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PREPARATION AND CHARACTERIZATION OF PURE AND SILVER NITRATE DOPED NANO HYDROXYAPATITE FOR BIOMEDICAL APPLICATIONS

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Abstract— **The use of biomaterials and bioceramics has been continuously increasing because of its potential medical applications. Hydroxyapatite (HAp) is the bioceramic material, which is widely used for biomedical application mainly in orthopedics and dentistry. Pure and silver nitrate doped nano particles was prepared using chemical precipitation method. The prepared samples was kept in the electrical furnace for three different temperatures (600°C; 400° C; 200° C) for annealing. These prepared samples was characterized using XRD, SEM, EDAX, FTIR analysis and Antimicrobial Sensitivity test. The grain size was calculated from XRD and the porosity of the as prepared sample was analyzed using SEM. Presence of dopants are observed using EDAX analysis and Compound formation was verified by FTIR analysis. The antimicrobial activity was also performed for the pure and silver nitrate doped samples. The results and discussion will be presented in detail.**

Index terms- **Hydroxyapatite; Bioceramic; Chemical precipitation; Antimicrobial.**

I. INTRODUCTION

During the last two decades, significant advances have been made in the development of biocompatible and biodegradable materials for medical applications. In the biomedical field, the goal is to develop and characterize artificial materials or, in other words, "spare parts" for use in the human body to measure, restore and improve physical functions and enhance survival and quality of life. Biomaterials play a vital role in replacement heart valve, hydroxyapatite material for hip implants and also it is used in everyday life for dental application, surgery and drug delivery. Biomaterial isa special material that has been used for pass 50 years in several medical applications. The major application are joint replacement bone plates bone cements heart valves artificial ligaments and tendons, dental implants and contact lenses. The important role of biomaterials is that they should be biocompatible with body and mechanically durable, which must be proofed before implanting into the body. Biomaterials can be in the forms of metals and alloys, ceramics, polymers and composite.

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is a main inorganic component of hard tissues like bone and dental reparation and also for the application in drug delivery systems [1][2]. HApexhibits excellent properties, like high osteo-conductivity and osteoinduction when implanted in the human body [3]. HAp-loaded with therapeutic concentration of antibiotics like cephalexin or norfloxactin, amoxicillin, ciprofloxacin, gentamycin has been tested for the treatment of osteomyelitis and hence hap can be a useful delivery system and also in recent year hap is used in treatment of bone fractures upon electrical stimulation. This response to electrical stimulation has been attributed to micro structure and porosity of HAp[4].

In this paper, we report the synthesis of pure and silver nitrate doped hydroxyapatite by chemical precipitation method for different annealing temperatures.The synthesized samples were characterized by XRD,FTIR,SEM,EDAX and Antimicrobial sensitivity test.

II. EXPERIMENT

The pure and silver nitrate doped hydroxyapatite was prepared using chemical precipitation method. The following description describes about the preparation of pure and silver doped hydroxyapatite nano particles.For the preparation of pure hydroxyapatite 4.723g of calcium nitrate tetrahydrate was made to dissolve in 100 ml of distilled water and 1.320g of diammonium hydrogen phosphate was made to dissolve in 50 ml of distilled water. These solutions was made to stir in the magnetic stirrer until the chemicals dissolve completely. Then the diammonium phosphate solution was added drop wise to the calcium nitrate tetrahydrate. This solution wasmade to stir for 2 hours. To maintain the pH level in the solution, sodium hydroxide (NaOH) was added until the solution reaches the pH level of 11, and made to stir for 1 hour. The solution was keptover night to get the precipitate. The precipitate was washed in the distilled water thrice to get rid of the impurities. The precipitate got by filtration, the precipitate is kept in the hot air oven for 10 hours kept approximately 500^0C. The precipitation is now moved to electrical furnace, maintained at three different temperature (200^0C, 400^0C, & 600^0C) kept for 2 hours. The powder is now grained nicely and we get the

desired hydroxyapatite powder. The same process is repeated for the doped silver hydroxyapatite, except for the fact that silver is added after the pH level is maintained.

