

## Synthesis of Composite of Hydroxyapatite using Chitosan Template

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### ABSTRACT

This work presents the synthesis of composite HA in chitosan polymer. The synthetic composite was investigated by using XRD, FTIR, SEM methods. The experiment for bio-compatibility was effectuated by the contacting of the extract solutions from synthetic composite and the cellular culture media. The obtained results confirmed the introduction of both HA and Chitosan in the structure of synthetic composite. The high values of cellular viability confirmed the good bio-compatibility of synthetic composite.

**Keywords:** Hydroxyapatite (HA), Mineral, Chitosan, Synthesis, Bone engineering.

### 1. Introduction

Hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  (HA) is a calcium phosphate biomedical material which is widely used as an artificial bone material in orthopedic surgery, bone grafts, dental fillings because this material is quite similar to inorganic mineral of natural bone in the human body. HA material is proven to be biocompatible because it is not mined waste when implanted in missing bone sites in the human body. Besides, HA material also exhibits biological activity due to dissolution by interaction with the environment, followed by the precipitation of ions such as  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$  in the environment to form a new HA layer on the surface.

New mineral HA serves as a bridge for the attachment of artificial material and natural bone, through which damaged bones are repaired and filled [1]-[2]. Chitosan  $(\text{C}_6\text{H}_{11}\text{O}_4\text{N})_n$ -a natural polymer which has biodegradable and biocompatible properties. Chitosan has a number of important applications such as a seed treatment agent and biopesticide; help plants fight fungal infections; use as a smoothing agent; a bacteriostatic agent; used in self-healing polyurethane coatings. In particular, in biomedicine, chitosan is usefully used in dressings to reduce bleeding and as an antibacterial agent; it can also be used to help deliver medication through the skin; using as a rapid healing agent in surgery due to its ability to stimulate the growth of osteoblasts around the implant site in the transplantation and surgery [3]-[4].

Hybrid materials combining bone material HA and chitosan polymer exhibit buoyant properties of each original individual material components of HA and chitosan. Combination of HA materials with polymer chitosan produces a composite that can easily to make different molds for bone pieces in surgical procedures. Moreover, the reaction medium containing chitosan with natural viscosity can control the distribution of HA material particles in the chitosan polymer matrix, creating a hybrid material system with porous properties while promoting the advantages of both starting materials [5]-[6].

This study is to synthesis of composite containing HA material in chitosan media. Initially characterizations such as morphology, properties of phase composition, and in vitro experiment were performed.

## 2. Experiment and method

### 2.1. Experiment

The HA/Chitosan composite was synthesized according the mass-composition ratio of 70/30 (HA/Chitosan). The synthesis protocols include several steps as below:

**Step 1:** Putting 6g of Chitosan into becher containing 500ml of acetic acid solution 1%, using a magnetic stirrer to stir the reaction mixture for 3 hours to obtain a transparent solution.

**Step 2:** Adding 14g of HA material to becher containing 5ml of 1% acetic acid solution, stir with a magnetic stirrer for 15 minutes to ensure that HA particles are evenly dispersed in the acetic acid solution.

**Step 3:** Placing the becher containing the chitosan solution under the magnetic stirring.

**Step 4:** Adding slowly the HA solution on the burette to the chitosan solution.

**Step 5:** Stirring well the mixture including HA and Chitosan for 15 minutes and then slowly add 5ml of  $\text{NH}_4\text{OH}$  into the reaction mixture to precipitate the product. After 15 minutes, the reaction mixture showed a resulting layer consisting of HA - Chitosan composite particles below and the upper solvent layer. When the above solvent layer was no longer viscous, the stirring process ends. To settle, then remove the solvent, we get the particles of composite HA - Chitosan.

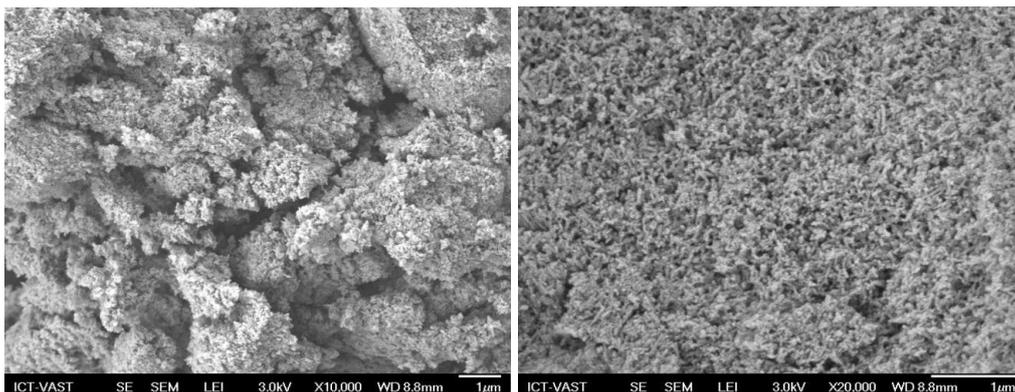
**Step 6:** Washing the precipitation several times with distilled water to neutralize the obtained composite particles. Then, the product was dried at  $50^\circ\text{C}$  for 24 hours. Finally, we obtained the HA/Chitosan composite in the form of dried powder.

