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Original Article

Fine Structure analysis and Antibacterial property of Strontium doped Hydroxyapatite

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ABSTRACT

Calcium phosphate-based bioceramics play a key role in several biomedical applications and have been expanding remarkably the recent years. Among different forms of bioceramics, particular attentions have been placed to calcium phosphates-based powders, granules, dense or porous bodies, and coatings for metallic or polymeric implants due to their excellent biocompatibility and osteointegration properties. In consequence, synthetic bioceramics have been considerably utilized in the fields of orthopaedic and dentistry. Strontium ion plays a vital role in enamel demineralization. Strontium ions can replace calcium in hydroxyapatite but in addition may provide antibacterial action. Pure Hydroxyapatite and Sr doped Nanopowders have been prepared by Sol gel method. The formed gel was dried at 110°C for 22 hours and then calcined at 900°C for 4 hours. The formation mechanism, elemental composition, particle Size and antibacterial action have been investigated using XRD, TEM, EDS and ANTIBACTERIAL ACTIVITY. The particles of all samples are of nano Size with average length of 18nm in diameter.TEM confirms the influence of Strontium on their Morphology. The elemental composition was affected by the addition of Sr.The Antibacterial property shows the increase in zone of inhibition at 6% Sr doped HAp.

Keywords: *Strontium, XRD, Hydroxyapatite, Solgel, TEM, Antibacterial activity.*

1. Introduction:

Hydroxyapatite (HAp) is a calcium phosphate similar to the human hard tissues in morphology and composition. Particularly, it has a hexagonal structure and a stoichiometric Ca/P ratio of 1.67, which is identical to bone apatite. An important characteristic of hydroxyapatite is its stability when compared to other calcium phosphates. Thermodynamically, hydroxyapatite is the most stable calcium phosphate compound under physiological conditions as temperature, pH and composition of the body fluids [2]. With the development of nanotechnology, a major impact on materials science has been noticed. Nano-hydroxyapatite (nano-HAp) is attracting interest as a biomaterial for use in prosthetic applications due to its similarity in size, crystallography and chemical composition with human hard tissue [2].

Bone and teeth enamel are largely composed of a form of this mineral. Hydroxyapatite makes up bone mineral and the matrix of teeth [3]. During the last 20 years process in dental research the consideration was only with the role of trace elements, other than fluoride, in the dental caries process. The element which has attracted attention is strontium, which have association with low caries prevalence which was confirmed from both human and animal studies.

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The properties of strontium and calcium are very similar in their properties so that Sr can be substituted for alternate Ca in the hydroxyapatite crystal of human enamel. When strontium is doped in HAp, those dopants are believed to have significant role towards improving cell-material interactions of HAp and strengthening its mechanical properties. . The process of cell interaction on materials is very dynamic, thus encouraging an exceptional tissue response at the biomaterials surface [4]. Strontium is one of the metallic elements found in bones and teeth.

Strontium appears to be one of the most effective substances for the treatment of osteoporosis and other bone related conditions [5]. Because calcium and strontium share the properties of group 2A elements, strontium can replace calcium in hydroxyapatite and hence in bone, without much difficulty [6].

The bone remodeling in women after menopause leads to the decreased bone mass, altered bone microarchitecture, and increased fracture risk. Current antiosteoporotic drugs increases bone formation. Strontium ranelate is a newly developed ant osteoporotic drug that reduces bone reabsorption and promots bone formation.

2. Experimental Procedure

Diammonium hydrogen phosphate is dissolved in deionized water and stirred in magnetic stirrer at 90˚C. Calcium nitrate tetra hydrate is dissolved separately in deionized water and added. The above two solutions are added drop wise pH is maintained at 10 by adding ammonium hydroxide. It is maintained at 90°C for 6 hours. Precipitates are filtered and washed then dried at 110°C for 22 hours and then calcined at 900°C for 4 hours.