III. RESULTS AND DISCUSSIONS

The XRD patterns of pure and silver doped samples synthesized by chemical precipitation method are as shown in figure 1 and figure 2. The XRD patterns were in good agreement with standard JCPDS value of HAp (74-0566). The average crystallite size, dislocation density and micro strain were calculated.

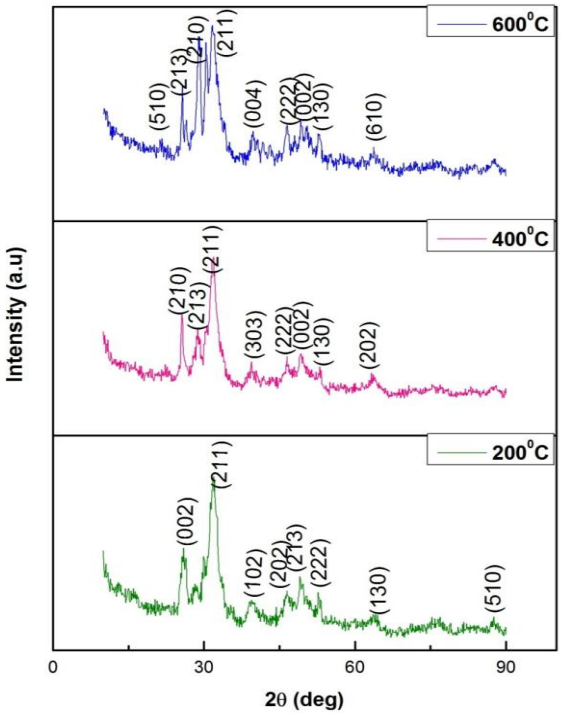


Fig 1 XRD pattern of pure hydroxyapatite

Table 1 The XRD results of samples for pure HAp

Sample no.	Temperature ^{0}C	2θ Values	JCPDS Data	Crystalline size (nm)	Dislocation Density ($\times 10^{16}$)	Micro Strain ($\times 10^{-3}$)
1	600	30.6,25.9,49.8	PDF# 74-0566	6.93	2.1	5.0
2	400	31.7,28.7,25.8	PDF# 74-0566	6.9	2.0	5.0
3	200	31.9,25.9,49.4	PDF# 74-0566	4.6	4.6	7.5

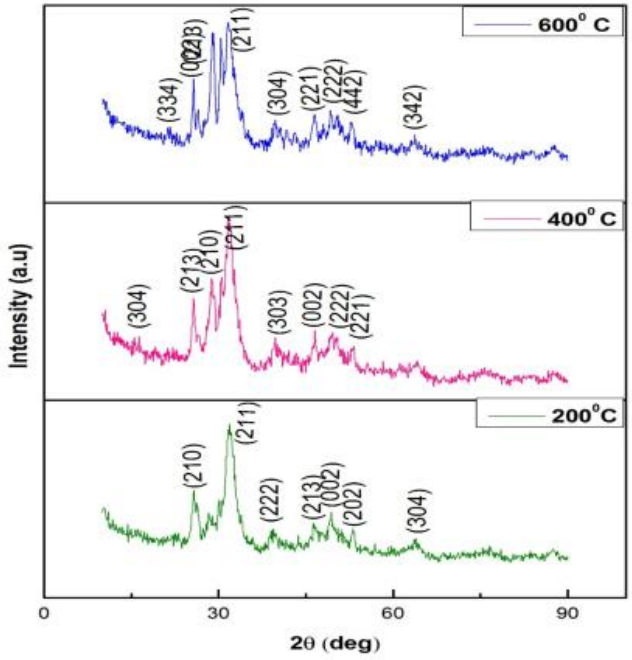
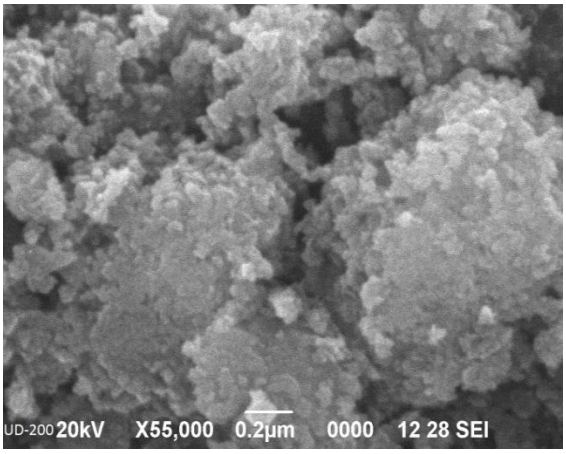


