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Synthesis And Characterization Of Zinc Doped Fluorapatite Nanoparticles Using Modified Wet Chemical Method

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Abstract — *Fluorapatite was doped with zinc oxide to investigate the possibility of enhancing its applications in the field of dentistry, drug delivery and bio materials. FA and Zn-FA nanoparticles were synthesized using modified wet chemical precipitation method and their structure and phase, morphology, elemental composition, thermal stability and in vitro assessment were studied using X-ray diffraction (XRD), Fourier transform infrared spectroscopy(FTIR), Transmission electron microscopy(TEM), Energy dispersive X-ray spectroscopy(EDX), Thermogravimetric analysis and in vitro antibacterial activity and anti-inflammatory activity. The XRD diffractogram confirms the presence of FA and Zn and their average crystallite size were calculated by using debye scherrer's formula and it ranges from 20nm to 31nm. The rod like morphology of the synthesized samples was confirmed using TEM. The chemical bonding was confirmed by FTIR. The TGA/DTA was carried out from 25ºc to 1200 ºc and analysed the change in composition and thermal stability for the samples. The in vitro antibacterial activity of the samples were studied against gram positive bacteria Staphyococcus aureus and gram negative bacteria Escherichio coli. The in vitro anti-inflammatory activity against standard drug diclofenac sodium for the samples were studied and found to be compatible with the standard values. The results show that zinc is incorporated well with fluorapatite structure and the antibacterial and anti-inflammatory activity confirms the enhancing properties of the sample. Thus the FA and Zn-FA samples can be used for dentistry and biomedical application.*

Keywords: *Fluorapatite, Zinc oxide, Wet chemical method, Characterization*

I. INTRODUCTION

Dental enamel consists of 95-96 wt% of the apatite phase accompanied by 3 wt% of water and 1 wt % of organic matter [1]. Hydroxyapatite and Fluorapatite with the chemical formula $Ca_{10}(PO_4)_6$ $[OH/F]_2$ [2] is naturally present in the teeth enamel [3], and it has gained many applications oriented interest in the field of research due to its properties like bioactivity, high thermal stability, corrosion resistance and crystallinity [4]. Due to the substitution of the F^- ion in the hydroxyl ion OH^- , the crystal structure of the hydroxyapatite forms more stable and it reduces the acid solubility of the crystals by restraining the ability of dental caries [5]. The apatite structure consists of Ca^{2+},PO_4^{3-}, OH^- and CO_3^{2-} [6]. The stability of the apatite structure depends on the trace elements and minor constituents [7]. The major and minor constituents of the tooth enamel are CO_3, P, K, Mg, Cl and Fe, Zn, Na, F, Sr, Ca. The trace elements present in the tooth enamel are Zn, Si, Sr, F, S, Al and Fe. During the process of mineralisation in the tooth enamel, the trace elements and minor constituents are incorporated [8].

As of Zinc, an essential trace element in the human body [9] and its natural presence in saliva and teeth has the properties of antimicrobial and anti bacterial effects [10-12]. Zinc is related with the reduction of enamel solubility and it also amends the calcium phosphate crystal growth during remineralisation. Hence the presence of zinc is essential for the re/demineralisation balance in the mouth [13-15]. Both zinc and fluoride has the ability to remineralise the partially dissolved crystal [9]. The addition of zinc in fluoride diminishes the formation of calculus in the teeth [16] and the anti caries effect of fluoride is not altered [17]. The crystal structure of zinc and Fluorapatite [6] are hexagonal, the epicacy in the incorporation of zinc in fluorapatite forms a strong physicochemical structure and it relinquishes significant applications in the field of dentistry, drug delivery [9] and biomaterials [4].

The synthesis of FA and Zn-FA can be done by using several techniques like sol-gel process, solid-state reaction, wet chemical processing, solvo-thermal process, flame synthesis and spray-drying process. Among the above mentioned techniques, the modified wet chemical precipitation is the less time consuming method [18]. The FA and Zn-FA nanopowders synthesized by this modified wet chemical precipitation method has been characterized to study the phase, morphology, chemical bonding and composition, thermal stability and *in vitro* assessment of the samples.

