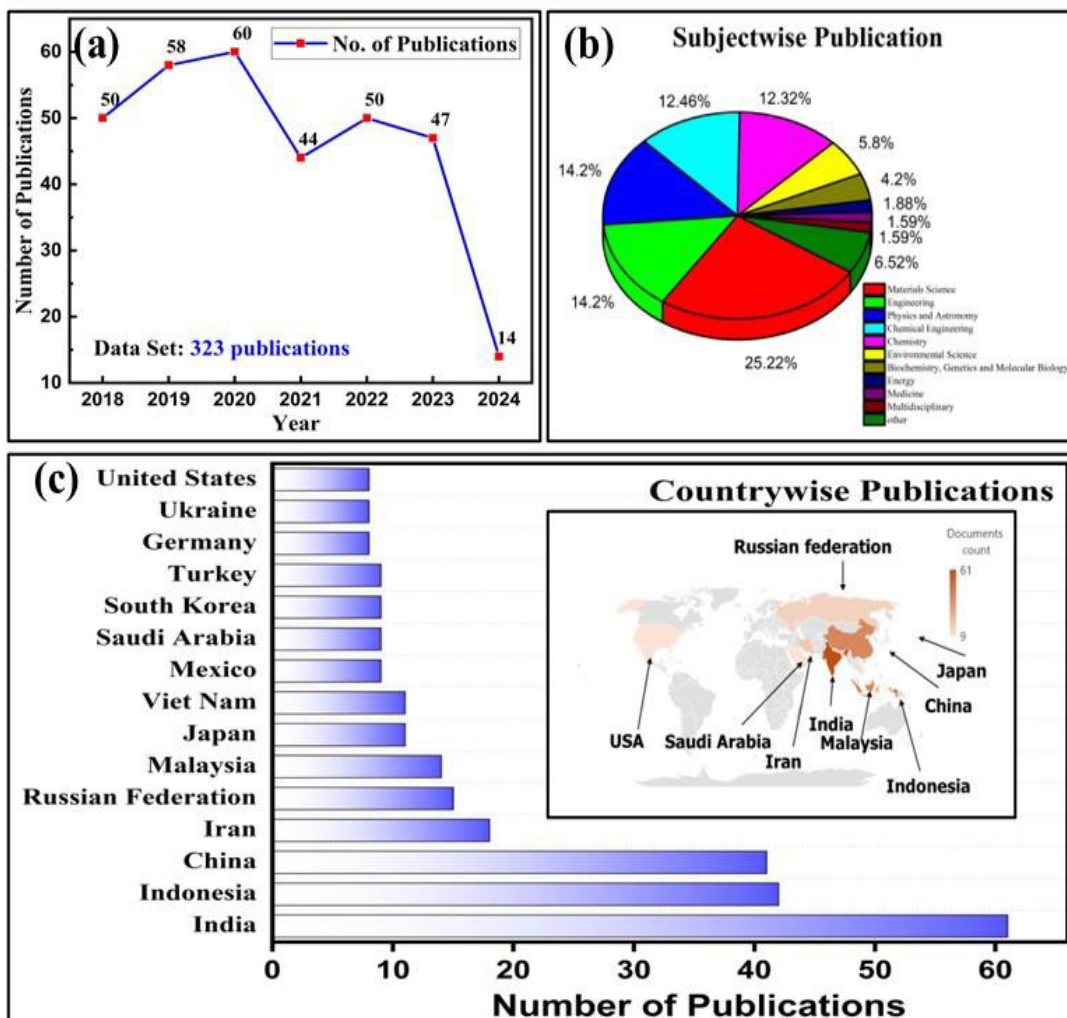


Fig. 2.1 (a) Yearwise, (b) subjectwise, and (c) countrywise number of publications on “synthesis of hydroxyapatite”

In the current literature review, the bibliometric analysis on “**Synthesis of Hydroxyapatite**” was performed using the Scopus database of the last 5 years (2018-

2024 (March), shown in **Fig. 2.1 (a)** that yielded a total of 323 publications. The subject-wise analysis report shown in **Fig. 2.1 (b)** reveals that the maximum no. of publications (i.e. 25.22 %) is in the material science domain followed by engineering (14.2 %), physics & anatomy (14.2 %), chemical and engineering (12.46 %), chemistry (12.32 %), environmental science (5.8 %), biology (4.2), and remaining in energy & multidisciplinary. It is interesting to observe that India (14.87 %) has had the maximum number of publications in the last 5 years on the “**synthesis of hydroxyapatite**” followed by Indonesia (10.24 %) and China (10 %). The top 15 countries in terms of quantitative publications are shown in **Fig. 2.1 (c)**.

Table 2.1. Top 15 Countrywise documents and citations on the “synthesis of hydroxyapatite”.

S. No.	Country	Documents	Citations	Total link strength
1	India	61	875	16
2	Indonesia	42	176	0
3	China	41	553	7
4	Iran	18	279	4
5	Russian Federation	15	151	4
6	Malaysia	14	414	2
7	Japan	11	70	6
8	Vietnam	11	241	5
9	South Korea	9	401	10
10	Saudi Arabia	9	123	5
11	USA	9	94	3
12	Mexico	9	142	1
13	Turkey	9	202	0
14	Germany	8	49	2
15	Ukraine	8	9	1

2.1.1.1 Co-authorship Analysis

The overlay, network and density visualization of the co-authorship analysis based on countrywise unit for the publications on “synthesis of hydroxyapatite” is shown in **Fig. 2.2 (a-c)**. Out of the 65 countries publishing research articles on “synthesis of hydroxyapatite”, top 15 based on number of documents are listed in **Table 2.1**. while for

co-authorship analysis based on countries, only those threshold countries are considered which has minimum 5 documents. As observed, India has maximum collaborative link strength (i.e. 16 links with 61 documents) (Table 2.1). that Indonesia has good number of publications (42 nos.) and citations (176) but it has zero link strength. China and South Korea also has good number of documents, citations, and international collaborations for their publications. The high total link strength helps to evolve higher research potentials and innovation strategies.

