Preparation and characterisation of fluorapatite by modified wet chemical method

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Abstract

Fluorapatite (FA, $Ca_{10}(PO_4)_6F_2$) has potential applications in dentistry and orthopedics. FA nanopowder was synthesized by modified wet-chemical precipitation method. Sodium fluoride was added to the hydroxyapatite preparation for the synthesis of fluorapatite. The synthesized samples were characterized by using FTIR, XRD, SEM, EDX, TEM, and *in vitro* antimicrobial activity. From the size and morphology analysis it is confirmed that the synthesized sample is FA nanopowder and from the *in vitro* activity it is confirmed that the FA nanopowder can be used for biomedical applications.

Keywords: Fluorapatite; wet chemical method; XRD; TEM; in vitro antimicrobial activity

I. INTRODUCTION

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6OH_2$) belongs to the calcium phosphate (CaP) family and is the main mineral component of human bone and teeth [1]. The application of calcium phosphates, especially HAp, for bone regeneration is known for a hundred years, but only last fifteen years CaP have been studied extensively as promoters of tooth enamel and dentine remineralisation. Since 96-97% of tooth enamel consists of mineral phase, mainly HAp [2, 3], and fluorine is known for its anti-caries effect [4], several scientific papers [5-7] are devoted to synthesis and characterization of F-doped hydroxyapatite (FHAp, $Ca_{10}(PO_4)_6OH_{2-x}F_{2x}$). FHAp is of high interest due to its bioactivity, mechanical properties and chemical similarity to teeth [4]. The structural and chemical stability of FHAp against chemical and physical factors are the main properties promising for remineralisation of tooth enamel. As reported in [8], too high intake of fluorine can lead to bone and teeth diseases like osteomalacia and dental fluorosis. Therefore it is necessary to control F^- content in the HAp structure and also release afterwards. Once the OH groups were partially substituted by the F^- ions, the existing hydrogen ions of the OH groups were bound to the nearby F ions because of the higher affinity of the F ions with respect to the oxygen ions, producing a quite well-ordered apatite structure, which caused an increase of the thermal and chemical stabilities of the HA matrix. Therefore, when a certain amount of F ions substituted the OH groups in the HA matrix, a certain level of chemical and thermal stability of the FHA ceramics was achieved. Theoretically, a F ion concentration of 50% in the FHA should be enough to remove the disorder of the crystal structure of HA and hence stabilize the structure F due to the alternating arrangement of the F ions between each pair of OH groups.[9] In this research, synthesis of fluorapatite nanopowder via modified wet-chemical method by using sodium fluoride and the resulting FA powder was characterized by instrumentation techniques

II.EXPERIMENTAL PROCEDURE

2.1 Synthesis of FA nanopowder

Modified wet chemical method was used to prepare the pure FA sample. The precursors for synthesis were calcium hydroxide ($Ca(OH)_2$)(Merck,India), ammonium dihydrogen phosphate $(NH_4)H_2PO_4$ (Merck, India)(ADP) and sodium fluoride (NaF) (Sigma Aldrich, India). 1.0M calcium hydroxide and 0.67M ammonium dihydrogen phosphate were dissolved in 100ml of double distilled water for 1hour in magnetic stir. The dissolved solution of ADP was added drop wise to the ($Ca(OH)_2$) solution for 35mins and continuously stirred for 18hours. The pH of the solution was maintained in 11. The obtained slurry was irradiated to microwave radiation for 30mins and the dried powder was grinded well by using mortar and pestle.

2.2 Characterisation

The size, morphology and *in vitro* activity for the synthesized FA nanopowder were characterized by using Fourier transmission infrared spectroscopy (FTIR), X-Ray diffraction(XRD), Scanning electron microscope (SEM), Energy-dispersive X-Ray spectroscopy (EDX), Transmission electron microscope (TEM) and *in vitro* antibacterial activity.

III. RESULTS AND DISSCUSSION

3.1 FTIR analysis

Figure 1.shows the FT-IR spectrum of FA nanopowder. The characteristic bands exhibited in the range 400cm^{-1} to 4000cm^{-1} are assigned here: a) the bands at 1095.47 cm^{-1} and 1044.69 cm^{-1} arise from ν_3 PO_4^{-3} , the bands at 604 cm^{-1} and 566 cm^{-1} arises from ν_4 PO_4^{-3} , the band at 473.4 cm^{-1} arises from ν_2 PO_4^{-3} (b) the bands at 1420.24 cm^{-1} and 1456.69 cm^{-1} arises from CO_3^{-2} (c) the band at 3438.56 cm^{-1} arises due to OH….F bond.