II. EXPERIMENTAL PROCEDURE

The synthesize of FA and Zn-FA nanoparticles was done via modified wet chemical method by using calcium hydroxide ($Ca(OH)_2$), ammonium dihydrogen phosphate ($NH_4H_2PO_4$), sodium fluoride (NaF)and zinc oxide (ZnO) as

the starting precursors. 1M $Ca(OH)_2$ and 0.6M $NH_4H_2PO_4$ were dissolved in separate 100ml distilled water. 0.2M of ZnO and 0.2M of NaF were added to the calcium and phosphate precursor solutions and stirred vigorously for 1hour. The ADP solution was added drop wise to the $Ca(OH)_2$ solution for about 30minutes. During the titration pH of the solution was maintained above 10. The final solution was stirred for 18hrs and irradiated in microwave oven (2KHz, 800W, Samsung) for 20minutes. The obtained dried Zn-FA sample was grinded well to a fine powder by using mortar and pestle. The synthesize of FA nanopowder was done by using the same procedure without the addition of ZnO.

III. CHARACTERISATION

The structural and the phase analysis of the synthesized FA and Zn-FA nanopowder was done by XPERT PRO diffractometer (40kV, 30mA) with Cu Kα radiation (λ = 1.54060Å). The functional group of the samples was identified using the Fourier transform infrared spectroscopy (Perklin elmer Spectrum two) ranging from 400-4000cm^{-1}. The thermal stability of the samples was analysed using TG analyser. The morphology and elemental composition of the synthesized samples were done by using TEM and EDAX. The *in vitro* anti-inflammatory and anti-bacterial activities were carried out using protein denaturation method and disc diffusion method to study the anti-inflammatory and anti-bacterial properties of the samples.

IV. RESULT AND DISCUSSION

A . FTIR ANALYSIS

The FTIR spectrum of the synthesized samples is shown in figure 1a & 1b. The peaks corresponding to the hydroxyl group was detected between the wavelengths 3436cm^{-1} - 3650cm^{-1} and that was assigned to OH stretching mode. The presence of PO_4^{3-} group was confirmed by the peaks ranging from 1035 cm^{-1}-1040 cm^{-1} and 550 cm^{-1}- 610 cm^{-1}. The peaks at 1036 cm^{-1} and 1039 are due to the ν_3 PO_4, the peaks at 605 cm^{-1} and 566 cm^{-1} are due to the ν_4 PO_4. The bands at 471.14 cm^{-1} is due to ν_2 PO_4. Due to the reaction of FA samples with the air atmosphere, there is a presence of CO_3^{2-} group in the spectrum which helps in the bioactivity of the samples. The peaks ranging from 1460 cm^{-1} - 1424 cm^{-1} represents the presence of carboxyl group. The presence of fluorine F^- and Zn^{2+} ions in the synthesized samples is confirmed by the broad stretching of the OH^- in the spectrum. The above mentioned explanation confirms that the synthesized nanopowder are fluorapatite and zinc doped fluorapatite.