To Obtain Sr doped HAp

Strontium doped hydroxyapatite nanocrystals were synthesized by sol gel method for two different concentrations of the strontium (2% and 6%).Diammonium hydrogen phosphate is dissolved in deionized water and stirred in magnetic stirrer at 90˚C. Calcium nitrate tetra hydrate and strontium nitrate are dissolved separately in deionized water and added. The above two solutions are added drop wise pH is maintained at 10 by adding ammonium hydroxide. It is maintained at 90°C for 6 hours. Precipitates are filtered and washed then dried at 110°C for 22 hours and then calcined at 900°C for 4 hours.

3. Results and discussion

3.1 X-ray diffraction (XRD)

The structural characterization has been done by XRD. X- ray powder diffraction measurements was performed at SAIF (Sophisticated Analytical Instrument Facility), Cochin using $CuK\alpha_1$ with 2θ range from 10^0 to 120^0 and 0.02^0 step sizes. The XRD profiles of the pure hydroxyapatite and Mg doped hydroxyapatite are given in Figs 1a-1c respectively. The comparative XRD profiles are given in Fig 1d. The crystallite size was calculated from full-width-at-half-maximum (FWHM) for the diffraction peaks and it is given Fig 1e. The crystallite size of the doped samples decrease with respect to pure HAp which may be due to the larger ionic radius of Strontium (1.12Å) when compared with Calcium (0.99Å).

