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Synthesis And Characterization Of Hydroxyapatite-Chitosan Scaffold Films Obtained From Oceanic Sand Lobsters Shells

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Abstract- A species of sand lobster available at the Bay of Bengal in abundance was chosen for the bio mineral extraction. Therefore, our aim was to prepare a film through bio mineral extraction from the species, *then us orientalis*. The lobsters were collected from the shore and its hard portions were separated. Then the hard portion was dried under sun for few days till it had become completely dry. The same was ground into powder in a mixer and taken for Hydroxyapatite (HAp) product preparations. The obtained bio mineral, HAp powder was white and crystalline. The HAp powder was added to Chitosan solution and casted in Petri dish and obtained as film. The film was removed and kept in a sealed cover for further analyses. The HAp and Chitosan film was characterised for their chemical functional group analyses by FTIR. The thermal behaviour and thermal stability were evaluated by DSC and TGA respectively. The future works such as good film formation of the blend and incorporating them into 3D printing for making film shall be worked upon. Both the conventionally prepared film and by 3D Printed films properties will be compared and studied.

Keywords: Hydroxyapatite, Sand Lobster, Chitosan, film, scaffolds, bio mineral**.**

I. INTRODUCTION

Skin tissue rehabilitation is a field under regenerative medicine. When the skin is injured, body induces a series of events to repair the wound. Nevertheless, the chronic wounds are open sores due to delay in healing processes. The synthesis of extracellular matrix provides structure and biochemical support to skin cells plays a pivotal role in the wound healing process. The ECM is likely to contribute to the fast healing due to the regeneration processes. ECM mimics the body's natural healing processes and helps promote healing fast. Scaffolds are used in regenerative engineering. Scaffold structure is either fibrous or porous in their architecture. Bio functional polymers have unique mechanical properties that allow few materials to embed over it acting as a matrix for cell functions. Naturally available polymers are highly biocompatible and bioactive.

Biomaterials are either available naturally or fabricated synthetically. They have a response in regenerative medicine. Regenerative engineering uses biomaterial scaffold and allows both the cellular access and neovascularisation. There have been numerous fabrication methodologies for scaffolds aiming on optimizing its performance through matrix pores [1]. Scaffolds are used to regenerate the structure of tissues internally and are used sometimes for controlled release of agents in tissue engineering applications. There are various conventional methods of fabricating scaffolds for regenerative medicinal applications [2]. Bioactive polymers that are implanted for regenerative purposes holds the cells onto the matrix till they grow through the skeletal structure of the scaffold.

Synthetic polymers such as Polyesters, polyurethanes, polyacetals, polyamides, polyanhydrides, polyphosphazenes, and pseudo polyamino acids can form porous sheets, interwoven mesh, rigid scaffold blocks supporting enhanced cell growth as compared to their film pair [3]. In order to repair a wound, the scaffold releases signalling molecules such as growth factors to develop tissue growth and repair it. Injection of growth factors into the regeneration site will be ineffective because of instability of growth factor toward physical and chemical inactivation [4].

Research shows self-repairing tissues and they need higher calcium deposits, greater alkaline phosphatise activity and up regulation of marker. None of the bioactive polymeric scaffolds should impose any detrimental effects. The material functions as a carrier for targeted and controlled delivery for oestrogenic regeneration [5]. Scaffolds that deliver drugs for formulations and allow for controlled release of

compounds. Common polymers are used in drug releasing scaffolds and most common fabrication methods are employed in synthesizing the scaffolds. This polymer blends and releases drug to achieve better delivery rate [6].

Scaffolds with the bone marrow cells under different basal media were evaluated for various osteogenic and chondrogenic markers. The chondrogenic markers expressed scaffolds. Bioactive scaffolds were able to support the osteogenic and chondrogenic synthesis even in the absence of growth factors [7].

Degradable polymeric biomaterials are usually preferred for developing the therapeutic devices such as temporary prostheses, scaffolds for tissue engineering and as controlled release drug delivery vehicle. Each and every element involves physical, chemical, biological, biomechanical and degradation properties. Polymers usually degrade by hydrolytic or enzymatic route [8].

Metal ions released from metals react and form a composite with nanoparticles. This in turn forms reactive oxygen species due to electron-hole formation under certain wavelength. Biocompatible and biodegradable ZnO NPs have more therapeutic and cosmetic field's applications. Biopolymers like cellulose, Chitosan and alginate are the cost-effective scaffold for regenerative medicine.