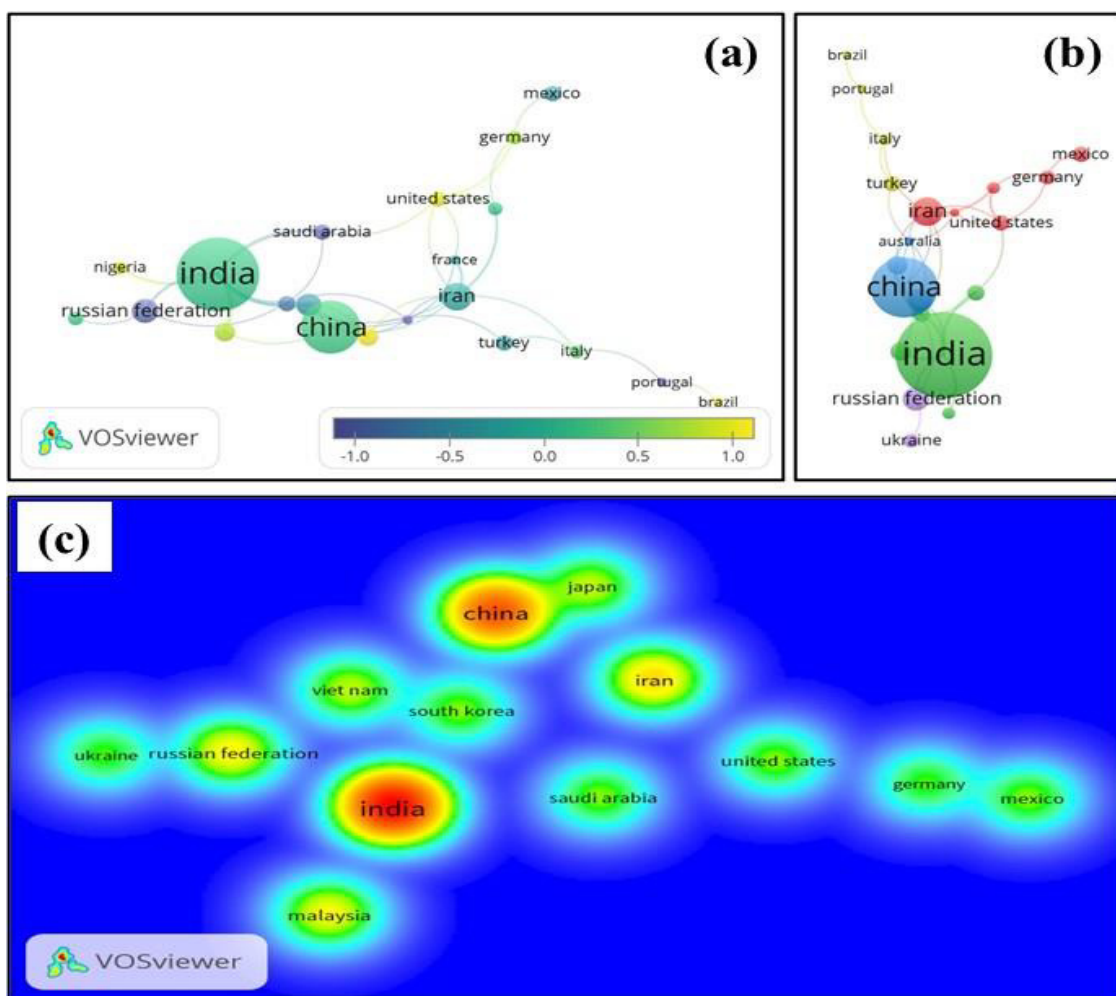


Fig. 2.2 (a) Overlay visualization, (b) Network visualization, and (c) Density visualization of the co-authorship analysis based on countrywise unit

2.1.1.2 Co-occurrence Analysis

Figure 2.3. highlights the co-occurrence analysis with all the keywords that surround the “synthesis of hydroxyapatite” as its major keyword. The overlay, network and density

visualization of co-occurrence analysis with all keywords related to synthesis of hydroxyapatite are shown in Fig. 2.3 (a-c). Some of the major keywords related to the current research includes synthesis, characterization, calcination, nanoparticles, crystallite size, scaffold, composites, etc. However, the characterization and biomedical relation of hydroxyapatite has maximum intervention as observed in the analysis. Therefore, the efforts are made to elaborate the literature related to the visualizations obtained using the analysis.

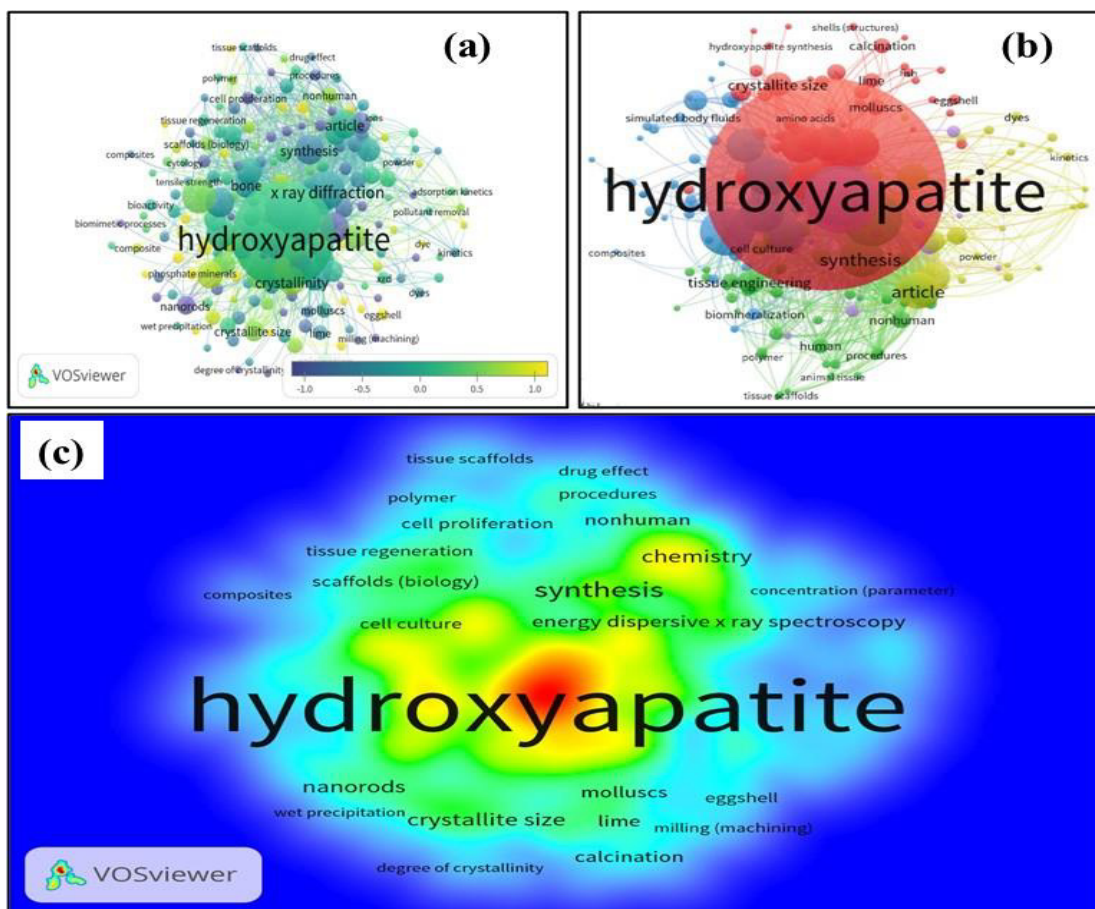


Fig. 2.3. (a) Overlay visualization, (b) network visualization, and (c) density visualization of the co-occurrence analysis of keywords related to “synthesis of hydroxyapatite”.

2.2 Literature review related to synthesis of Hydroxyapatite

Synthesis and yielding capacity of hydroxyapatite using several methods and sources depends on the type of synthesis method adopted and the source of synthesis. The

pure form of hydroxyapatite has several ideal characteristics as tabulated in **Table. 2.2.** Agbeboh et al. [38]. reviewed the efficient synthesis of hydroxyapatite along with environmental sustainability. The major concern was to develop an eco-friendly method in biomedical applications related to material extraction, coating, and optimization. The investigation followed particle size, geometry, morphology, mechanical properties, biocompatibility, toxicity, thermal stability, stoichiometry etc. as the governing parameters to deliver cost-effective processing and synthesis routes for the development of HAp. Synthesis of HAp was done using various organic and inorganic sources including natural resources (plants, animals, mammals, bioshells and limestone) [39] and synthetic laboratory preparation using several methods [40–42] adopted for the synthesis of hydroxyapatite is systematically tabulated by Balhuc, S. et al. [44].

Table. 2.2. Properties of pure hydroxyapatite [43].