Fig 2 XRD pattern of silver nitrate doped hydroxyapatite

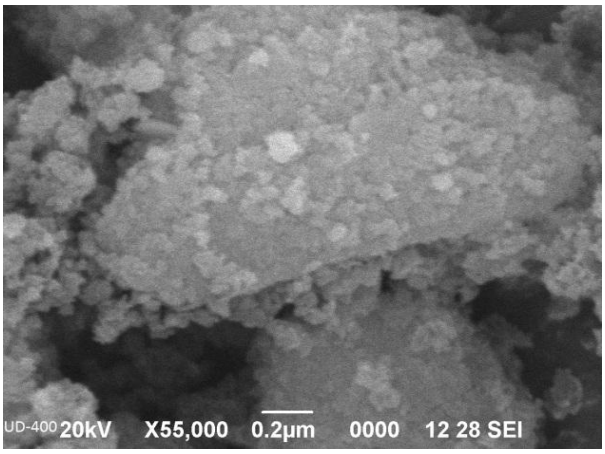
Table 2 The XRD results of samples for silver nitrate doped Hap

Sample	Temperture ^{0}C	2θ Values	JCPDS Data	Crystalline Size (nm)	Dislocation Density ($\times 10^{16}$)	Micro Strain ($\times 10^{-3}$)
1	600	30.6,25.9,49.8	PDF# 74-0566	6.93	2.1	5.0
2	400	31.7,28.7,25.8	PDF# 74-0566	6.9	2.0	5.0
3	200	31.9,25.9,49.4	PDF# 74-0566	4.6	4.6	7.5

SEM photograph of the as prepared pure and silver nitrate doped HAp sample annealed at three different temperatures (600^{0}C,400^{0}C,200^{0}C) for 2hrs is shown in Figure 3 and Figure 4. The SEM image of the prepared pure and silver nitrate doped hydroxyapatite annealed at three different temperature (200^{0}C, 400^{0}C, 600^{0}C) shows that prepared samples are similar to pure Hap with high porosity and agglomerated with the sperical like shape.



(a)



