CHAPTER 3 MATERIALS AND METHODS

This chapter deals with the description of all the materials and their systematic synthesis routes. The explanation of precursors, substrates, synthesis method, machines, characterizations, testing methodologies and standards are elaborated.

3.1 Materials

Various consumable/ non-consumable materials used in the current research include waste eggshells, orthophosphoric acid, silver nitrate, PMMA, sodium hydroxide, etc. The related details are listed in **Table 3.1.** Also, the ISO/ ASTM standards used in current thesis are listed in **Table 3.2.**

Table 3.1. List of consumables and non-consumables in the current research.

S. No	Item	Specification	Supplier	Consumable/ Non-consumables
1	Eggshells	Waste from various sources	-----	C
2	Orthophosphoric Acid (H_3PO_4)	Extra pure, 85%, M. W. 98.00, product number 36678, 1.70g weight per ml (20^0C)	Sisco Research Laboratories Pvt. Ltd. Turbhe, New Mumbai, India.	C
3	Silver Nitrate ($AgNO_3$)	Extra pure, MW 169.87g/mol, assay 99.8%	Srichem Research Laboratories Pvt. Ltd. India.	C
4	Polymethyl methacrylate (PMMA)	Cold cure, consisting of pre-polymerized powder and the liquid monomer	Dent Ganga, New Delhi	C
5	Alumina boat	40ml (136x27x21 mm), Max Temp-1750°C	Ants Laboratory	NC

6	Sodium Hydroxide	Product Code: S0290, MW 40g/mol Assay 97%	Avantor Materials India Limited, Thane, Maharashtra.	C
7	Distilled Water	Medical grade, purified, PH	Laboratory distillation system	C
8	Laboratory Equipment	Glasswares, PH meter, veils, Beakers, etc.	Ants Laboratory	NC
9	Magnetic Stirrer	MS with hot plate, RPM and Temperature regulator, Dimension: 200 x 225 x 185 mm, Model- Q-19A	REMI Sales and Engineering Ltd.	NC
10	Digital Electric Oven	Ambassador - 220/230VAC, MT:250 ^{0}C	B. P. Industries, Delhi, India	NC
11	Muffle Furnace	Max Temp 1250 ^{0}C	Sunrise Lab Solutions, India	NC
12	Weighing Machine	WENSAR high precision WM, LC: 0.1 mg	WENSAR India Pvt. Ltd	NC

Table 3.2. ISO/ASTM Standards

S. No	Parameter	standardization	Remark
1	Tap density of powder	ISO 3953 (2011), ASTM D7481-09	Tapped in container using Tap density analyzer.
2	Experimental Density (Archimedes)	ASTM B962	For compacted and sintered powder.
3	Angle of repose of bioceramics powder	ISO 8398: 1989	Using a cone and funnel.
4	Hydroxyapatite as biomedical material (Ca/P ratio)	ISO 13779-3: 2018(en)	Determine the range of Ca/P for biomedical applications.
5	Implant for Surgery-Hydroxyapatite	ISO 13779-6: 2015 (en)	Determine characteristics for Hap.
.6	Contact Angle	ASTM D5946	Model DSA 25s, Kruss, Germany.
7	Compressive Strength	ISO 17162:2014	UTM (ASI Sales Pvt. Ltd). The length and diameter of the samples were in ratio ½.

8	Particle Size Analysis	ASTM D6913	Using sieve analysis.
9	Water Absorption Test	ASTM D570	Test for 24 h minimum.
10	Density and Specific Gravity of sintered samples	ASTM D792-13 ASTM	Archimedes principle.
11	Hardness	ISO 6507-02	Vickers microhardness using Mitutoyo tester.
12	Thermal Analysis	ASTM E1131-20	TGA and DTA Analysis available at CIF, IIT (BHU).

3.2 Synthesis of Eggshell-derived Hydroxyapatite

Synthesis of biocompatible hydroxyapatite from waste eggshells that are disposed into a dustbin is done using multistage calcination and chemical precipitation method at four different stirring timings i.e. 6 hours, 12 hours, 18 hours and 24 hours and the final HAp prepared are nomenclate as HAP06Hr, HAP12Hr, HAP18Hr and HAP24Hr, respectively as shown in **Fig. 3.1.**

Fig. 3.1. Final prepared samples of hydroxyapatite synthesized for 6, 12, 18 and 24 h.

Firstly, the raw eggshells are washed using light warm distilled water (DW) and ethanol to remove all the contaminations, eatable yolk, and dirt. The thin shell layer present between the egg white and eggshell was removed. Then the eggshells are heated at 200^0C, 450^0C, 650^0C and 900^0C using a multistage calcination method with

20 min halt timings and 5^0C/min heating rate. The process converts $CaCO_3$ to pure CaO powder. CaO is then preserved in glass desiccator for 24 hrs. Further, converting the CaO into $Ca(OH)_2$ by adding 1/3rd amount (by weight) of water and stirring at 1100 rpm for 1 hour using the magnetic stirrer. The reaction is shown in equation (3.1). The reaction is exothermic, and the temperature of the prepared $Ca(OH)_2$ rises to 70 ^{0}C as measured. The conversion of calcium oxide to calcium hydroxide is essential as the solubility of $Ca(OH)_2$ is better in the acidic and water medium.