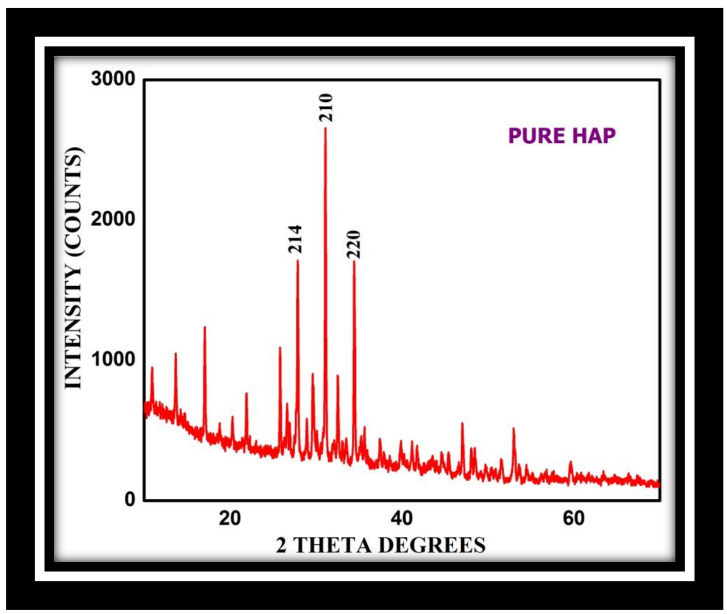

Fig1a. Pure HAp

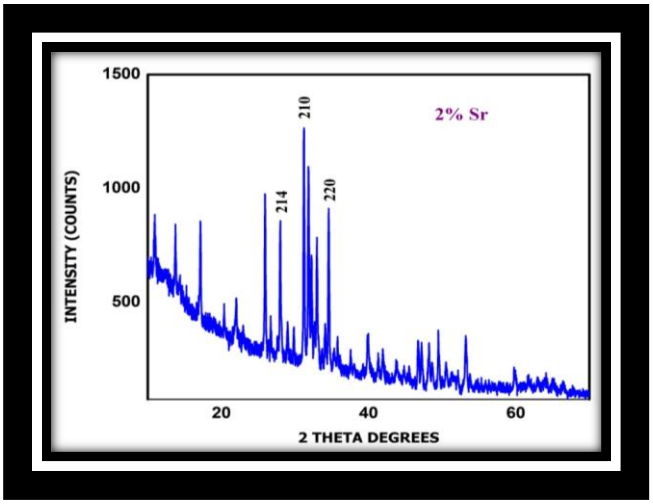

Fig1b. 2% Sr doped HAp

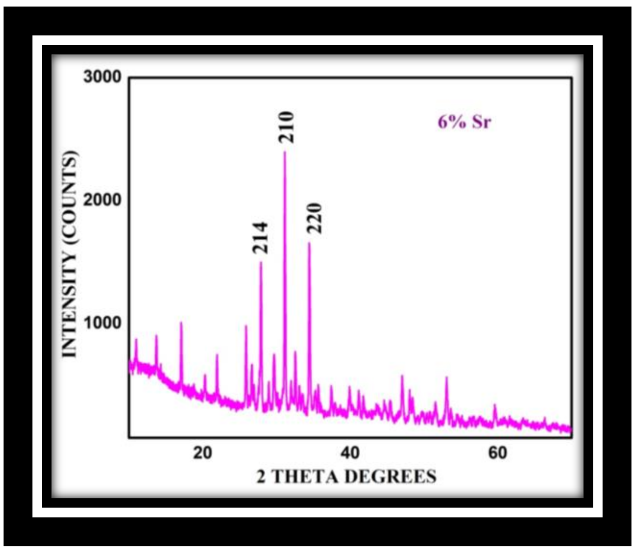

Fig1c. 6% Sr doped HA

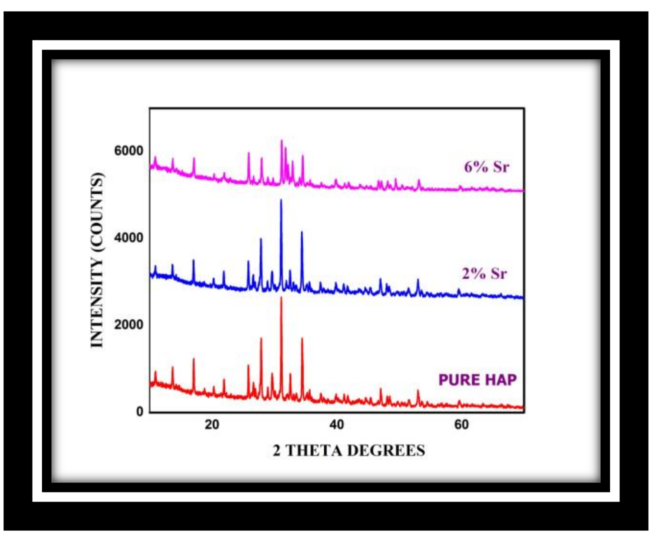

Fig1d. Comparative XRD profiles of Pure and doped Samples

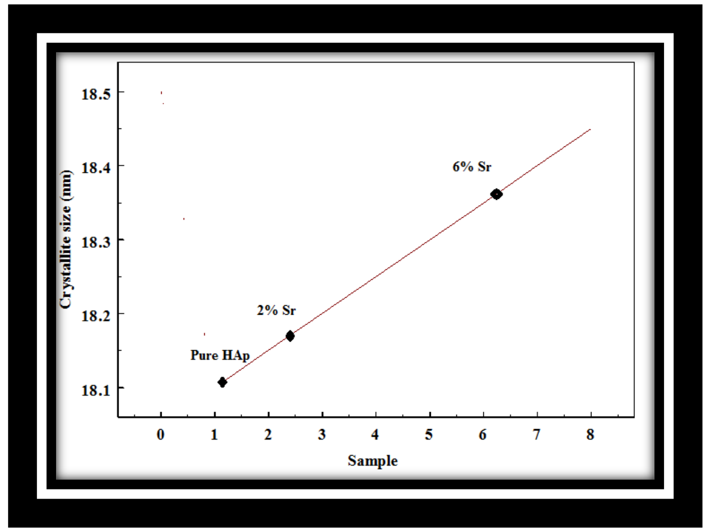

Fig 1e Crystallite Size from XRD

Table 1 Crystallite size from XRD

S. No	Sample	d in nm
1	PureHAp	18.09
2	2% Sr doped HAp	18.23
3	6% Sr doped HAp	18.36

3.2 EDX

The elemental composition of the synthesized Sr samples were studied and evaluated by Energy dispersive (X –ray) analysis. This characterization was done at Kalasalingam academy of research and education, Krishnankoil. It is relies on an investigation of sample through interaction between electromagnetic radiation and matter in response with charged particle. Energy Dispersive X-ray spectroscopy (EDS) is an analytical technique used for the elemental analysis of the sample. At 2% and 6% of the Sr dopants, the Ca-deficiency increases significantly. Fig 2a to 2c confirms the presence of all constituent elements of the Sr HAp powders (Sr, Ca, P and O). The table 2 shows the calculated atomic ratios of the constituent elements for each powder are presented. These results suggest that Strontium ions were incorporated in the hydroxyapatite structure. This study confirms the increase of the Sr concentrations in the samples.