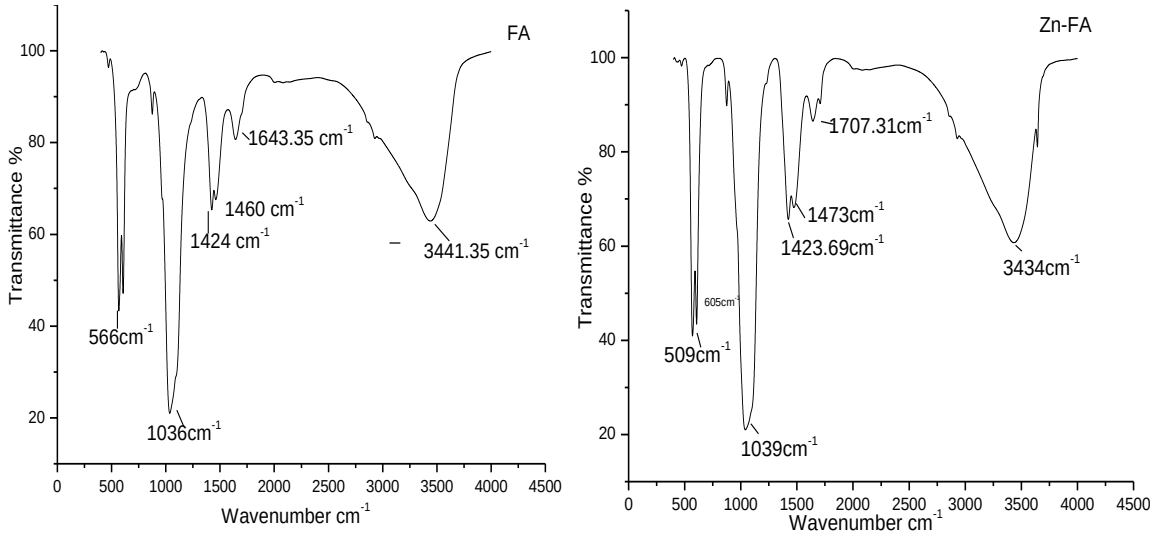


Fig 1a & 1b. FTIR spectrum of FA and Zn-FA

B . XRD ANALYSIS

The XRD pattern for the FA and Zn-FA samples are shown in fig 2a & 2b. The diffraction peaks corresponds with the JCPDS card no. FA = 15-0876 and ZnO = 36-1451. In figure 2a the crystalline nature of the FA samples is indicated by the diffraction peaks in the range from 32º to 41º as (211), (222), (213) and (004). The substitution of ZnO in FA diminishes the crystalline nature of FA sample. The diffraction peaks at 34.31º (002) and 47.35º (102) confirms the presence of Zn^{2+} ions in fluorapatite. The crystalline size was calculated by using the debye scherrer formula. The average crystalline size of the synthesized FA and Zn-FA are 20.38nm and 32.78nm. From the crystalline size, it is perceived that the incorporation of zinc in fluorapatite reduced the crystallite size and promotes the formation of apatite structure.

C. ELEMENTAL ANALYSIS

The elemental composition of the synthesized sample obtained by the EDAX spectra are shown in figure 3a & 3b. The presence of the Ca, P, F is confirmed by the peaks in the spectra 3a. The incorporation of zinc in FA is confirmed by the presence of elements Ca, P, F, Zn and O in the given spectra 3b. Hence the elemental composition of the synthesized samples is confirmed by EDAX analysis.

