



**Preparation and Characterization of Synthesized Hydroxyapatite, Poly Lactic Acid & Cotton (Gauze) Composite Film.**

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## Abstract

In biomedical research, fabrication of porous scaffolds from advanced biomaterial for healing bone defects represents a new approach for tissue engineering. Hydroxyapatite (HAp) is a biologically active ceramics have been recognized as substitute material for bone and teeth in orthopedic and dentistry field due to their chemical and biological similarity to human hard tissue. It is biocompatible and bioactive material that can be used to restore damaged human calcified tissue. Hydroxyapatite, Poly Lactic Acid & Cotton (Gauze) Composite Scaffold film were produced by natural drying in-situ synthesized hybrid suspension. Matrix mediated precipitation of hydroxyapatite particles in the polymer, controlled the particle size in nanometer range. The chemical and thermal properties of composite were investigated by Simultaneous Thermal Analyzer (STA)/ (TGDTA). Crystallographic characterization by X-Ray Diffraction and Mechanical properties by tensile testing are calculated as tensile strength.

This systematic study was carried out to assess the possibility of developing Hydroxyapatite, Poly Lactic Acid & Cotton (Gauze) Composite film as a new approach for tissue engineering and improved understanding of its characteristics.

## 1. Introduction

Biomaterials is a most rising discipline, due to its direct relation with the healthcare and impact on human health related issues. [1]. So development and improvement of orthopedic related biomaterials is a very active and growing research field.

Tissue engineering is an interdisciplinary and multidisciplinary field that aims at the development of biological substitutes that restore, maintain, or improve tissue function [2]. From the viewpoint of tissue engineering, tissue formation in three dimensions (3D), a considerable approach to make porous scaffold that provides the microenvironment (synthetic temporary extracellular matrix) for regenerative cells, supporting cell attachment, proliferation, differentiation, and neo tissue genesis [3]. Therefore, the chemical composition, physical structure, and biologically functional moieties are all important attributes to biomaterials for tissue engineering. Biodegradability being one of the most important requirements, limits the choice of materials to a few ceramics and polymers. [4] Ceramics are considered as ideal materials because of their osteoinductive properties, the ability to induce bone formation. Synthetic ceramics such as  $\beta$ -tricalcium phosphate and synthetic hydroxyapatite (HAp) have been used [5-9]. That's why; research on bioceramics (ceramics, carbon, glass and glass-ceramics) has attracted significant attention because of their potential for biocompatibility and bioactivity with the human body. An important research on different forms of biocompatible calcium phosphate, hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , HAp] is one of the most well-known phases studied for a number of biomedical applications, due to its similar chemical composition and crystal structure to apatite in the human skeletal system. The bioactivity and osteoconductivity of HAp offer a suitable surface for new bone growth and integration as well as reinforcement to polymer scaffold material for tissue regeneration [1,10]

Use of calcium phosphate polymer composites creates a highly biocompatible product by increasing cell-material interactions compared to the polymer alone. [11]. Furthermore, the sustained release of calcium and phosphate from those composites, where the two ions serve as substrates in the remodeling reactions of mineralized tissues is an added benefit. [12] Due to the extensive use of PLAs in the biomedical field, their composites with HAp improved the bending strength of the composite but also had an active role in new bone formation. [13]. The HAp surface was found to be highly reactive and led to

favorable attachment to tissue and bioactivity in bone repair.[14] Solvent casting was first described by Mikos et al.[15] to prepare highly porous biodegradable polymer membranes used as scaffolds with tunable porosity and desired crystallinity. Phase Inversion is similar to solvent casting technique is not useful because of the low mechanical properties.[16] Besides, Melt Molding is not viable because of highly porous scaffolds inhibit in controlling pore distribution. Fiber bonding method produces scaffolds variable pore size. That's why; it is not beneficial due to immiscibility might harm cells and organs.[17] High pressure processing is a novel technique suggested for fabricating macro porous sponges of biodegradable polymers without the use of organic solvents. Mooney et al.[18] have employed this technique to fabricate poly (D,L-lactic-co-glycolic acid) by saturating them with high pressure CO<sub>2</sub> and then by rapidly reducing the gas pressure to atmospheric levels. These porous sponges have been suggested as ideal candidates for tissue engineering. The process has a few disadvantages such as, low mechanical properties and closed pore structure. Three-dimensional printing (3DP) is a technique to produce scaffolds[19] have limited layer thickness is one of the major disadvantages of this technique. Freeze-drying process has been used to prepare hybrid collagen-gelatin polymer networks by Mao et al.[20] by utilizing ice micro particles as pyrogen. This process depends on thermally induced phase separation. Precise control of parameters is required to fabricate the scaffolds. Electrospinning, invented by Formhals[21] in 1934 to fabricate fibrous structures of polymers by the application of a high voltage to a viscous polymeric based solution, has recently emerged as a leading technique to fabricate fibrous structures for tissue engineering[22-24]

as the process has the unique capability to fabricate three-dimensional scaffolds that can mimic the extracellular matrix (ECM) with tunable porosity and morphology [25]. Electro spun fibers have various diameters ranging downward from 5 μm to 0.05 μm. The small diameter of the fibers provides high surface area to volume ratio making it suitable for scaffolds for biomedical applications.[26] Electrospinning has emerged as a promising one-step technique to fabricate scaffolds for bone tissue engineering.

Most of these techniques described do not have the capability to produce three dimensional scaffolds with controlled amount of porosity and pore size in a single step. Electrospinning, however a remarkable Nano manufacturing technique has the ability to

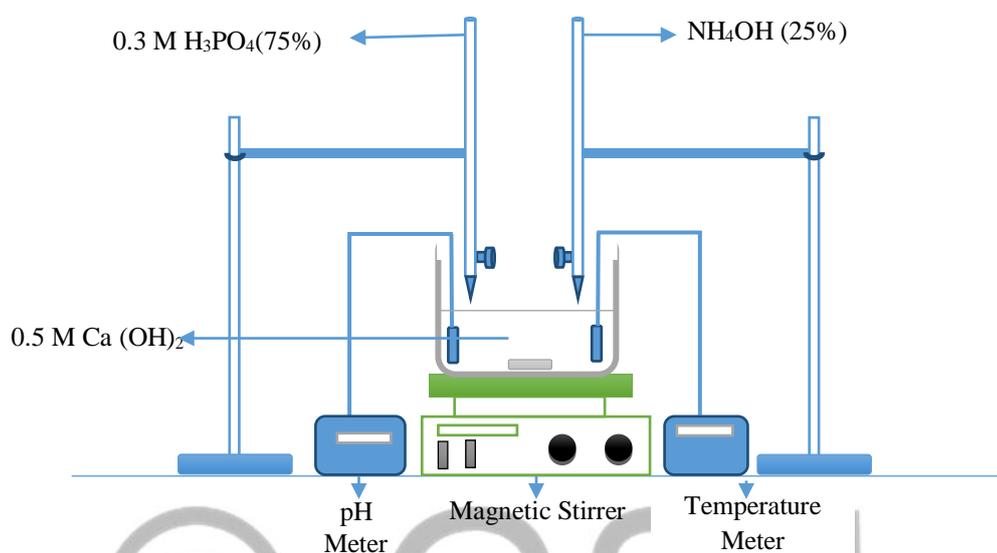
produce three dimensional bio mimicking scaffolds with layered configuration with complex pore structures and controlled pore size[25]

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## 2. Experimental

### 2.1. Synthesis of Nanohydroxy Apatite

nHAp powders are produced via wet chemical precipitation technique.



**Figure 2.1.1:** Schematic drawing of the synthesis apparatus for nHAp powders using precipitation technique.

nHAp powders were prepared via, precipitation technique. In brief, 50 ml aqueous suspension of 0.5 M calcium hydroxide [Ca (OH)<sub>2</sub>] was prepared and vigorously stirred for 30 minutes. Then 50 ml of 0.3M Orthophosphoric acid [H<sub>3</sub>PO<sub>4</sub>] was slowly added drop wise (0.5 ml/min) into the Ca (OH)<sub>2</sub> suspension. And simultaneously temperature and pH are measured and the pH (10.5) is adjusted by adding drop wise 1 M ammonium hydroxide [NH<sub>4</sub>(OH)] solution. The suspension were well stirred (1000 rpm) using magnetic stirrer for 2 hour and aged for overnight at room temperature (as shown in figure 3.5. In the second day the suspension is heated at 150<sup>0</sup>C for 2 hour and stirred at 1000 rpm then cooled at room temperature. After that the suspension is sonicated at ultra-sonication bath with 30 minutes period for 6 times for dispersion and finally high-speed stirring at 1500 rpm for 6 hour, and then aging for 24 hour. On the fourth day the precipitates were subjected vacuum filtrating using Buchner funnel, repeatedly washed to obtain pH 7 with deionized water and filtered again. The precipitates were dried at 80<sup>0</sup>C for 48 hours. Dried lumps of powders were ground by clean pestles and mortars.

