

# Review on the characterization methods of Hydroxyapatite and its Bio-composites

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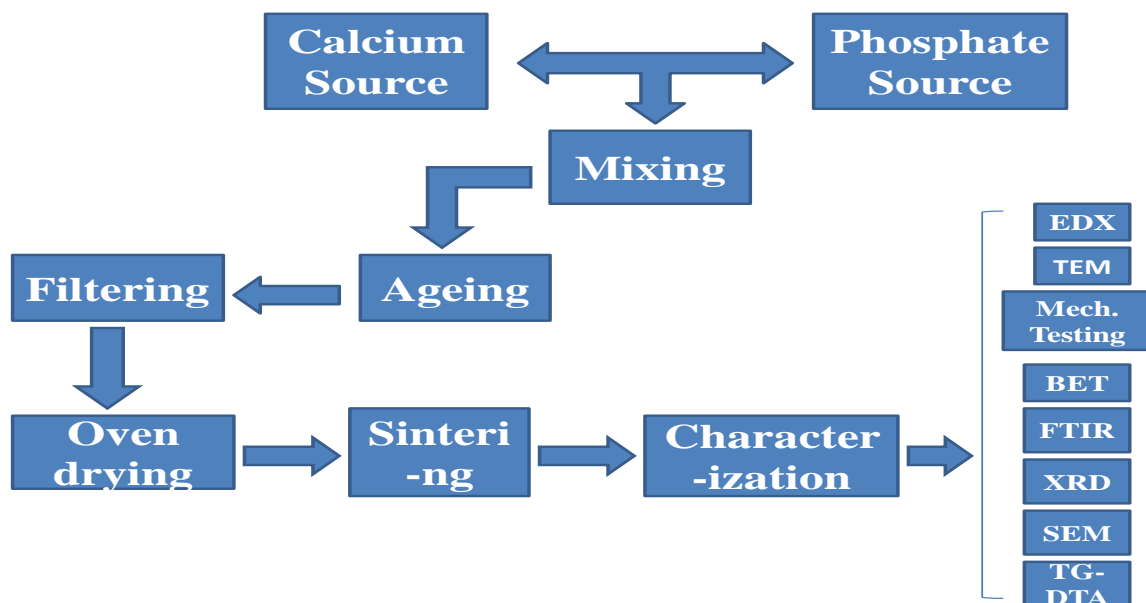
**Abstract-** Natural bone constitutes 67% of its weight as Hydroxyapatite (HAp) and is a bioactive substance having ability to form chemical bond with body tissues. It can be synthesized in laboratory using calcium and phosphate reagents and can be one of the potential bone graft material. The only factor that prohibits its use as natural bone is in its poor mechanical strength. Research is being conducted to enhance its compressive strength by formation of biocomposites using Carboxymethyl Cellulose, Gelatin, Chitosan and other polymeric materials. The characterization of HAp and its biocomposites synthesized is an important topic. The various characteristics analyzed include various functional group using Fourier Transform Infrared Spectroscopy (FTIR), morphology and surface properties using Scanning Electron Microscopy (SEM) / Transmission Electron Spectroscopy (TEM), crystallinity and chemical composition of compound using X-ray diffraction Spectroscopy (XRD), thermal stability using Thermogravimetric/ Differential Thermal Analysis (TG-DTA), surface area, pore size & pore volume using Brunauer–Emmett–Teller (BET), compressive strength using mechanical testing. This paper gives an elaborative review on these analytical methods reported in literature along with the detailed observations and interpretation. The suitability of analytical technique to be adopted and the interpretation of the findings would be eased by this review paper for prospective researchers working in this area.

**Index Terms –** Hydroxyapatite, Characterization methods, FTIR-SEM-TG-DTA, Biocomposite testing methods.

## I. INTRODUCTION

HAp is an inorganic biomaterial constituting about 67% mineral matter of bone. Hence it can be used in bone repair such as to fill small defects or voids or as coatings on metallic implants. Due to some limitations in its mechanical properties such as it has poor tensile strength and brittleness HAp cannot be used in most load bearing application. Composites of HAp are prepared using various natural polymers such as Chitosan, Starch, Gelatin, etc which act as HAp binders. The composites thus obtained can be used to repair larger bone defects with enhanced load bearing properties. HAp and its biocomposites are being used as a better source of bone grafting in near future.