Nan composites (NCs) increase antibacterial properties of wound-healing tissue scaffolds. Targeted drug release of metal ions conjugated with a physiological medium is a factor in the industrial fabrication [9]. Electrospinning technique is used to synthesize nanoscale fibres from polymers. Fibres are porous and provide larger surface to volume ratio. Integrating materials such as metallic nanoparticles or ceramics upon electro-spun fibres emerges a route to new nanoscale composite materials with enhanced functional properties. Nanoparticles on or within the nanofibrous scaffold provides many functional property in the tissue engineering [10]. Electro spun polymer made nanomaterial composites have relevance to tissue engineering, wound dressing and drug delivery.

A fibrous polymer blend made with the help of electros pinning is indulged in tissue regeneration applications. Polymers that use quite larger electric field to create a meshwork is applied in the regenerative medicine basically in the wound dressing applications [11]. The nucleation behaviors of electrospun nano fibers also find applications in tissue engineering [12]. A traditional food, tofu can be used as a natural scaffold as it has porous built-up and has proved biological safety. It is easy to be improved by surface modification. In addition, the collagen has been coated to enhance the surface compatibility of the edible material-based scaffold. It was having porous structure and cytocompatibility. Food based biomaterial is being a sustainable natural porous scaffold for regenerative medicine applications with excellent bioactivities [13].

In this study we are planning to use a technique to create 3D scaffolds made of Chitosan, a natural polysaccharide derived from the shells of crabs, and investigates their potential use in making matrix to load a drug and to apply to the patient's wounded part. 3D printing is a method to make polymers, ceramics, metals and composites. 3D printed objects are used in wider applications such as wound healing, cosmetics turbine blade, jewellery designing, mould making, building, tissue engineering. The 3D printed materials pose many advantages such as desired shape, pore size, mechanical properties, etc. 3D printing uses the technology and precise control of the fabrication process.

Tissue engineering plays an important role in research. An engineered scaffold should control the environment to create desired scaffolds with pre-designed shape, structure and function for tissue regeneration [16]. Among various methods, the technology has been considered as a novel method in fabricating tissue engineering scaffolds, as the printed structure could mimic the multiscale structure of human body tissue [17]. Many investigations have been performed to obtain 3D printed scaffolds for various applications.

II. EXPERIMENTAL

A. Materials and Methods

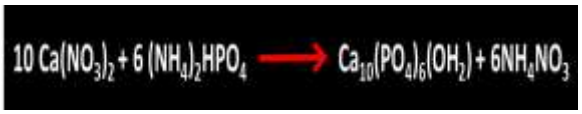
Sand-Lobsters were collected at the sea shore of North Chennai of Bay of Bengal. Nitric Acid, Di-ammonium hydrogen phosphate, Acetic acid and Chitosan (Low Molecular weight) were purchased from M/s Aldrich Chemical Company.

B. Preparation of Hydroxyapatite (HAp)

Hydroxyapatites (HA) have been explored considerably over the last many decades due to its potential Bio-medical and industrial applications. 50% of bone is made up of a modified form of the inorganic mineral Hydroxyapatite [13]. Moreover, recycling of waste material is widely assumed to mitigate resource scarcity, which decreases environmental pollution and minimize energy consumption. Nano structured Hydroxyapatite was extracted from *then us orientalis.* They were dried for 2 hours in Hot air oven. Dried shells were powdered using Mortar-Pestle. Grounded powder was kept in Muffle furnace for 3 hours. At 900°C, CaO Phase was formed by the following reaction:

Obtained CaO was treated with 25% Nitric Acid.

0.06 M of $(NH_4)_2HPO_4$ was added to the solution slowly and the result obtained amount of CaP ratio was 1.67. pH was maintained at 9 and the solution was filtered through Whitman 40 filter paper.



It was dried in a hot air oven and then calcinated at 900 °C for 3 hours to obtain Hap, which details are given in table 1.

C. Fabrication of Hydroxyapatite-Chitosan (CS-HA) film

A 1.2Wt% of HAp was dissolved in 20%V/V diluted acetic acid by stirring for 2 hours. Then 4.8Wt% aqueous solution of Chitosan was added to the solution. Combined mixture was kept under constant stirring till it became homogeneous. The solution was poured into a Petri plate and dried it in oven at 80°C till it became dry and formed as film (Figure. 1) [14].