3.2 XRD analysis

The XRD analysis was performed using the X-Ray diffractrometer. Figure 2.shows the XRD spectrum of the synthesized FA nanopowder. The pattern matches the FA (JCPDS PDF#15-0876) data indicating that the synthesized were FA. The average crystalline size of the synthesized powder was calculated by using Debye Scherer equation. It is observed that the value of average crystallite size calculated from the reflection of the planes: (002),(211), (300) and (202) are 43.31nm,35.12nm, 22.02nm and 29.44nm.

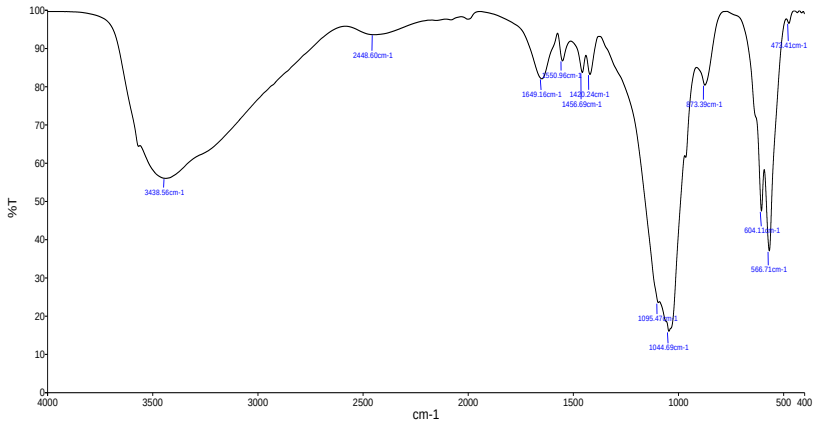

Fig 1. FTIR spectrum of FA nanopowder

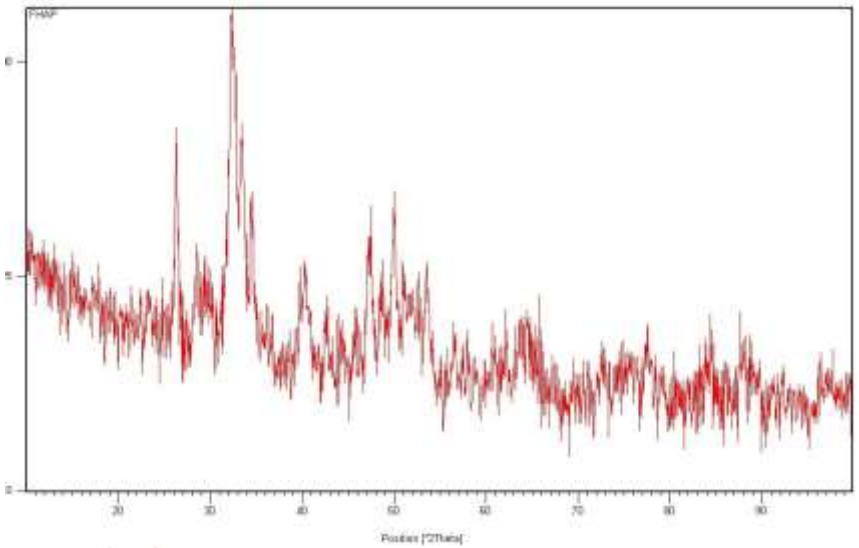

Fig 2. XRD spectrum of FA nanopowder

3.3 SEM and EDX analysis

The SEM analysis was observed using VEGAS TESCAN SEM instrument. The morphology of the synthesized FA nanopowder was shown in figure 3. The elemental composition of the FA nanopowder is observed in the figure 4. The chemical composition of the synthesized FA Nano powder is Ca, P, Na, F and O.