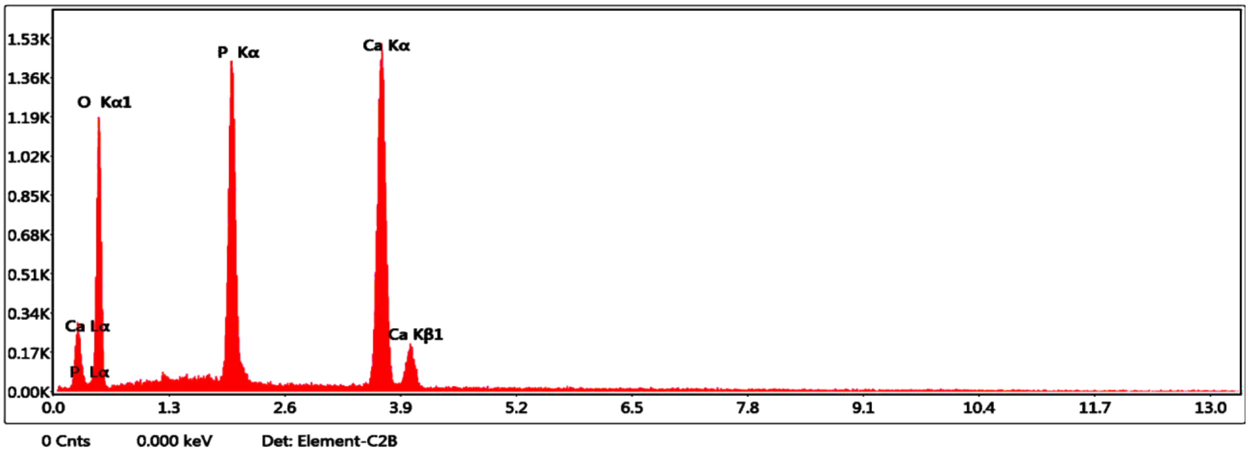

Fig 2c EDAX spectrum of 6% Sr doped HAp

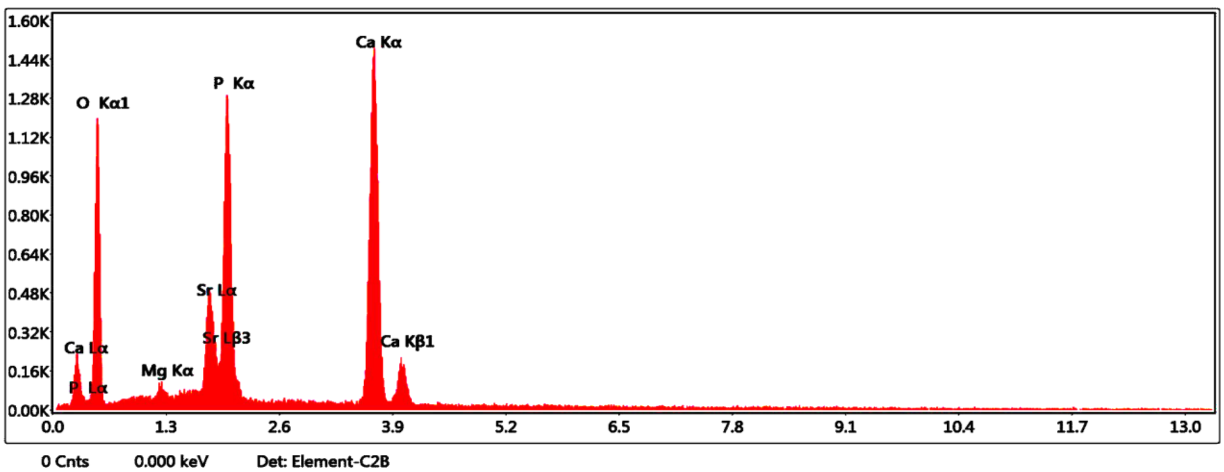

Fig 2b EDAX spectrum of 2% Sr doped Hap

S.NO	Element	Pure HAp	2% Sr doped HAp	6% Sr doped HAp
1	O	49.3	42.2	45.3
2	P	17.4	18.0	14.4
3	Ca	33.3	35.7	27.3
4	Sr	-	2.6	7.0

Table 2 Constituent elements of pure and Sr doped HAp

3.3 TEM

Transmission electron microscopy is a microscopy technique in which a beam of electrons is transmitted through a specimen to form image. TEM images of Pure HAp and doped Mg samples were taken at. Sophisticated Analytical Instrument Facility (SAIF), Cochin. The TEM images clearly demonstrated the morphologies of Sr HAp nanoparticles. Fig. 3a-3c shows TEM micrographs of Pure HAp and Sr doped HAp. An increase in the concentration of doped Sr made the particles larger due to Strontium's crystal growth accelerating effect which enhances the enlargement of HAp crystals.

