Synthesis of Hydroxyapatite-Nanorods with the Effect of Non-Ionic Surfactant as a Drug Carrier for the Treatment of Bone Infections

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Abstract— **Hydroxyapatite (HAP) was synthesized using wet chemical method through surfactant assisted route. Microstructural features such as size and morphology of the resulting HAP were studied using Field Emission Scanning Electron Spectroscopic (FESEM) analysis. The physical properties of HAP were investigated by using X-Ray diffraction (XRD), Fourier Transform Infra-Red Spectroscopy (FTIR). The HAP with better microstructural features was used for loading an antibiotic, Ciprofloxacin (CPF). The CPF, loaded HAP (HAC), exhibits a bacterial growth hindrance against a staphylococcus aureus (S aureus) and Escherichia coli (E coli) compared to a pure HAP. Drug loaded HAC was subjected to an antibacterial susceptibility test and antibacterial efficacy test. The results were corroborated to understand the change in the microstructure towards the usefulness of drug carrier properties and subsequently avoiding bacterial infections on the implant**.
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Keywords-; Hydroxyapatite;Brij-35; ciproflaxin; Nanorods

I. Introduction

Bone is composed of organic collagen fibrils (20 wt%),crystalline inorganic phase (69%),and water(9%).The inorganic component is formed from carbonated hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) with lower crystallinity. Hydroxyapatite (calcium phosphate) is a biomaterial comes under the category bioceramics and has an excellent biocompatibility and bioactivity. Clinical and biological studies of this material give a 3D interconnected structure porous structure, which provide a pathway for biofluids [1-5].

Conventional drug delivery systems like tablets, injections, suspensions, ointments, which deals with a rapid release of a drug, and do not regulate the targeted area of the drugs. The overall drug consumption and side effects can be reduced by deliver an active ingredient to the targeted region [9, 10]. Therefore there is a need for targeted drug delivery system, in order to achieve low toxicity, bioavailability.

Hydroxyapatite have been widely used in delivery systems for genes, proteins and various drugs due to their nontoxicity as have been experimentally proven by recent reports [6-8]. There are so many methods to synthesis a nanosized HAP. They are micro emulsion method, chemical precipitation, hydrothermal method, ultrasonic irradiation, microwave irradiation and surfactant assisted routes [9]. Among the various methods, chemical precipitation method is found to have a high phase purity and targeted morphology[10]. Although, surfactant assisted method gives a hydroxyapatite in a nano dimensions [11].

The severe complications in orthopaedic surgery and traumatology are osteomyelitis, osteitis and septic arthritis. Treatment for above complications is deals with a implant. Due to the presence of adherent micro-organisms, biofilm is formed on the surface of the implant. This biofilm is difficult to remove. Therefore antibiotic loaded implant is needed, in order to avoid the above complications.

Therefore in this study, we focus on the synthesis of HAP using surfactant and to load the ciprofloxacin, an antibiotic drug in the synthesized HAP. Also characterisation of both HAP and drug loaded HAP is studied.

II.EXPERIMENTAL SECTION

II.I . METHODS

1. Chemicals and Materials

Reagent grade calcium nitrate tetra hydrate ($Ca(NO_3)_2.4H_2O$), ammonium dihydrogen ortho phosphate, liquid Ammonia, Brij 35(Polyoxyethylene lauryl ether),and Ciprofloxacin Hydrochloride, ethanol from Aldrich chemicals were used.

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2. Synthesis of HAP

Synthesis of Hydroxyapatite by direct precipitation method involves solutions of 1M calcium nitrate and 0.67M of NH_4H_2PO. Both solutions were maintained at a pH of 11.0 using aq.ammonia solution. Different quantities of Brij-35 were used as a surfactant. The Brij-35 was added to the 1M $Ca(NO_3)_2.4H_2O$, and the solution was continuously stirred for 1 hour. Drop wise addition of 0.67 M of $NH_4H_2PO_4$ at PH 11.0 to the above solution. After addition of $NH_4H_2PO_4$,. the aqueous solution was continuously stirred for about 2 hours. The white precipitate obtained was then collected by centrifugation and washed repeatedly with distilled water. The product was dried in an oven at 80ºC for 24 hr. The HAP powders thus obtained were ground with a mortar and pestle. Finally, the HAP powders were calcined at 800ºC in a conventional furnace under an air atmosphere for 2 hr.