$$CaO + H_2O \rightarrow Ca(OH)_2 + Heat \quad \ldots (3.1)$$

$$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \quad \ldots (3.2)$$

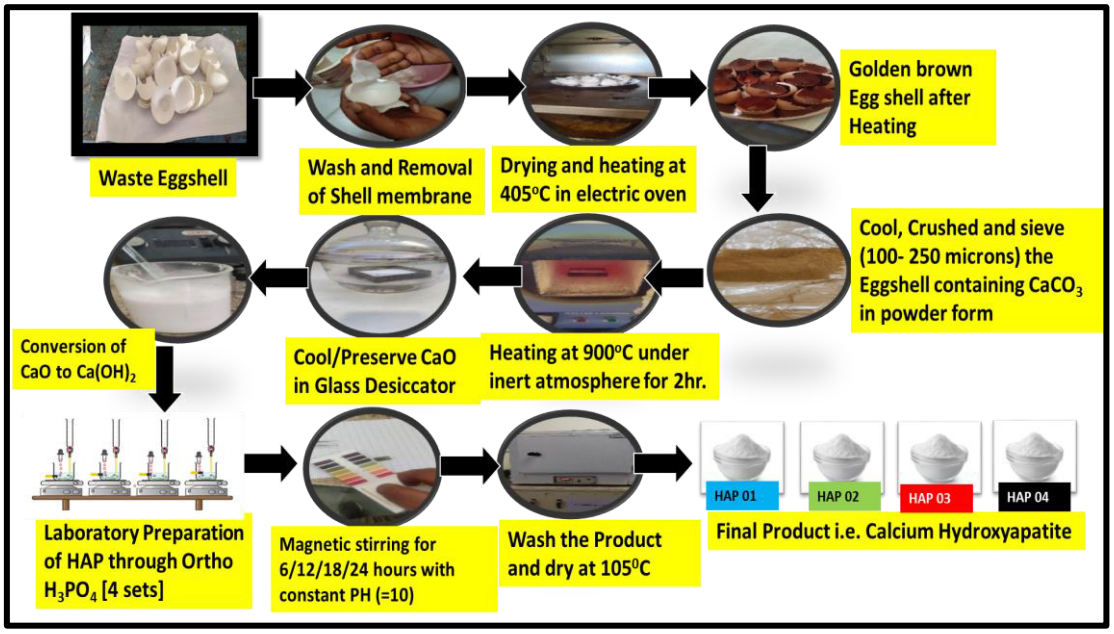

Fig. 3.2. Step by step methodology for HAp preparation.

After the conversion of CaO to $Ca(OH)_2$, the chemical precipitation process is performed for the synthesis of 4 different HAp samples following the parameters tabulated in **Table 3.3** using equation (3.2). **Figure 3.2.** provides the elaborative pictorial view of the experimental setup utilized for the simultaneous synthesis of HAp during the chemical precipitation method. The experimentation is done in similar

environmental conditions, and the reaction timing is varied with 6, 12, 18 and 24 hours of magnetic stirring.

Table 3.3. Synthesis Parameters for Optimizing Reaction Rate.

Sample Name	Moles of Ca	Moles of P	pH	NaOH (M)	External Temp. Range (^{0}C)	Stirring Speed (RPM)	Reaction timing (hr)
HAP06Hr	10	06	10	0.1	45 – 65	660	6
HAP12Hr	10	06	10	0.1	45 – 65	660	12
HAP18Hr	10	06	10	0.1	45 – 65	660	18
HAP24Hr	10	06	10	0.1	45 – 65	660	24

3.3 Synthesis of Eggshell-derived Silver-doped Hydroxyapatite

The synthesis of HAp requires calcium and phosphorous as the major substrates to amalgamate in proper proportion and yield the desired purity and quantity. Here, the calcium precursor is waste eggshell collected from hostel mess, and the phosphorous precursor is orthophosphoric acid (H_3PO_4) extra pure, 85%, M. W. 98.00, product number 36678, density 1.70 g/cm^3 (20^{0}C), purchased from Sisco Research Laboratories Pvt. Ltd. Turbhe, New Mumbai, India. For Ag^+ doping, silver nitrate ($AgNO_3$) extra pure with molecular weight 169.87, assay 99%, product number 94118, from Srichem Research Laboratories Pvt. Ltd was used. India. One molar aqueous solution of Sodium Hydroxide (NaOH), Product Code: S0290, M. W 40.00, Assay 97% for maintaining pH equals 10 during the reaction was obtained from Avantor performance materials India Limited, Thane, Maharashtra. Distilled water, pH meter, magnetic stirrer with heating plate and temperature controller and all other laboratory products as desired were used for chemical dispersion synthesis.

The well-defined pictorial representation of the synthesis of silver-doped hydroxyapatite using a modified method, i.e., multistage calcination and chemical dispersion method, is shown in **Fig. 3.3.** Also, the detailed representation of chemical

dispersion is highlighted in the enlarged image, as shown in **Fig. 3.4.** Standard and optimized process parameters i.e. pH 10, stirring speed 660 rpm and temperature 55 ^{o}C, are used to obtain high-purity HAp samples. The stirring time for chemical synthesis of modified HAp is kept at 18 hours, which is similar to the optimum timing that was determined in the previous experiments in the same laboratory. Four different combinations of powdered samples were prepared by doping $AgNO_3$ in dispersed calcium phosphate hydroxyapatite with 0.0, 0.1, 0.2 and 0.5 wt% termed HAP0.0Ag, HAP0.1Ag, HAP0.2Ag and HAP0.5Ag, respectively. The final prepared powdered samples are shown in **Fig. 3.5.** The samples prepared are analyzed using physical, morphological, and antibacterial characteristics.

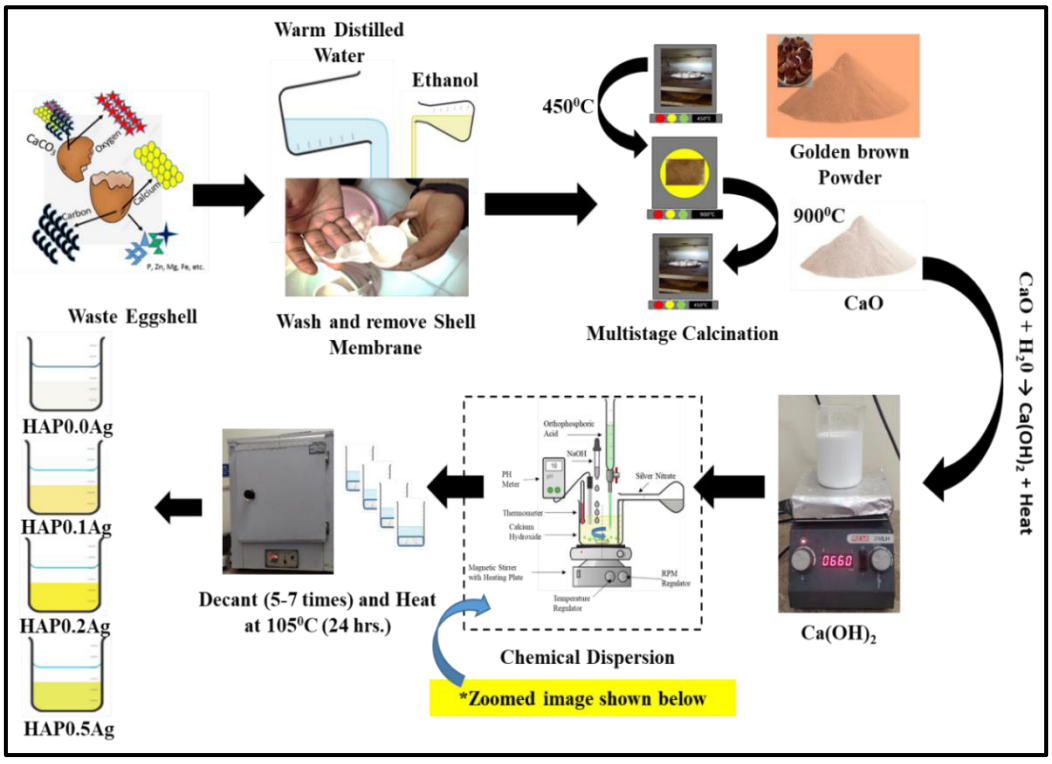

Fig. 3.3. Systematic representation of Step-by-step synthesis of silver-doped hydroxyapatite.