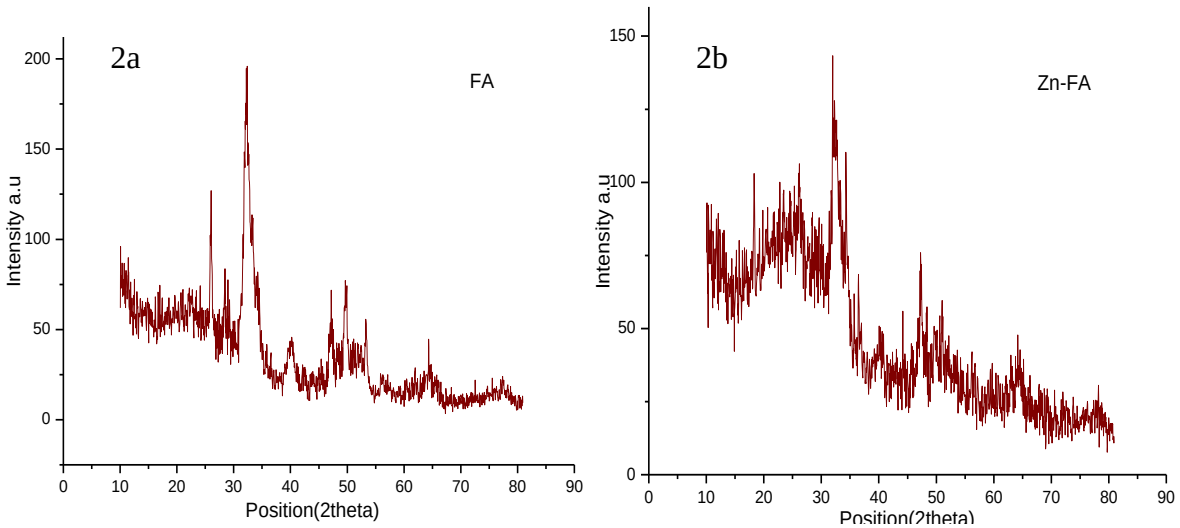


Fig 2a &2b. XRD Pattern of as-synthesized FA and Zn-FA

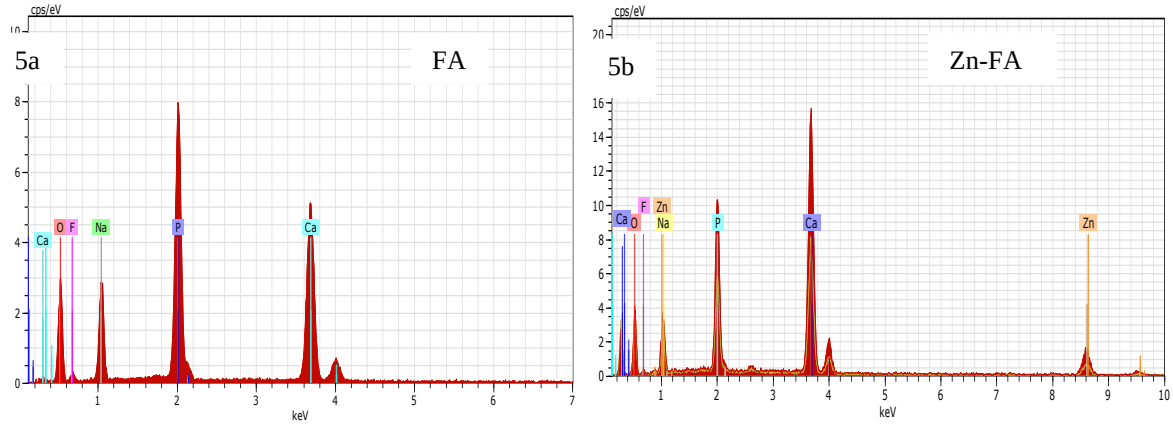


Fig 3a & 3b.EDAX spectrum of FA and Zn-FA

D. MORPHOLOGY ANALYSIS

The transmission electron microscope (TEM) images of the synthesized samples are shown in figure 4a & 4b. Both the FA and Zn-FA nanoparticles are highly agglomerated. The FA nanoparticles evinces rod like morphology between 8nm -60nm and the incorporation of Zn in FA decreases the length of the nanoparticles. The TEM image of Zn-FA indicates the addition of Zn^{2+} ions in the FA nanoparticles structure.

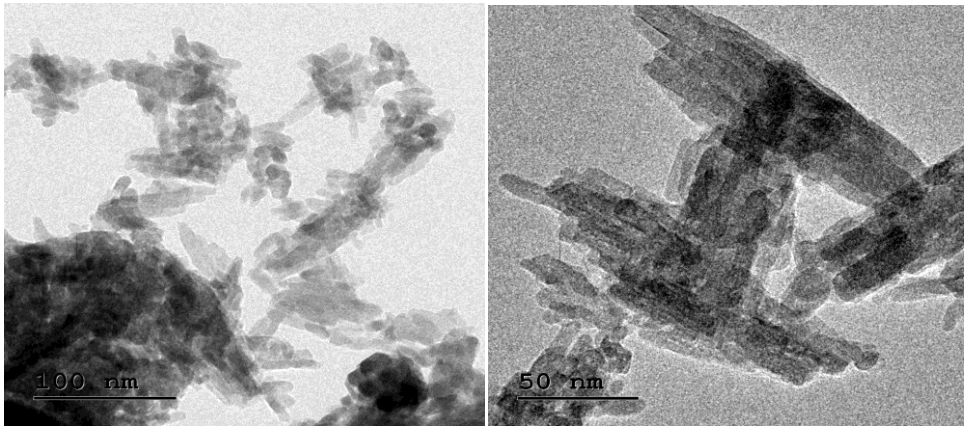
E. THERMOGRAVIMETRIC ANALYSIS

The change in the chemical composition and thermal stability of the FA and Zn-FA samples were studied using the TGA/DTA analysis is shown in figure 5a and 5b. The dehydration of the water molecules is the reason for weight loss in both the samples FA and Zn-FA between 25ºC - 450ºC. The second weight loss is due to the decomposition of the mineral carbonates present in the apatite structure from 400ºC -700ºC.The observation from the literature proved that fluorapatite is more stable till 1400ºC than hydroxyapatite. In the current work the FA and Zn-FA are stable till 650ºC - 750ºC. The zinc incorporation in FA has decreased the thermal stability of Zn-FA sample. The variation in the level of substitution alters the thermal stability of a compound.