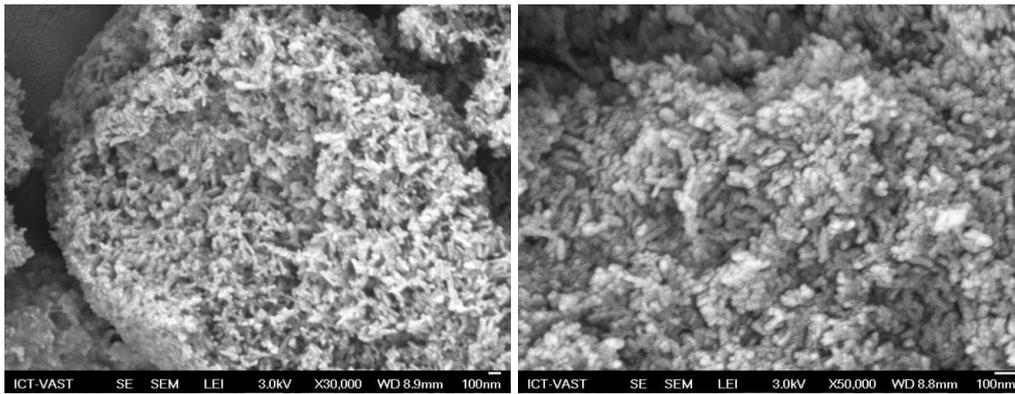
### 2.2. Characterized Method

Morphology of composite HA/Chitosan was investigated by using Scanning Electron Microscope (SEM) observation. The structural composition of resulting composite was analyzed by X-Ray diffraction (XRD) and Fourier Transformed Infra-Red (FTIR) spectroscopy. The cellular bio-compatibility of synthetic composite was tested in cell culture medium.

## 3. Results and discussion

### 3.1. Morphology analysis

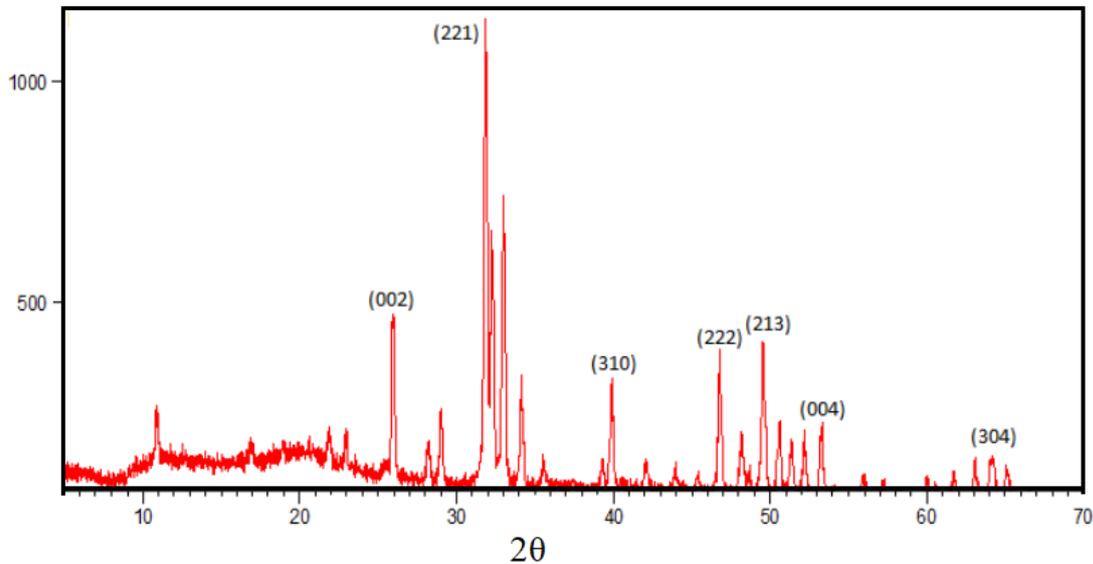




**Fig.1.** SEM observation of HA/Chitosan composite at different magnifications

Fig.1 presents SEM images of HA/Chitosan composite material at different magnifications of 10.000; 20.000; 30.000 and 50.000 times. It was observed that the HA particles were dispersed quite uniformly in the chitosan polymer matrix. HA particles are in the form of rods arranged alternately to create gaps and pores for synthetic composite material. Thus, chitosan polymer as a template to disperse HA particles to create porous composite material.