## 2.2. Preparation of HAp/PLA/Cotton scaffold

The materials are weighted as follows.

**Table 2.2.1:** Sample weighting for the hot press scaffold preparation.

Sample Name	HAp (gm.)	PLA (gm.)	Cotton (gm.)	1,4-Dioxane (ml)
5% nHAP/PLA	0.107	2.043	0.362	20
10% nHAP/PLA	0.207	2.080	0.360	20
15% nHAP/PLA	0.303	2.118	0.380	20
20% nHAP/PLA	0.409	2.031	0.388	20

**Table 2.2.2:** Sample weighting for the cold press scaffold preparation.

Sample Name	HAp (gm.)	PLA (gm.)	Cotton (gm.)	1,4-Dioxane (ml)
5% nHAP/PLA	0.105	2.049	0.367	20
10% nHAP/PLA	0.211	2.046	0.363	20
15% nHAP/PLA	0.311	2.016	0.366	20
20% nHAP/PLA	0.403	2.010	0.365	20

The required quantity (given at the table) of HAp powder was dispersed in 1,4-Dioxane by sonication, then following the table required amount of PLA was added to the mixtures, and stirred at 50<sup>0</sup>C until complete dissolution. Four solutions with different content in HAp were prepared, 5%, 10%, 15%, 20% particle by weight with respect to PLA. The HAp/PLA solution was poured in petri dish containing Gauze film. Then the scaffolds were placed in a vacuum dryer at 40<sup>0</sup>C in order to remove the

1, 4-Dioxane. After extraction of Dioxane, scaffolds were dried in atmospheric temperature. These samples were then stored in desiccator.

For Hot Press sample, after removing from the vacuum dryer it scaffold kept in mold of Hot Press machine at 40°C temperature and 20 bar Pressure for 15min. Then it is stored in desiccator.

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### 3.1 Measurement of Water Absorption:

Water absorption, % by mass, after 24 hours immersion in water is given by the formula:

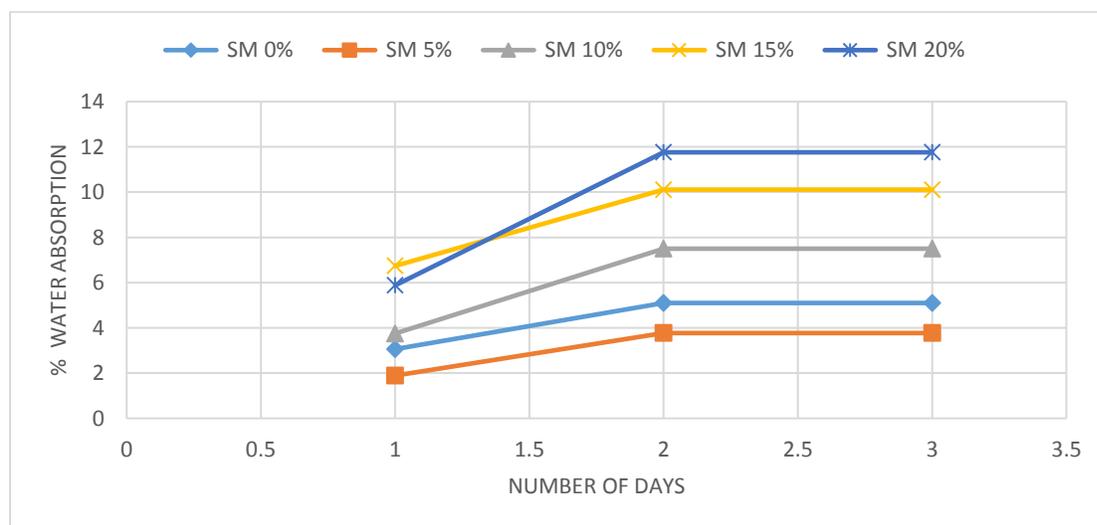
$$W = \frac{M_2 - M_1}{M_1} \times 100$$

Where  $M_1$  = Initial weight &  $M_2$  = Final weight

#### Cold press:

**Table 3.1.1:** Measurement of Water Absorption (Cold Press)

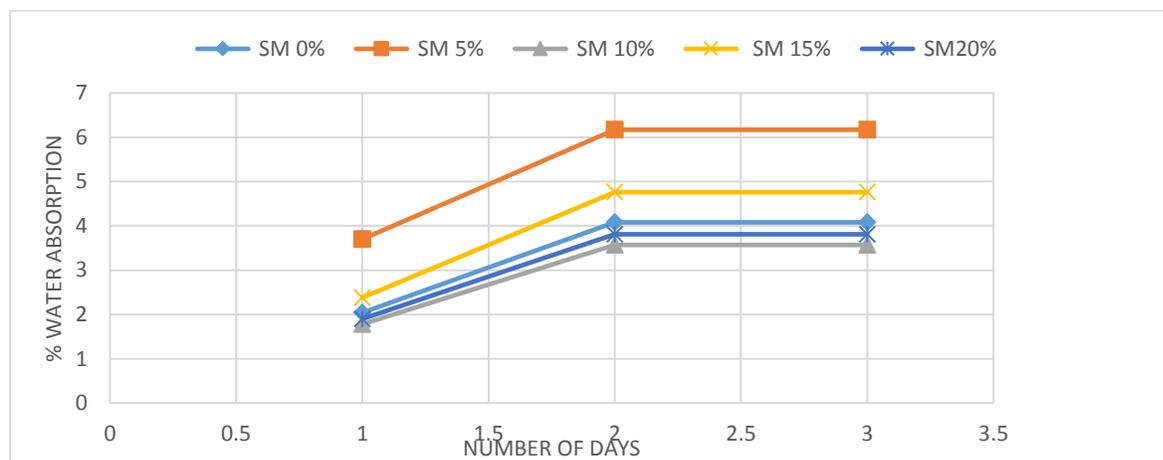
Sample ID	Initial Weight gm.	Water Absorption, %		
		Day 1	Day 2	Day 3
SM 0%	0.098	3.06	5.10	5.10
SM 5%	0.106	1.89	3.77	3.77
SM 10%	0.080	3.75	7.50	7.50
SM 15%	0.089	6.74	10.11	10.11
SM 20%	0.068	5.88	11.76	11.76



**Figure 3.1.1(a):** Measurement of Water Absorption of Composite (Cold Press)

**Hot press:****Table 3.1.1(b):** Measurement of Water Absorption (Hot Press)

Sample ID	Initial Weight	Water Absorption, %		
		Day 1	Day 2	Day 3
%	gm.			
SM 0%	0.049	2.04	4.08	4.08
SM 5%	0.081	3.70	6.17	6.17
SM 10%	0.056	1.78	3.57	3.57
SM 15%	0.084	2.38	4.76	4.76
SM 20%	0.105	1.90	3.81	3.81

**Figure 3.1.1(c):** Measurement of Water Absorption of Composite (Hot Press)

It reveals that the water absorption depends on filler content and immersion time. Result shows that the rate of water absorption is very low with time. This is due to the fact that degree of cross-linking reaction, which diminishes the void spaces i.e. with the increase of degree of polymerization, the composite becomes more dense or reinforced materials are distributed properly eliminating all voids. The water absorption is positively co-related with the porosity of sample. That means the higher the porosity the higher the water absorption. For 20% composite the absorption is higher because of its higher porosity.

## 3.2 Measurement of Tensile Properties:

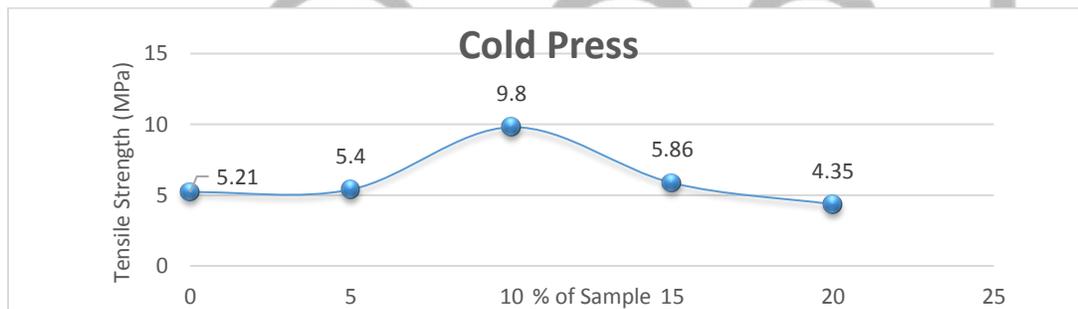
### 3.2.1 Measurement of Tensile Strength:

Tensile Strength is the maximum stress that a material can withstand while being stretched or pulled before failing or breaking. It measure the force required to pull something such as rope, wire or a structural beam to the point where it breaks.