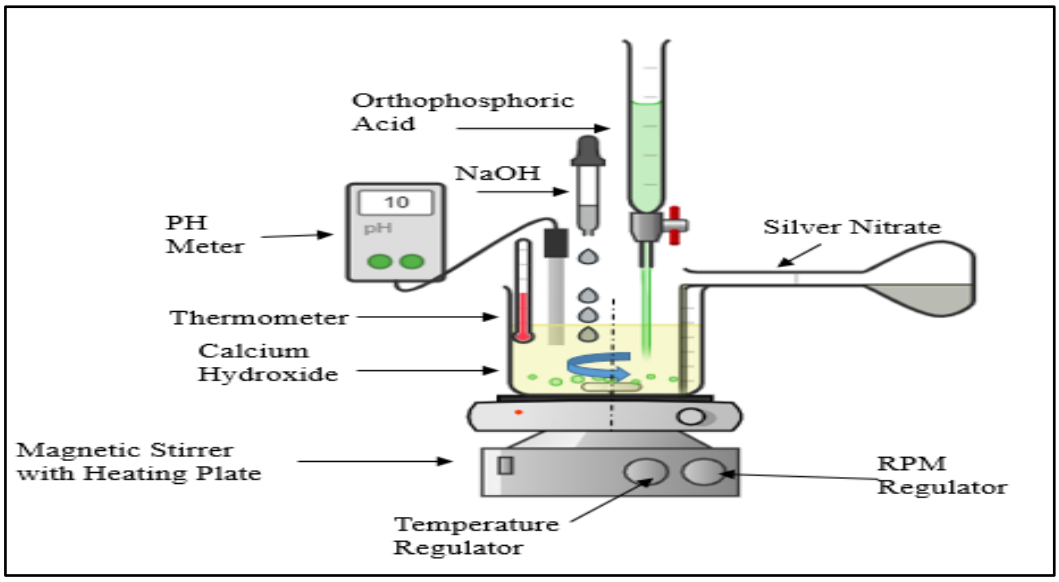

Fig. 3.4. Enlarged image for the chemical synthesis of silver-doped Hap.

Fig. 3.5. Prepared HAP0.0Ag, HAP0.1Ag, HAP0.2Ag and HAP0.5Ag powder samples.

3.4 Compact HAPAg Sample Preparation

Before preparing compact and sintered HAp samples, particle size uniformity of eggshell-derived silver-doped HAp powder was attained through its preprocessing in the Synthano 2S high-energy planetary ball milling machine by Scinomat Solutions Pvt. Ltd. Ball milling was done in six cycles of 4 hours each at the speed of 300 rpm to resist the sticking of HAp powder on the periphery of the zirconia vial and 05 mm ball. The inert atmosphere is maintained using argon (99.9 % purity) to avoid

oxidation. After that, the powder was uniaxially cold pressed to form pellets (10mm dia) at 318 MPa compaction pressure for one minute in the closed cylindrical die. Then the compacted samples were sintered in air at 900 ^{0}C for 1h at a ramp rate of 5 ^{0}C/min and 2 h soaking time. The optimum sintering temperature and compaction pressure are taken as recommended by Muralithran & Ramesh [137] which provides maximum compaction along with desired porosity in the powdered particles. For the characterization of compressive strength and Young's modulus, the height of the pellets (20 mm) is machined as per ISO 17162:2014 (for advanced ceramics). High surface finish with 400- 2000 grit size grinding followed by cloth polishing is done before indentation for measuring Vickers's microhardness.

3.5 Fabrication of PMMA/ Hydroxyapatite composite

Firstly, the eggshell-derived, silver-doped HAp nanopowders (HAPAg) were synthesized with 0.2 wt% Ag doped in waste eggshell-derived calcium and Orthophosphoric acid precursors using the chemical precipitation method at 660 rpm, 18 h stirring and 55^{0}C (discussed above) [138]. The varying weight percentages of HAPAg (0, 2.5, 5 and 7.5%) were uniformly mixed with PMMA powder using a four-jaw metallic mixture for 120 min at 500 rpm. Liquid monomer with a solid/ liquid ratio of 10/7 is mixed for 30 s, and the sticky dough is formed immediately. The dough was cold compressed at 100 MPa with uniaxial loading for 60 s in a die-steel cylindrical die. The hardened composite was removed from the mold after 60 s of compression. The prepared samples were cleaned and desiccated in a glass desiccator until it is used for testing and characterization. The process was conducted as per the recommendation and instruction of the PMMA manufacturer.