### 3.2. XRD analysis

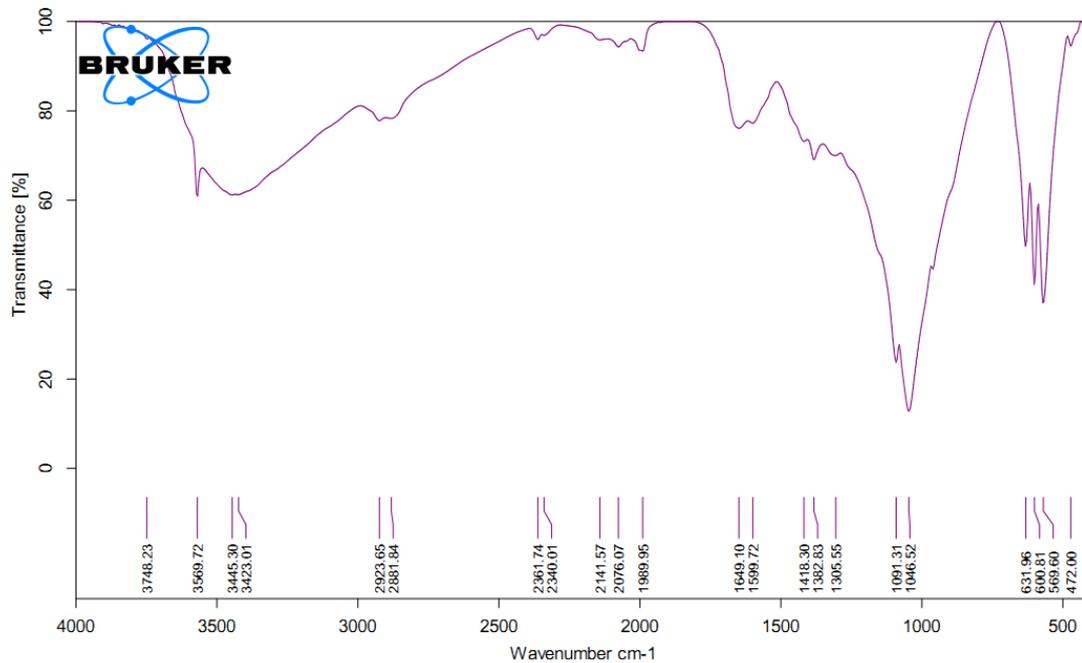


**Fig.2.** XRD diagram of composite HA/Chitosan

According to previous studies [7]-[8], Chitosan's X-ray diffraction pattern showed it to be a poorly crystalline material. The diffraction pattern shows only one broad peak at position  $19^\circ$  ( $2\theta$ ). XRD spectrum analysis of HA shows that this material is characterized as a lattice crystalline material with main characteristic peaks at  $26^\circ$  and  $32^\circ$  ( $2\theta$ ). XRD spectrum analysis of composite HA/Chitosan shows that the composite system has all the characteristic peaks of individual HA. At the same time, there is no shift in peak positions, indicating that the composite product formed between the two starting materials which does not react strongly enough to change their own structures. The resulting product is formed by the dispersion of HA in the chitosan polymer matrix. At the region of  $19^\circ$  ( $2\theta$ ), the composite can show the existence of chitosan in the appearance of a broad peak in low

intensity. Thus, the HA particles dispersed in the chitosan network to create the HA/Chitosan composite, but the HA particles still retain its structure and ensure the bioactivity and biocompatibility of this biomedical material.