S. No	Properties	Experimental data
1	Chemical composition	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$
2	Ca/P molar	1.67
3	Crystal system	Hexagonal
4	Space group	$\text{P6}_3/\text{m}$
5	Cell dimension (Å)	$a = b = 9.42, c = 6.88$
6	Young's modulus (GPa)	80–110
7	Elastic modulus (GPa)	114
8	Density (g/cm^3)	3.16
9	Relative density (%)	95–99.5
10	Fracture toughness ($\text{MPa m}^{1/2}$)	0.7–1.2
11	Hardness (HV)	600
12	Decomposition temperature ($^\circ\text{C}$)	> 1000
13	Melting point ($^\circ\text{C}$)	1614
14	Dielectric constant	7.40–10.47
15	Thermal conductivity ($\text{W}/\text{cm K}$)	0.013
16	Compressive strength (MPa)	400–900
17	Bending strength (MPa)	115–200
18	Biocompatibility	High
19	Bioactivity	High
20	Biodegradation	Low
21	Cellular compatibility	High
22	Osteoinduction	Nil
23	Osteoconduction	High

Figure. 2.4. briefly shows different synthesis methods in a hierarchical method. The complete synthesis methods are divided into 5 categories: **Dry, wet, high temperature, biogenic source, and combination of methods.** The dry synthesis method includes solid state synthesis, composite development synthesis [45,46] and mechanochemical synthesis method. Wet methods include sol gel, chemical precipitation, hydrolysis, emulsion, hydrothermal and sonochemical method. High-temperature method includes combustion, pyrolysis, and variable temperature sintering method. Synthesis from biogenic and natural sources includes all calcium-rich plants, animals, mammals, livestock waste, shells, etc. Also, as per the requirement and application, the combination of methods and sources are being used for the effective synthesis of HAp [47]. Hydroxyapatite is a frequently employed biomaterial due to its high osteoconductivity, biocompatibility, and bioactivity.

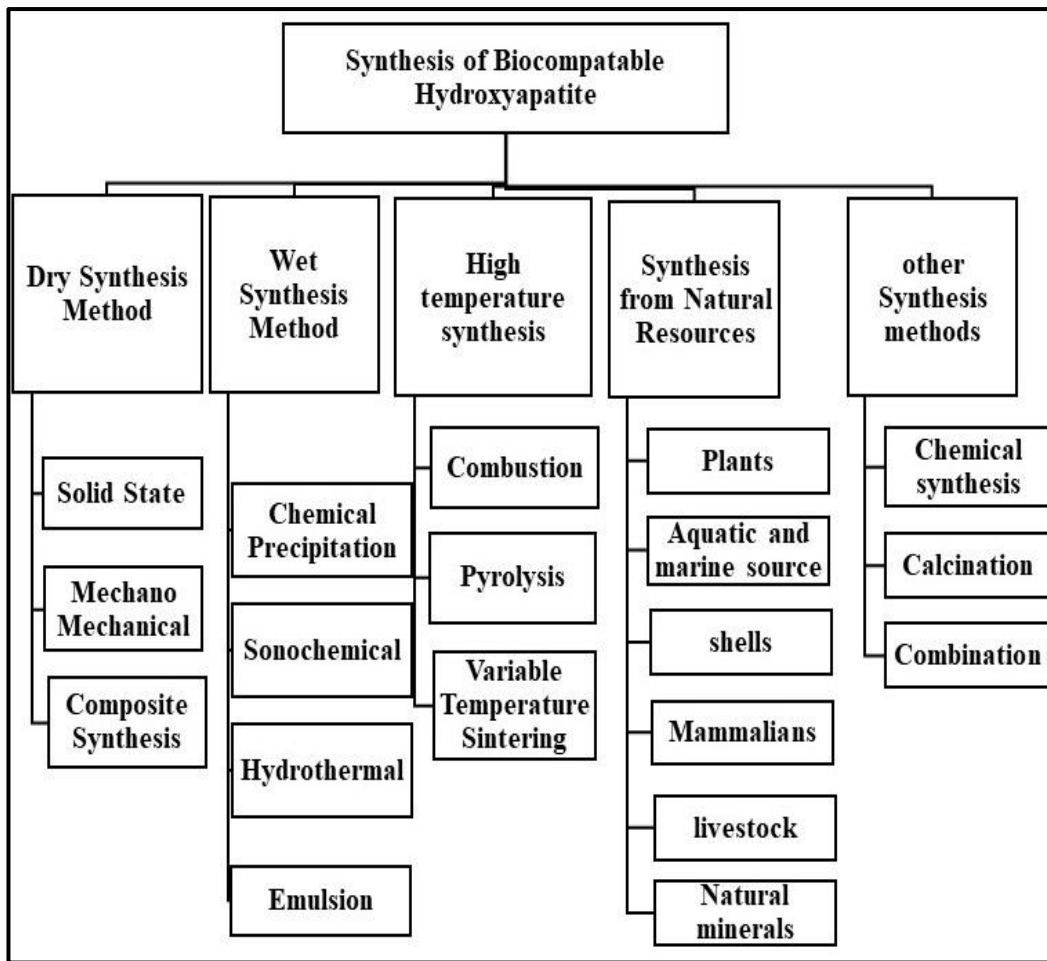


Fig. 2.4. Synthesis methods for biocompatible Hydroxyapatite

Eggshells and animal bones are commonly used as raw materials for synthetics. Calcium carbonate, a common mineral present in eggshells, has the ability to be converted into hydroxyapatite. This study will describe the manufacture of hydroxyapatite powder from used eggshells, as well as its potential applications in biomedicine, the environment, and agriculture. The synthesis of HAp can be done through various methods as shown in **Fig. 2.4.**

Table. 2.3. The main synthesis methods and properties of synthesized HAp [48].

Synthesis Method	Morphology	Size	Characteristics
Sol-gel Method	Spherical, rods, needle, tube	3-1000 nm	Capacity to synthesize homogeneous materials, mechanical properties enhanced due to nano-crystalline grain structures and high purity.

Solid state dry synthesis	Irregular, rod, needle, whisker	5nm - 1000 μ m	Simple procedure, environment friendly, scalable, purity & stoichiometry control, high temp. stability and time efficient.
Co-precipitation	Rod, sphere, fiber, tube, filament, flower, whisker	5-1000 nm	It provides effective doping possibilities. Improved and homogeneous outputs can be achieved. The reactions are carried under controlled pH and temp. It produces fine particles. HAp synthesized exhibits excellent biocompatibility.
Hydrothermal	Rod, needle, spherical, rollers, wire, spherulites, leaves, belts	4-140 nm	Low costs, good homogeneity and solubility of the precursors, tailorable nucleation, and growth of the crystal
Chemical Precipitation	Needle, whisker, rods, spherical, platelets	5-130 nm	Low costs, a controlled environment, and enhanced porosity.
Biosources	Irregular, sphere, flakes, needles, rods	6-180 nm	Capacity to retain trace element, increase bioactivity, and be eco-friendly.
Microemulsion	Spherical, rods, irregular, needle, flakes	5-100 nm	Good anti-aggregation property, outstanding stability, and self-controlled development of apertures
Thermal Decomposition	Flakes, plate, sheet, irregular	5-200 nm	This method provides good particle control and crystallinity
Combustion	Needle, rods, irregular, spherical	40-200 nm	Simplicity, high purity of the obtained products, low energy consumption, and high purity of synthesized material.
Mechanochemical	Irregular, spherical and flakes	25-50 nm	Particle size decreased with increasing mechanical activation duration. High crystallinity of HAp after sintering at high temperature

HAp is a sophisticated functional material and a valuable biomaterial due to its remarkable combination of properties. It is essential to comprehend these properties in

order to maximize their potential in a variety of applications. At the microscopic level, HAp demonstrates an intricate behavior that is impacted by various factors, including surface chemistry, crystal structure, and particle size. A variety of synthesis techniques exist for HAp, each providing distinct capabilities in regulating its properties (refer to **Table. 2.3**). Common synthesis methods consist of the following:

❖ **Wet chemical precipitation:** The chemical precipitation synthesis of HAp involves the controlled aqueous solution combination of calcium and phosphate precursors, which facilitates the precipitation-induced formation of HAp nanoparticles. Phosphate sources, such as ammonium phosphate or diammonium hydrogen phosphate, and calcium sources, such as calcium chloride or calcium nitrate, are typically dissolved in water and combined. In order to facilitate the formation of HAp, the pH of the solution is frequently altered through the addition of a base, such as sodium hydroxide or ammonium hydroxide. Calcium and phosphate ions combine as the pH rises to form HAp nuclei, which subsequently undergo expansion and coalesce to generate larger particles [49]. Following these steps, the suspension is dried, rinsed, and filtered to obtain the HAp powder. In the biomedical and materials science fields, chemical precipitation synthesis is a prevalent technique utilized in the development of bone tissue engineering materials, drug delivery systems, and dental materials. Preferred due to its straightforwardness, capacity for expansion, and ability to regulate the dimensions and morphology of HAp nanoparticles [49].