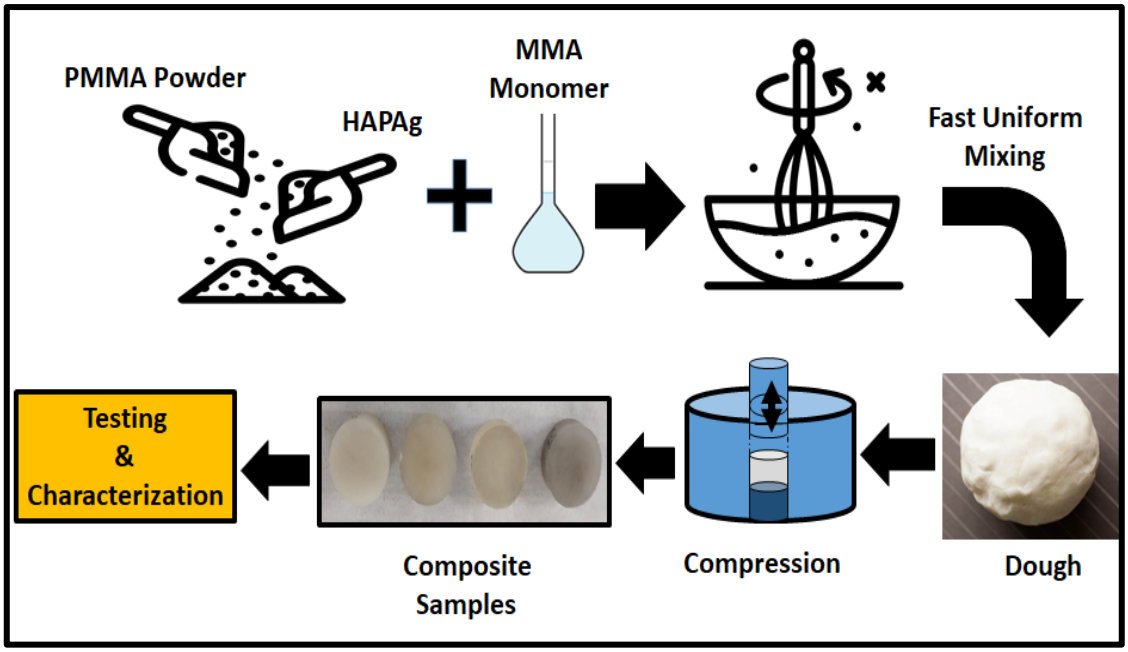

Fig. 3.6. Schematic diagram of composite sample preparation.

3.6 Preparation of Ti6Al4V Substrate

Ti6Al4V alloy substrate (25 x 50 x 1.5 mm^3) was machined from the larger alloy bar using a CNC wirecut EDM machine (Expresscut Series -Ex 4032C, Medha Enterprises). Ti-alloy has high strength and fatigue resistance characteristics and is preferred in load-bearing bone implant applications [139]. The samples were chemically etched using Kroll's reagent (100 mL distilled water, 1-3mL hydrofluoric acid and 2-6 mL nitric acid) under a high precautionary environment. The RMS roughness value changes from 0.235 µm to 0.473 µm. Before deposition, the Ti6Al4V substrates are degreased using acetone and distilled water in an ultrasonic bath for 15 minutes and dried for 1 hour at 90^0C in an electric oven.

3.7 Preparation of PMMA / Ag-Doped HAp Sol

The as received PMMA pellets were cleaned using acetone and dried in an electric oven at 55 ^{0}C for 30 min before processing. The PMMA pellets were dissolved in toluene at a 1:5 weight ratio and stirred at 800 rpm and 45^0C for 3 h on a magnetic stirrer (REMI, 2MLH) in a top-covered glass flask. A 20 % solution of PMMA in

toluene is used in the current research. Toluene shows effective solubility for PMMA [140] and accelerates coating using sol-gel dip coating methods. The synthesized Ag-doped HAp powder was dispersed in liquid PMMA in different weight fractions (10, 15 and 20%) at 0.1 g/min using a chemical dispersion technique with continuous magnetic agitation for 2 hours. **Table 3.4.** represents the nomenclature as per varying wt% of PMMA and HAPAg in preparation of sol for dip coating.

Table 3.4. Nomenclature and composition of the coating solution.

Nomenclature	PMMA (%)	Ag-Doped HAp (%)	No. of Dip cycles
PMMA/H0	100	00	5
PMMA/H10	90	10	5
PMMA/H15	85	15	5
PMMA/H20	80	20	5

3.8 Deposition of PMMA/ Ag-Doped HAp on Ti6Al4V

The deposition process was performed using a dip coating machine (S. N. Scientific Equipment Pvt. Ltd). The coating on the Ti6Al4V substrate was performed with a down speed of 60 mm/min, up speed of 450 mm/min, delay time 30 s, tank dwell 10 s, heating time 1 min and heating temperature 60^0C. The process is carried out at constant speed with five cycles per composition without any lateral shaking for effective coating thickness. The coated samples were air-dried in an isolated environment for 24 hours. Heating was avoided of the coated samples as it creates bubbles on the surface [128]. The final coated samples are shown in **Fig. 3.7.**

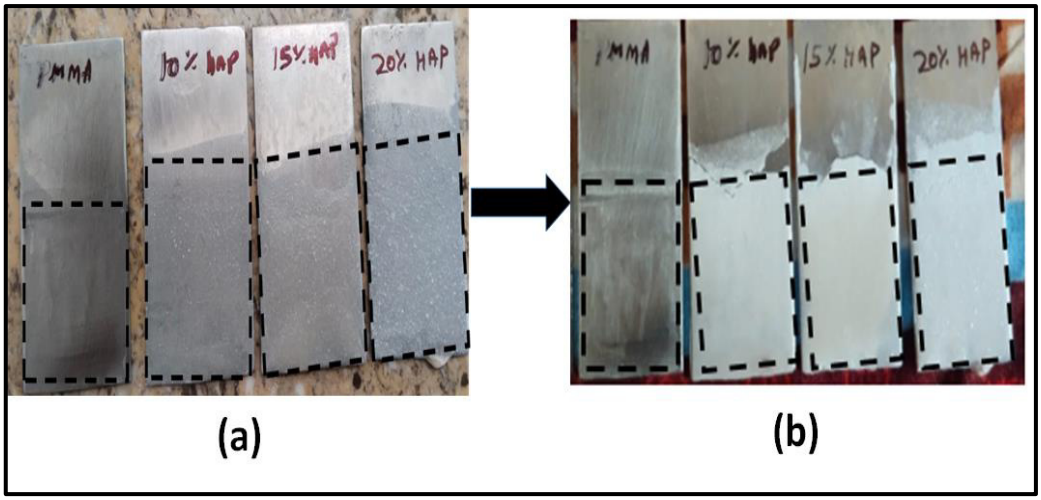

Fig. 3.7. Samples with a coating of PMMA/Ag-Doped Eggshell Derived HAp on Ti6Al4V (a) immediately after coating and (b) after 24 hours of coating.

3.9 Characterizations

After the synthesis and fabrication of the desired samples in the form of powder, pellet, or composite, the characterization of the samples was performed and analyzed. The following (shown in **Fig. 3.8)** characterizations were performed on the prepared samples.