There are various methods of HAp synthesis such as precipitation technique, sol-gel approach, hydrothermal technique, multiple emulsion technique, biomimetic deposition technique, electrodeposition technique, etc each employing various process parameters<sup>[1]</sup>. The general process of the synthesis of HAp includes the reaction between the calcium source and phosphate source in specific stoichiometric ratio of 1.67. The calcium source and the phosphate source may vary. The reactants mixing is employed with various temperature conditions followed by ageing and precipitation of solution. The precipitate is oven dried and sintered in furnace. The schematic is as shown in fig 1.1.



**Fig 1.1** General block diagram of the process

The HAp thus obtained needs to be analyzed using various characterization methods such as:

- Fourier Transform Infrared Spectroscopy (FTIR) - This method is used to analyze the various functional groups present in the any compound.
- Scanning Electron Microscopy (SEM) – This method is used to analyze the surface morphology of any compound whether porous or crystalline.
- Energy Diffraction Spectroscopy (EDX) – This method is used to determine the elemental composition of the compound.
- Thermogravimetric/ Differential Thermal Analysis (TG-DTA) – This method is used to determine the thermal stability of the compound i.e; the change in the weight of the compound with respect to temperature.
- X-ray diffraction Spectroscopy (XRD) - This is used to determine the components of compound and the crystallinity of compound.
- Brunauer–Emmett–Teller (BET) – It is used to determine the specific surface area of the material.
- Transmission Electron Microscopy (TEM) - It is used to determine the crystal orientation, electronic structure, modulation in chemical identity of any compound.
- Mechanical testing – Mechanical testing involves the testing of tensile strength, Compressive strength, Hardness test, etc

The paper is an effort to take a stock of various research papers published and is divided into two parts:

- The first part is denoted for the review on characterization methods for HAp.
- The second part addresses to review on characterization of biocomposites.

## II. REVIEW ON CHARACTERIZATION OF HYDROXYAPATITE

Gre'ta Gergely et al <sup>[2]</sup> studied the preparation of HAp from egg shell. The obtained morphological structure of HAp were dependent on the two mechano chemical activation methods i.e; Attrition milling and Ball milling employed during the mixing of the reactants, CaO obtained from egg shell and phosphoric acid. The structures of the HAp were characterized by XRD, SEM and FTIR. The characterization results are given in table 2.1.

Characterization methods	Remarks	
	Attrition milling	Ball milling
<b>SEM</b>	Nanosized HAp with size 100nm were obtained	Coagulated samples with smooth surfaces were obtained
<b>XRD</b>	After milling- hydroxyapatite , calcite (CaCO <sub>3</sub> ), monetite (CaHPO <sub>4</sub> ) and hydrogen phosphate (H <sub>3</sub> PO <sub>3</sub> )  After heat treatment at 900 <sup>0</sup> C- the main phase was hydroxyapatite with some calcite and calcium-oxide CaO	After milling- Powder produced by ball milling included montetite, hydrogen phosphate and portlandite (Ca(OH) <sub>2</sub> )  After heat treatment- calcite, calcium-oxide, calcium phosphate (Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> ), and whitlockite (Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> ), HAp
<b>FTIR</b>	PO <sub>4</sub> asymmetric mode : 1021 and 1087 cm <sup>-1</sup> , Non degenerated symmetric stretching mode of PO <sub>4</sub> at 962 cm <sup>-1</sup>  Components of the triplet of PO <sub>4</sub> bending mode: 599 and 562 cm <sup>-1</sup> .  Carbonate bands: 1550– 1350 cm <sup>-1</sup> , 873 cm <sup>-1</sup> and 712 cm <sup>-1</sup> .  Traces of water incorporated in the structure 3000–3400 cm <sup>-1</sup>  H–O–H bending- 1640 cm <sup>-1</sup>  -OH of the hydroxyapatite, surface OH band at 3644 cm <sup>-1</sup>	Peaks of carbonate: 1466 and 1409 cm <sup>-1</sup> , 874 cm <sup>-1</sup> , 713 cm <sup>-1</sup> .  Surface –OH :3640 cm <sup>-1</sup>  PO <sub>4</sub> :1086 and 1018 cm <sup>-1</sup> , 961 cm <sup>-1</sup>  Triplet of PO <sub>4</sub> : 626, 599 and 561 cm <sup>-1</sup>
<b>Concluding remarks:-</b> From the SEM results , it is observed that smaller particle size with homogeneous size distribution is obtained with attrition milling as compared to ball milling.The XRD of the samples shows that the unwanted peaks disappears		

after heat treatment of the sample at high temperature. The FTIR of both the samples are similar showing all the peaks for the functional groups present in the molecular formula of HAp.