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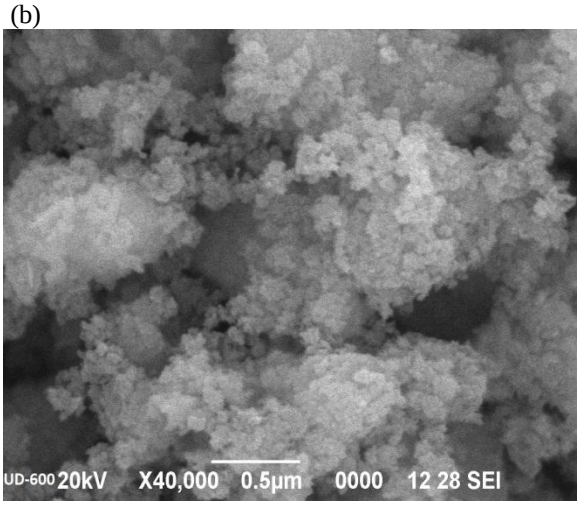
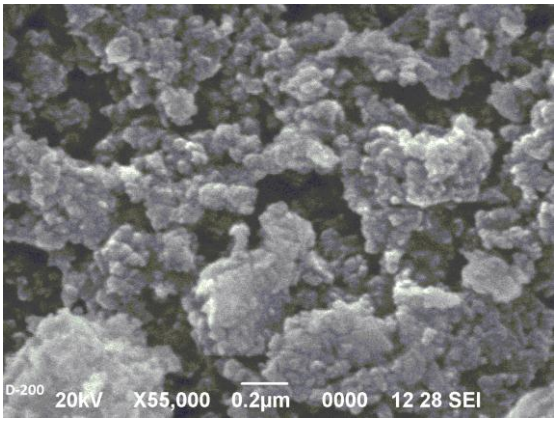
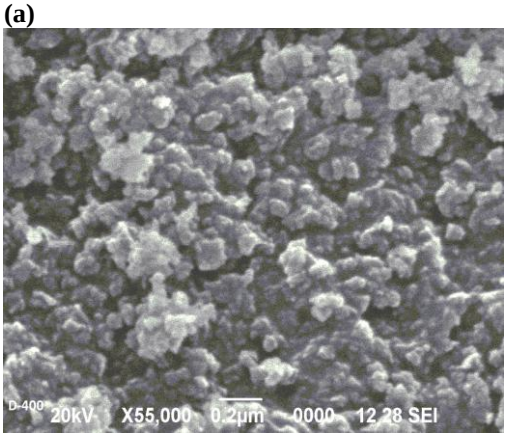


(c)

Fig 3 SEM images of pure hydroxyapitate for (a) 200^{0}C (b) 400^{0}C (c) 600^{0}C



(a)



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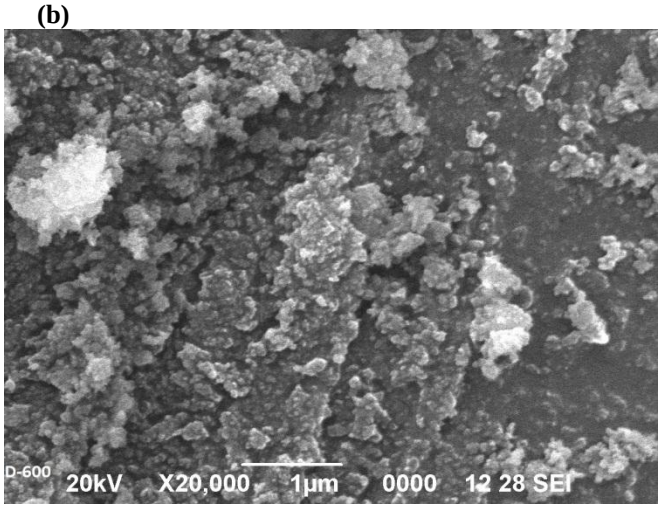

(c)

Fig 4 SEM images of silver nitrate doped hydroxyapitate for (a) 200^{0}C (b) 400^{0}C (c) 600^{0}C

As prepared pure HAp and silver nitrate doped HAp is subjected to EDAX analysis ,the EDAX spectrum of the pure HAp was obtained.The presence of the constituent elements of the pure HAp was confirmed by the occurrence of their respective peaks as shown in Figure 5 and Figure 6 and the Table 3,4 shows the percentage of the elements present in the pure HAp compound.