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3. Drug loading

A700 mg of HAP with suitable morphology (sintered HAP obtained with 25 ml 0f Briji-35) is mixed with 300 mg of CPF, hereafter these powders will be referred as a, HAC300. This mixture powder was continuously stirred for 24 hrs by the using, ethanol as a solvent. The mixture was then dried, the drug loaded powder is used further studies**[13].**

III CHARACTERIZATION

Phase analysis was performed using BRUKER D8 advance X-ray Diffractometer (XRD).Synthesized HAP powder were characterized using XRD to determine the fraction of crystallinity, crystallite size, specific surface area. The crystallite size of the sample was calculated from the Scherrer's equation [12],

$$Xs = 0.9\lambda / \beta\cos\theta$$

Where, λ is the wavelength CuKα radiation source (λ=1.54A°)
β is the full width half maximum,
θ is the angle of diffraction.
Schimadzu FT-IR 8300 series were recorded for HAP,The samples were scanned at the range of 4000 to 400 cm-1 by KBr pellet technique, in order to ensure the various functional group frequencies and chemical structure. FTIR spectra also recorded for pure drug (CPF) and compared the result with the Indian pharmacopeia spectra. In addition, FTIR spectra for HAC also taken, in order to ensure the drug interaction in HAP. The morphology of the sample was investigated using FESEM HITACHI SU6600 analysis.

III.RESULTS AND DISCUSSION

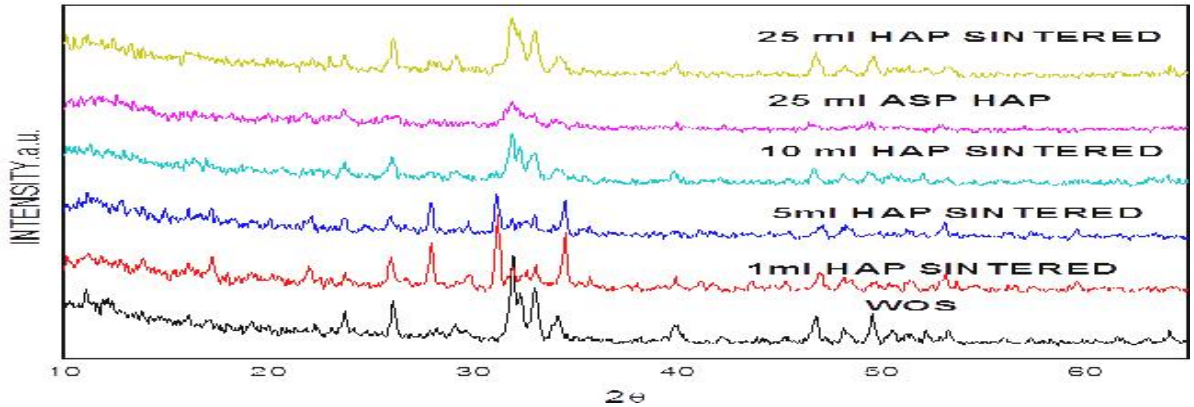

FIGURE.3 1. XRD PATTERN OF HAP SAMPLES.

XRD pattern for the HAP samples were shown in Fig 3.1. All the samples were sintered for phase change and to remove the residual non-ionic surfactant. The 2θ peaks from 31 to 31.9° were observed, which corresponds to the peak of hydroxyapatite. The HAP prepared without surfactant peak was found to deviate slightly from the standard one, although it refers to the hydroxyapatite with "d" spacing value of 2.71. All samples were in typical hydroxyapatite structure and matched with the JCPDS card no. 09-0432. The peaks observed with all samples were in good agreement of crystallinity except as prepared sample prepared with 25 ml of surfactant. The decrease in crystallinity may be due to the masking effect of the nonionic surfactant. Crystallite size of every sample were calculated using Scherer's eqn. for the 2θ peak (211) :

$$D = K\lambda/\beta \cos\theta$$

However, the crystallite structure of n-HAP prepared with and without surfactants were well looked into and compared with ICDD VALUES. The diffraction peaks at 2θ = 25.9, 28, 29.1, 31.76, 32.9, 34.1, 39.8, 46.73, 49.5, 50.5, 53.25, 61.59 and 64.07 are designated as (002), (102), (211), (112), (300), (202), (310), (222), (213), (321), (004), (214), and (304) plane of HAP respectively [**14**].