F. *IN VITRO* ANTI BACTERIAL STUDIES

The antibacterial activity for the synthesized FA and Zn-FA is shown in figure 6a and 6b. The activity was done by using disc diffusion method. Petri plates were prepared by pouring 30ml of nutrient agar medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes. 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

4a)



4b)

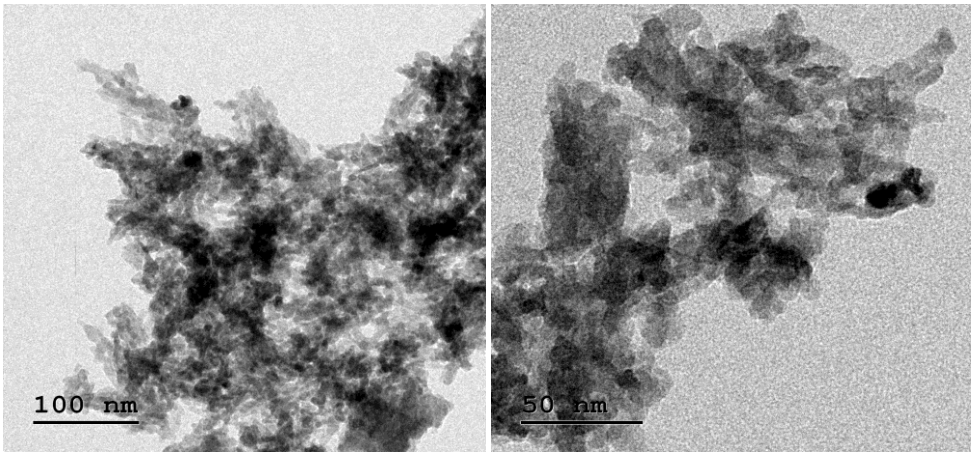


Fig 4a & 4b. TEM images of FA and Zn-FA

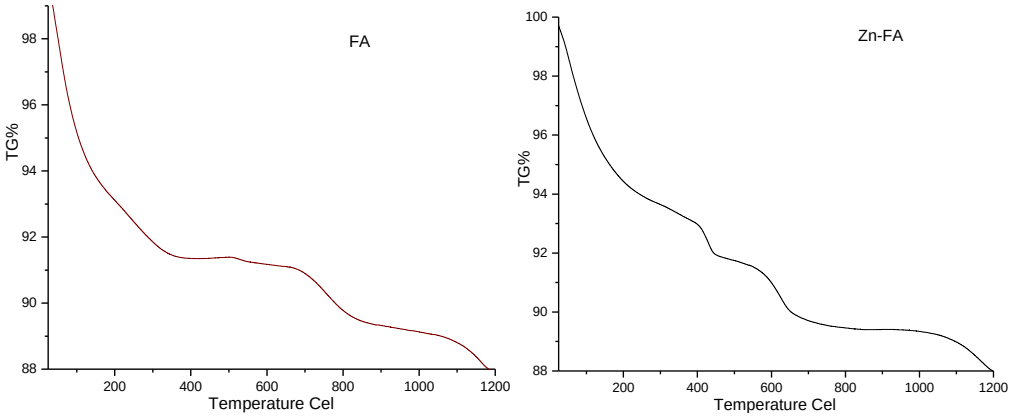


Fig 5a & 5b. TGA thermograms of FA and Zn-FA

to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing gram – negative bacteria *Escherichia coli* and gram – positive bacteria *Staphylococcus aureus* were spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the FA and Zn-FA (50µl, 100µl and 150µl) was laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Control used as solvent (Ethanol : Water). 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 millimetres. The zones of inhibition of the tested microorganisms by FA and Zn -FA were measured using a millimetre scale. The antibacterial activity of zinc incorporated fluorapatite against *E.coli and S.aureus* is found to be effective than fluorapatite. The Zn-FA is found to be more active in the organism *S.aureus* .