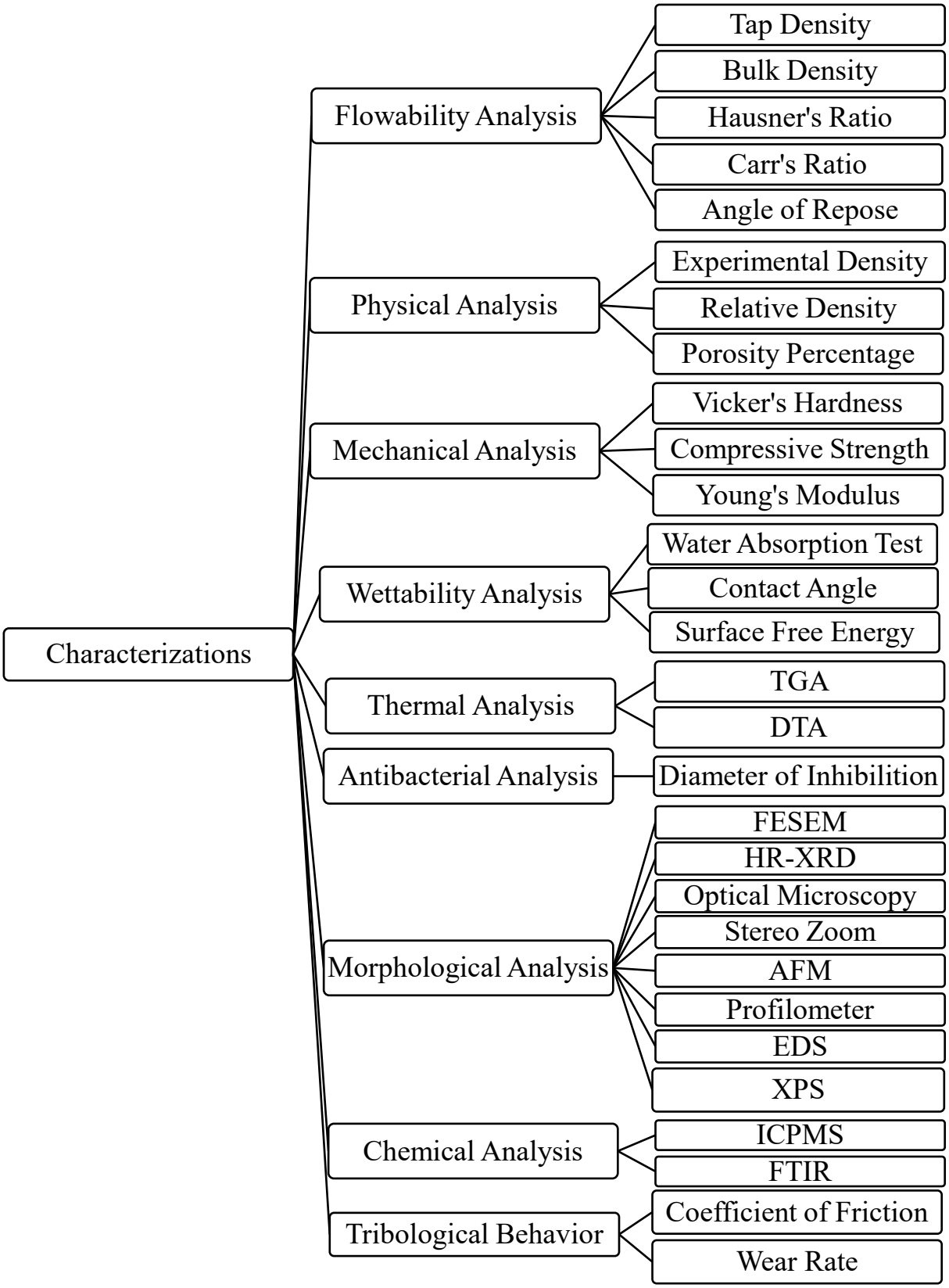

Fig. 3.8. Various characterizations performed in the current thesis.

3.10 Flowability Analysis

Flowability characteristics of the powdered samples were determined using bulk density, tap density, angle of repose, Hausner ratio and Carr's Index (Compressibility Index) of the powdered samples are highly applicable in pharmacy applications. The process adopted is as per Amidon et al. [141]. The flow property of synthesized biocompatible powders are interpreted using the standard quantified chart shown in **Table 3.5 .**

Table 3.5. Standard range to determine flow property of powdered sample [142].

Angle of Repose (Q Degrees)	Hausner Ratio	Carr's Index	Flow Property
≤30	- 1.11	<10	Excellent Flow
31- 35.99	1.12 – 1.18	11- 15	Good Flow
36 – 40.99	1.19- 1.25	16- 20	Fair flow- external assistance not needed
41 – 45.99	1.26- 1.34	21- 25	Travelable- may need assistance
46- 55.99	1.35- 1.45	26- 31	Poor – must agitate, vibrate
56- 65.99	1.46- 1.59	32- 37	Very Poor
≥ 66	>1.6	>38	Awfully Poor Flow

3.10.1 Bulk Density and Tap Density

The tap density testing machine (22V/50HZ/45W) with drop position 3 ± 0.2 mm, tapping speed 60 per minute, tap count 2-9999 and cylinder size 25 and 50 mL was used at room temperature to determine the bulk and tap density. ISO 23145-1: 2007 (en) standard is followed for tap and bulk density of fine ceramics. The bulk and tap density of the powdered samples are determined as per the customized conditions and it varies with its utility, application and the method used. It also determines the flow and compressible characteristics of the powdered sample. Currently, for tap density, a

minimum of 1250 taps per sample were made, and the sample's tap volume (V_{1250}) was measured to calculate tap density using equation (3.3) and 2-3 taps are made for bulk density, then the powder's volume is estimated to evaluate its bulk density using equation (3.4). The process was repeated five times per composition, and the average reading with standard deviation was considered.

$$Tap\ Density\ (\rho_t) = \frac{m}{V_t} \quad \ldots (3.3)$$

$$Bulk\ Density\ (\rho_B) = \frac{\mathrm{m}}{V_b} \quad \ldots (3.4)$$

Where, m = mass of the powder, V_b = bulk volume of the known mass of powder and V_T = tap volume of the known mass of powder.