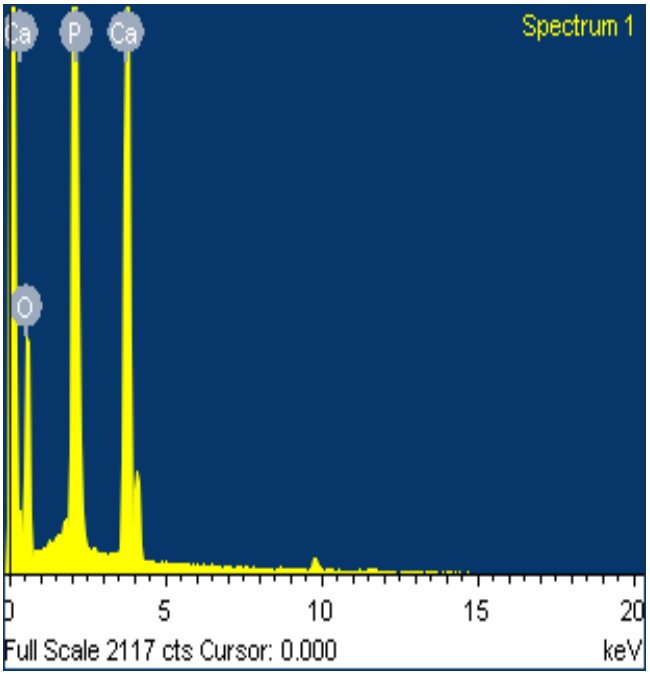

Fig5 EDAX Spectrum of pure HAp nanoparticles

TABLE 3:The presence of the constituent elements of pure HAp nanoparticles

Element	Weight%	Atomic%
O K	54.58	72.89
P K	18.50	12.76
Ca K	26.92	14.35
Total	100.0	100.00

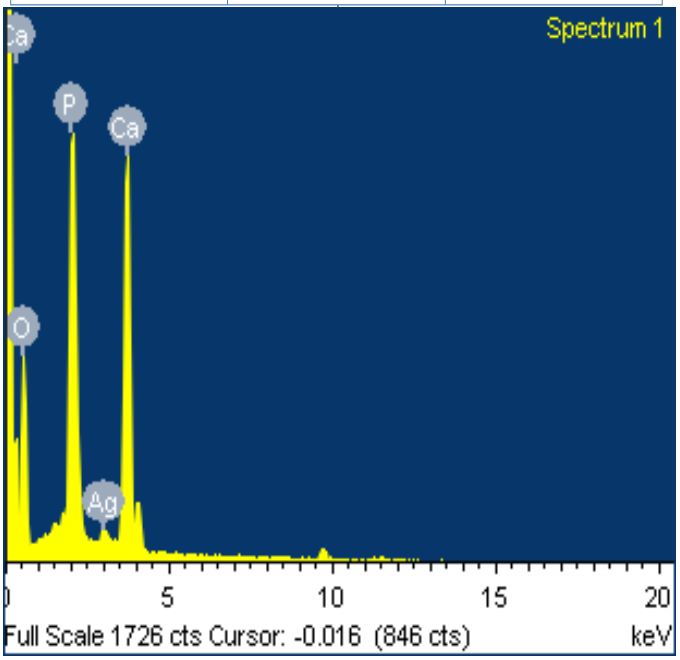

Fig 6 EDAX Spectrum of silver nitrate doped HAp nanoparticles

TABLE 4:The presence of the constituent elements of silver nitrate doped HAp nanoparticles

Element	Weight %	Atomic %
O k	47.72	67.85
P K	19.52	14.34
Ca K	30.58	17.35
Ag L	2.18	0.46
Total	100.00	100.00

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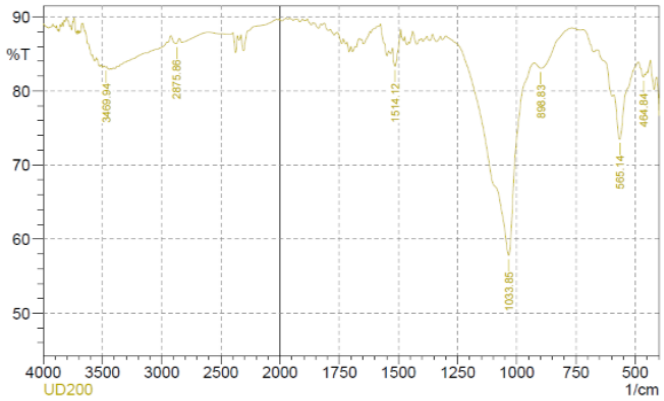