❖ **Sol-Gel:** The sol-gel method is utilized in the synthesis of HAp to generate HAp nanoparticles through the induction of condensation and hydrolysis of precursor molecules in a solution [50]. Precursors for calcium and phosphate, such as calcium nitrate and ammonium phosphate, are typically dissolved in a solvent such as alcohol or water. A mineralizer or stabilizer is also incorporated in order to control the rate of the

reaction and the formation of crystals. A sol is produced as a consequence of the hydrolysis process in which the solution is transformed into a colloidal suspension of nanoparticles. The sol is composed of species of calcium and phosphate. The nanoparticles undergo increased condensation and aggregation with time, leading to the formation of a gel network. Following the drying process and subsequent thermal treatment of the gel, any residual solvent and organic constituents are removed, resulting in the formation of HAp nanoparticles. By means of this methodology, the HAp nanoparticles' composition, morphology, and crystallinity can be precisely modified, making it suitable for an extensive array of applications in materials science and biomedicine, including drug delivery and bone tissue engineering.

❖ **Hydrothermal/ Solvothermal synthesis:** The Hydrothermal synthesis, also known as solvothermal synthesis, is a technique that involves the reaction of calcium and phosphate precursors in a solution that is both high-pressure and high-temperature and contains water. A highly crystalline structure is produced as a result of this process, which makes it possible to create HAp (HAp) with exact control over the particle size and form of the deposited material [51,52].

❖ **Electrochemical Synthesis:** The electrochemical synthesis of HAp entails the utilization of an electrochemical cell to expedite the creation of HAp from calcium and phosphate precursors in a water-based solution. Usually, calcium ions and phosphate ions are supplied by calcium and phosphate-containing salts that are dissolved in the electrolyte solution. Within the cell, an anode and a cathode are submerged in the electrolyte solution, and a voltage is supplied between them to facilitate the intended chemical processes. The cathode supplies electrons, which cause the reduction of calcium and phosphate ions in the solution, resulting in the formation and enlargement of HAp crystals on its surface. By manipulating the electrolyte composition, pH, and applied

potential, one may precisely regulate the characteristics of the resultant HAp, including its particle size, shape, and crystallinity [53].

❖ **Microwave Synthesis:** Microwave irradiation accelerates the chemical reactions that form HAp, the main component of bones and teeth. This synthesis method has shorter reaction times, higher purity, and better particle size and morphology control than others. Synthesis of HAp using microwave uses calcium and phosphate precursors in stoichiometric proportions $[(\text{NH}_4)_2\text{HPO}_4]$ and calcium nitrate $[\text{Ca}(\text{NO}_3)_2]$ [54]. The solution is microwaved. Surfactants or template molecules may be needed to control HAp nanoparticle crystal formation and shape in addition to solvents like water or ethanol. Microwave synthesis parameters including power level, irradiation period, and reaction temperature can be adjusted to get the desired HAp nanoparticle properties and improve the process. Hydroxyapatite nanoparticles are dried, washed, and sometimes calcined or annealed after microwave irradiation to improve their characteristics.

❖ **Mechano-chemical Synthesis:** The mechanochemical synthesis of HAp involves the initiation of chemical interactions between calcium and phosphate precursors by applying mechanical energy, typically through grinding or milling. This process reaches its highest point with the deposition of HAp nanoparticles. Mechanical energy enhances the disintegration of chemical bonds and aids the dispersion and interaction of the precursor particles during the milling process. The described approach has several advantages, including simplicity, scalability, and the ability to produce very pure HAp nanoparticles without the need for high temperatures or complex reaction setups. Mechanochemical synthesis not only allows for control over particle size, shape, and crystallinity, but also produces HAp that is well-suited for various applications in materials science and biomedicine. These applications include bone tissue engineering, drug delivery systems, and bioactive coatings.

Figure. 2.5. shows that the formation of HAp depends on the **type of synthesis route** (hydrothermal, microwave, micro-emulsion, solid-state, sol-gel, biomimetic, etc.), **process parameters** (hydrothermal temperature/time, pH, concentration, precursors, calcination temperature/time, dopants, etc.), **desired applications** (dental, biocoating, nano catalysis, antibacterial, water purification, drug delivery, bone tissue, etc), **and the morphology** (rods, plates, flakes, wire, needles, whiskers, fibers, sheets, irregular, etc.). Therefore, it is very interesting that the customized HAp material can be obtained as per the availability of resources and desired requirements.