**Table 2.1 Greta Gergely et al characterization methods**

Eric M. Rivera et al <sup>[3]</sup> studied the synthesis of Hydroxyapatite from egg shell and the process was carried out at elevated temperature of 1050<sup>0</sup>C for 3 h. The Phosphate source was Tri calcium phosphate (Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>). The characterization results are given in table 2.2.

Characterization methods	Remarks			
XRD	Egg Shell after calcinations	Tricalcium Phosphate		Product HAp
	Mineral phase observed	Ca <sub>10</sub> (PO <sub>4</sub> ) <sub>6</sub> OH <sub>2</sub> , CaHPO <sub>4</sub>		HAp, CaO, Ca(OH) <sub>2</sub>
FTIR	-	-		Peaks of (PO) <sub>4</sub> - 605cm <sup>-1</sup> , 1050cm <sup>-1</sup>
SEM	Inner part of eggshell	HAp treated at 450 <sup>0</sup> C	HAp treated at 900 <sup>0</sup> C	HAp treated at 1050 <sup>0</sup> C in Phosphate solution
	Fiber like morphology observed	Fiber like morphology maintained	Fine powder with porous morphology observed	Irregular shaped agglomerates were formed
<b>Concluding remarks:-</b> The XRD shows the additional peaks in the product along with the main product HAp which may be due to the incomplete transformation of the reactants. The SEM of the step by step conversion stages i.e; starting from egg shell to final HAp formation is helpful in distinguishing the morphological characteristics of the raw material, the intermediate product and the final product.				

**Table 2.2 Eric M. Rivera et al characterization methods**

Khelendra Agrawal et al <sup>[4]</sup> synthesized hydroxyapatite Powder by Sol-Gel method. The calcium source used was calcium nitrate tetra hydrate Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O and the phosphate source used was Phosphorous pentoxide (P<sub>2</sub>O<sub>5</sub>). The reaction was carried out at 80<sup>0</sup>C. The results of the characterization are given in table 2.3.

Characterization methods	Remarks	
XRD	All the peaks corresponding to HAp are present in both the HAp samples sintered at 400 <sup>0</sup> C and 750 <sup>0</sup> C with increase in the width of the peak with temperature showing the increase in the crystallinity with temperature.	
DTA-TG	Upto 220 <sup>0</sup> C-10% wt loss 220 to 350 <sup>0</sup> C-40% wt loss 600 <sup>0</sup> C- 5% wt loss 600-1200 <sup>0</sup> C- No weight loss	
FTIR	<b>HAp Calcined at 400<sup>0</sup>C</b>	<b>HAp Calcined at 750<sup>0</sup>C</b>
	OH Stretching band-3580.74 cm <sup>-1</sup> CO <sub>3</sub> <sup>2-</sup> - 1567.78 cm <sup>-1</sup>  PO <sub>4</sub> <sup>3-</sup> stretching mode-976.89 cm <sup>-1</sup>  Bending mode- 623.02 cm <sup>-1</sup> and 560 cm <sup>-1</sup>	OH - 3575.02 cm <sup>-1</sup>  CO <sub>3</sub> <sup>2-</sup> - 1554.23 cm <sup>-1</sup> , 834.65 cm <sup>-1</sup>  PO <sub>4</sub> <sup>3-</sup> Bending mode - 589.7 cm <sup>-1</sup>
SEM	The powder appears to be of crushed annular shape. Further magnification reveals single particle of HAp is made of agglomeration of nano sized grains.	
<b>Concluding remarks:-</b> The XRD shows that the crystallinity of HAp increases with increase in the sintering temperature. The DTA-TG technique indicates the thermal stability of the HAp with no weight loss beyond 1200 <sup>0</sup> C. 10% reduction in weight upto 220 <sup>0</sup> C is due to crystallization of HAp. 40% weight loss upto 350 <sup>0</sup> C is due to the evaporation of water in Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O.		

**Table 2.3 Khelendra Agrawal et al characterization methods**

Hrvoje Ivankovic et al <sup>[5]</sup> studied the synthesis of highly porous hydroxyapatite through hydrothermal transformation of aragonitic cuttlefish bones. The phosphate source used was NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>. Mechanism of hydrothermal transformation of bones was investigated by DTA/TG analyzer coupled online with FTIR spectrometric gas cell equipment (DTA-

TG-EGA-FTIR analysis), X-ray diffraction analysis (XRD) and scanning electron microscopy (SEM). The characterization results are given in table 2.4.

