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# Synthesis of Pure Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) by the Sol –Gel Method and the Doxycycline Loaded in Presence of Gelatin for the Application of Drug Delivery

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**Keywords:** hydroxyapatite, sol gel process, PXRD, FT-IR, SEM, drug loading and releasing.

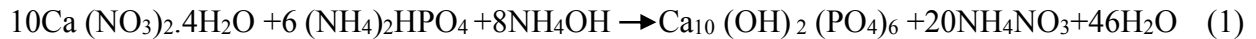
**ABSTRACT.** Hydroxyapatite (HAp) is the most widely accepted biomaterial for the repair and reconstruction of bone tissue defects. The current study is based on HAp was synthesized using sol - gel method. The drug was loaded in presence of gelatin with pure HAp. Precursors like calcium nitrate tetrahydrate and diammonium hydrogen orthophosphate were used and ammonia solution was added to maintain the pH value at 10.5 throughout the reaction. The synthesized HAp and drug loaded HAp with gelatin were characterized using PXRD, FTIR, SEM, Drug loading, drug release studies. Results show that the average crystallite size for prepared HAp and drug loaded HAp with polymer are ~ 30 to 300 nm respectively was calculated using PXRD and morphology of pure HAp and drug loaded HAp with polymer was found using SEM. Drug loading and release percentage was calculated. Keeping the above points in the present study was aimed to produce the biocompatibility and bioactivity of HAp.

**Introduction.** Hydroxyapatite (HAp) has been extensively investigated and used in bone clinical application for more than four decades. The increasing interest in HAp is due to its similar chemical composition to that of inorganic component of natural bone [1-2]. Doxycycline is most widely used for bacterial bone infections which are most frequently found in infected bone or a patient with osteomyelities. The present study describes the synthesis and characterization of DOX loaded hydroxyapatite nanoparticles in presence of gelatin intended to be used as drug delivery system. The associations of doxycycline with hydroxyapatite nanoparticles and the nature of interfacial process occurring as a result of coupling between doxycycline molecule and hydroxyapatite surface and the orientation of the DOX on the hydroxyapatite surface are also investigated[3]. The hydroxyapatite/gelatin combined is an ideal vehicle for the delivery of cells, proteins and drugs in the treatment of defective tissues and their regeneration. This kind of composite drug delivery system can release therapeutic molecules in situ to produce an action associated with the osteoconduction. It is used in the prevention and treatment of bone infections [4-5].

## Materials and Methods:

**Materials Preparation.** Hydroxyapatite is prepared by using sol-gel process. Calcium nitrate tetrahydrate and diammonium hydrogen orthophosphate were used as a precursor, for pure hydroxyapatite. The molar concentration of calcium nitrate tetrahydrate and diammonium hydrogen orthophosphate is adjusted to have a theoretical value of Ca/P ratio 1:67. The precipitation process is carried out by drop wise addition of diammonium hydrogen orthophosphate into calcium nitrate solution. The process is carried out under continuous stirring at 70°C for 4 hours and an ammonium hydroxide solution was used to adjust pH of 10.5. The white precipitate is formed and washed with

7-8 times with double distilled water and finally with ethanol and dried in a hot air oven till it gets dry.



**Drug loading.** In order to load drug on hydroxyapatite, doxycycline powder was dissolved in double distilled water. The concentration of DOX is kept constant (10mg/ml). HAp particles were added to the drug solutions at different ratios (1:1, 1:2, 1:4, 2:1, 4:1) and stirred using magnetically stirred for 60 min at 50°C. Then the solution was left undisturbed overnight. The suspension was then centrifuged (2500 rpm for 15 min) and the supernatant and precipitate were separated. The amount of drug loaded in HAp was determined by finding the difference in DOX concentration in the aqueous solution before and after loading. The percentage of drug loading is calculated using,

$$\% \text{ of drug loading} = \frac{A-B}{A} \times 100 \quad (2)$$

where  $A$  is initial concentration,  $B$  is final concentration

The maximum loading was found to be 87.41 % for 4:1 ratio.

**Doxycycline loaded HAp with gelatin.** Aqueous solution of 10% gelatin content was prepared using double distilled water at 37°C. After that the gelatin was dispersed in 200ml of vegetable oil in a beaker in order to produce emulsion. Then the DOX loaded HAp with gelatin in a beaker is made of continuous stirring at 200 rpm and repeat the same procedure as the above, then the amount of drug loading can be calculated using the above equation (2). The maximum loading was found to be 70.34%.

## Results and discussion

**Powder X-ray diffraction (PXRD).** PXRD pattern indicates that all the synthesized samples are composed of pure apatite phase. The sharp peaks confirm they were highly crystalline. Fig. 1 shows that the intensity of diffracted X-rays as a function of  $2\theta$ . The presence of characteristic HAp peaks is represented in the Fig. 1 confirms the hexagonal structure of HAp. PXRD spectrum of pure HAp (a), DOX loaded HAp (b), DOX loaded HAp with gelatin (c) has shown peaks characteristics of pure HAp and confirmed with JCPDS [3]. A comparison between powder X-ray diffraction patterns of a, b and c did not show any major differences in the diffraction patterns even after loading doxycycline is shown in Fig. The average crystallite size was determined using Scherer formula around 40 nm, 100 and 300 nm.