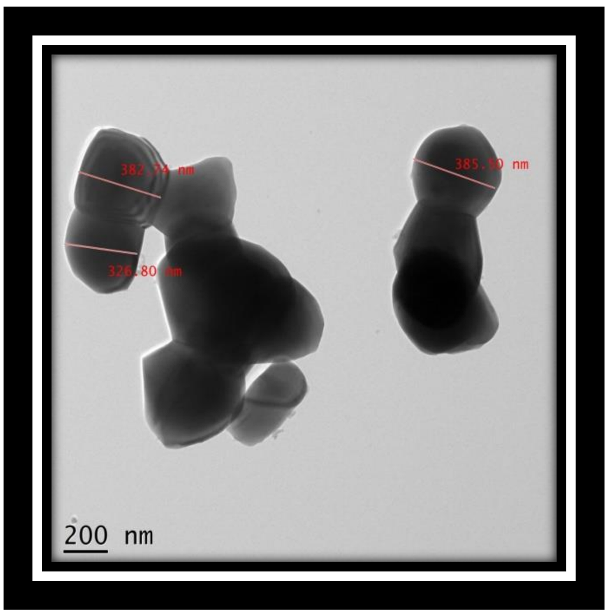

Fig 3a TEM image of pure HAp

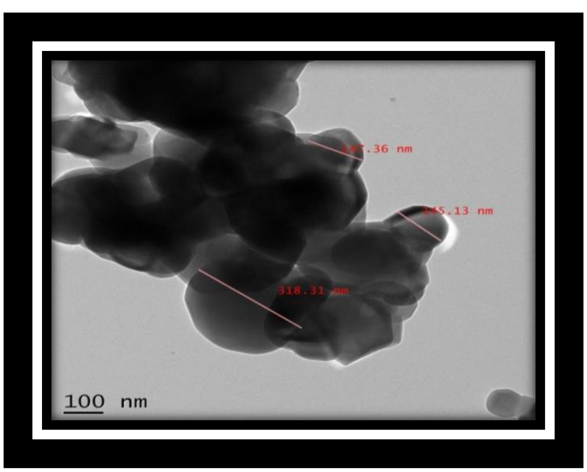

Fig3b TEM image of 2% Sr doped HAp

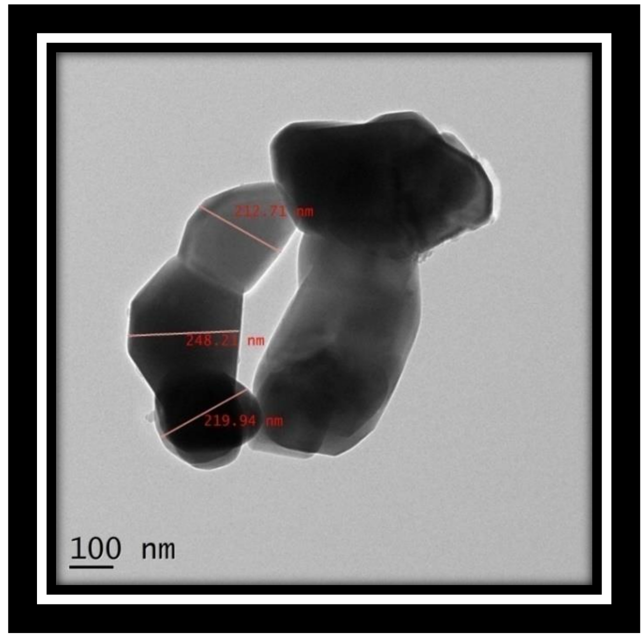

Fig3c TEM image of 6% Sr doped HAp

3.4 ANTIMICROBIAL SUSCEPTIBILITY TEST

By using Agar-disk diffusion method antimicrobial susceptibility testing was done at Venture institute of biotechnology and bioinformatics research, Madurai. Antimicrobial activity was carried out using disk diffusion method. The bacterial strains were inoculated in nutrient broth and incubated for 24 hours before used in antibacterial assay. Sterile Muller Hinton agar (high –media) plates were prepared and allowed to set. The cultures to be screened were swabbed on tap of the solidified media. Disk impregnated with the nanoparticles were placed on the swabbed plate. Methanol was used as a control. Levofloxacin (antibiotic) was used as standard. The plates were incubated at 37°C for 24 hours. After incubation, the inhibition zone was measured. Assay was carried out in triplicates and control plates were maintained. Zone of inhibition was measured from the edge of the disc to the clear zone in millimeter (mm). The antimicrobial activity of the Strontium substituted hydroxyapatite with 2% and 6% Sr was compared to that of pure HAp.