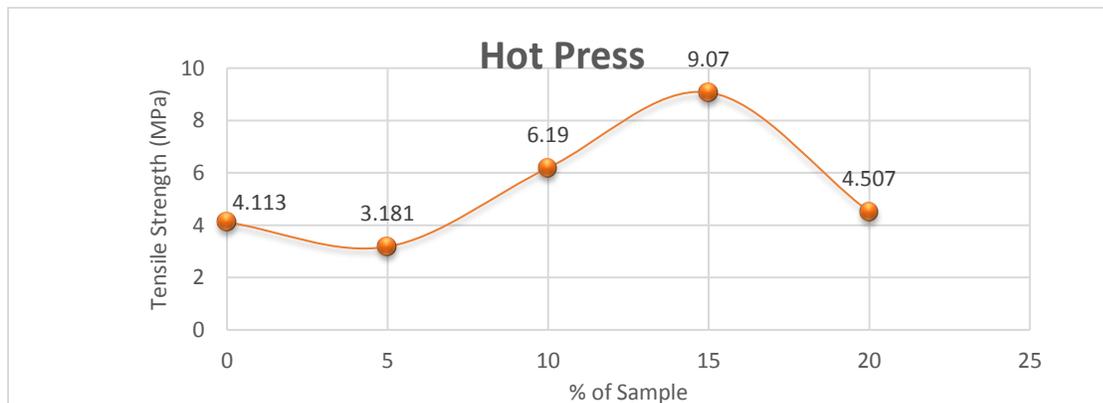
Tensile Strength is measured by the formula:

$$\text{Tensile strength} = F/A \text{ (N/mm}^2\text{)}$$

The 0% composition has a high tensile strength value due to its high elasticity. With the addition of filler tensile strength value show a downward inclination due to its rigidity and non-elastic property.



**Figure 3.2.1(a):** Tensile strength of % Sample composite (Cold Press)

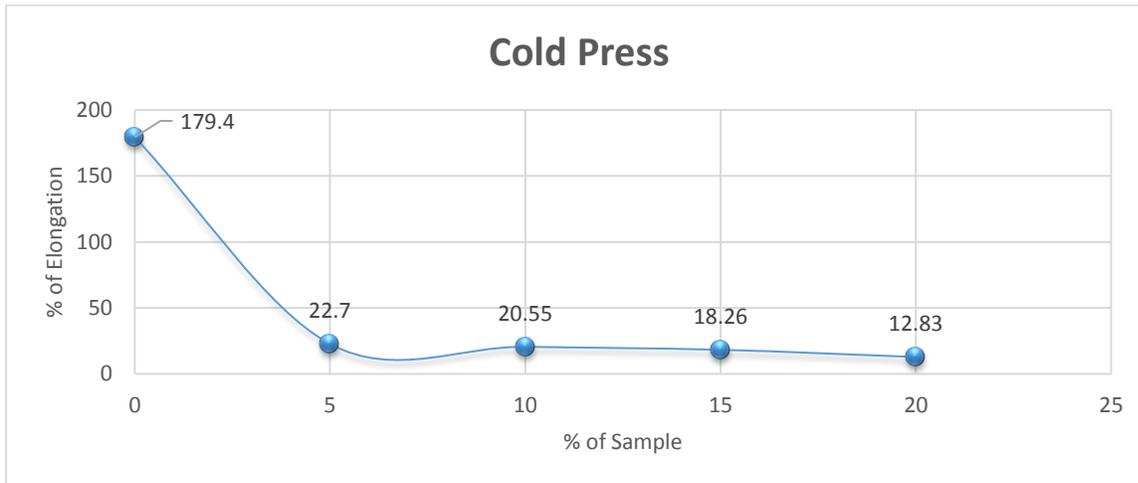


**Figure 3.2.1(b):** Tensile strength of % Sample composite (Hot Press)

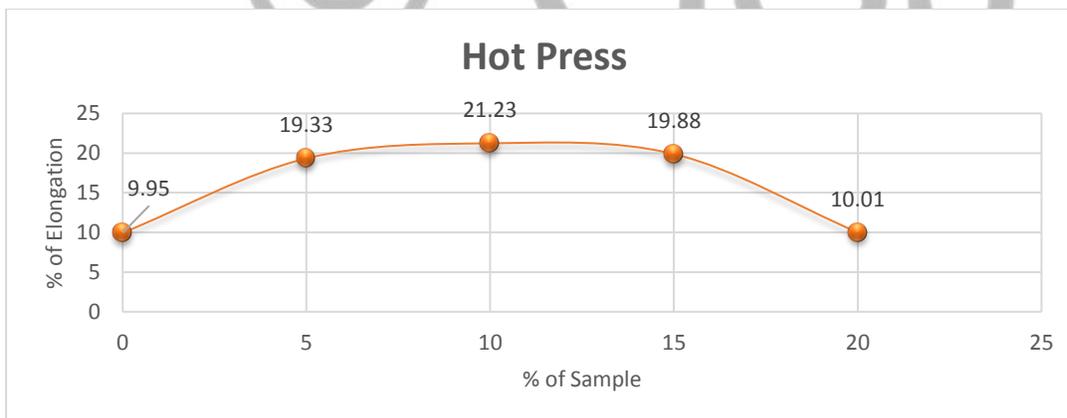
### 3.2.2 Measurement of % of Elongation

The elongation of the test specimen expressed as a percentage of the gage length.

$$\% \text{ of Elongation} = \frac{(\text{Final length} - \text{Original length})}{\text{Original length}} \times 100$$



**Figure 3.2.2(a): % of Elongation of composite (Cold Press)**



**Figure 3.2.2(b): % of Elongation of composite (Hot Press)**

### 3.2.3 Measurement of Ductility:

Ductility is the ability to deform before braking. It is the opposite of brittleness. Ductility can be given either as percent maximum elongation  $E_{\max}$  or maximum area reduction.

$$\%EL = E_{\max} * 100\%$$

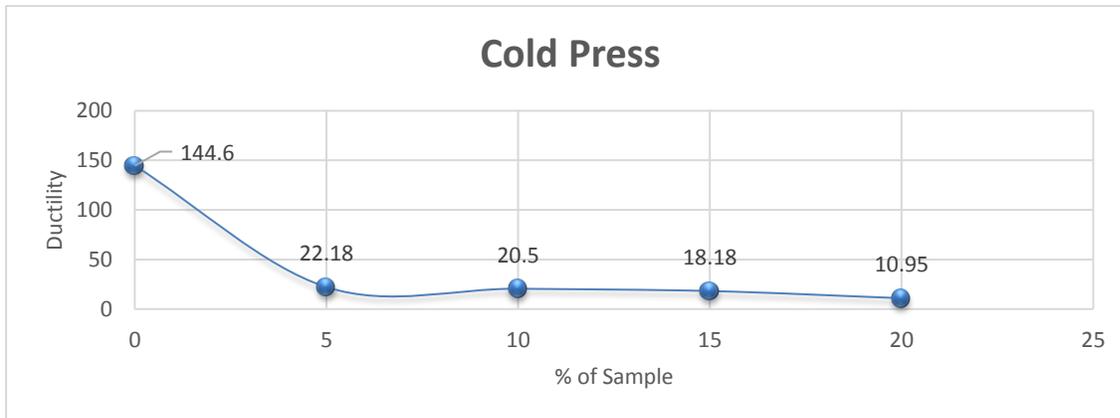


Figure 3.2.3(a): Ductility of composite (Cold Press)