Characterization methods	Remarks	
<b>XRD</b>	<b>Temperature of heat treatment of sample (HAp)</b>	<b>Components detected</b>
	(Heat treated for 20 min) 140-160 <sup>0</sup> C	Poorly crystalline HAp and well Crystalline Brushite (CaHPO <sub>4</sub> · 2H <sub>2</sub> O)
	180 <sup>0</sup> C	Discernible amount of Brushite
	200 <sup>0</sup> C	No Brushite observed
	Heat treated at 180 <sup>0</sup> C for 48 hrs	95.4 wt% of HAp and 4.6 wt% of untransformed aragonite
	Heat treated at 200 <sup>0</sup> C for 24 hrs	Aragonite transformed completely into hydroxyapatite
	Heat treated at 220 <sup>0</sup> C for 24 hrs	97.9 wt % of HAp, and 2.1 wt% of untransformed aragonite
Heat treated at 220 <sup>0</sup> C for 48 hrs	The amount of hydroxyapatite decreased on account of monetite, CaHPO <sub>4</sub> , which was formed in quantity of 3.2 wt %	
<b>FTIR (samples heat treated at 180<sup>0</sup>C)</b>	<b>Functional group</b>	<b>Vibrational frequency</b>
	PO <sub>4</sub> <sup>3-</sup>	1042 and 1088 cm <sup>-1</sup> , 602 and 563cm <sup>-1</sup> , 960 cm <sup>-1</sup> and 470 cm <sup>-1</sup>
	OH band	3570 cm <sup>-1</sup>
Carbonate ions on B- site of HAp structure	1412 and 1446 cm <sup>-1</sup> and the peak at 872 cm <sup>-1</sup>	
<b>DTA- TG of cuttlefish bone</b>	<b>Temperature of CO<sub>2</sub> release</b>	<b>Components decomposed</b>
	400 <sup>0</sup> C	Oxidation of organics
	690 <sup>0</sup> C	Decomposition of Aragonite
800 and 1060 <sup>0</sup> C	CO <sub>3</sub> <sup>2-</sup> groups incorporated in the structure of HAp	
<b>SEM</b>	<b>Before Heat treatment</b>	<b>After heat treatment</b>
	Dandelion-like HAp nanostructures with diameter from 3 to 8 μm	Nano rods with an average diameter of about 200-300 nm and an average length of about 8-10 μm.
<b>Concluding remarks:-</b> In the XRD of HAp it is observed that with increase in the heat treatment temperature the crystalline Brushite decreases while for longer heat treatment time of 48hrs the amount of HAp is decreased on account of monetite. The FTIR results show that with increase in the heat treatment time the intensities and resolutions of PO <sub>4</sub> <sup>3-</sup> and OH band increases.		

**Table 2.4 Hrvoje Ivankovic et al characterization methods**

P. Hui et al [6] studied the synthesis of Hydroxyapatite by hydrothermal method using egg shell as calcium source and Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> as phosphate source. The process was carried out at high temperature. Different calcium phosphate phases were obtained by changing the thermal treatment. The final product was characterized by XRD, SEM and FTIR. Thermal analysis (TG-DTA) was carried out to investigate the thermal stability of HAp. The details are given in table 2.5.

Characterization methods	Remarks	
FTIR	PO <sub>4</sub> <sup>3-</sup> asymmetric stretching	1047.9 cm <sup>-1</sup>
	PO <sub>4</sub> <sup>3-</sup> bending	604.1cm <sup>-1</sup> ,566.7cm <sup>-1</sup>
	PO <sub>4</sub> <sup>3-</sup> symmetric stretching	961.4 cm <sup>-1</sup> , 470.4 cm <sup>-1</sup>
	-OH Stretching	3571 cm <sup>-1</sup> , 1637 cm <sup>-1</sup>
	CO <sub>3</sub> <sup>2-</sup> stretching	1425.1 cm <sup>-1</sup> ,876 cm <sup>-1</sup>
	H <sub>2</sub> O adsorbed	3413.3 cm <sup>-1</sup> , 1637.5 cm <sup>-1</sup>