There was no new peak at 2θ corresponding to surfactant after sintering the samples. Only, the intensity peaks referring to HAP and crystallite size decreases as a function of increase in surfactant quantity. The synthesized sample showed similar peak to that of natural bone.

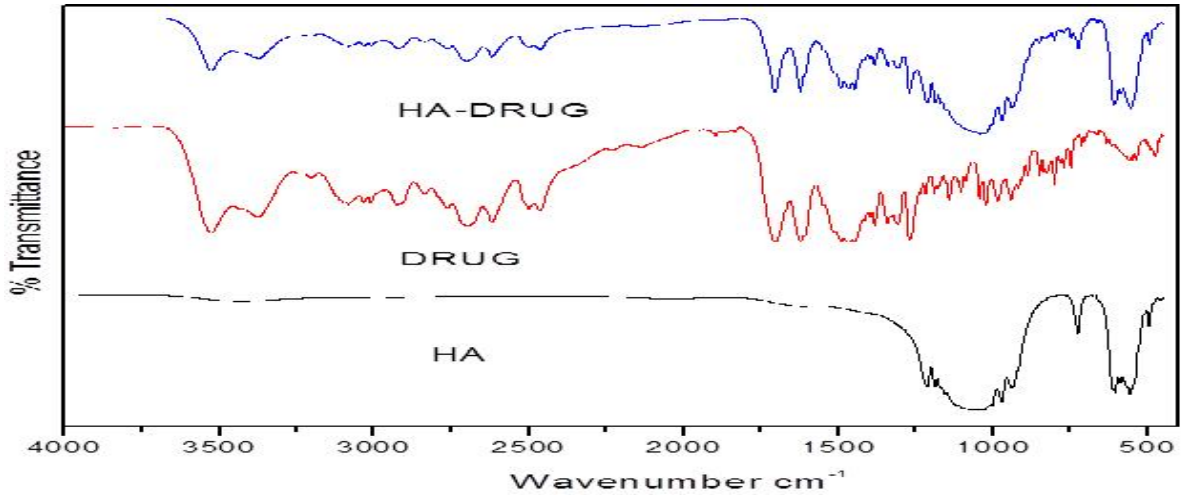

FIGURE 3.2. FTIR PATTERN OF HAP, DRUG, HAC.

The FTIR spectra for HAP and Drug-HAP samples were shown in fig.3.2. The intense peak observed at 3572 $cm^{-1}$and weak peak at 632cm^{-1} belonging to stretching (υ_s) and vibrational (υ_L) modes of hydroxyl anions of HAP crystals. The broad peaks occurring approximately at 3412 cm^{-1} and 1629 cm^{-1} will be assigned to adsorbed peak of water within Hydroxyapatite **[15]** . The weak band detected at about 1466 cm^{-1}, indicates that there is a partial substitution of CO_3^{2-} for PO_4^{3-}. The band occur at 1039 cm^{-1} is due to the triply degenerate (υ_3)

antisymmetric P-O stretching mode. Band at 963 cm^{-1} is assigned to non-generate symmetric P-O bond stretching **[15]**. The peaks arised at 601$cm^{-1}$and 566 cm^{-1} referring the triply degenerate (υ_4) O-P-O bending mode. The peak at 468cm^{-1} is due to doubly degenerate (υ_2),O-P-O bending mode. The drug loaded HA showed peaks similar to pure HAP and pure drug and hence, it confirmed that the drug exist along with HAP.