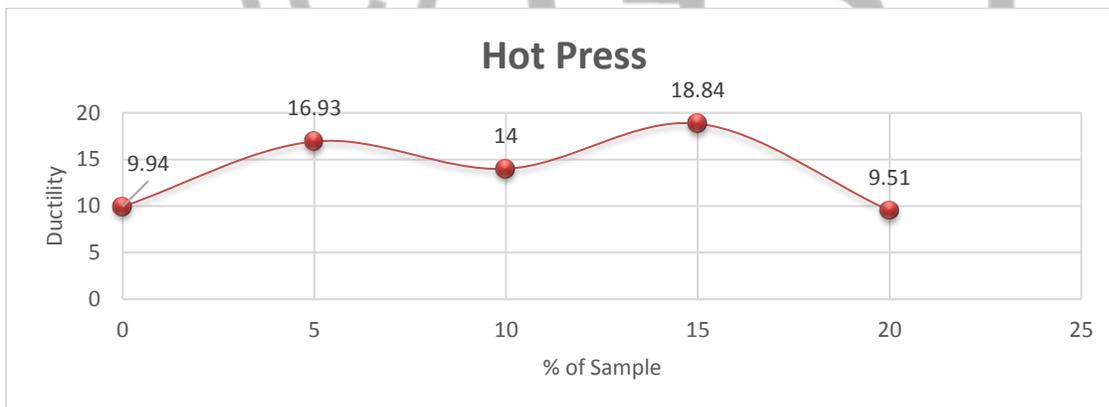


Figure 3.2.3(b): Ductility of composite (Hot Press)

### 3.2.4 Measurement of E-modulus:

The E-modulus is a measure of stiffness of materials. It was calculated from the tangent of stress-strain curve. It is related to the second derivative of the interatomic potential, or the first derivative of the force vs. intern clear distance.

Due to thermal vibrations the elastic modulus decreases with temperature. E is large for ceramics (stronger ionic bond) and small for polymers (weak covalent bond).

In case of tensile modulus the higher the elongation the lower will be the modulus for the same stress. In case of 0% of the sample the elongation is higher therefore possessing the lowest E-modulus. On the other hand the E-modulus continuously increases with the addition of filler in it due to its reduction in elongation at yield

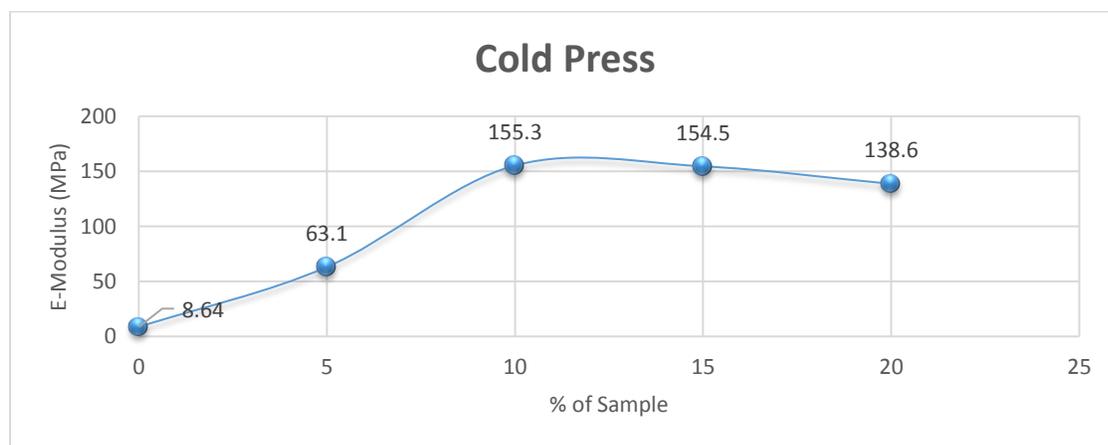


Figure 3.2.4(a): Comparison of E-Modulus of composite (Cold Press)

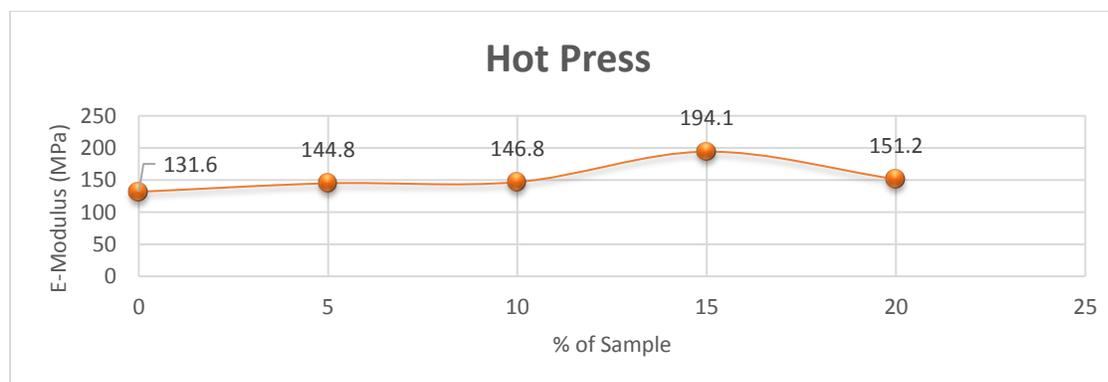
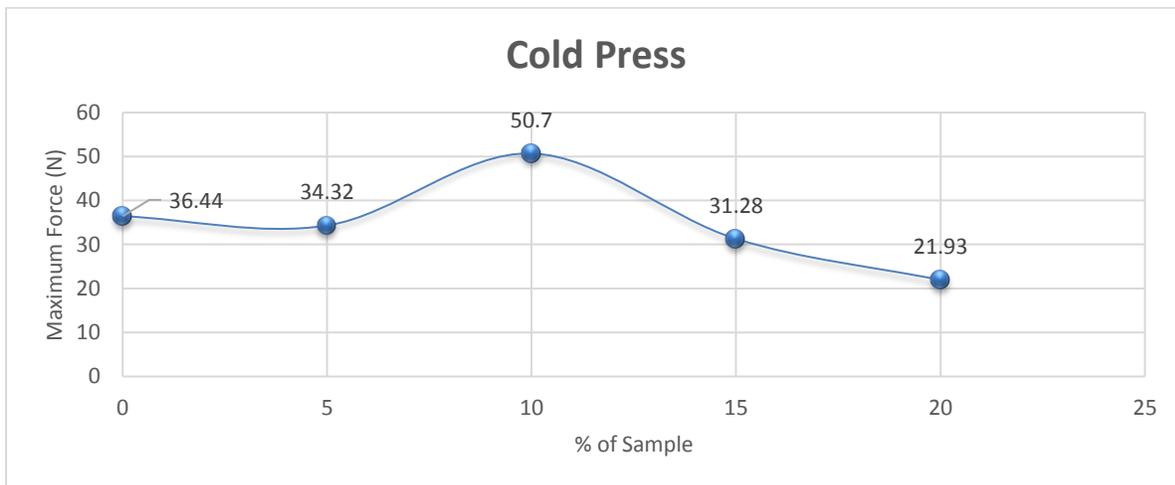


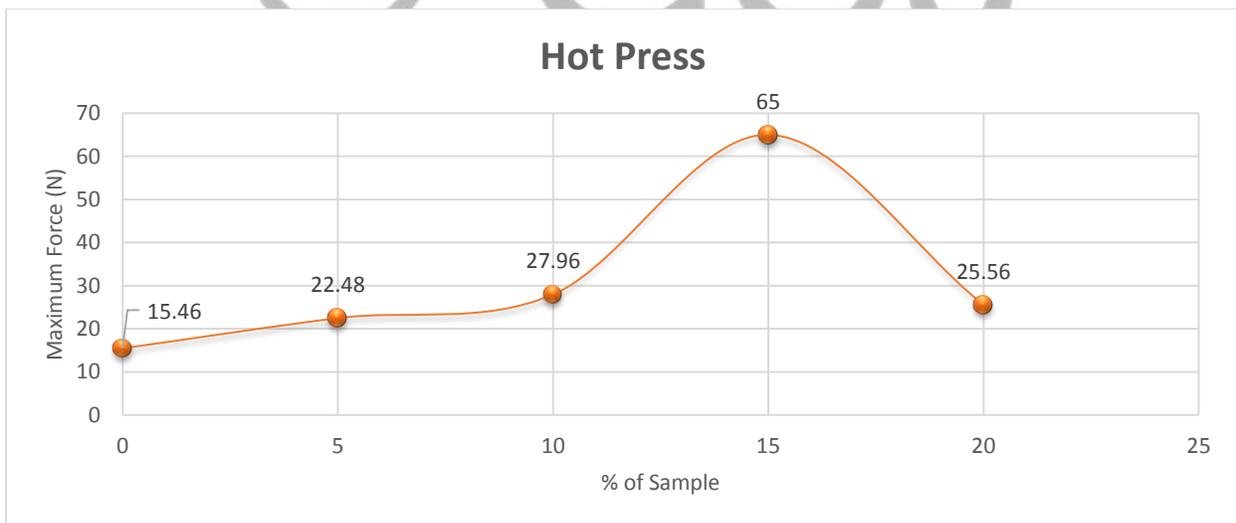
Figure 3.2.4(b): Comparison of E-Modulus of composite (Hot Press)