XRD	31.8 <sup>0</sup> -32.5 <sup>0</sup> corresponds to the characteristic peak of apatite phase. The crystalline phases was identified as HAp , with some phases of calcium oxide and calcium hydroxide .	
DTA-TG	Temperature range	Weight loss
	380 <sup>0</sup> C 380-420 <sup>0</sup> C 800 <sup>0</sup> C 800-1400 <sup>0</sup> C	6% 10% 9% No weight loss
SEM	The synthesized HAp consists of agglomerates the shapes of which were oval and spherical and the size was between 3µm and 5µm, built up from fine particles about 400 nm in size.	
<b>Concluding remarks:-</b> The DTA result shows that product is thermally stable beyond 1400 <sup>0</sup> C. XRD results shows that the product is crystalline. SEM result shows that the particles are spherical and nano size		

Table 2.5 P. Hui et al characterization methods

Rozita Ahmad Ramli et al <sup>[7]</sup> synthesized nano porous HAp in a slightly different way by preparing HAp - Chitosan (CS) composite. HAp was first synthesized using Calcium hydroxide and Phosphoric acid. The synthesized HAp was then blended with chitosan . The Chitosan - HAp template was then calcined to obtain nano porous HAp. The characterization methods followed were FTIR, XRD, SEM, BET and the results are given in table 2.6.

Characterization methods	Remarks		
	HAp-CS composite	Calcined HAp-CS composite	Calcined HAp
FTIR	All peaks present in HAp were observed along with Chitosan aliphatic CH stretching bands -2877 cm <sup>-1</sup> NH <sub>2</sub> of chitosan- 1639 and 1258 cm <sup>-1</sup> C-O stretching mode of the primary alcohol group (CH <sub>2</sub> -OH) of chitosan-1384 cm <sup>-1</sup>	PO <sub>4</sub> <sup>3-</sup> bands- 569, 603, 963, 1045 and 1091 cm <sup>-1</sup> Peaks for structural OH of the apatite : 3570 and 633 cm <sup>-1</sup>	Same peaks as present in Calcined HAp-CS composite were observed along with CO <sub>3</sub> <sup>2-</sup> peaks at 874, 1421 and 1466 cm <sup>-1</sup> .
BET			
Surface area (m <sup>2</sup> /g)	16	20	24
Pore Size (nm)	12	44	35
Pore volume (cc/g)	0.0174	0.3450	0.2264
SEM	Scaffold structure with an average pore size of 26.7 ± 11.7 µm	100nm	100nm
XRD	The CS peak was detected at approximately 20° (2θ), while HAp peaks occurred in the 32–35° (2θ) region.		
<b>Concluding remarks:-</b> The calcined HAp - CS composite has higher pore size and pore volume than calcined HAp sample. In the FTIR of the calcined HAp - CS composite peaks corresponding to CS are not observed due to the decomposition of CS at 280-300 <sup>0</sup> C. Also it does not show the peaks for carbonate ions due to the dissolution of the ions in acidic medium employed during the composite preparation. The XRD result reveals the increase in the crystallinity of the product due to increase in temperature.			

Table 2.6 Rozita Ahmad Ramli et al characterization methods

### III. REVIEW ON CHARACTERIZATION OF BIOCOMPOSITES OF HAp

Subhadra Garai et al <sup>[8]</sup> synthesized HAp - Carboxymethyl cellulose composites (CMC) composites varying the CMC concentration as 5, 10, 15, 20%. The composites were molded in mold of dimension 10 cm × 10 cm × 5 cm and dried in an oven at 50 ± 2°C for 72 h to obtain the three dimensional structures. The table 2.7 shows the characterization results of the composites.

Characterization methods	Remarks
1) XRD	The XRD patterns of the four composites were same but there was a difference in the peak width due to different crystallite size of HAp in polymer matrix with different concentration. The average crystallite size of HAp decreases from 50nm to 15nm with increase of CMC concentration.

2) SEM	There was a formation of interconnected micro porous three dimensional structure comprising HAp nano particles aggregates forming a bead like structure, embedded in a CMC matrix. There was an increase in bead agglomeration and decrease in bead size with increase of polymer content and uniform distribution of irregular shaped pores of size range 0.75–2.0 micrometer interconnected three dimensionally.
3) TEM	The TEM studies of the four nano composites manifested similar micro structure on nano scale as well as an average crystallite sizes in the range of 15–35 nm.
4) FTIR	Hydrogen Bonding between HA and CMC: HAp-5 : - 3418cm <sup>-1</sup> HAp-10:- 3424cm <sup>-1</sup> HAp-15:- 3512cm <sup>-1</sup> HAp-20:- 3166,3514,3128cm <sup>-1</sup> PO <sub>4</sub> <sup>3-</sup> :- 1037,603,566cm <sup>-1</sup>
6) Mechanical testing	Compressive strength (MPa): HAp-5 :-12.4 HAp-10:-4.0 HAp-15:-1.74 HAp-20:- ----
<b>Concluding remarks:-</b> The crystalline size of HAp in the polymer matrix is of the order of that found in the natural bone. The absorption bands of HAp-5 & HAp-10 are broad which indicates the presence of strong bonding between HAp and CMC as compared to HAp-15 and HAp-20. sample HAp-5, HAp-10 & HAp-15 deformed through an elastic-cum-visco elastic deformation process while sample HAp-20 followed a visco elastic process for its deformation i.e; first three samples got ruptured after crossing their breaking load, while no rupture could be seen in the sample HAp-20.	