Fig 7 FTIR spectrum of pure hydroxyapatite sample annealed at 200˚C

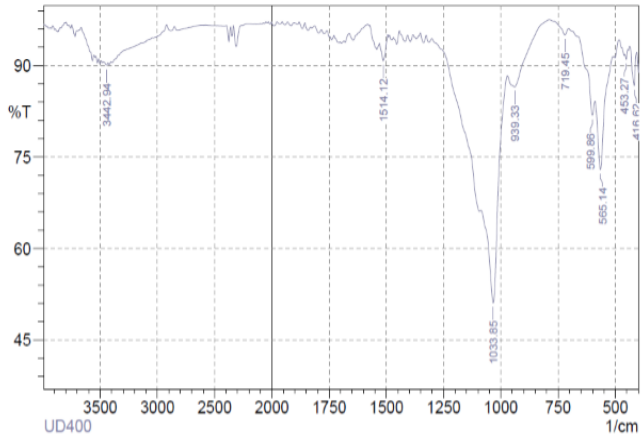

Fig 8 FTIR spectrum of pure hydroxyapatite sample annealed at 400˚C

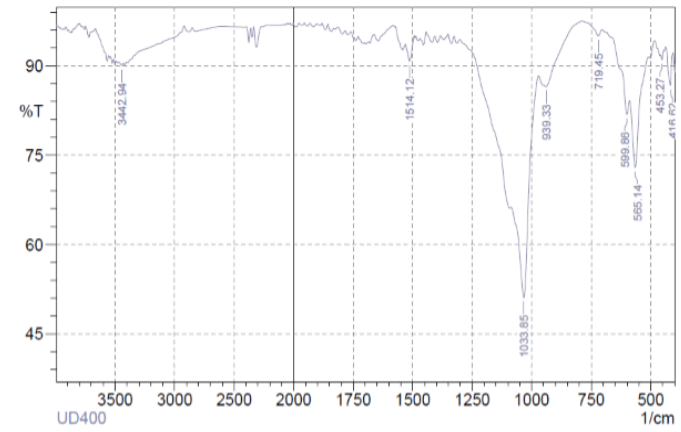

Fig 8 FTIR spectrum of pure hydroxyapatite sample annealed at 400˚C

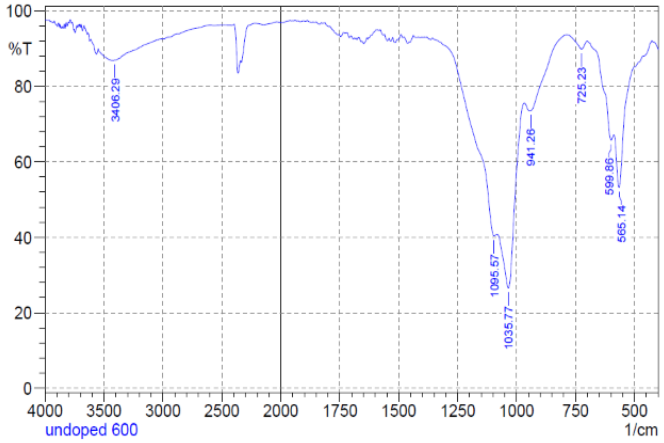

Fig 9 FTIR spectrum of pure hydroxyapatite sample annealed at 600˚C

The absorption bands at 610 and 3406 cm−1 can be ascribed to OH stretching. The peak at 1650 cm^{-1} may be attributed to the water molecules present in the HAp lattice. The peak at 1400 cm^{-1} could be assigned to the vibrational mode of carbonate ions which might have been incorporated from the atmosphere during preparation. The peak observed at 939.33 cm^{-1} corresponds to υ1 mode of nondegenerate P−O symmetric stretching. The band at 453.27 cm^{-1} is attributed to the doubly degenerate of υ2 bending of O−P−O mode. The peak at 565.14 and 599.86 cm^{-1} are assigned to the triply degenerate υ4 bending of O−P−O mode.