### 3.2.5 Measurement of Maximum Force required at yield point:

With the increase in elongation at yield for a composite the maximum force required will be higher and vice-versa. For this reason, the maximum force required to bring yield point is highest for 0% sample with lowest value for 50% sample



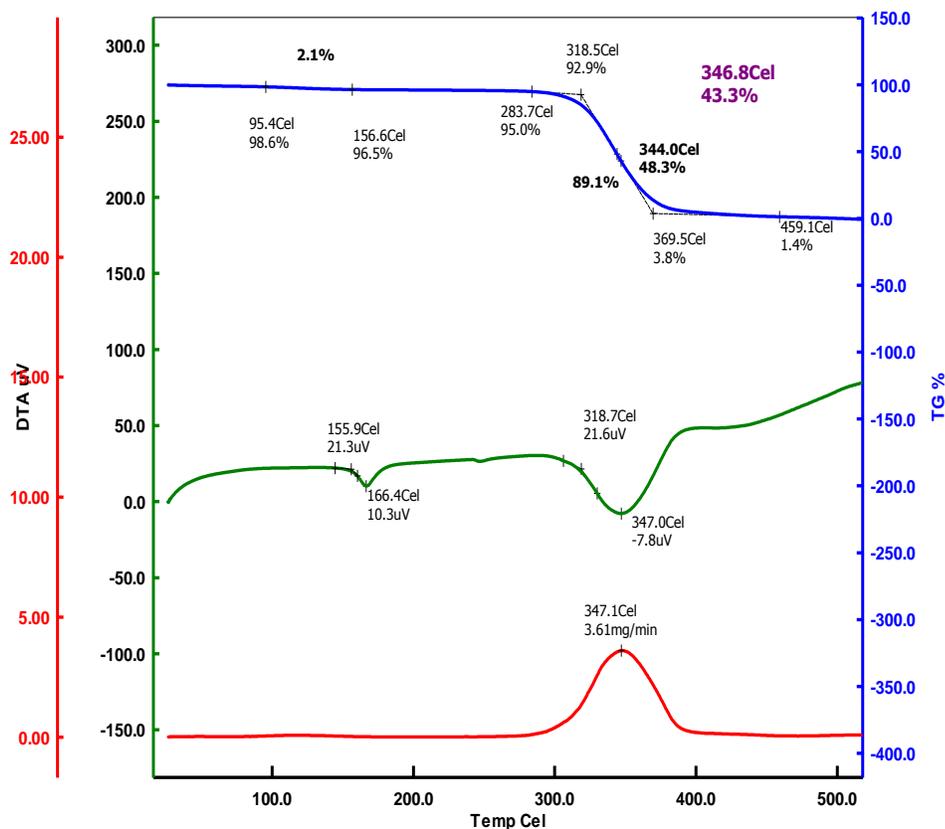
**Figure 3.2.5(a):** Maximum Force required at yield point for Composite (Cold Press)



**Figure 3.2.5(b):** Maximum Force required at yield point for Composite (Hot Press)

### 3.3 TG/DTA Analysis

#### Cold press

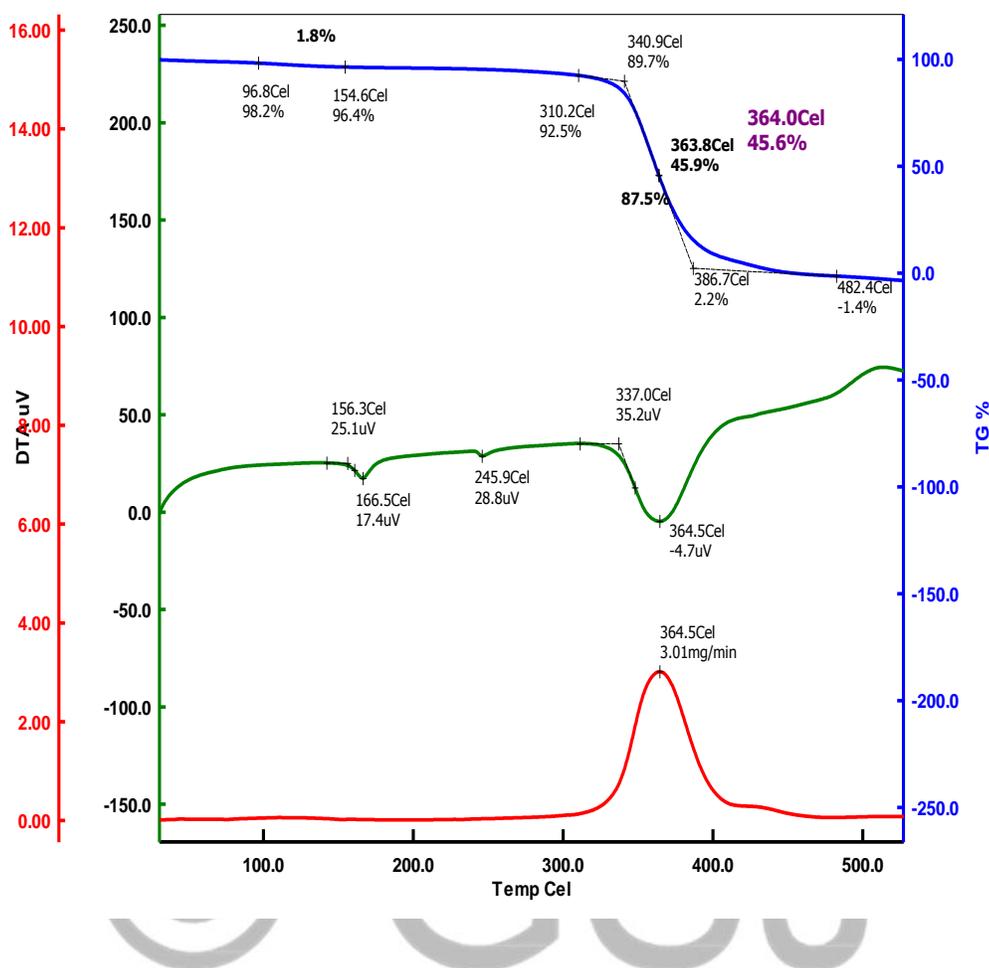


**Figure 3.3.1:** TG/DTG/DTA/ curve of 0% Sample (Cold Press)

Figure shows the TG, DTA and DTG curves for 0% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 2.1% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 318.5°C, 344.0°C and 369.5°C. The total degradation is 89.1%. The maximum peak is at 346.8°C where the degradation is 56.7%.

The DTA curve shows two endothermic peaks at 166.4°C and 347.0°C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 347.1°C means that there is one-step degradation. The maximum degradation occurs at a rate of 3.61 mg/min.