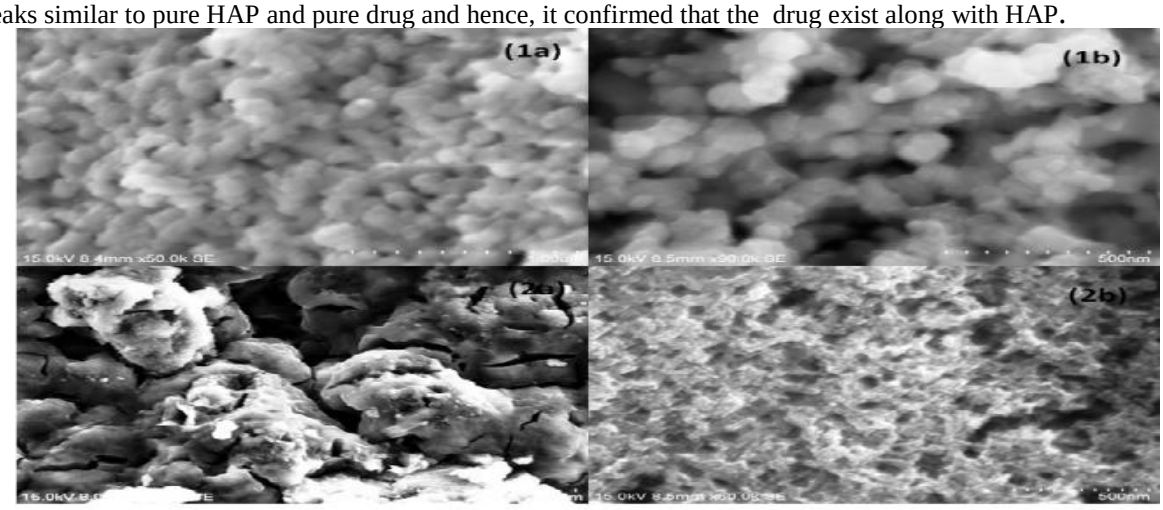

Fig.(3) FESEM FOR THE SAMPLES (1a) Without Surfactant Asp (1b) Without Surfactant Sintered (2a) With Surfactant Asp and With Surfactant Sintered HAP

FIGURE 3.3. FESEM IMAGES PATTERN OF HAP.

The Figure (3.3.1a) refers to FESEM micrographs of as-prepared samples using no surfactant, which revealed highly agglomerated HAP particles. The sintered sample prepared without surfactant, showed a morphology change to give a discrete particle (Fig 3.3.1b) with very little agglomeration. The (Fig 3.3.2a) refers to the image of as prepared samples obtained with 25 ml of Briji-35, it shows template assisted form , whereas sintered sample with the same synthetic condition yielded, particles as Nano rods in the (Fig 3.3.2b)

ANTIMICROBIAL STUDIES

HAP and HAC were subjected to antimicrobial studies with gram positive and gram negative bacteria.Antibiotic loaded HAP shows more inhibition zone than the HAP without antibiotic load

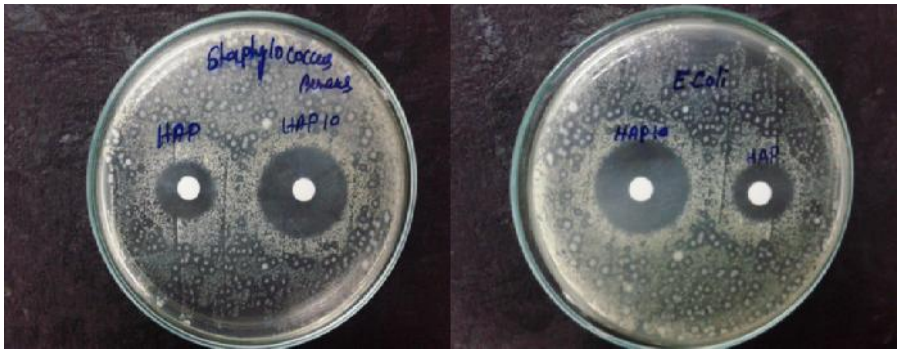

FIGURE 3.4.ANTIMICROBIAL STUDIES OF HAP, HAC.

CONCLUSION

Nano sized HAP was prepared using Brij35 as a surfactant by direct wet chemical synthesis method. Functional group analysis revealed the formation of pure HAP. The information on crystallinity and the phase transformation was availed with XRD analysis. The antibiotic drug was successfully loaded in a HAP matrix. A change in morphological feature was observed form agglomerated HAP particle to discrete particles and finally nano-HAP rods as the condition changes. The results of anti bacterial efficacy test, intro drug release will be under investigation.

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