The silver nitrate dopedHAp samples were subjected to FTIR analysis and the corresponding spectrum was recorded. The FTIR spectrum of the sample prepared and annealed at three different temperatures (600^0C,400^0C,200^0C) are shown in Fig 10, Fig 11 and Fig 12 respectively.

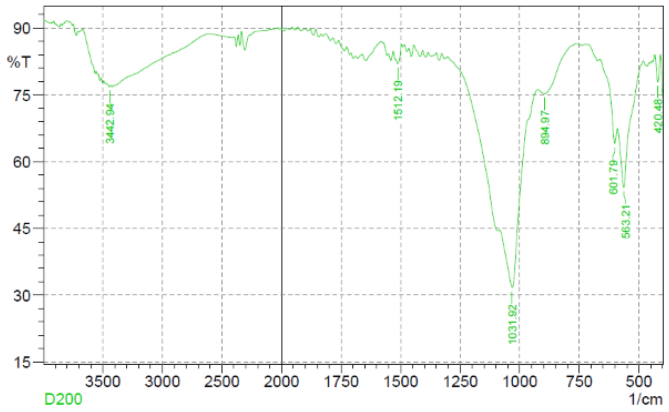

Fig 10 FTIR spectrum of silver nitrate doped hydroxyapatite sample annealed at 200˚C

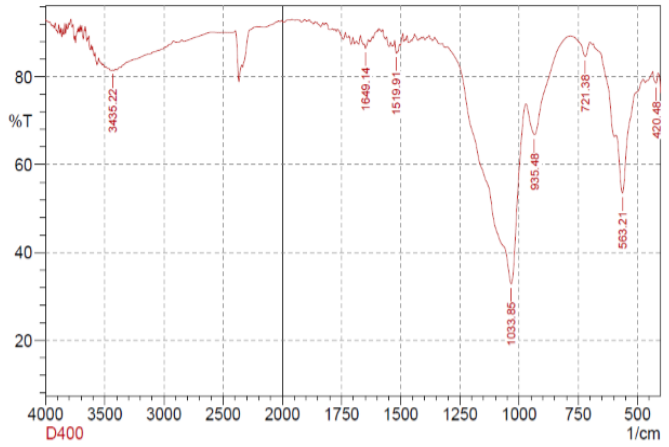

Fig 11 FTIR spectrum of silver nitrate doped hydroxyapatite sample annealed at 400˚C

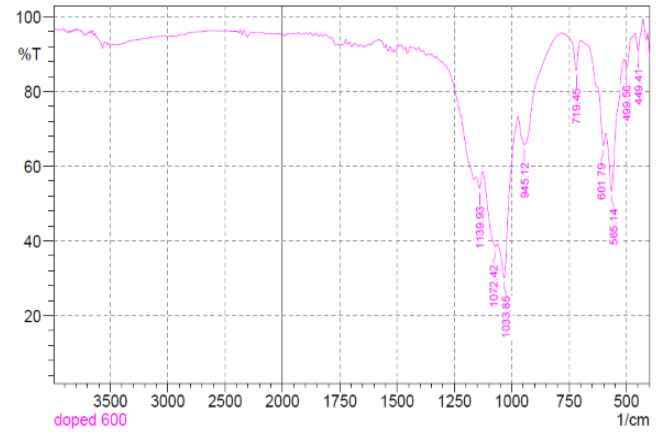

Fig 12 FTIR spectrum of silver nitrate doped hydroxyapatite sample annealed at 600˚C