3.10.2 Hausner's Ratio and Carr's Ratio

Hausner ratio (equation (3.5)) and Carr's Index (equation (3.6) or (3.7)) determine the flow property of powdered materials [143]. The same set of samples were further cross verified using an angle of repose (Q) [formula in equation (3.8)].

$$\text{Hausner Ratio (HR)} = \frac{\rho_t}{\rho_b} \quad \ldots (3.5)$$

$$\text{Carr's Index (\%)} = \frac{\rho_t - \rho_b}{\rho_t}\ x\ 100 \quad \ldots (3.6)$$

$$= \left[1 - \frac{1}{\mathrm{HR}}\right] \mathrm{x}100 \quad \ldots (3.7)$$

3.10.3 Angle of Repose

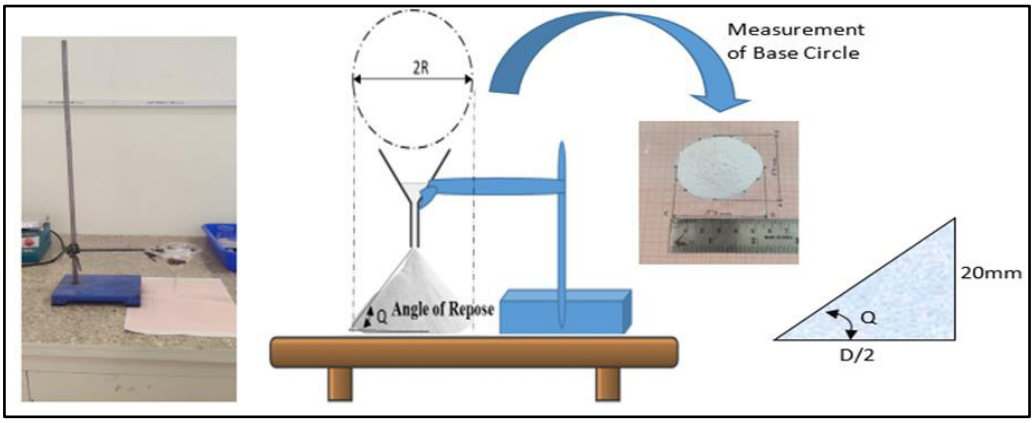

Fig. 3.9. Schematic Diagram to determine Angle of Repose.

The angle of repose of the synthesized bioceramic powdered sample was experimentally evaluated as per ISO 8398:1989 standard (a standard test method for assessing the angle of repose of the free-flowing mold powders) using equation (3.8). The typical range chart for comparison is shown in **Table 3.5**. Flow property is evaluated as per the measured angle. An angle less than 30^0 shows excellent flow, whereas the angle of repose greater than 66^0 indicates extremely poor powder flow. The angle of repose is evaluated for all the HAp powder samples (minimum 5 times for each sample) using equation (3.8) and the average value with standard deviation was tabulated.

$$\text{Angle of Repose (Q)} = \tan^{-1}\left(\frac{h}{R}\right) \quad \ldots (3.8)$$

Where, h = Distance between funnel tip and horizontal plane (here, 20±0.10mm) and R = radius of the powder's pile base.

3.11 Physical Analysis

3.11.1 Experimental Density

Experimental density measurement using Archimedes' principle neglects the volume of pores during measurement. It can be expressed by equation (3.9). The density of distilled water (ρ_w) is taken as 1 g/cm^3.

$$\rho_{TA} = \frac{W_{air}}{W_{air} - W_{water}}(\rho_w) \quad \ldots (3.9)$$

W_{air} = Weight of sample in air (g)

W_{water} = Weight of sample in water (g)

3.11.2 Relative Density

The theoretical density of the silver-doped HAp samples was evaluated from the weight fraction of $AgNO_3$ and the remaining powdered sample using equation (3.10). The weight % is estimated through the molar concentration formula. The theoretical density of HAp powder is taken as 3.16 g/cm^3 and silver dopant as 4.35g/cm^3. Relative density is calculated using equation (3.11).

$$\frac{1}{\rho_{TH}} = \frac{Wf_{AgNO_3}}{\rho_{AgNO_3}} + \frac{Wf_{HAP}}{\rho_{HAP}} \quad \ldots (3.10)$$

$$\rho_R = \frac{\rho_{TA}}{\rho_{TH}} \text{ x } 100 \quad \ldots (3.11)$$

ρ_{TH} = Theoretical Density or density of particle (g/ cm^3)

ρ_{TA} = True Density of sample using Archimedes Principle (g/ cm^3)

ρ_{AgNO3} = Theoretical Density of Silver Nitrate (4.35 g/ cm^3)

ρ_{HAp}= Theoretical Density of undoped HAp (3.16 g/cm^3)

Wf_{AgNO3} = Weight fraction of $AgNO_3$

Wf_{HAp}= Weight fraction of HAP

3.11.3 Porosity Percentage

The porosity of the prepared solid sample quantifies the percentage of pores present in and on the sample prepared using the compaction and sintering process. Porosity percentages are calculated using equation (3.12)

$$P(\%) = 100 - \frac{\rho_A}{\rho_{TH}} x100 \quad \ldots(3.12)$$

3.12 Mechanical Behaviour

3.12.1 Vicker's Microhardness

Vickers microhardness (HVN) of the composite samples was determined using the Vickers Hardness Testing Machine (Mitutoyo, HM-200) with 5 inline indents per sample following ISO 6507-2 standard. The part programming panel with automatic measurement tool was used with pitch length 0.2 mm, normal load 100 g and dwell time 10 s. The values obtained were the average ± SD.

3.12.2 Compressive Strength

Compression properties of cylindrical samples prepared as per ISO 17162:2014 (for advance ceramics) standard were done using 25 kN servo controlled Universal Testing Machine (ASI Sales Pvt. Ltd) with aspect ratio 2, crosshead speed 1mm/min and under displacement control mode. The sample pellets were prepared using uniaxially cold pressing at 318 MPa for one min. in the closed cylindrical die followed by sintering at 900 ^{0}C for 1 h at 5 ^{0}C/min ramp rate and 2 h soaking time. The temperature recorded during the compression test was ≈ 30°C and relative humidity (RH) was ≈52 %. The compressive stress-strain relationship, maximum compression strength and young modulus were experimentally determined.

3.12.3 Adhesion Strength

The evaluation of adhesion strength was performed using Ducom Scratch tester TR-101 following ASTM D 7187-05 test standard. The Leica stereozoom S9i with LAS X imaging software was used for the macrograph imaging. Here, three scratches per sample were made using scratch tester for determining the adhesion strength between the coating and the substrate. The Rockwell C diamond stylus with 200 μm spherical tip and 120^0 cone angle was used to perform the progressive scratch test at room temperature at 53 % relative humidity. The test parameter includes, starting load (01 N), finish load (50 N), stroke length (5 mm), scratch offset (1 mm), loading rate 10 N/mm and scratch velocities (0.1, 0.5 and 1.0s mm/s). At the controlled normal load (F_n), the stylus scratches the surface, and the tangential load (F_t) is measured along with the coefficient of friction ($R = F_t/F_n$). Here the F_n is continuously increased until an articulated coating failure is reached. The adhesion was determined using the magnitude of critical normal load [144]. At this moment, the scratch shows the abrupt change in the pattern in the form of delamination, crack or other visible sign of damage.