Table 2.7 Subhadra Garai et al Characterization methods

Vitor M. Correlo et al <sup>[9]</sup> synthesized the composites of HAp using Chitosan and polyesters such as poly(e-caprolactone) (PCL), poly(lactic acid), poly(butylene succinate) (PBS), and poly(butylene terephthalate adipate). These blends were injection molded and evaluated for thermal, morphological, and mechanical properties. The characterization results are given in table 2.8.

Characterization Methods	Remarks
1) DSC	<b>PLA/Chitosan – HAp blend:</b> With the addition of HAp (30%) to the 50/50 wt of chitosan and PLA blend the glass transition temperature (T <sub>g</sub> ) drops to 53.6 <sup>0</sup> C from 60.1 <sup>0</sup> C. The Cold crystallization temperature (T <sub>c</sub> ) drops from 89.2 <sup>0</sup> C to 83.9 <sup>0</sup> C. The composite presents a lower melting temperature (T <sub>m</sub> = 148.2 <sup>0</sup> C) than the pure PLA (155.3 <sup>0</sup> C)  <b>PCL/Chitosan HAp blend:</b> The reinforcement of chitosan/PCL by HAp increased the degree of crystallinity in the PCL fraction. In this case, the nucleating effect of the HAp particles may be the origin of the enhancement of the degree of crystallization.  <b>PBS-Chitosan-HAp blend:</b> The introduction of 30% of HAp to PBS/Chitosan blends tends to increase the melting temperature. The increase of the HAp content tends to increase the degree of crystallinity.
2) Mechanical testing	The addition of HAp decreased the tensile strength over chitosan/ polyester blends. The elongation at break shows a marked decrease upon the addition of hydroxyapatite. The composites displayed brittle failure.
3) SEM	SEM of tensile stretched specimen shows that there are two sets of cavities one having diameter less than 4micrometer and other with diameter greater than 4 micrometer.
<b>Concluding remarks:-</b> Various composites have been synthesized by combination of PLA, Chitosan, PCL, PBS. PCL-Chitosan-HAp composite has shown lower melting point than PLA. PCL-Chitosan-HAp has enhanced degree of crystallization and PBS-Chitosan-HAp tends to increase the melting temperature and degree of crystallinity. HAp decreases the tensile strength of the polyester blends.	

Table 2.8 Vitor M. Correlo et al characterization methods

Edgar B. Montufar et al <sup>[10]</sup> synthesized HAp- Gelatin composite scaffolds by mixing  $\alpha$  – tricalcium phosphate ( $\alpha$ - TCP) with HAp and the Liquid phase of gelatin was prepared adding Na<sub>2</sub>HPO<sub>4</sub> as an accelerant. Both the liquid and the powder phase were mixed and were molded or placed into the syringe at room temperature. The characterization were carried out before and after immersion of the composites in Ringer's Solution i.e; 0.9 wt% of NaCl solution and 100 % humidity. The results are given in table 2.9.