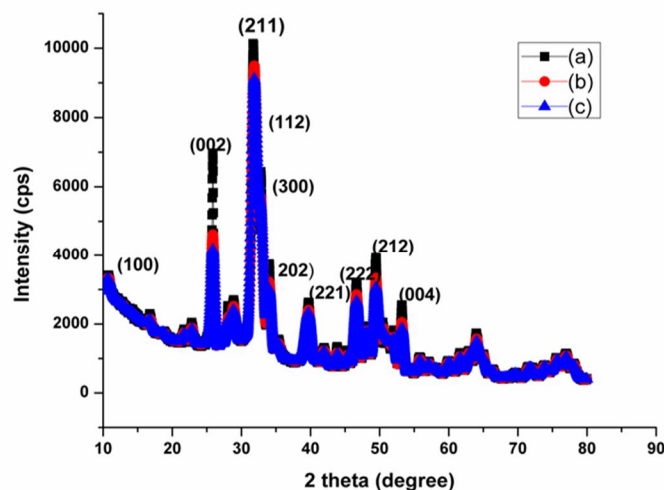


Fig. 1. PXR: a) Pure Hydroxyapatite b) DOX loaded hydroxyapatite c) DOX loaded HAP with gelatin.

**Fourier transform of infrared analysis of doxycycline loaded HAP with gelatin.** The spectra were recorded over the range of 500 - 4000 $\text{cm}^{-1}$ . The FTIR spectrum of pure HAP, doxycycline loaded with HAP, drug loaded HAP with gelatin is shown in Fig. 2 and the characteristic absorption peaks are shown in table 1. The drug loaded HAP showed peaks similar to pure HAP hence it confirmed that the drugs exist along with HAP [6].

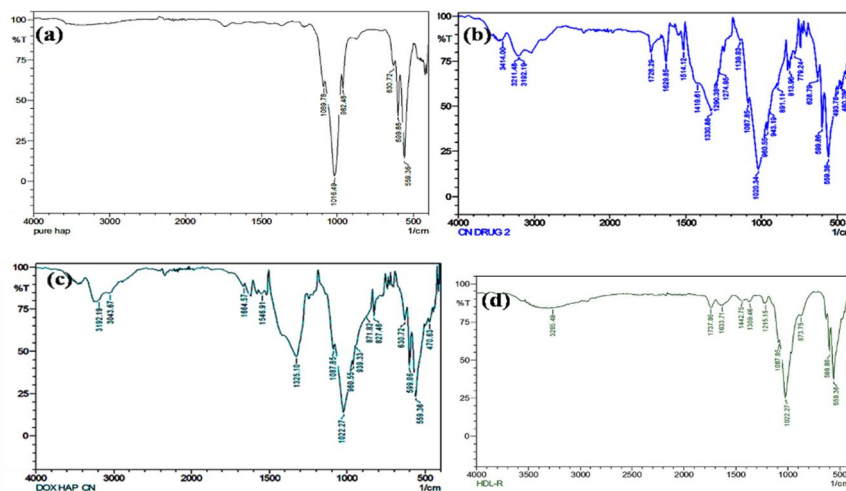


Fig. 2. FTIR: a) Pure Hap, b) DOX loaded hydroxyapatite, c) DOX loaded HAP with gelatin, d) after release.

**Drug release percentage.** The release profiles of doxycycline from DOX loaded with pure HAP, DOX loaded HAP with gelatin is shown in the Fig. 3. It is clear that DOX loaded with pure HAP shows initial burst release within 24 hours maximum of 45-50% of drug were released and most of the remaining drug was released during 150 hours with a slightly lower rate [8]. The fast release of DOX is due to the fact that the physical interaction between doxycycline and HAP is not that much stronger. The doxycycline release from HAP with gelatin shows that a maximum of 20% release during 24 hours and the remaining percentage of release was released during 150 hours and with these

two combinations the release rate of the drug is significantly slowed down hence it attains sustained release.

Table 1. IR characteristic absorption.