Figure 1. Hydroxyapatite-Chitosan film

D. Fabrication of Hydroxyapatite-Sodium Alginate film

Hydroxyapatite and sodium alginate were taken in the ratio of 1:2 (i.e.,) 1g of HA and 2g of SA. Distilled water was poured into beaker with constant temperature. 1 gram of Hydroxyapatite was slowly mixed in 20 mL of water and stirred well for 2 hours. Alginate powder was put in solution and stirred for about 2 hours. Then the obtained mixture was dried at room temperature for 24 hours (figure. 2) [15].

Figure 2. Hydroxyapatite-Sodium Alginate film

III. RESULTS AND DISCUSSION

A. Evaluation of Hydroxyapatite (HAp) by FT-IR Spectral analyses

FTIR was effectively employed in detecting functional groups present in hydroxyapatite and characterizing their covalent bonding information. Figure 3 shows the FTIR characteristics spectra of HAp. The spectral graph showed typical characteristics absorption bands of HAp.

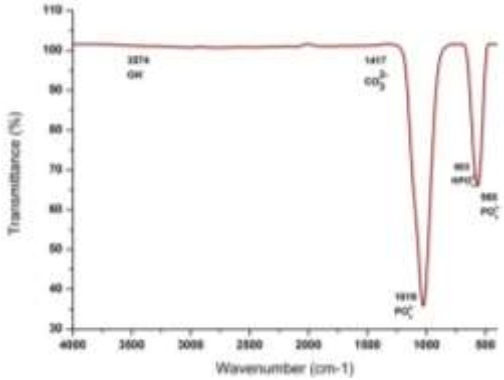


Figure 3. IR spectra of nano structure of hydroxyapatite

Table 1. Characteristics of Functional Group

FUNCTIONAL GROUP	CHARACTERISTICS	WAVE NUMBER
Po_4^{3-} (phosphate)	Asymmetric stretching Sharp intense peak	1019 cm^{-1}
Po_4^{3-} and HPo_4^{3}	Bending mode	603 cm^{-1} and 565 cm^{-1}
OH- (hydroxyl)	Presence of hydroxyl group Sharp peaks due to vibration mode	3574 cm^{-1}
CO_3^{2-} (carbonated apatite)	It is always used as a carbonate substitute for phosphate group	1417 cm^{-1}

B. Evaluation of Hydroxyapatite (HAp) by DSC analyses

DSC is used to measure the changes in enthalpy. It happens due to changes in the mechanical properties of a material with respect to temperature or time. Thermal behaviour of film was studied by DSC analysis. The DSC curves of HAp, CS and HAp-CS films are shown in figure 4.

The HAp shows glass transition temperature around 95°C which attributed to the thermal behaviour of the HAp particles. Chitosan polymer exhibited Tg temperature around 118°C was confirmed the Chitosan thermal behaviour of the polymer. The HAp-CS polymer film Tg temperature found to be around 109°C. The film showing greater than 100°C is confirms the thermal behaviour of the Chitosan as well (figure. 5).