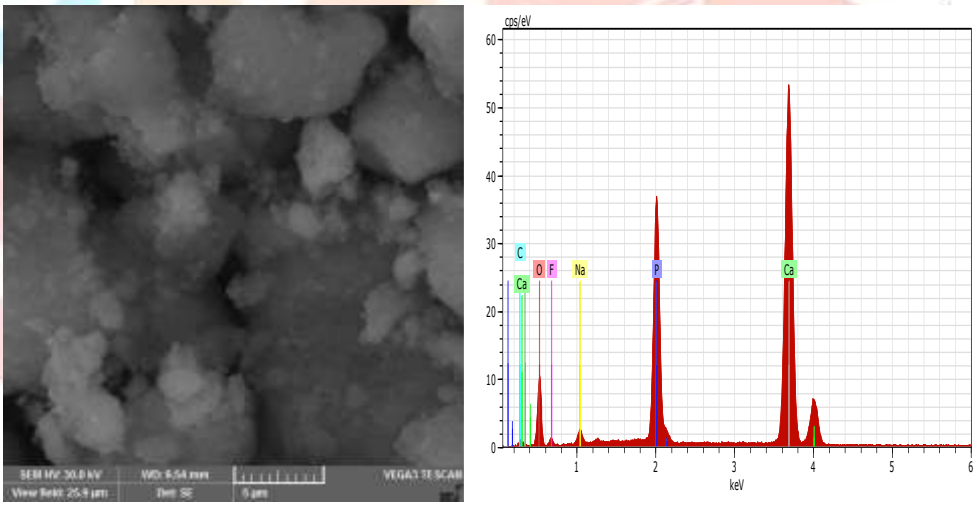

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Fig 3 SEM and EDX spectrum of FA nanopowder

3.4 TEM analysis

The size and morphology of the fine powder may be determined with TEM. The bright field transmission electron microscopic images of FA are shown in figure 4. The FA nanoparticles are rod like morphology and do not possess regularity in shape due to the porous surface. The particles show a high tendency towards agglomeration.

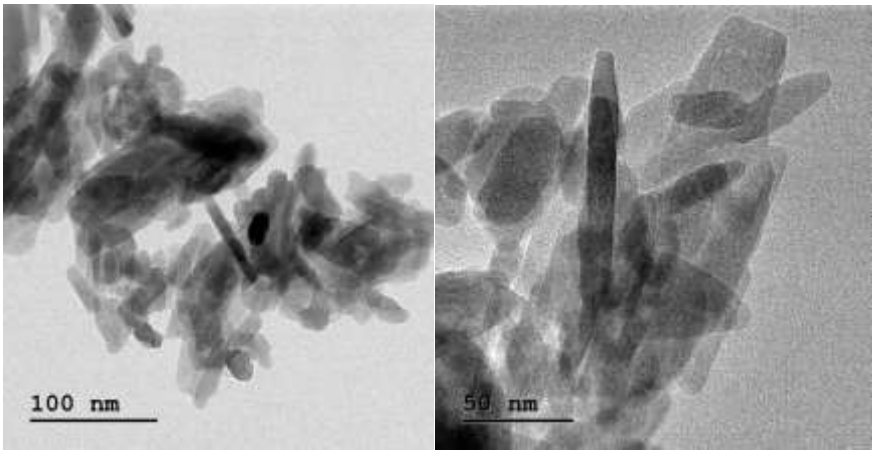

Fig 4. TEM images of synthesized FA nanopowder

3.5. In vitro anti microbial activity

The antimicrobial activity was performed by disc diffusion method.

Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007) using sample. Petri plates were prepared by pouring 30 ml of NA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Staphylococcus aureus* and *Escherichia coli* specie of bacteria were spread on Nutrient agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Antimicrobial activity

	Escherichia coli		*Staphylococcus aureus*		
Microorganisms	(50µl)	(100µl)	(150µl)	Standard (30µl)	Control (solvent) (30µl)
Bacteria					
Escherichia coli (mm)	0.90±0.06	3.10±0.21	5.90±0.41	8.70±0.60	0.20±0.01
Staphylococcus aureus (mm)	0.50±0.03	2.70±0.18	5.30±0.37	8.50±0.59	0.10±0.01

Table 1. Antimicrobial activity of FA nanopowder

IV. CONCLUSION

Fluorapatite nanopowder was synthesized successfully by using modified wet chemical method by using sodium fluoride in hydroxyapatite suspension. The synthesized FA powder was characterized by FTIR, XRD, SEM, EDX, TEM and *in vitro* antimicrobial activity. The FTIR analysis reveals that the characteristics band corresponding to the FA appears in the synthesized sample. The disappearance of the hydroxyl vibration band in the FTIR spectrum confirms the fluorine substitution in the HA. The XRD analysis reveals that the particles of the FA nanopowder are of nano size and homogeneous in composition. The EDX spectra confirm the presence of chemical composition of the FA sample. The morphological evaluation shows that the morphology is rod like and agglomerated. The *in vitro* antimicrobial activity was done by disc method by using gram positive and gram negative bacteria like E.coli and S.aureus. From the *in vitro* studies it is confirmed that the synthesized FA nanopowder has antimicrobial property and the sample can be used for biomedical applications.

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