Table 1a. Antibacterial activity (Escherichia coli)

Samples Code	50 (µl) (mm)	100 (µl) (mm)	150(µl) (mm)	Standard (30µl) (mm)
FHA	2.30 ±0.21	6.10±0.42	8.50±0.56	8.20±0.52
FHA-ZnO	2.80±0.22	7.10±0.43	9.00±0.63	8.80±0.58

Table 1b. Antibacterial activity (Staphylococcus aureus)

Samples Code	50 (µl) (mm)	100 (µl) (mm)	150(µl) (mm)	Standard (30µl) (mm)
FHA	3.10 ±0.26	5.80±0.369	8.20±0.51	9.10±0.55
FHA-ZnO	3.60 ±0.26	8.60±0.58	10.20 ±0.74	11.10±0.82

FHA **FHA ZnO**

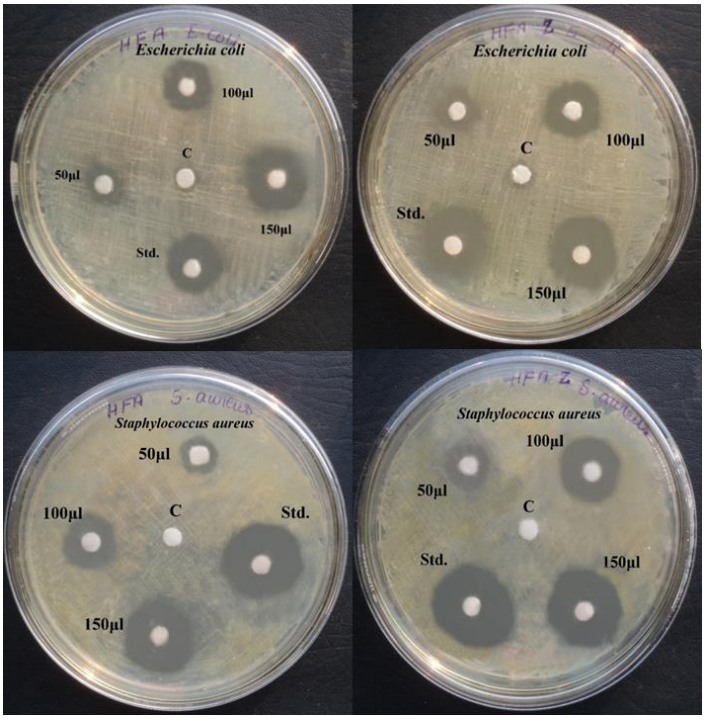


Fig 6a & 6b. Antibacterial activity (Escherichia coli, Staphylococcus aureus)

G. *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity evaluated by protein denaturation method . The reaction mixture (5ml) consisting of 2 mL of different concentrations of **FA and Zn-FA** (100,200,300,400and 500 µg/ml) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Diclofenac sodium (100,200, 300, 400and 500 µg/ml) used as standard drug and similarly for determination of absorbance. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{At - Ac}{Ac} \times 100$$

Where, At=absorbance of test sample; Ab=absorbance of control

Tests were carried out in triplicate for 3 separate experiments. The anti-inflammatory activity of sample was expressed as 50% effective concentration (EC_{50}), which represented the concentration of sample having 50% of anti-inflammatory effect. The Zn-FA sample showed better activity than FA sample with the standard value.

Table 2. Anti-Inflammatory activity of FA and Zn-FA

S. No.	Concentration (µg/ml)	FHA	FHA-ZnO	Standard (Diclofenac sodium)
1	100	15.56 ±1.05	17.78 ±1.24	20 ±1.4
2	200	28.88 ±2.02	33.34 ±2.33	35.56 ±2.52
3	300	40 ± 2.8	44.45 ± 3.11	57.78 ± 4.06
4	400	53.34 ± 3.71	60 ± 4.20	73.34 ±5.11
5	500	68.89 ± 4.83	75.56 ± 5.28	88.89 ±6.23

V. CONCLUSION

Modified wet chemical method using microwave oven is a viable and cost effective method for the synthesis of fluorapatite and zinc doped fluorapatite. The synthesized nanoparticles were highly agglomerated and exhibits rod like morphology by the TEM analysis. The FTIR and XRD showed the chemical bonding and phase analysis of the samples. The decrease in the crystallinity of the FA due to the incorporation of Zn^{+} ions is confirmed by the XRD diffractogram. The average grain size of FA and Zn-FA are 20.38nm and 32.78nm. The elemental composition shows the presence of the Ca, P, F, Zn and O in the samples with a thermal stability till 750ºC. The *in vitro* antibacterial activity shows good response of Zn-FA towards the most common bacterial organisms *E.coli and S.aureus.* The samples showed relatively closer anti-inflammatory properties while comparing with the standard Diclofenac sodium. Thus the prepared samples are applicable for biomedical applications in drug delivery, dental and orthopaedic implantations.

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