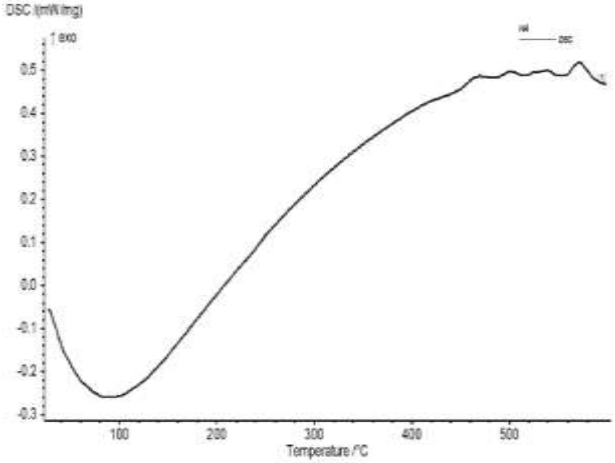


Figure 4. DSC analysis HAp

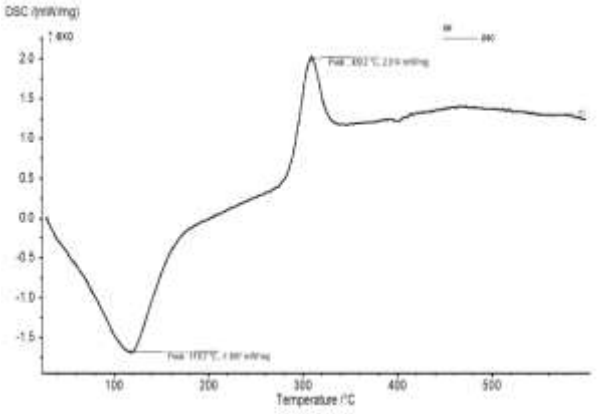


Figure 5. DSC analysis of Chitosan

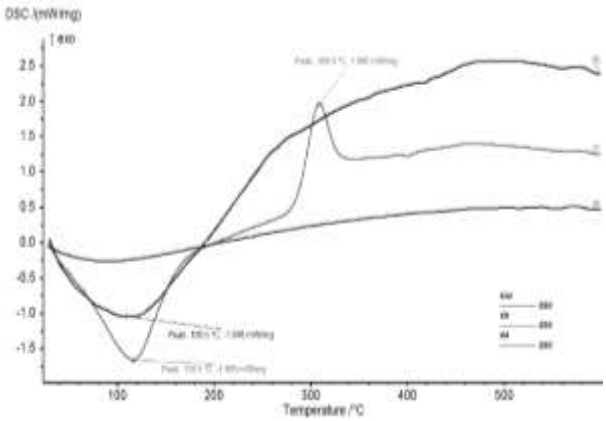


Figure 6. DSC analysis of HAp-CS film

C. Evaluation of Hydroxyapatite (HAp) by TGA analyses

Thermo gravimetric analysis was performed by TGA analyser with scan range from 50-900 degree Celsius at constant heating rate of 10 degrees Celsius minute with continuous nitrogen flow. The HAp mineral TGA curve shows a linear curve even beyond 500°C, exhibits its excellent thermal stability. There appears 5Wt% loss is due to the moisture present in the Hap, Figure 6. Shows the DSC analysis of HAp-CS film

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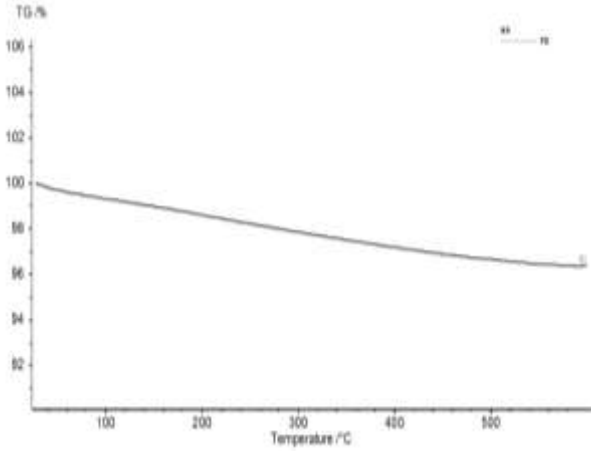

Figure 7. TGA analysis Hap

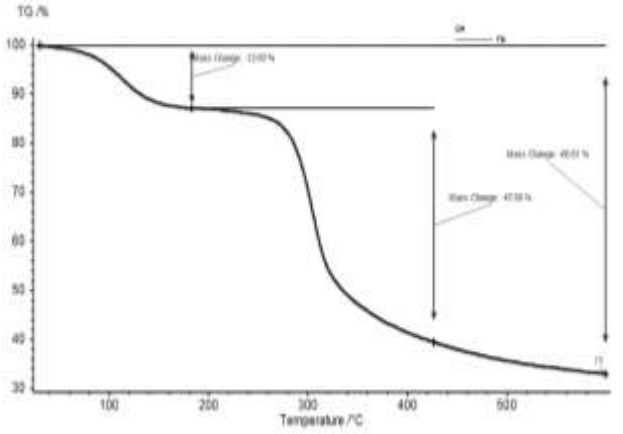

Figure 8. TGA analysis CS

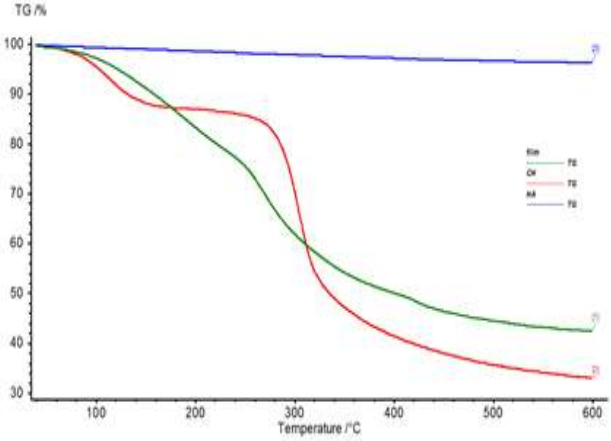

Figure 9. TGA analysis of HAp-CS film

The Chitosan polymer shows thermal stability up to 300°C. The thermal stability showing intermediate temperature between HAp and CS around 250 confirms the blended film formation. The film is showing 50% thermal stability up to 600°C Figure 7.shows the TGA analysis Hap.