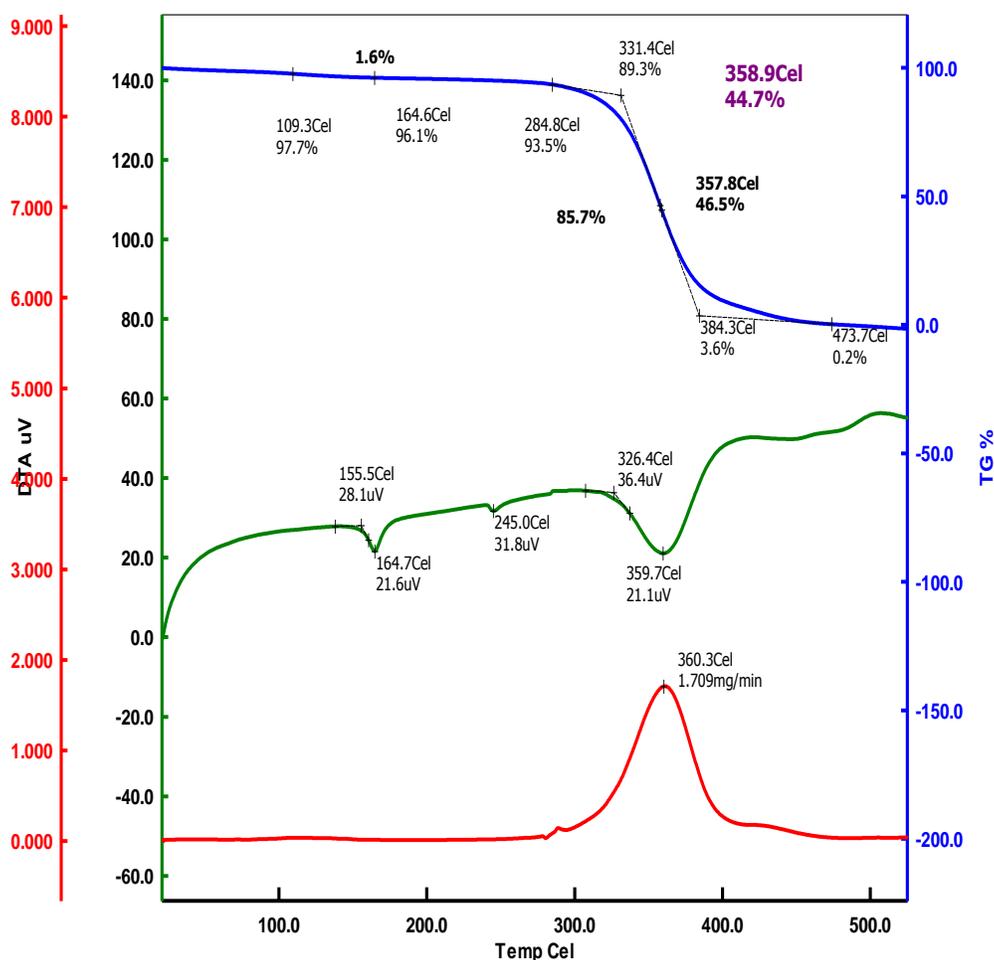


**Figure 3.3.2:** TG/DTG/DTA/ curve of 15% Sample (Cold Press)

Figure shows the TG, DTA and DTG curves for 15% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 1.8% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 340.9<sup>o</sup>C, 363.8<sup>o</sup>C and 386.7<sup>o</sup>C. The total degradation is 87.5%. The maximum peak is at 364.0<sup>o</sup>C where the degradation is 54.4%.

The DTA curve shows two endothermic peaks at 166.5<sup>o</sup>C and 364.5<sup>o</sup>C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 364.5<sup>o</sup>C means that there is one-step degradation. The maximum degradation occurs at a rate of 3.01mg/min.



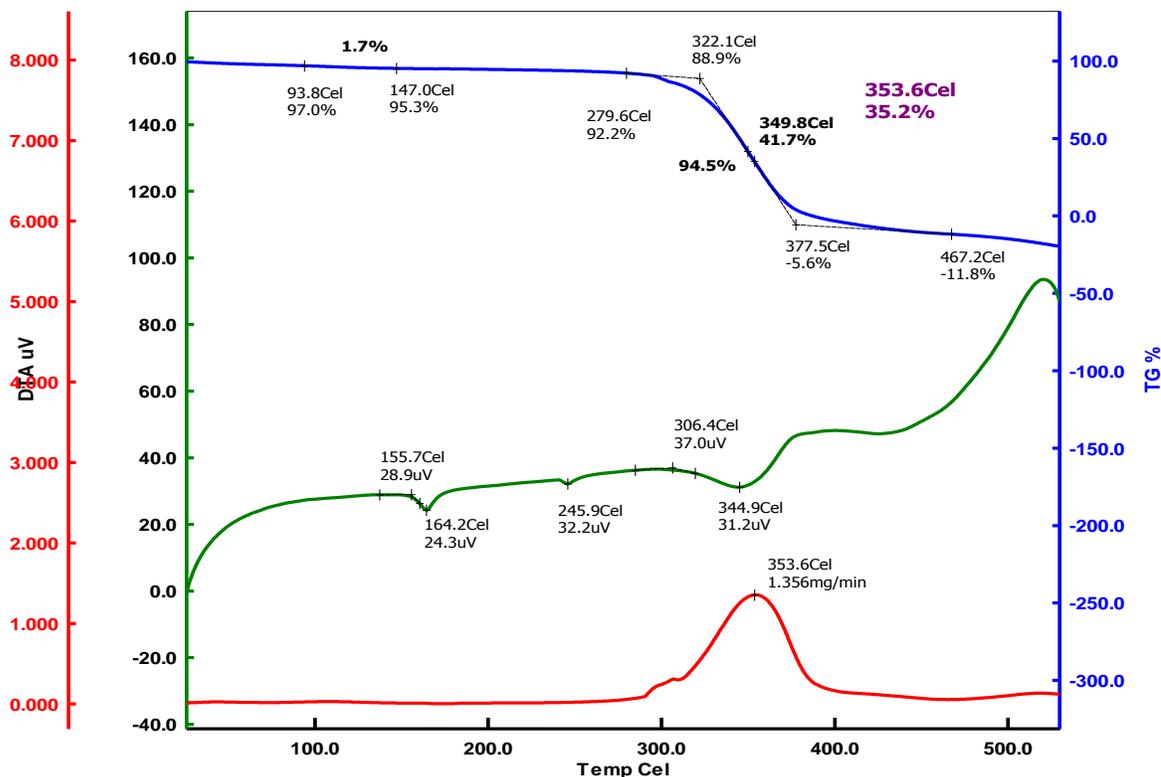
**Figure 3.3.3:** TG/DTG/DTA/ curve of 20% Sample (Cold Press)

Figure shows the TG, DTA and DTG curves for 20% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 1.6% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 331.4°C, 357.8°C and 384.3°C. The total degradation is 85.7%. The maximum peak is at 358.9°C where the degradation is 55.3%.

The DTA curve shows two endothermic peaks at 164.7°C and 359.7°C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 360.3°C means that there is one-step degradation. The maximum degradation occurs at a rate of 1.709 mg/min

## Hot press

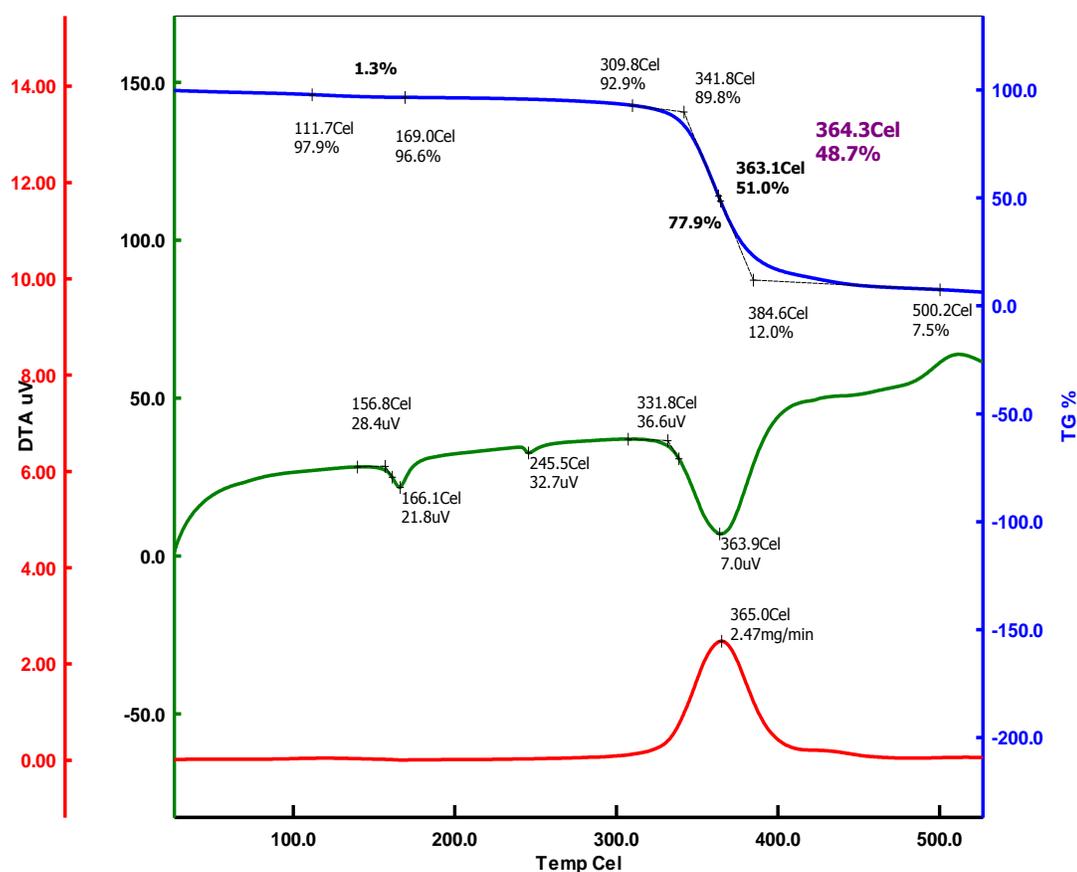


**Figure 3.3.4:** TG/DTG/DTA/ curve of 0% Sample (Hot Press)

Figure shows the TG, DTA and DTG curves for 0% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 1.7% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 322.1°C, 349.8°C and 377.5°C. The total degradation is 94.5%. The maximum peak is at 353.6°C where the degradation is 64.8%.