S.No	Peak position (cm <sup>-1</sup> )	Assignment
1	Around 3200	Absorbed peak of water (bending vibration of O-H (strong))
2	3414, 3535, 3484	N-H stretching
3	1633.71, 1629.95, 1664, 1633.71	N-H Bending
3	1737, 1726, 1753.2	C =O Stretching
4	1400-1600	C=C Stretching
4	1411, 19, 42	-C-H Bending vibration
5	1325, 11, 30, 69	-C-H bending vibration
6	1024, 1089, 1039, 1016, 962	Y <sub>3</sub> Vibration mode of phosphate group Y <sub>1</sub> vibration mode of phosphate group
7	630, 601, 599, 563	Y <sub>4</sub> bending mode of phosphate group

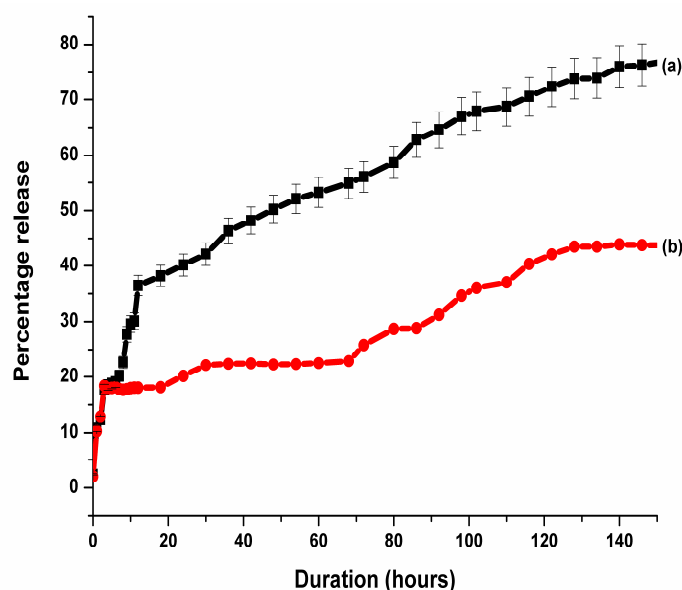
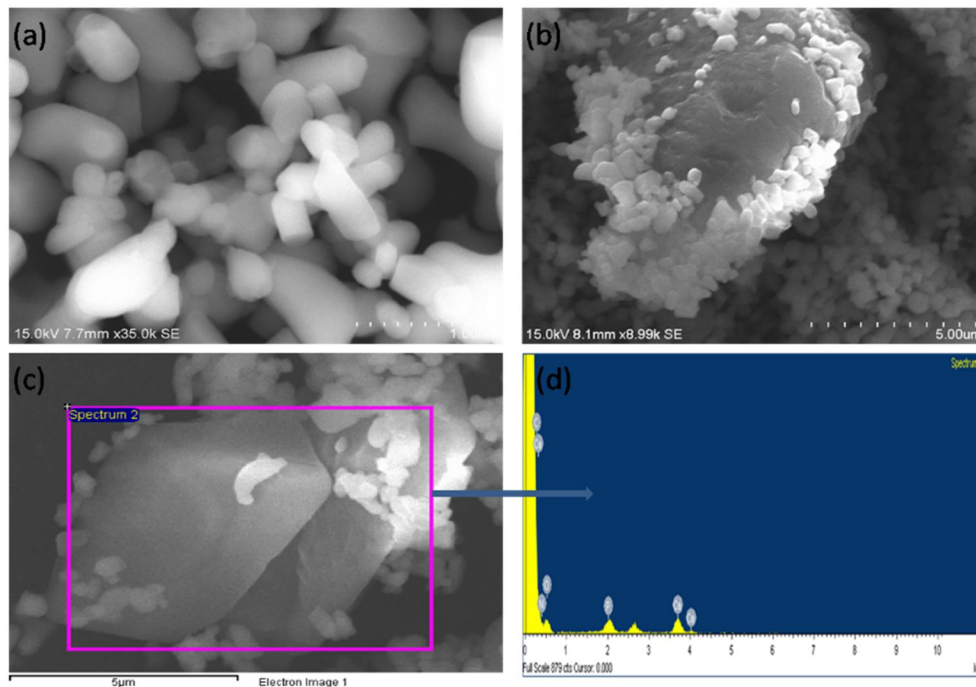


Fig. 3. Drug release: a) DOX loaded hydroxyapatite b) DOX loaded HAp with gelatin.

**Scanning Electron Microscope.** Fig. 3 a) shows the morphology of synthesised HAp and shows rod like morphology. b) shows that the drug loaded HAp shows some morphological changes when compared to that of pure HAp. It is found that drug loaded HAp particles are aggregated into large clusters and also formed of separate units gathered into a whole which consists of aggregated beads of approximately 100nm to 300nm. Fig. 3c shows that the drug loaded HAp with gelatin shows that the drug loaded HAp is coated with gelatin [7] hence the pores are filled with drug particles.



*Fig. 4. SEM: a) Pure hydroxyapatite, b) DOX loaded hydroxyapatite, c) DOX loaded HAp with gelatin.*

**Summary.** HAp synthesized by sol-gel method and the synthesized HAp, drug loaded HAp, drug loaded HAp with gelatin were characterized using PXRD, FTIR, SEM, Drug loading, drug releasing. Results show that the prepared HAp and drug loaded HAp with polymer are ~ 30 to 300 nm respectively and morphology of pure HAp and drug loaded HAp with polymer was found using SEM. Drug loading and release % were calculated as 87.41 %, 75.73% and 76.22%, 44.23% respectively and in-vitro drug release of doxycycline from these methods has done for the period of 150 hours. The release percentage shows slow and sustained for these days.

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