The FTIR spectra of pure silver HAp and reduced silver HAp show considerable variation in the peaks of spectra (Figure 4.7). Regarding the silver nitrate about 23 peaks were found where as in the purified silver nanoparticles only 19 peaks found. The reduction of certain peaks is the clear indication of the loss of certain groups. A sharp peak at 1649.14 cm^{-1}which is present in the spectrum of $AgNO_3$ is not found in the spectrum of Ag-Nanoparticles. It is due to the loss of nitrate group from the silver species. Molecules containing NO_2 groups, such as nitro compounds, nitrates, and nitramines,

commonly exhibit asymmetric and symmetric stretching vibrations of the NO_2 group at 1649.14 cm^{-1} to 1519.91cm^{-1} and 1370cm^{-1} to 12480 cm^{-1} region. The band of carboxyl or carbonyl groups also comes under the same region. This may be the reason for the reduction of the transmittance at this region in the case of spectrum of nanoparticles since the NO_3 group lost for it. The shift of the band from 1649.14 cm^{-1} to 1519.91cm^{-1} indicates the formation of metal carbonyl groups. It is due to the stabilization of Ag nanoparticles by the –coo- group of trisodium citrate. This asymmetric shift can be comparable with the data presented by previous works (AnupamGiri et al, 2010). According to them, when the citrate ligand bound to magnetite nanoparticles surfaces the antisymmetric stretching of COO- at 1595 cm^{-1} almost remains the same but the symmetric COO-stretching mode of citrate becomes red shifted and appears sharply at 1378 cm^{-1}.

Antimicrobial sensitivity test was taken for prepared pure hydroxyapatite and silver nitrate doped hydroxyapatite annealed at three different temperatures (200^{0}C, 400^{0}C, 600^{0}C) against E.*coli*, S. *epidermidis*, and S.*aureus* are shown in the below Figure 13 and Figure 14. The antimicrobial sensitivity test shows a zone of inhibition which confirms that the prepared pure hydroxyapatite has antimicrobial activity

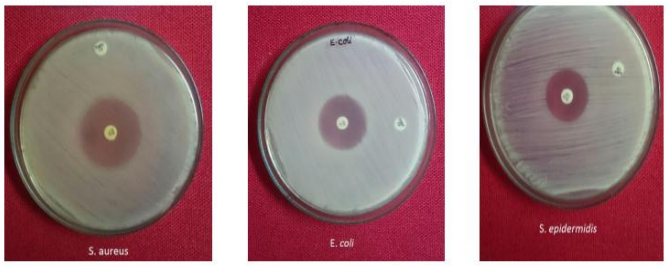

Fig 13 Antimicrobial sensitivity test for pure HAp with S. aureus, E. coli, S. epidermidis

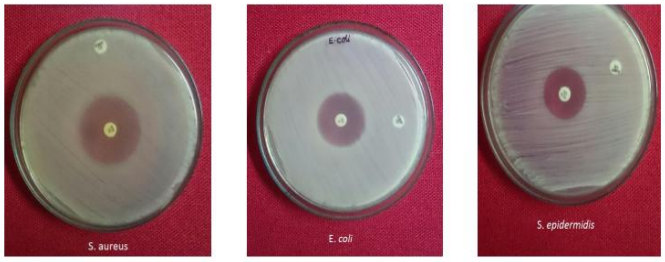

Fig 14 Antimicrobial sensitivity test for silver nitrate doped HAp with S. aureus, E. coli, S.epidermidis

IV. CONCLUSION

XRD pattern confirms that when temperature decreases crystalline size also decreases.FTIR peaks confirm that the functional group ofnHAp.EDAX confirms the presence of constituent elements ofHAp was confirmed by the occurrence of their respective peaks.SEM images confirm that the prepared doped and undoped samples have high porosity.Antisensitivity test confirms that for both doped and undoped samples have antibacterial effect.

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