3.13 Wettability Analysis

3.13.1 Water Absorption Test

The ASTM D 570-98 standard was followed for the water absorption test. The absorbed water content of the composite samples was determined after immersing the sample in 150 mL DI water for prearranged duration at room temperature (≈37-41 ^{0}C). The accurate weight of the sample was measured using an electronic weighing machine (Model AR2140, Essae Teraoka Ltd.) with an accuracy of 0.0001 g. The dry weights (W_{dry}) of the composite samples were measured prior to immersion, and their wet weights (W_{wet}) were measured after immersion in water for 8 h, 16 h, 24 h, 48 h, 72 h, 96 h, 120 h and 144 h. The weight measurement was taken at least three times

per observation per sample and the readings were taken till the equilibrium is attained i.e. till water absorption percentage (W_a (%)) becomes constant. The W_a (%)) was determined using equation (3.13).

$$W_a\ (\%) = (W_{wet} - W_{dry})/\ W_{dry} \times 100\ \% \quad \ldots (3.13)$$

3.13.2 Contact Angle

The surface wettability of the coated surface was measured using a contact angle measurement machine (Model: DSA 25S, Kruss Germany) following ASTM D5946 standard. The characterization was conducted using sessile drop orientation of water/ ethanol (40/60) liquid with tangent fitting and manual baseline correction. A minimum of five measurements per sample determined the average CA with standard deviation.

3.13.3 Surface Free Energy (SFE)

The SFE of the coated samples was examined using a CA measurement machine (Model: DSA 25S, Kruss Germany) using Diiodomethane and distilled water sessile droplets. The magnitude of SFE was obtained directly and by using Owens Wendt Equation [145,146]. The average from the 5 readings of contact angle (θ) of deionized water and diazomethane droplets were used for evaluating the SFE of a material (E_s) using equation (3.15) termed as OW Equation. Equation (3.14) shows the relation between the CA and SFE. The constant values of dispersive, polar and total SFE of the two liquids (distilled water and Diiodomethane) were taken from Mandolfino et al. [147].

$$\frac{1+\cos\theta}{2} = \sqrt{E_S^d}\left(\frac{\sqrt{E_l^d}}{E_l}\right) + \sqrt{E_S^p}\left(\frac{\sqrt{E_l^p}}{E_l}\right) \quad \ldots(3.14)$$

$$E_s = E_S^d + E_S^p \quad \ldots(3.15)$$

Where,

$E_l =$ surface free energy of liquid droplet $\left(\text{diiodomethane} = \frac{50.8\text{mJ}}{\text{m}^2} \text{and water} = \frac{72.8\text{mJ}}{\text{m}^2}\right)$

$E_S^d =$ Dispersive component of SFE

$E_S^p =$ Polar component of SFE

$E_l^d =$ Dispersive component of liquid $\left(\text{diiodomethane} = \frac{50.8\text{mJ}}{\text{m}^2} \text{and water} = \frac{20.8\text{mJ}}{\text{m}^2}\right)$

$E_l^p =$ Polar component of liquid $\left(\text{diiodomethane} = 0 \text{ and water} = \frac{51\text{mJ}}{\text{m}^2}\right)$

3.14 Thermal Analysis

Thermal Gravimetric Analysis (TGA) was performed to analyse the degradation of material with temperature using TGA-50, 220/240V machine manufactured by M/s Shimadzu (Asia Pacific Pvt. Ltd.). The temperature range was taken from room temperature to 1000 ^{0}C, ramping rate 10 ^{0}C/min under a nitrogen-based inert atmosphere with a maximum 10 mg sample weight placed in a platinum crucible. Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability.

TGA graph can be interpreted as the weight changes as a function of temperature. TGA detects the temperature at which a material loses weight or does not lose weight. It creates the rate of change in weight as a function of time or temperature. The purpose of DTG analysis is to see the temperature at which the material loss is the most.

3.15 Antibacterial Analysis

Antibacterial characterization against gram-positive and negative strains were performed using the well diffusion method in six different bacteria culture - *S. epidermidis, S. aureus, B. subtilis, E. Coli DH5α, P. aeruginosa* and *E. Coli.* The zone of inhibition, also known as Kirby- Bauer's Zone [148] for each sample, was measured

after 24 hours of incubation at 37ºC in a closed incubator. The organisms, microbial type culture collection (MTCC) and gene bank number, strain type, source, laboratory code and effect are shown in **Fig. 10.** All the strains involved in the characterization are linked with human diseases and coagulations. A cleaned and autoclaved Petri dish was filled with 0.028 gm/mL nutrient agar medium and solidified. Then the bacteria culture (1 mL) was dispersed over the medium and incubated. After 24 hours, circular well (5 mm dia) were created equidistant from each other, and the diluted HAp samples (250 µL) was poured inside the wells. Samples were incubated for 24 h at 37 ^{0}C and analyze the zone of inhibition for antibacterial characteristics of silver-doped HAp samples was analysed.

S. No	Code	Organism Name	MTCC * No.	Gram Strain	Source of Bacteria	Effects
1	{10}	S. epidermidis	3382	(+)	Marine sponges	Boils, wound infections, etc.
2	{12}	P. Aeruginosa	2582	(-)	Moist environment: Soil water	Infection in body with weak immunity
3	{18}	E. Coli	68	(-)	Contaminated water or food	Infection and damages small intestine
4	{18α}	E. Coli DH5α	484	(+)	Artificially engineered by D. Hanahun	Combine DNA fragments
5	{37}	S. Aureus	737	(+)	Environment: water, soil In nose and skin of humans	Skin and soft tissue infection
6	{41}	B. Subtilis	441	(-)	Soil	Causes immunity degradation, ropiness in food items

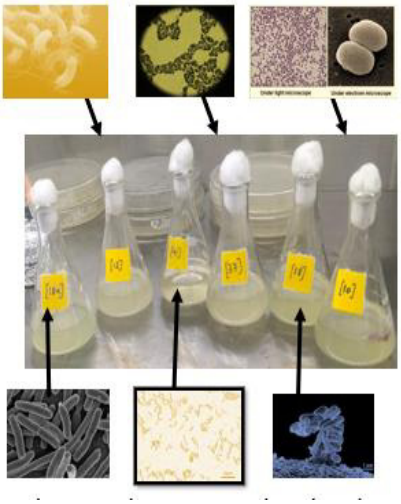

Fig. 3.10. Details of bacteria sample used for antibacterial characterization.