D. Evaluations of Hydroxyapatite (Hap) – Sodium Alginate (SA) film by FT-IR Spectral analyses

FTIR was effectively employed in detecting functional groups present in hydroxyapatite and characterizing their covalent bonding information. Figure shows the FTIR spectra of Hap-SA film. The spectrum showed typical characteristics absorption bands of Hap-SA film Figure 8. Shows theTGA analysis CS. The FTIR spectral analysis, carried out for sodium alginate films prepared by keeping SA constant and HAp content was varied from 1, 3 and 5Wt% to the 100Wt% of SA. Thus, prepared HAp-alginate composite film spectral images are shown in Fig 10 respectively. The PO_4 Characteristic peak band stretching vibration occurred at 1025.62, 1025.53 & 1023.42 Cm^{-1} and the Carboxyl group present in the alginate shown as asymmetric peak at 1602, 1603 & 1605 Cm^{-1} were for the films of SA with 1, 3 & 5Wt% respectively. The –OH group pertained to the sodium alginate showed a broad peak at 3275, 3277 & 3290 Cm^{-1} were for the films of SA with 1, 3 & 5Wt% respectively. The bonding of SA with Hap was confirmed by the presence of a peak at 1409, 1410.55 &1410.52 Cm^{-1} occurred due to the bond between Calcium in HAp and Oxygen in the alginate thus confirmed the chemical bonding within in the composites Figure 9.shows the TGA analysis of HAp-CS film.

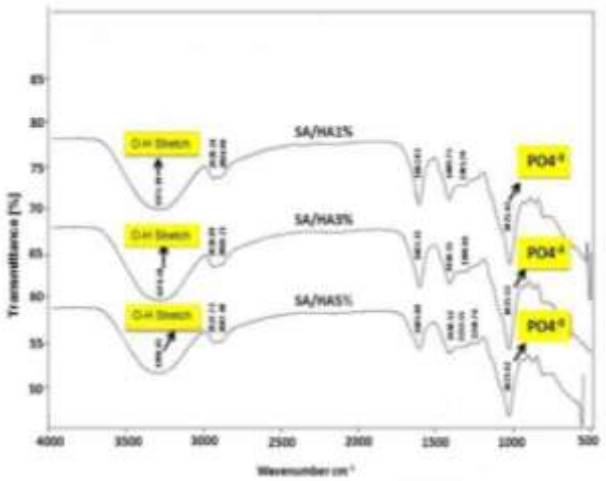

Figure 10. FT-IR analyses of HA-SA film

E. Evaluation of Sodium Alginate (SA) by DSC analyses

DSC is used to measure enthalpy changes due to changes in the physical and chemical properties of a material with respect to temperature or time. Thermal behaviour of film was studied by DSC analysis Figure 10.shows the FT-IR analyses of HA-SA film. The DSC curves of SA, and HAp-SA film shown figure 11. The glass transition (Tg) temperature of the SA and SA-HAp were 75°C and 120°C respectively. A 45°C increase in the Tg value of SA-HAp film when compared to SA alone, which attributed to the HAp incorporation in the film. The presence of HAp in SA has increased the Tg temperature. Hence, the SA-HAp film has service temperature up to 120°C which confirms good thermal stability of the HAp incorporated SA film.

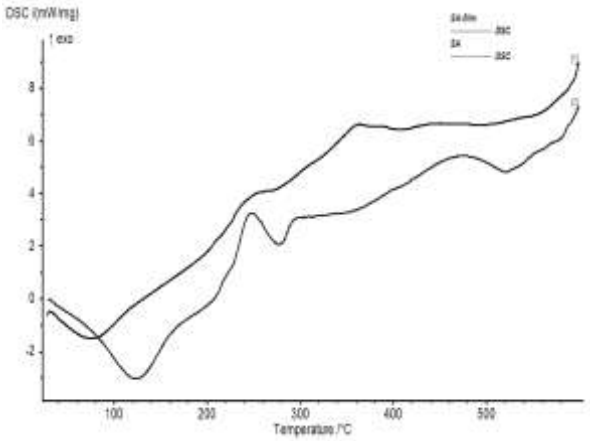

Figure 11. DSC of HA-SA film

Thermo gravimetric analysis was performed by TGA analyser with scan range from 50-900 degrees Celsius at constant heating rate of 10 degree Celsius minute with continuous nitrogen flow. The SA mineral TGA curve shows a linear curve even beyond 500°C, exhibits its excellent thermal stability. There appears 5Wt% loss is due to the moisture present in the SA.