The DTA curve shows two endothermic peaks at 164.2°C and 344.9°C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 353.6°C means that there is one-step degradation. The maximum degradation occurs at a rate of 1.356 mg/min.

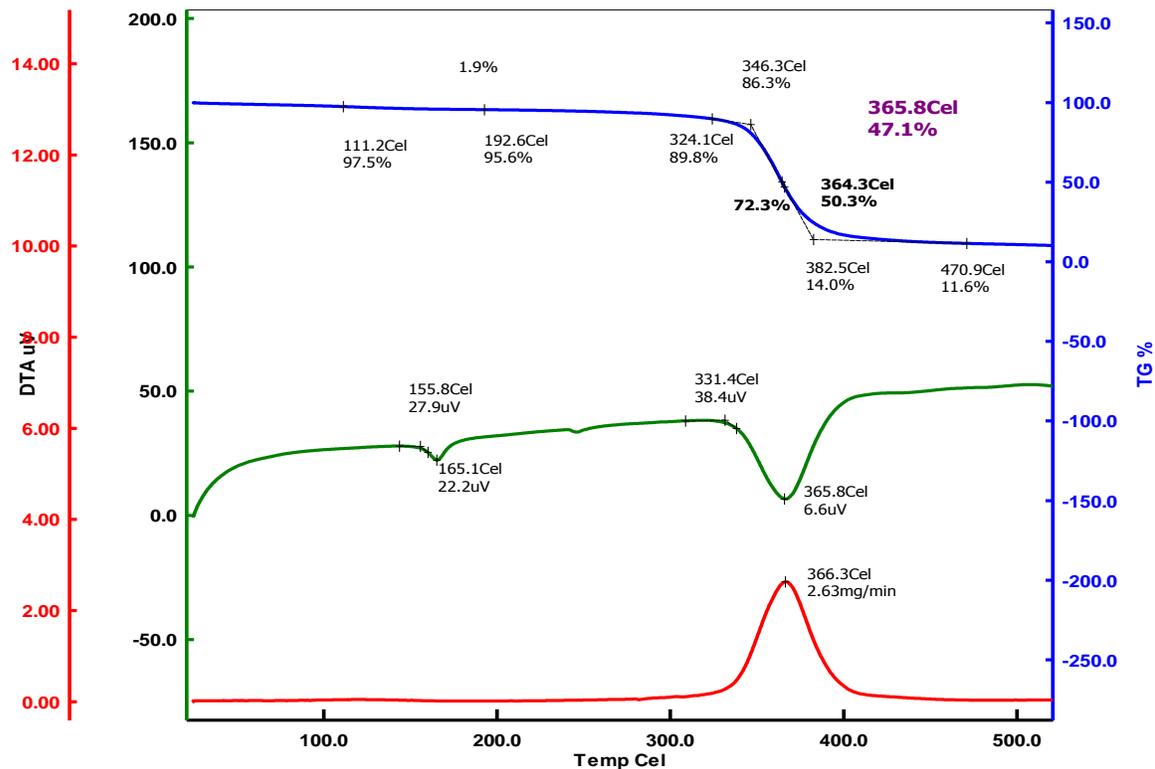


**Figure 3.3.5:** TG/DTG/DTA/ curve of 15% Sample (Hot Press)

Figure shows the TG, DTA and DTG curves for 15% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 1.3% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 341.8°C, 363.1°C and 384.6°C. The total degradation is 77.9%. The maximum peak is at 364.3°C where the degradation is 51.3%.

The DTA curve shows two endothermic peaks at 166.1°C and 363.9°C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 365.0°C means that there is one-step degradation. The maximum degradation occurs at a rate of 2.47mg/min



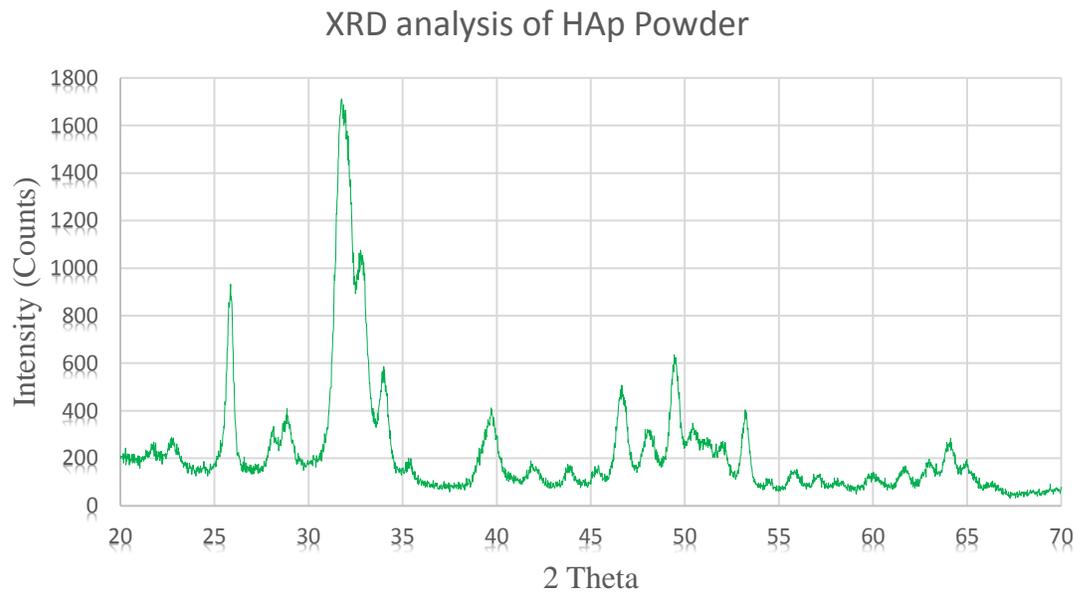
**Figure 3.3.6:** TG/DTG/DTA/ curve of 20% Sample (Hot Press)

Figure shows the TG, DTA and DTG curves for 20% sample. The blue curve for % TG, the green one for DTA and the red one for DTG. TG shows for initial loss 1.9% due to moisture. The onset temperature, 50% of major degradation and the maximum slope are at 346.3°C, 364.3°C and 382.5°C. The total degradation is 72.3%. The maximum peak is at 365.8°C where the degradation is 52.9%.

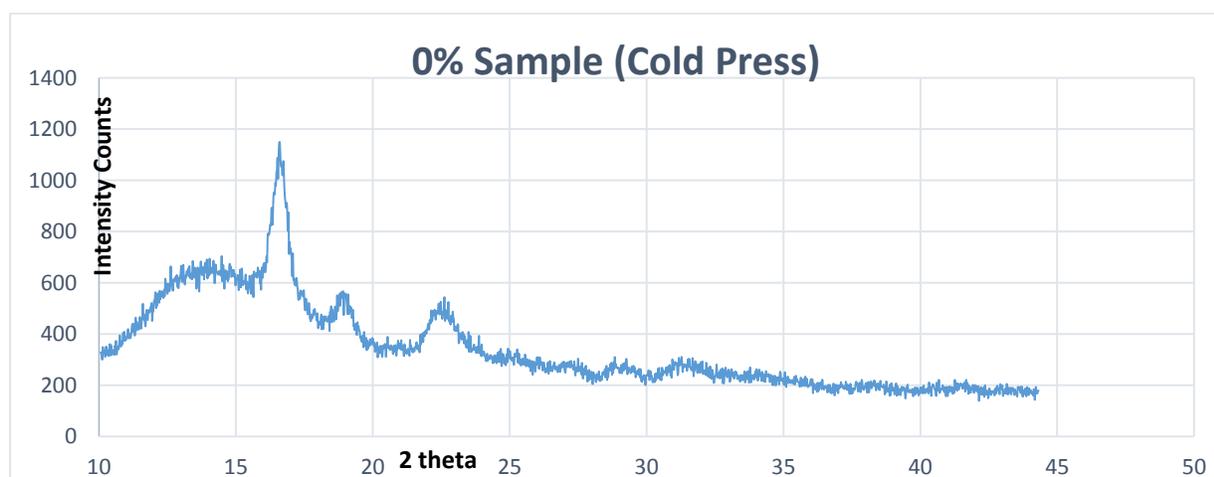
The DTA curve shows two endothermic peaks at 165.1°C and 365.8°C are due to moisture and thermal degradation respectively.