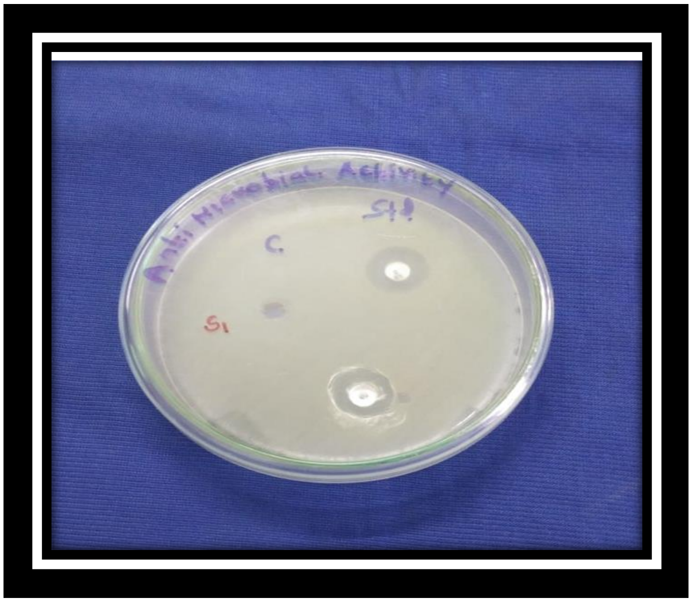

Fig 4a Pure HAp

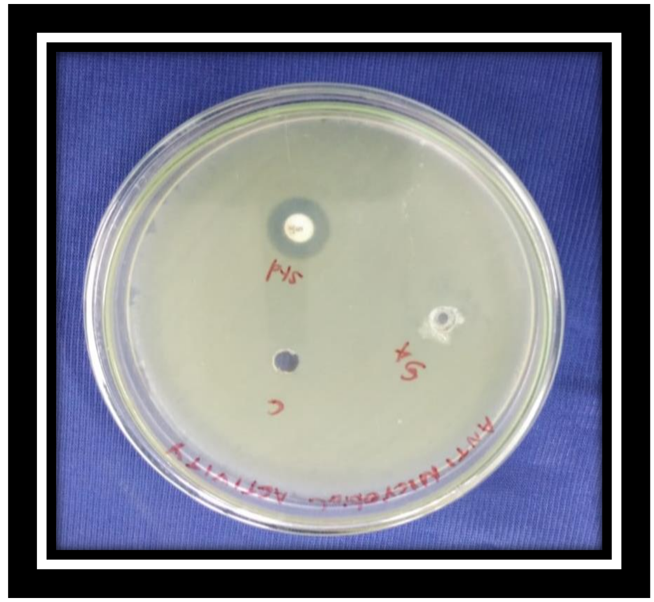

Fig 4c 6% Sr doped HAp

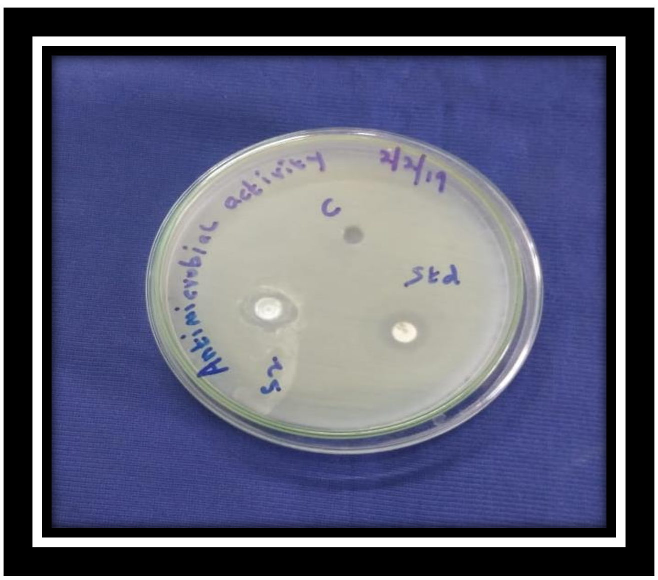

Fig 4b 2% Sr doped HAp

Table 3 Antimicrobial activity of the samples treated against bacterial pathogens

S. No	Sample	Diameter of the inhibition zone(in mm)
1	Pure HAp	15
2	2% Sr doped HAp	13
4	6% Sr doped HAp	9

Conclusion

Strontium Doped Hydroxyapatite Nanocrystals were successfully synthesized by a simple and easy method, sol-gel method. Hydroxyapatite was doped with two different concentrations (2% & 6%) of Strontium. Structural parameters were analyzed using XRD. The XRD profiles were compared with JCPDS, the space group and the lattice parameters were determined. From XRD, using Debye Scherer's formula the average crystallite size was determined as 18.22 nm. The crystallite size increases with increase in doping concentration of Strontium. Main peak 2theta value decreases with increase in Strontium concentration. This shows that Strontium was doped in the Calcium site. The elemental composition was determined using EDX. The surface morphology of the samples were studied and the particle size was confirmed using TEM.Antibacterial effect of Strontium doped HAp was confirmed from antimicrobial susceptibility testing.

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