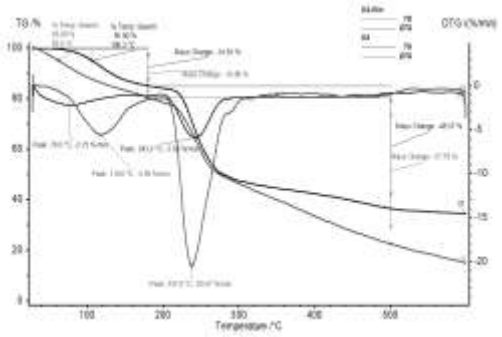

Figure 12. The Combined DSC and TGA analyses of SA

The TGA analysis shows two step degradation for both SA powder and as well SA-HAp Film. The degradation patterns are similar looks alike. There is a 30Wt% loss and 5Wt% loss before reaching 100°C in SA and SA-HAp film respectively. The huge quantity of loss observed in SA is due to the highly hygroscopic nature of SA when compared to 5Wt% moisture absorption observed in SA-HAp film which shows that the SA-HAp posses good moisture resistance property. There is a mass loss between seen for the both the samples around 200- 300°C. The SA loses 15Wt% and SA-HAp loses 20Wt%, the 15-20Wt% loss observed in both the sample owes to the Alginate chain scissoring and fragmentations. Beyond 300°C both the samples undergoes continuous degradation and nearly 50-60Wt% of the samples gets degraded. At the end of the heating, SA leaves 10Wt% loss and SA leaves 20Wt% and SA-HAp leaves 40Wt% residue, the high char residue and High thermal stability up to 243°C attributes to the excellent thermal stability of the SA-HAp film. The HAp has influenced to the raise in the thermal stability of the SA-HAp film, Figure 12.shows the Combined DSC and TGA analyses of SA

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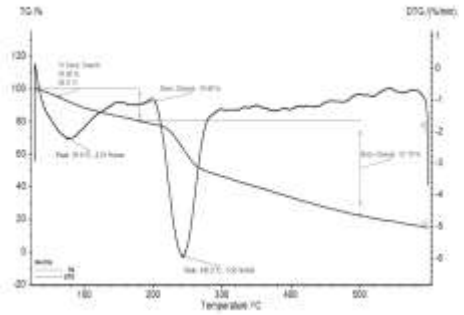

Figure 13. The Combined DSC and TGA analyses of HAp-SA film

F. Optical microscope analysis

To magnify images of small samples with visible light. Figure 5 shows, the well homogenised blends of HAp and CS is seen, The Crystallisation of HAp also witnessed as white flowers, Figure 13. Shows the Combined DSC and TGA analyses of HAp-SA film, Figure 14.shows the Optical microscopy of HAp-CS film

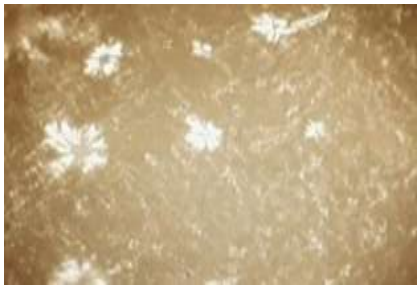

Figure 14. Optical microscopy of HAp-CS film

IV. Conclusion

Sea shell is widely used raw material for synthesis of hydroxyapatite. Our project observed a simple, inexpensive and biocompatible method of scaffolds synthesis. A novel chitosan with hydroxyapatite composite film was prepared by hydrothermal method of synthesis to imitate the function of extracellular matrix of the shell and thereby employed in various biomedical applications. Based on mechanical and biological properties of composite film it was observed that it has greater porosity, good thermal stability, uniform dispersion. The obtained SA-HAp will be compatible with the bone tissues and therefore find application as scaffold material for regeneration of tissue between the bone fractures. The future works such as good film formation of the blend and incorporating them into 3D printing for making film shall be worked upon. Both the conventionally prepared film and by 3D Printed films properties will be compared and studied. After subjecting it to series of tests to analyse the compatibility of the scaffolds, it shall be subjected to wound healing applications.

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