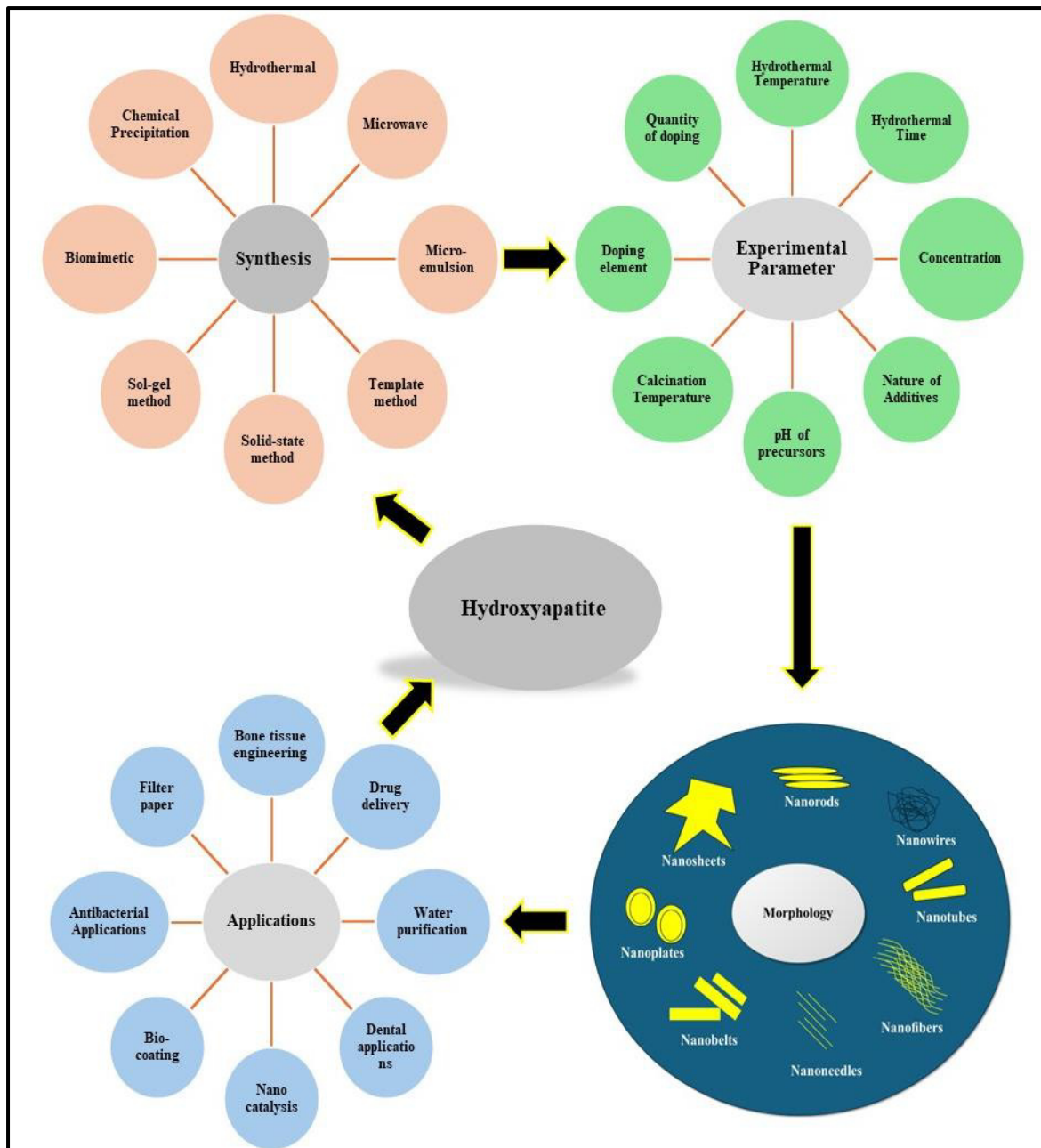


Fig. 2.5. Experimental parameters, synthesis, application, and morphology of nanocrystal HAp [55]

In the current experimental synthesis, the waste eggshell was used to extract an attractive bioceramic material known as Hydroxyapatite (HAp), chemically known as Penta Calcium Tri-Phosphate hydroxide i.e. $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ or in general $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. The modified synthesis method adopted is multi-stage calcination followed by the chemical dispersion method [56,57]. Multi-stage calcination performed at the beginning of the

synthesis helps to eliminate the excess unwanted particles, elements, residues, and contaminations on the waste eggshell.

HAp is the most stable bioceramic material having characteristics mirrored as human bone. It is helpful in the repair, regeneration, coating [58–65] and adhesion of bioimplants in different reinforcing combinations. The inorganic phase of bone consists of almost 60- 70% of HAp [20]. HAp possesses crystallographic and morphological similarities to human bone and due to its excellent osteoconductivity and biocompatibility, researchers are immensely working on the synthesis of HAp from various calcium-rich sources like mammalian bone, shells, chemical extracts, plants, natural minerals, etc. using different synthesis methods like dry, wet, high temperature, low temperature, chemical route etc. as systematically reviewed by Balhuc et al. [44].

Biomedical engineering utilizes different combinations of composite materials based on the strength, compatibility and characteristics requirements [66]. Synthesis of HAp from both natural and synthetic sources are done as per need. Design, synthesis and fabrication of artificial bioimplant has become the necessary direction for the biomechanical and biomedical researchers to focus upon [67,68]. Rapid increase in the accident and trauma cases in the world has forced to develop the high quality and customized implant with high level of accuracy and aesthetics. Researchers from material background are working on identifying the most biocompatible, nontoxic, non-reactive, osteointegrative, bioactive and high strengthen material composites [69,70]. All kind of composites like polymer based, metal based, ceramic based, fiber reinforced, particulate reinforced and hybrid composites are being synthesized, fabricated, and analysed based on the applications. Some of the major Bioimplant applications includes Total Hip Replacement [71], Knee Joint , Elbow Joint, Dental Implant, etc. [27,72]. HAp can be synthesized from various natural sources as listed in **Table. 2.4.**

Table. 2.4. Synthesis method of HAp using various Sources.

Source		Synthesis Method	Reference	
Foliage and Algae	Leaves of Moringaoleifera	Rapid microwave irradiation	[73]	
	Wood: rattan and pine wood	Pyrolysis and hydrothermal	[74]	
	Potato peel	Biochemical Precipitate preparation	[75]	
	Leaves of Papaya	Biochemical Precipitate preparation	[75]	
	Waste Peel of orange	Biochemical Precipitate preparation	[75]	
	Calendula flower	Biochemical Precipitate preparation	[75]	
	Wastewater of potato Processing	Biochemical Precipitate preparation and biological Pretreatment	[76]	
	Catha edulis (Khat)	In vitro high temperature calcination	[77]	
	Basil herb	In vitro high temperature calcination	[77]	
	Scenedesmus sp. Microalgae	Hydrolysis-hydrothermal mineralization	[78]	
	C. officinalis red algae	Pyrolysis and alkaline hydrothermal	[79]	
	Mammal Based	Bovine Bone of mammals	Hydrothermal and Calcination	[80]
		Bone of Pig	Alkaline Hydrolysis and Calcination	[81]
		Bone of fish and Scale	Alkaline Hydrolysis and Calcination	[82–84]
Bone of Camel		High Temperature Calcinated	[85]	
Cone of Horse		High Temperature Calcinated	[85]	
Bio Shell	Cockleshell	High Temperature Calcinated and sol gel	[86]	
		High Temperature Calcinated and hydrothermal	[87]	
	Clamshell	High Temperature Calcinated and mechanochemical	[88,89]	
	Seashell	High Temperature Calcinated and chemical precipitated	[90]	

	Eggshell	High Temperature Calcinated and chemical precipitated	[91]
Sandstone/ Limestone	Musselshell	Calcination and RMI	[92]
	Sandstone	High Temperature Calcinated and chemical precipitated	[41]
	Sandstone	High Temperature Calcinated and hydrothermal	[93]
	Sandstone	High Temperature Calcinated and ultrasonic irradiation	[94]

2.3 Doping in Hydroxyapatite

Doping is the process of introducing metallic ions, known as **dopants**, into the crystal lattice of a substance with the intention of modifying its properties. This procedure is frequently employed in order to impart the material with the targeted properties. Doping HAp with metallic ions alters its properties for several biomedical applications. Bone and tooth enamel primarily are composed of HAp, an inorganic form of calcium apatite that occurs naturally in minerals. Bone transplants, implant coatings, and drug delivery systems can all benefit from this material. When some calcium ions inside the HAp lattice are replaced with metal ions, this process is called **metallic doping**. The replacement has the potential to impart many desirable properties onto HAp, including **enhanced bioactivity, magnetic properties, antibacterial capabilities, and mechanical robustness**. Some of the important cations and anions used for doping in biomedical applications are listed in **Table 2.5**. The following are examples of some of the common metal ions used to dope HAp:

- ❖ **Strontium (Sr):** It has been investigated for its ability to improve bone formation and prevent bone resorption, which shows promise for the treatment of osteoporosis and other bone-related conditions.

- ❖ **Magnesium (Mg):** The addition of magnesium to HAp can enhance bone formation and enhance the biological reaction to HA implants. Additionally, it enhances the mechanical characteristics and biodegradability.
- ❖ **Zinc (Zn):** The addition of zinc can improve the ability of HAp to kill germs and stimulate the regrowth of bones. Additionally, it contributes to enzymatic pathways that are essential for bone metabolism.
- ❖ **Silicon (Si):** The use of silicon-doped HAp has been studied to determine its potential in promoting bone formation and biological activity. Silicon has been observed to promote the process of osteoblast development and mineralization.
- ❖ **Iron (Fe):** The addition of iron to HAp can impart magnetic characteristics to the material, making it advantageous for applications such as targeted medication delivery and the development of contrast agents for magnetic resonance imaging (MRI).
- ❖ **Copper (Cu):** Copper-doped hydroxyapatite demonstrates antimicrobial characteristics and has the ability to promote the growth of blood vessels (angiogenesis) and the formation of bone tissue (osteogenesis). This makes it valuable for applications in the field of bone tissue engineering.
- ❖ **Silver (Ag):** Silver doping in HAp adds antibacterial qualities, making it more suitable for biomedical applications needing infection control, such as bone implants and wound dressings.