The DTG curve shows one peak at 366.3°C means that there is one-step degradation. The maximum degradation occurs at a rate of 2.63 mg/min.

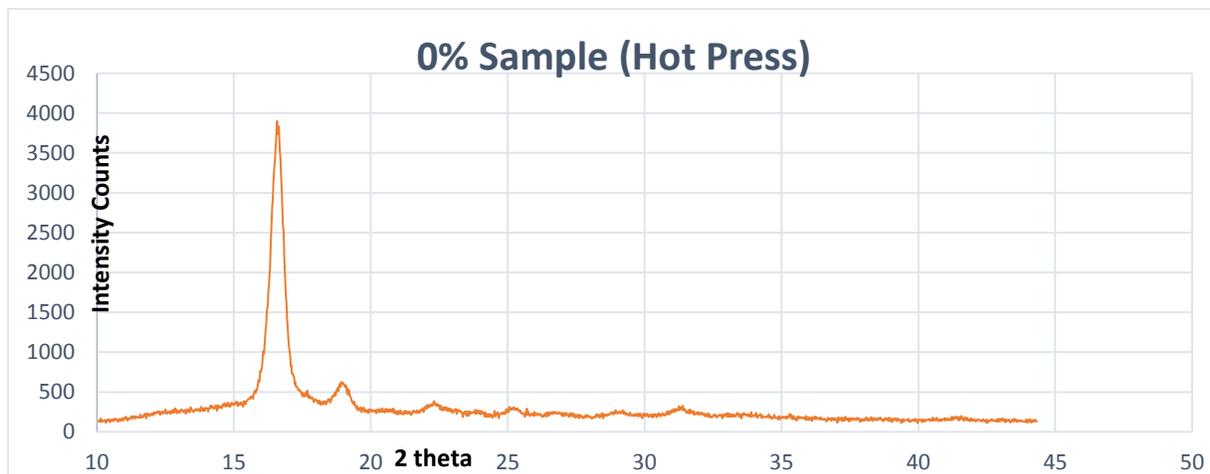
### 3.4 X-ray diffraction (XRD) Analysis



**Figure 3.4.1:** XRD pattern of Hydroxyapatite particle showing characteristic peak

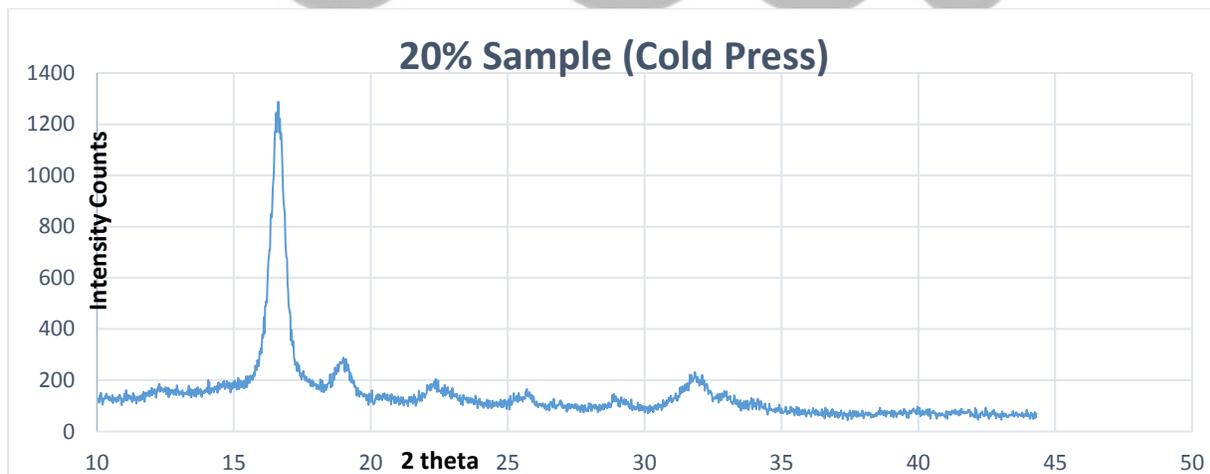


**Figure 3.4.2:** XRD pattern of 0% Sample showing characteristic peak

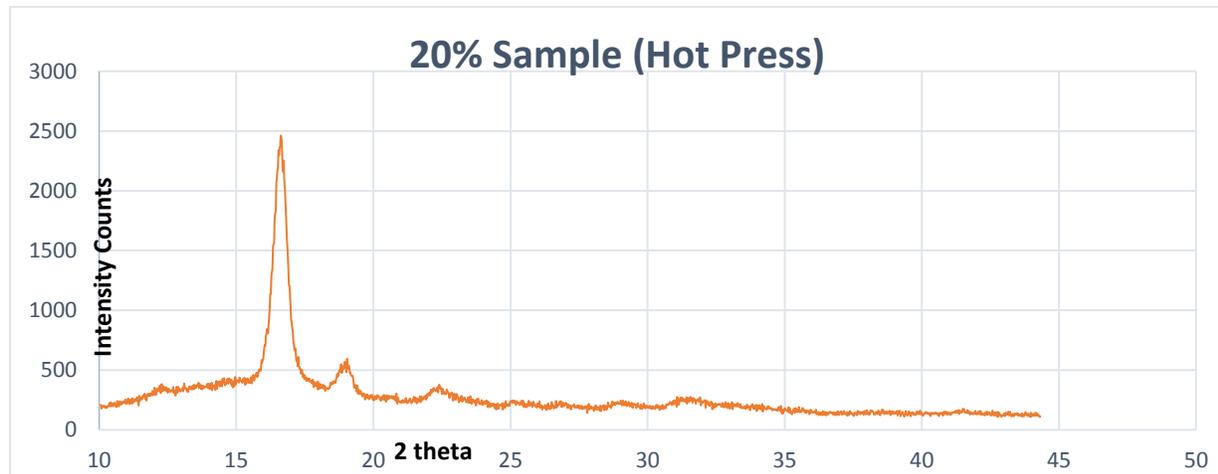


**Figure 3.4.3:** XRD pattern of 0% Sample showing characteristic peak

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**Figure 3.4.4:** XRD pattern of 20% Sample showing characteristic peak



**Figure 3.4.5:** XRD pattern of 20% Sample showing characteristic peak

From the above figures it can be said that, the Synthesized HAp sample exhibit almost same diffraction pattern with the characteristic peaks of Hap. The prepared samples are amorphous structure with some crystalline structure found due to percentage of Hydroxyapatites.

## CONCLUSION

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Lack of osteoinductivity and the problem of inherent brittleness are the two major challenges associated with hydroxyapatite (HAp), the main mineral component of bone. This imposes a limitation in its application as a load bearing orthopedic implant material. The present work aims to overcome the problem of inherent brittleness of HAp by incorporating HAp/PLA/Cotton composite matrix. This work focused on the investigation of composites in two main areas: (1) the production and characterization of composite and (2) the evaluation of mechanical properties and thermal properties.

In this work we try to present some mechanical properties of composite such as tensile strength, E-modulus, %elongation. TG/DTA analysis, X-ray diffraction (XRD). The study was involved to prepare composites containing HAp, Poly Lactic Acid and Cotton by natural drying process after proper mixing in 1,4 Dioxine at about 50°C and also the effect of percentage of PLA and HAp on the composite. The composite are used to replacement of bone. From this study the following can be conclude that-

- The tensile properties of the composite are reduced with increasing HAp.
- 10% HAp composite shows better tensile properties for Cold Press.
- 15% HAp composite shows better tensile properties for Hot Press.
- From the TG/DTA data it is observed that sample is highly stable.
- The prepared samples are amorphous structure with some crystalline structure found due to percentage of Hydroxyapatites.

Though HAp has good biocompatibility it lacks the strength. The composites are found to complement each other and improve the required properties. This study indicates that, HAp, PLA and Cotton can be used as a bone replacement material. Therefore this is an attempt to bring out the ideas in this emerging field, to kindle the research in this line, to modify the composition of the composite and to explore the highly potential applications

### Recommendations for Future Research:

1. Cytotoxicity test can be done to analyze cytotoxicity effects.
2. Cell Culture test can be done to investigate acute biocompatibility of materials and solutions used in the composite synthesis for the prediction of toxicity potential.

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