3.16 Morphological Analysis

3.16.1 Field emission scanning electron microscopy

The SEM images were obtained from EVO – SEM MA15/ 18 model, manufactured by Carl Zeiss Microscopy Ltd, having 39 ºC ambient temperature with 42% RH. Further, ImageJ and Origin tool was used to predict the average particle size of the powdered HAp samples using histogram curves.

3.16.2 X-ray diffraction (XRD)

XRD diffractograms were generated using Rikagu SmartLab powder type XRD machine manufactured by RIKAGU corporation private. Ltd. Working parameters included scan rate 5 degree /min, theta range 10^0- 75^0, step size $.01^0$, 40 kV tube voltage, 15mA tube current and Cu-Kα radiation.

3.16.3 Optical Microscope

Leica DM 2700 M optical microscope along with K3C coloured camera and HC PL FLUOTAR objective lens (5x, 10x, 20x, 50x and 100x) available at CoEMTD, IIT (BHU) Varanasi was used for capturing the micro images in the current thesis.

3.16.4 Stereo Zoom Microscope

Stereo zoom microscope (Leica S9i) with LAS X version 5.2.0.26130 Greenough (0.61x – 5.5x) available at CoEMTD, IIT (BHU) was used to capture the high-definition images of the scratch test and wear deformation. The obtained images from scratch tests were further converted to 2D and 3D images using Gwyddion 2.65 software, an SPM data visualization and analysis tool developed and supported by Czech Metrology Institute.

3.16.5 Profilometer

The depth and area of wear surface was evaluated using Taylor Hobson Profilometer available at Tribology Lab, IIT (BHU). Three readings per scratch were taken for the analysis.

3.16.6 Atomic force microscopy (AFM)

The Atomic force microscopy (AFM, Model: NTEGRA Prima, NT-MDT Service & Logistics Ltd.) was used to determine the average roughness (R_a and S_a), root mean

square roughness (R_q and S_q), roughness profile, skewness, and kurtosis values of the dip-coated samples. The obtained results were analyzed using Nova Px 3.4 software following ASME B46 standard. The 2D and 3D topography curves and the roughness profiles are generated to quantify the surface characteristics.

3.16.7 Energy-dispersive X-ray spectroscopy (EDS)

SEM-EDS used here is a 51N000 EDS model manufactured by Oxford Instruments Nanoanalysis. The molecular and atomic weight percent of the elements (calcium, phosphorous and oxygen) present in the final HAp samples were identified. The experimental Ca/P ratio is considered as 1.67. The expected elements during EDS were labelled as Ca, C, O, Na, Mg, P, Fe and Zn.

3.17 Chemical Analysis

3.17.1 Fourier transform infrared Spectroscopy (FTIR)

FTIR spectroscopy were used to identify functional groups present in a sample and were performed using KBr Technique on the powdered samples in Nicolet iS5 FTIR spectroscopy available at CIF, IIT (BHU). Operating parameters included two spectrums with $LiTaO_3$ detector, 5^0C- 45^0C temperature range, and eight split frequency ranges of 4250- 250 cm^{-1}. The IR spectrum of transmission, absorption and photoconductivity of the samples are experimentally diagnosed.

3.17.2 Inductively Coupled Plasma Mass Spectroscopy (ICPMS)

The Inductively Coupled Plasma Mass Spectroscopy (ICPMS- Nexion 2000, PerkinElmer) was used for the elemental analysis of the synthesized samples. The predetermined quantity (S:L = 1:20) of all the samples, including the initial (HAP0.0Ag), was dissolved in freshly prepared aqua regia (HCl (16 ml) and HNO_3 (4 ml)) at 55 ^{0}C and 500 rpm for 1.5 hr. After complete dissolution, it was filtered using

Whatman-42 filter paper and the prepared liquid samples were sent for ICPMS Analysis.

3.17.3 X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) of pure PMMA and silver-doped HAp reinforced PMMA composite was examined using K-Alpha model XPS machine manufactured by Themo Fisher Scientific company available at CIF, IIT (BHU).

3.18 Tribological Analysis

The wear and friction analysis of composite samples were done using reciprocating friction monitor available at mechanical engineering department, IIT (BHU).The setup was used to perform tribological characterization of the prepared samples using standard parameters.

3.18.1 Coefficient of Friction

The ball on disc reciprocating friction monitor ((ASTM G99, Magnum Engineers, Bangalore, India, TE 200ST) with 62 HRC chrome steel ball (10 mm dia) was used to determine the coefficient of friction of composite samples at varying loads and particulate reinforcement percentage. The operational parameters are tabulated in **Table 3.6.**

Table 3.6. Experimental process parameters of wear tests.

Parameters (units)	**Experimental conditions**
Normal load applied (Newton)	20, 40 and 60
Track length (mm)	1.5
Frequency (rps)	14
Sliding distance (m)	50.4
Sliding time (min)	20
Temperature (°C)	35 ± 2
Humidity (%)	55 ± 1
Surface Roughness, Ra (μm)	0.015

3.18.2 Wear Rate

The wear rate of the composite samples placed under tribological testing was examined using the Taylor Hobson 3D surface profilometer. The profile generated along the transverse direction helped to determine the surface area, the width of the worn track, the depth of the worn surface and FWHM values. The profile obtained determines the average depth value using equation (3.16), volume loss (equation (3.17)) and average wear rate (equation (3.18)) illustrated by Doni et al.[149].

$$\text{Average Depth Value } D_{avg} = \frac{A_w}{W} \quad \ldots(3.16)$$

Aw = Average wear loss area (using profilometer)

W = Average width of each worn track (using profilometer)

$$\Delta V = \text{Volume Loss } (mm^3)$$

$$\text{Volume Loss } \Delta V = \left[\frac{1}{3} * \pi * D_{Avg}^2 \left(3R - D_{Avg}\right)\right] + A_w * l \quad \ldots(3.17)$$

We, R = radius of Ball (5 mm), l = length of wear track (1.5 mm)

$$Average\ Wear\ Rate\ W_v = \frac{\Delta V}{S} \quad \ldots(3.18)$$