Table. 2.5. Doping material introduced into HAp structures and their improved properties [1].

Doping Ion		Biological Activity
Cation	Na ⁺	Enhanced osteoconductivity and improved cell proliferation.
	K ⁺	Enhanced thermal stability.

	Mg^{2+}	Improved crystal growth, crystallization, thermal stability, and dissolution rate.
	Sr^{2+}	Enhanced bone regeneration and formation, and inhibited bone resorption.
	Mn^{2+}	Enhanced cell adhesion.
	Zn^{2+}	Enhanced bone formation and regeneration, increased antimicrobial activity.
	CO_3^{2-}	High specific surface area, reduced crystallite size, enhanced osteoconductive properties, and increased solubility.
Anion	SiO_4^{4-}	Enhanced bioactivity, improved dissolution rate.
	F^-	Improved stability, reduced solubility, and increased remineralization.
	Cl^-	Enhanced osteoconductive properties, and increased solubility.

Several factors, including the type and amount of dopant ions, the synthesis method, and processing conditions, determine the doping process and the properties that arise. The incorporation of metals into hydroxyapatite offers a versatile way to tailor its properties to specific medical applications.

Modifying HAp using metallic ion doping outperforms its properties and provides improved results depending on the dopants. Cacciotti [95] reviewed doping characteristics of various cations (Na^+ , Ag^+ , Mg^{2+} , K^+ , Zn^{2+} , Al^{3+} , Sr^{2+} and Ba^{2+}) and anions (Cl^- , F^- , CO_3^{2-} and SiO_4^{4-}) adsorbed or incorporated within the HAp appetite lattice and summarized the physiochemical properties of doped samples. Researchers engaged in diverse material characterization justify silver doping as a suitable antibacterial substrate component. Mangal et al. [96] performed several interesting research on the characterization of silver-doped HAp coating through the plasma spray method for orthopedic and biomedical applications. 2, 4 and 6 wt% of Ag^+ were doped in HAp at

800°C. Adhesion strength upto 15MPa was achieved. Bacterial resistance was analyzed against *pseudomonas aeruginosa*. Results obtained confirmed the appreciating antibacterial nature of the prepared Ag-doped coatings. Later, strontium-doped coating on titanium substrate was also characterized to offset the cytotoxic effect of Ag [97]. According to studies, Ag⁺ ions can get through the bacterial cell wall and cause DNA to change into a condensed state, which combines with proteins with thiol groups to trigger cell death. It has been shown that the antibacterial efficacy of a material improves with the amount of silver included in it, albeit at the expense of increasing cytotoxicity.

2.4 Coating of Hydroxyapatite

The process of coating materials includes the application of a material layer onto the surface of a substrate material in order to impart desired functional, protective, or cosmetic characteristics. Numerous sectors, including manufacturing, biomedical, construction, automotive, aerospace, and electronics, make extensive use of this method [98].

Coatings comprise composites, metals, pigments, polymers, and metals, among others. The motivation behind the development of composites is its customized composition as per the requirement of the applications. Researchers working in the field of advanced material technology are working to develop the material as per the characteristics requirements [99]. The various characteristics that govern a material, include its size, form, strength, texture, morphology, compatibility, and reactivity, can be effectively regulated, and produced by utilizing different combinations of matrix and reinforcement. The application of sophisticated coating processes has made it possible to modify the contact surface by adopting the same attitude and implementing upgrades. Hydroxyapatite, when used as a biocomposite coating on metal, polymer, and ceramic based composites, improves the surface morphological features and topography. The

governing parameters of HAp coating include the nature of the substrate, its adhesive qualities, compatibility with the coating material, durability, and application. Coating methods includes dip coating [100], sputter coating, electrophoretic coating, pulsed laser deposition coating, sol gel coating and thermal spraying [101].

Out of all the coating methods mentioned, Sol gel coating method provides minimum coating thickness of less than one micron. The advantages, disadvantages, adhesion thickness and strength of several coating methods are mentioned in **Table 2.6**.

In order to modify the surface morphology, characteristics, hardness, bio compatibility, chemical insulation and decrease degradation rate, HAp coating is an effective solution [102]. It is interesting to note that, every coating method has its own advantages and disadvantages. Galindo et al. [103] worked on the coating of nano sized HAp on the bio compatible polymer matrix substrate to enhance the bioactivity of polymer based implant. The results revealed the improved rheological, thermal and mechanical properties of the final composite along with biocompatibility and bioactivity [103]. Maximum Bioceramics [104] have inert nature and requires osteogenesis as it does not form any bonding with the bone[105]. Thus, adding a fibrous HAp coating on the surface enables osteoconduction nature of the implant and the natural bone. Hydroxyapatite is a bioactive bioceramic with improved biocompatibility[93,106–108]. Size, shape, composition, and morphology of different graded HAp regulates the coating characteristics. Pepla et al. [109] effectively reviewed the nano sized HAp and elaborated the efficient adhesion, proliferation and mineralization on regenerative, restorative and preventive dentistry applications [109].

Coating of HAp on metallic, polymeric, ceramic or hybrid substrate requires some standardize parameters as directed by Food and drug administration and ISO standards. These parameters include coating thickness ($>1\mu\text{m}$), approx. 62% crystallinity, 95%

purity, constant Ca/P ratio (between 1.67 – 1.76), weight density (2980 kg/m³), tensile strength (more than 50 MPa), shear strength (more than 20 MPa) and permissible abrasion.[110–112].

Table 2.6. Characteristics of various coating methods adopted for Hydroxyapatite Coating [113].

Coating Method	Coating Thickness	Adhesion Strength (MPa)	Advantages	Disadvantages
Sol Gel Method [62][113]	100 nm - 1µm	25- 35	Works at Low temperature, can coat complex shapes, Thin coating and Cost effective	It requires control atmosphere and some costly raw materials which are difficult to obtain
Pulsed Laser Deposition Method [89]	Less than 5µm	30 - 40	Uniform coating on simple and flat surfaces	Costly, time consuming, produce amorphous coating, cannot coat complex geometries.
Sputter Coating [114]	0.02 - 1µm	55- 65	Uniform coating on simple and flat surfaces	It uses line of sight technique, costly, cannot coat complex geometries, produce amorphous coatings
Dip Coating [100]	0.05- 0.5 mm	28- 32	Fast, low cost and can coat complex shapes	Requires high temperature for sintering, mismatch in thermal expansion
Electrophoretic deposition Method [115]	0.1 – 2 mm	15-18	Fast process, uniform coating, can coat complex substrates	Requires high thermal temperature, difficult to produce defect less coating
Thermal Spray Coating [116]	30 – 250 µm	25-35	Fast and high deposition rate	Line of sight coating, requires high temperature, it

Biomimetic Coating [12,89,113,117,118]	~1mm	10	Fast Process, can be done on complex geometries	induces deposition, amorphous coating occurs due to rapid cooling Low adhesion strength, Surface treatment required, Applicable for limited substrate
---	------	----	---	--

2.4.1 Advantages of PMMA/ HAPAg coating on Ti6Al4V substrate

The literature suggests that, the incorporation of PMMA/ HAPAg on Ti6Al4V substrate can offer following advantages:

- ❖ The incorporation of HAPAg powdered particles into PMMA coatings on Ti-alloy substrates can result in an enhancement of antibacterial and biocompatibility properties of the coated surfaces, which is comparatively better than those surfaces that are without coatings or are already coated with only PMMA.
- ❖ By encouraging bone-forming cells (osteoblasts) to adhere, proliferate, and differentiate, the utilization of PMMA/HAPAg coatings on Ti-alloy substrates could enhance the integration capability of dental or orthopedic implants with bone tissue. Consequently, enhanced integrity of the implant and progression of bone formation will ensue.
- ❖ Silver-doped HAp nanoparticles incorporated into PMMA coatings on Ti-alloy substrates will reduce the likelihood of implant-related infections and peri-implantitis by imparting long-lasting antibacterial properties against prevalent pathogenic microorganisms.
- ❖ Orthopedic implant durability and resistance to mechanical loading and frictional wear in the physiological environment can be significantly improved through the

application of a PMMA/HAPAg coating onto Ti-alloy substrates. This enhancement will ultimately lead to an extension of the implants' service life.