Characterization methods	Remarks																					
1)Effect of Accelerant addition on setting time of cements	The initial setting time decreased with accelerant addition and further decreased with the increase in gelatin concentration in the paste.																					
2)Injectability and cohesion test	Gelatin addition improved the cohesion and injectability (i.e; mass of extruded paste with respect to the original mass of the paste in the syringe) of the cement paste. The foaming process further increased the injectability . Increase in gelatin content increased the cohesion of cement paste when injected in water at 37 <sup>0</sup> C.																					
3)XRD (0 and 15 wt% of gelatin in the liquid phase after 7 days of immersion in Ringer's solution)	The main phase was in both cases hydroxyapatite, and only residual amounts of $\alpha$ -TCP were detected. Due to leaching of gelatin only 7.07 wt% was remaining in the cement paste.																					
4) FTIR ( after 12 days immersion in Ringer's solution)	The band for amide group of gelatin was observed at 1527 cm <sup>-1</sup> which indicates that there was no crosslinking of gelatin with HAp . The gelatin was leached after immersion in Ringer's solution leaving behind small amount of gelatin entrapped in cement paste.																					
5)SEM Foamed (F) and non foamed (NF) cement samples for 10% and 15% gelatin concentrations	In non foamed cement micro pores were observed.The foaming process incorporated spherical macropores into the cement. A higher gelatin concentration resulted in smaller macro pores and in a decrease of the total macroporosity																					
6) Compressive testing  a) After storing in 100% humidity  b) After storing in Ringer's solution	<p>The compressive strength increased with the addition of gelatin</p> <p>Gelatin dissolves in the solution leading to increase in microporosity thus reducing the compressive strength</p> <table border="1"> <thead> <tr> <th>Gelatin content</th> <th>Reaction medium</th> <th>Compressive strength (MPa)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>Ringer's</td> <td>29..0 ± 4..2</td> </tr> <tr> <td>10</td> <td>Ringer's</td> <td>15..5 ± 2..4</td> </tr> <tr> <td>15</td> <td>Ringer's</td> <td>11.2 ± 2.2</td> </tr> <tr> <td>0</td> <td>100% humidity</td> <td>29.8 ± 2.8</td> </tr> <tr> <td>10</td> <td>100% humidity</td> <td>35.3 ± 3.4</td> </tr> <tr> <td>15</td> <td>100% humidity</td> <td>32.5 ± 5.9</td> </tr> </tbody> </table>	Gelatin content	Reaction medium	Compressive strength (MPa)	0	Ringer's	29..0 ± 4..2	10	Ringer's	15..5 ± 2..4	15	Ringer's	11.2 ± 2.2	0	100% humidity	29.8 ± 2.8	10	100% humidity	35.3 ± 3.4	15	100% humidity	32.5 ± 5.9
Gelatin content	Reaction medium	Compressive strength (MPa)																				
0	Ringer's	29..0 ± 4..2																				
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0	100% humidity	29.8 ± 2.8																				
10	100% humidity	35.3 ± 3.4																				
15	100% humidity	32.5 ± 5.9																				
<b>Concluding remarks:-</b> Thus gelatin addition increased the cohesion and injectability and decreased the initial setting time of cements. There was no cross linking between gelatin and HAp. The immersion of composite in Ringer's solution leads to the dissolution of gelatin in the solution and thus shows the decreased compressive strength as compared to when stored in 100 % humidity where the strength increases with gelatin addition.																						

Table 2.9 Edgar B. Montufar et al characterization methods

Khaled R. Mohamed et al <sup>[11]</sup> synthesized nano HAp / Chitosan – Gelatin composites (HACG composites) without and with the addition of Citric acid solution (HACGCA composites) in various compositions. The composites were tested for its in vitro properties using Simulated Body Fluid (SBF) and also analyzed by other methods as given in table 2.10.

Characterization Methods	Remarks
1)SBF solution analysis after immersion of composites for several period	Concentration of calcium ions and phosphate ions recorded low values for HAp sample and composites proving the deposition of Ca <sup>2+</sup> ions and PO <sub>4</sub> <sup>3-</sup> ions as a result of interaction of Ca <sup>2+</sup> & PO <sub>4</sub> <sup>3-</sup> with amino groups of both chitosan and gelatin polymers . The deposition of Ca <sup>2+</sup> ions and PO <sub>4</sub> <sup>3-</sup> ions due to the presence of CA enhanced the interaction with Ca <sup>2+</sup> ions forming calcium citrate complex.