### 3.3. FTIR analysis



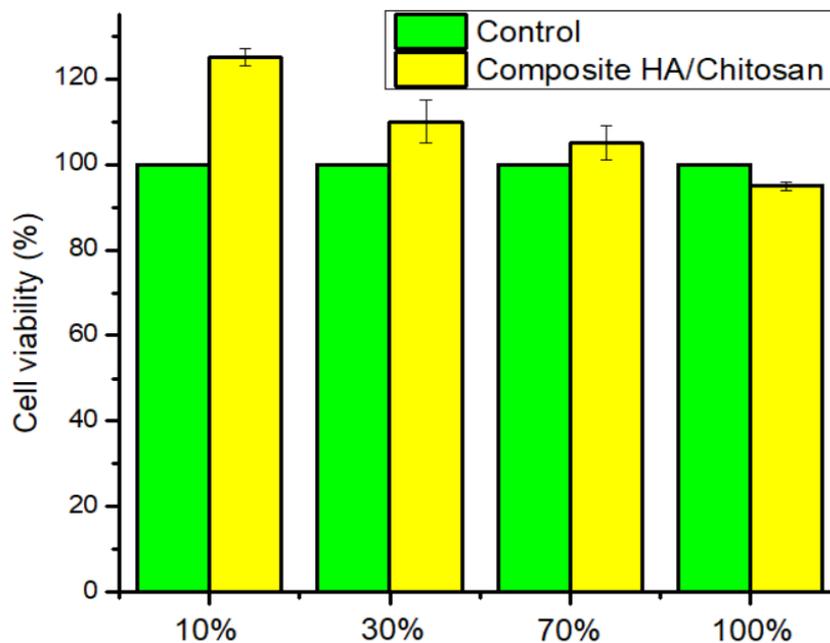
**Fig.3.** FTIR spectrum of composite HA/Chitosan

Fig.3 presents the FTIR spectrum of synthetic composite HA/Chitosan. The resulting product has spectral bands at the following characteristic wavelengths [7]-[8]. The absorption peak at 3423.01 cm<sup>-1</sup> corresponds to the fluctuation of -OH group in Chitosan. Absorption peak at 1649.10 cm<sup>-1</sup> is attributed to the fluctuation of -CONH<sub>2</sub> group in Chitosan. Absorption peak about 1599.72 cm<sup>-1</sup> is related to the fluctuation of -NH<sub>2</sub> group in Chitosan. The absorption peak of 1046.52 cm<sup>-1</sup> corresponds to the fluctuation of the C-O-C group in Chitosan. Absorption peak of 631.96 and 3569.72 cm<sup>-1</sup> corresponds to the fluctuation of -OH group in HA. Absorption peaks at 1091.31, 601 and 600.81 cm<sup>-1</sup> correspond to fluctuations of -PO<sub>4</sub><sup>3-</sup> group in HA. The absorption peaks of 1418.30 and 1599.72 cm<sup>-1</sup> are related to the fluctuations of -CO<sub>3</sub><sup>2-</sup> group in HA. From the analysis results, we can see that the FTIR spectrum of HA/Chitosan composite does not appear the peaks of CH<sub>3</sub>COOH and NH<sub>4</sub>OH, indicating that these substances have been completely removed in the precipitation during the washing process. At the same time, there is a full spectrum of bands characteristic of the original precursors. This confirms the contribution of both HA and Chitosan in the structure of the synthetic composite. On the other hand, some spectra of Chitosan are lost and some characteristic bands of HA are distorted in the spectrum of synthetic composite. This result shows that there is the interaction between the two initial precursors in the synthesis process. These interactions increase the cohesion of the two starting materials in the structure of the resulting composite.

### 3.4. Cellular bio-compatibility

The composite HA/Chitosan was verified for biocompatibility in cell culture medium. The cells (Eahy926) were directly contacted to extracts from composite powder at several concentrations during 24 h. The results of cell

viability are presented in the Fig.4. Cell viability without contacted to synthetic material was selected as a control (100%) [9]-[10].



**Fig.4.** Bio-compatibility of composite HA/Chitosan in contact with Eahy926 cell

The obtained results confirmed the non-toxicity of synthetic material in contacting with cellular media. The values of cell viability are high for all extracts. In detail, the cell viabilities are 122; 110; 104; and 98% for 10%; 30%; 70%; and 100% extracts, respectively. Experimental results confirmed that the composite HA/Chitosan exhibits a good biocompatibility with culture cells.

#### 4. Conclusion

A process has been built, and successfully using for synthesis of composite materials HA/Chitosan. XRD shows typical peaks for HA materials crystallized in chitosan matrix. SEM confirmed the dispersion of HA particles on the chitosan polymer. FTIR confirmed the functional groups of the two initial precursors in the structure of the synthetic composite. Test in cell culture medium confirmed the non-toxicity of synthetic composite.

#### Declarations

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##### *Consent for publication*

*Author declares that he/she consented for the publication of this research work.*

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