- ❖ By promoting the attachment of cells and their integration with surrounding tissue, the incorporation of HAP into the coating will inhibit the formation of a biologically active layer on the surface of the implant. A further benefit of silver is that it inhibits the development of biofilms and the proliferation of microorganisms, which contributes to improved clinical outcomes and increased success rates of implants.
- ❖ By securing the underlying metal against corrosive deterioration in biological fluids and harsh environmental conditions, the PMMA/HAPAg coating that is applied to Ti-alloy substrates will exhibit advantageous corrosion resistance properties. As a result, adverse effects on the living organism will be diminished and the longevity of the implants will be prolonged.
- ❖ In regard to adhesion strength, cohesion, and elastic modulus, the PMMA/HAPAg coating will exhibit mechanical characteristics that meet or exceed the requirements for implant coatings. Implementation and service will be facilitated by this, ensuring stability and integrity.

2.5 Fabrication of HAP reinforced PMMA composite

Poly (methyl methacrylate) (PMMA) is a thermoplastic material that is transparent, strong, highly resistant to UV radiation, and rigid. It is commonly used as a substitute for glass because of its several technological benefits over other transparent polymers. PMMA is a polymer that attracts a lot of attention because of its mild physical and mechanical qualities, ease of manufacturing, low cost, and possible uses. Regrettably, unaltered, or pristine PMMA is more fragile, particularly when subjected to greater impact force, and is more susceptible to scratching than traditional inorganic glass. Furthermore, when PMMA is changed with different fillers, it can exhibit superior scratch

and impact resistance, along with other desirable qualities, under specific circumstances. On the other hand, HAp is a mineral composed of calcium phosphate, with the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. It has chemical and crystallographic characteristics which are similar to the inorganic components of bone matrix and teeth. It has exceptional osteoconductivity and osteoinductivity, as confirmed by research. It has been scientifically employed as bioactive coatings on dental and orthopedic implants, facilitating the attachment and growth of osteoblast cells on the artificial surface, and leading to the establishment of a biological connection between bone tissues and the implant. Nevertheless, a significant drawback was observed when the material was subjected to substantial load carrying, mostly due to its inadequate mechanical characteristics, including low fracture toughness and tensile strength. The most commonly used materials for cranioplasty (i.e. it is a surgical procedure that involves repairing a bone deficiency in the skull caused by a previous operation or accident) are currently HAp and PMMA [119]. Furthermore, PMMA is preferred because it offers greater fracture resistance and is easier to process. Conversely, its osteoconductivity is relatively weak. On the other hand, HAp has good osteoconductivity but is inherently brittle, making it susceptible to shattering from external impacts. Consequently, the combination of PMMA with HA enhances their mechanical characteristics, including the ability to harden on their own and a high level of elasticity [120].

Several researchers in the past have worked on the fabrication of PMMA reinforced with commercially available HAp and performed different characterizations. **Table 2.7.** highlights some of the publications on application of PMMA/ HAp composites in dental applications. Wijesinghe et al. [121] synthesized PMMA/HAp nanocomposite through industrial methods using dolomite and performed only morphological characterization. Zebarjad et al. [122] studied wear, compression and three-point bending tests of PMMA/

HAp composite. The results reveal that the wear rate decreases with the increase in HAp content. Karthick et al. [123] performed mechanical and tribological characterization of PMMA and sea shell-based HAp (2- 20 wt%) for biomedical applications. The result shows the increase in wear resistance with increasing wt% of nanopowders till 12 wt%. Similarly, Fouly et al. [124] used PMMA/ HAp nanocomposite for denture application and performed tribological characterization using a pin-on-disc Tribometer. Raheem & Majid [125] developed a composite with PMMA/Mg-HAp/ Collagen for orthopedic application and characterized for osteointegration properties. Akhtar et al. [126] reinforced HAp in denture-based acrylic PMMA and performed tribological characterization. Gratiela et al. [127] diagnosed the hydrophobicity and hydrophilicity of PMMA reinforced with commercial HAp. In the same way, several researchers have performed various characterizations on PMMA/ HAp composite and used the chemically prepared HAp or the commercially available HAp for the development of HAp-reinforced composite with PMMA matrix. **Consequently, from the existing literature, it was observed that the reinforcement of eggshell-derived silver-doped HAp in PMMA has not been synthesized and characterized by any researcher till date.** Therefore, the **PMMA/HApAg** composite materials possess a harmonious blend of mechanical robustness, compatibility with living organisms, ability to stimulate biological activity, visibility under X-rays, and antibacterial characteristic i.e. ability to combat microbial growth. As a result, they hold great potential for various medical uses, such as bone implants, dental prosthetics, and drug delivery systems [128].

Table. 2.7. PMMA/ HAp composite for dental implant.

Authors	Title	Journal	Findings	Reference
Aldabib et al. (2020)	Effect of HAp filler concentration on mechanical properties of poly	S N Applied Sciences	Study investigates the effect of different filler concentrations on the physical and mechanical	[129]

	(methyl methacrylate) denture base		characteristics of PMMA/HA composite. Tensile, flexural and fracture toughness tests were conducted to evaluate the mechanical performance. Data analyses was performed to diagnose the effect of HAp wt% and loading on mechanical properties of acrylic denture base material.	
Kang In-Gu (2018)	Hydroxyapatite Microspheres as an Additive to Enhance Radiopacity, Biocompatibility, and Osteoconductivity of Poly(methyl methacrylate) Bone Cement	MDPI, Materials	Study demonstrates the utility of HAp microspheres as an additive to enhance the radiopaque properties, biocompatibility, and osteoconductivity of PMMA- based bone cements. The biocompatibility and osteoconductivity of PMMA/HA bone cement was significantly enhanced	[130]
Muhammad Sohail Zafar (2020)	Prosthodontic Applications of Polymethyl Methacrylate (PMMA): An Update	MDPI, Polymers	PMMA is commonly used for prosthetic dental applications, including the fabrication of artificial teeth, denture bases, dentures, obturators, orthodontic retainers, temporary or provisional crowns, and for the repair of dental prostheses. The unique properties of PMMA, such as its low density, aesthetics, cost-effectiveness, ease of manipulation, and tailorable physical and mechanical properties,	[131]

			make it a suitable and popular biomaterial for these dental applications. Modification is needed for further enhancement in properties.	
Rosa et al. (2022)	Application of Tribology Concept in Dental Composites Field: A Scoping Review	Journal of Functional Biomaterials	Overview of tribological application in resin dental composites. The lack of a standardized methodological approach makes the laboratory findings poorly informative for clinicians.	[132]
Srinivasan et al. (2022)	Wear behavior of HAp-reinforced polymer composite for biomedical applications	Surface topography: Metrology and Properties	Tribological properties of HAp powder reinforced composite were investigated. Taguchi, DOE and computational analysis was performed to study the influence of wear rate with HAp wt% in epoxy resin.	[133]
Ramkumar & Anoj (2022)	Comparative investigation of tribological behavior of hybrid dental restorative composite materials	Ceramics International	Nano HAp, alumina and titanium oxide were reinforced in dental resin. ANOVA analysis was performed. The results showed that incorporating high amounts of nHAp nanoparticles can improve the wear properties of dental composites.	[134]
Navarro et al. (2012)	Friction and wear properties of poly(methyl methacrylate)-HAp hybrid coating on	Wear	Dip coating was performed, and the samples were tested with wear characteristics. The incorporation of HAP	[135]