1)Weight loss after immersion in SBF	The increase in chitosan–gelatin polymeric matrix content in the composites leads to the increase in weight loss for HA80CG20 composite compared to HA70CG30 and HA60CG40 composites. In presence of CA the weight loss of the composites decreased.
2)Water absorption	The water absorption capability of composites increased with increasing Chitosan and Gelatin contents and it further increased with addition of Citric acid
3)FTIR	After immersion in SBF for 7 days and 28 days it was observed that the HAp bands such as phosphate, carbonate and OH groups in HAp decreased after the post-immersion for 28 days compared to 7 days denoting high solubility properties of the precipitated HAp. Increment in the intensity of the phosphate, carbonate, and OH groups at 7 and 28 days for HA70CG30CA composite compared to HA70CG30 composite indicates the enhancement of apatite formation in the presence of CA.
4) SEM HA sample	<p><b>Pre-immersed sample:-</b> White rods and few needle shapes on the surface</p> <p><b>Post immersed sample:</b> After 7days:- Spherical particles with many pores and some nucleation of apatite</p> <p><b>After 28 days:-</b> Apatite particles were deposited, concentrated, and covered the surface with many minute pores.</p>
HA70CG30 composite	<p><b>Pre-immersed sample:-</b> Rough surface with embedded particles into the composite .</p> <p><b>Post – immersed sample:</b> <b>After 7days:-</b> Aggregated spherical particles and small particles on the surface with bright color and few pores denoting effect of mineralization of calcium phosphate ions and immersion</p> <p><b>After 28 days:-</b> Spherical particles formed on the surface denoting the growth of apatite crystals with increase in immersion time.</p>
HA70CG30CA composite	<p><b>Pre-immersed sample:-</b> Spherical geometry and high dispersion of particles on the surface of the composite</p> <p><b>Post-immersed sample:</b> <b>After 7days :-</b> Accumulated and concentrated spherulites with number of tiny crystals which deposited on the surface and many pores between the particles as a result of immersion effect.</p> <p><b>After 28days:-</b> Increase in the number of deposited particles forming spongy structure.</p>
<p><b>Concluding remarks:-</b> From the results of the weight loss it can be concluded that the decrease in the weight loss due to increase in the chitosan, gelatin content of the composite is due to the increase in the stability of the composite due to interaction between HAp particles and chitosan and gelatin molecules via their functional groups. The FTIR, SEM analysis results shows that the increase in the chitosan and gelatin content leads to the increase in the apatite formation on the surface of composites.</p>	

Table 2.10 Khaled R. Mohamed et al characterization methods

Mehdi Kazemzadeh Narbat et al <sup>[12]</sup> synthesized HAp – Gelatin (GEL) composite with 30, 40 & 50 wt % HAp composition. The composites were then molded into Teflon molds and further characterized using different characterization methods mentioned in table 2.11.



Characterization methods	Remarks
1) FTIR	N-H stretching:- 3270-3370 $\text{cm}^{-1}$ C-H stretching:- 2920 $\text{cm}^{-1}$ , C=O stretching:- 1670-1650 $\text{cm}^{-1}$ , N-H deformation:-1500-1550 $\text{cm}^{-1}$ , $\alpha$ - helical configuration of composite:-1540 $\text{cm}^{-1}$ , -OH stretching:- 4000-3200 $\text{cm}^{-1}$ , Phosphate band:-900 and 1200 $\text{cm}^{-1}$
2) Mechanical Test	Compressive strength of composite : With 30 wt% HAp: 9.7MPa, With 40wt% HAp: 25.6MPa, With 50wt% HAp: 32.1MPa
3) Water absorption	The water absorption of HAp-GEL composite reduces with HAp content due to formation of a temporary HAp barrier preventing water permeating into the scaffold.
4) SEM	The scaffolds are interconnected and their size range from 80 to 400 micrometer.
5) Porosity	70%
6) Apparent Density	1.17 $\text{g}/\text{cm}^3$
7) Cell attachment evaluation	Cells exhibited rather good proliferation and partially covered the composite surface.
<b>Concluding remarks:-</b> The increase in the HAp content of the scaffolds resulted in the increase in compressive strength. The addition of HAp results in more dense and thicker pore walls with lower porosity. In the FTIR analysis the shift of the 1337 $\text{cm}^{-1}$ band in GEL has been effectively used to confirm the chemical bond formation between carboxyl ions in GEL and HAp phases.	

Table 2.11 Mehdi Kazemzadeh Narbat et al characterization methods

#### IV. CONCLUSION

Hydroxyapatite is mineral component present in bones & imparting it mechanical strength. However the organic matter contributing 30% of bones act as binder and gives it the load bearing strength. There are continuing efforts to synthesize HAp using different sources and techniques. Similarly various organic compounds are blended with HAp to make it mechanically strong and similar to natural bones. The properties of HAp and its biocomposites are important in deciding its suitability in various applications. Hence there is a need for selection of suitable characterization method followed by proper interpretation of findings of analytical method. In this paper various characterization techniques reported in the literature that are employed for HAp and its biocomposites are summarized and would be helpful for researchers working in this area.

#### V. ACKNOWLEDGEMENT

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