Virginia et al. (2018)	UHMWPE substrates Tribological and mechanical characterization of PMMA/HAp nanocomposites obtained by free-radical polymerization	MRS Advances © 2018 Materials Research Society	in PMMA enhances wear properties. PMMA/ HAP [136] composite with three different concentrations of benzoyl peroxide (PBO) of 3, 6, and 12 wt.% were fabricated using free-radical polymerization synthesis. The nanocomposite with the highest concentration of PBO presented the best mechanical and tribological behavior, as well as the lowest values of water absorption and porosity percent.
------------------------	--	--	--

2.6 Research Gaps identified from the current literature

After reviewing the existing literature following research gaps were identified:

- ❖ It was found that, 2.5 million tons of eggshell waste is produced every year and effective utilization of eggshell waste is still a major concern; hence, **waste to wealth** can be generated using eggshell waste.
- ❖ It was found that **calcination process** gives good phase purity. **But no published research has highlighted the effect of multi-stage calcination** on eggshell based HAp at the beginning of the synthesis. It is expected that it can yield a high level of purity and can remove all volatile impurities, dirt, contaminations, unwanted residues, etc. from the waste eggshell.
- ❖ Researchers working on synthesis of HAp have used **varying stirring timings (say 2 hours to 24 hours)** without any justification. Proper optimized stirring parameters are still not justified.

- ❖ It was found that, **silver doping** in various substrates yields good **biocompatibility and antimicrobial characteristics**. So, optimized eggshell-derived HAp can be modified using various **wt%** of silver doping.
- ❖ Silver doped, eggshell derived HAP is not synthesized using **multistage calcination** and characterized by any researcher so various characterizations can be done.
- ❖ **Biocompatibility** and **cell viability** for silver doped materials are done only for bacteria namely *E. Coli* and *S. Epidermides*. Some other bacteria cultures can be researched and explored.
- ❖ Several other bacteria cultures related to biological application that can be explored are *S. epidermidis*, *S. aureus*, *B. subtilis*, *B. cereus*, *P. aeruginosa*, *E. Coli* and *K. Pneumoniae*.
- ❖ Biocompatible composite with eggshell derived HAp reinforcement in PMMA was not **fabricated and characterized** by any researcher.
- ❖ Effect of **Dip coating** of PMMA/ HAPAg on Ti6Al4V substrate was not analysed by any researcher.
- ❖ **Tribological behavior** analysis of PMMA/HAPAg composite samples prepared using cold moulding method was also not analysed by any researcher till date.

2.7 Objectives of the study

Following are the objectives that need to be fulfilled in the current thesis:

- ❖ Synthesis of HAp from waste eggshell using multi-stage calcination and chemical precipitation method with varying reaction timings (6, 12, 18 and 24 hours).
- ❖ Process optimization and morphological characterization of synthesized HAp derived from waste eggshell.
- ❖ Synthesis of optimized eggshell derived HAp using silver doping at various molar concentrations (0.1, 0.2 and 0.5 wt%).

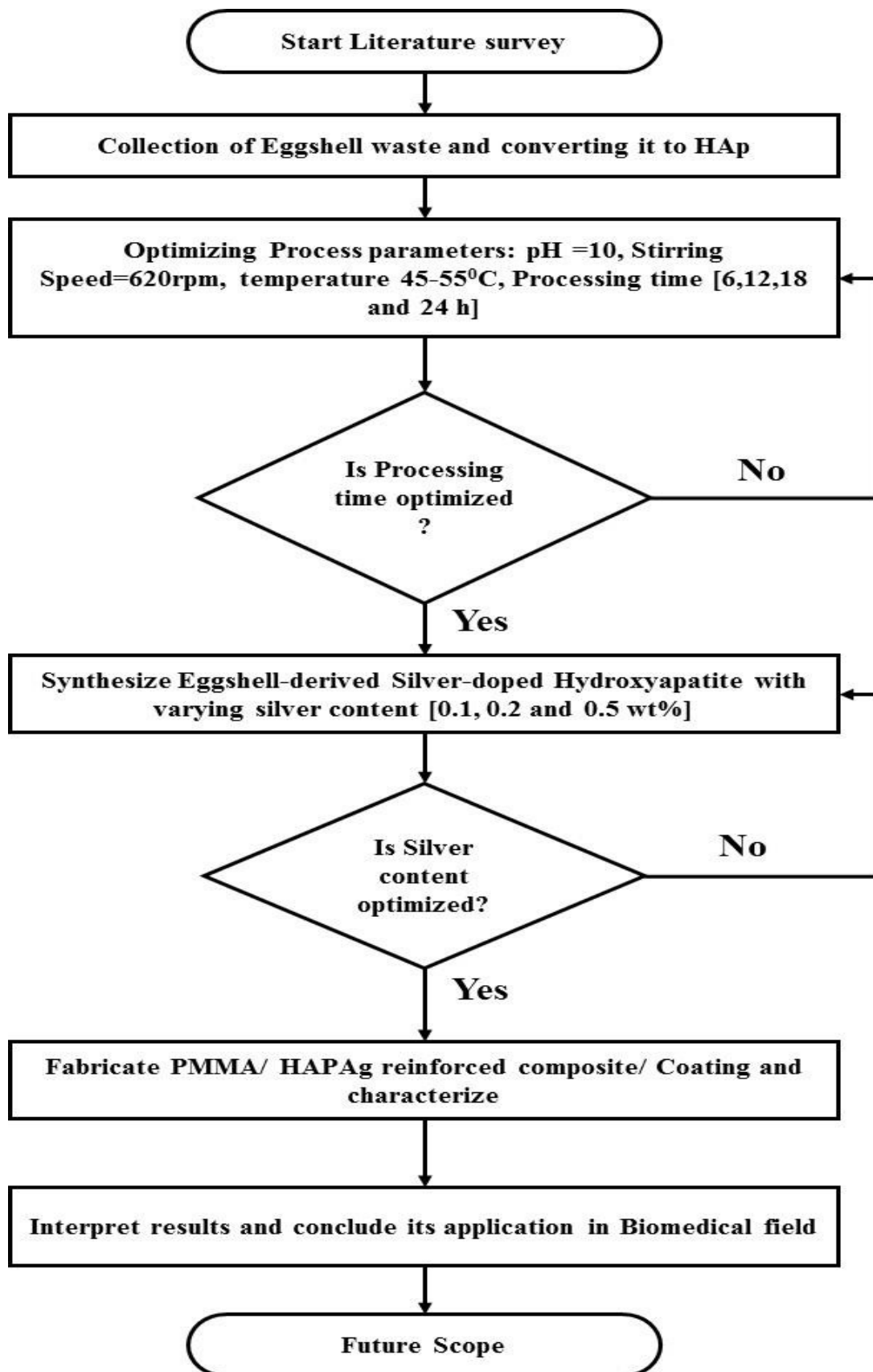


Fig. 2.6. Flowchart of the current study