RESEARCH ARTICLE

Synthesis, Characterization of Silver and Zinc doped Nano Biomaterial Hydroxyapatite in Simulated Body Fluid

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ABSTRACT: Hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2$ is the most suitable biocompatible material for bone and tooth replacement, the HAp powder was synthesized using biological waste of Mussel-shell as calcium source and using di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ as phosphate source. The grained powder of mussel shell was dissolved in diluted Nitric acid (HNO_3) and di-ammonium hydrogen phosphate was slowly added to the mixture while retaining the pH at 10 with ammonium hydroxide (NH_4OH), followed by microwave irradiation for 30 min. After the residue calcined at 900° C for 2h, then the HAp soaked in simulated body fluid (SBF) at 37°C for several period of time like 7, 14 and 30 days respectively. After the specific period of soaking; the remainder was collected, washed and dried at hot air oven. The processed powder was described by numerous investigative techniques.

KEYWORDS: Mussel shell, Hydroxyapatite, Microwave irradiation, Calcium Phosphate, SBF.

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1. INTRODUCTION

Hydroxyapatite $(Ca_{10}(PO_4)_6(OH)_2)$ is one of the most important bio-material which has been commonly used for exchanging the injured portion of the human body, primarily for bones and tooth etc [1,2]. Exactly in current times, it has been proved that the usages of calcium phosphates in biomedical field is an wonderful tool which is importance plentiful uses on biomaterial division as dentistry, bone substitution, bone renovation provisions and drug relief systems [3].

The produced Ag/Zn co-doped nano HAp initiates to have greater bio-activity and bio-compatibility to the expected calcium phosphate for suitable applications in the field of medicine [4, 5]. The technique linked with microwave-irradiation is one of the best processes to synthesize nano size HAp, which covers shorter reaction time and lesser-energy utilization etc. In the previous period, it has been creating much evidence which covenant the fabrication of nano sized HAp over microwave-irradiation. However, this route involves being novel, tolerable to recover the biological destroy and regulated over the pollution [6].

In addition, agreeing to Kokubo and Takadama's new results, a bioactivity of HAp could be proved by soaking in simulated body fluid for allowing to access apatite growth. Expected by the above effort, we tried and established the bio activity and biocompatibility of as synthesized HAp to be predictable to bone joining species and to form *in-vivo* apatite events. The formation can be imitate in a simulated body fluid with ion concentrations almost identical to that human blood plasma. The prepared powder sample, a few grams were immersed in 50 ml of SBF at 37°C for various timings and check their bioactivity [7].

2. MATERIAL AND METHODS

2.1 Synthesis of HAp

The skeletons of mussel shells were collected and washed with tap water, followed by distilled water to get rid of surplus deposits and wastes. They were dried in direct sun light for 24h and creased by pestle and mortar [8,9], 200 size net used to sieve then acquire fine powder [10].The mussel-shell powder was dissolved in dilute Nitric acid and the mixture was added to a solution of di-ammonium hydrogen phosphate. $ZnCl_3$ and $AgNO_3$ each 0.1gm were added to the solution prepared until that time and the pH was maintain at 10 using ammonium hydroxide. The mixed solution was stirred for 1hr and quickly shifted to a domestic microwave oven (LG, India) and irradiated at 800W energy of frequency retaining 2.45 GHZ for 30 min frequently. After the irradiation, the deposit was washed with de-ionized water three times and then dried in a vacuum air oven at 60° C for 12hr [11].

2.3. Synthesis of Simulated Body Fluid (SBF)

SBF is well-known as a metastable buffer solution containing calcium and phosphate ions previously supersaturated with respect to the apatite. The Ion Concentration of SBF and human blood plasma value as depicted in Table 1. The SBF is prepared as follows, and even a small, undesired difference in both of the preparation steps and storage temperature may drastically affect the phase purity and high temperature stability of the prepared HAp powder, as well as the preparation of kinetics of the precipitation process. Merck-grade NaCl (99.5%), $NaHCO_3$ (99.5%), KCl (99.0%), $Na_2HPO_42H_2O$ (99.5%), $MgCl_26H_2O$ (99.0%), $CaCl_22H_2O$ (99.0%), Na_2SO_4 (99.5%), $(CH_2OH)_3CNH_2$ (99.5%) and HCl were used in the preparation of SBF for this study as tabulated in Table 2. Add 750 ml of deionized water in to a 1000ml of beaker (polyethylene beaker). Stir the water and maintain its temperature at 37^{o}C with magnetic stirrer. Add each chemical given in the Table 2, into water until Na_2SO_4, one by one in the order given in the Table 2, after each reagent

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was totally dissolved.

Add $(CH_2OH)_3CNH_2$ little by little with less than about 1g ,in order to avoid local increase in pH of the solution. Place the electrode of the pH meter in the solution measure its pH value while the temperature at 37°C. If we add 1M-HCl solution with pipette to adjust the pH 7.40.Then add adjust the total volume of the solution to 1000 ml and store the solution at 5-10°C in the refrigerator [12].

Table.1 Ion Concentration (mM) of SBF and Human Blood Plasma.

Ion	Simulated Body Fluid	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103.0
HCO^{3-}	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

Table.2 The following - chemicals were used in the preparation of SBF solution with deionized water

Order	Reagent	Amount (in gm per liter).
1	NaCl	7.996
2	$NaHCO_3$	0.350
3	KCl	0.224
4	$Na_2HPO_42H_2O$	0.228
5	$MgCl_26H_2O$	0.305
6	1M-HCl	40 ml
(About 90% of HCl to be added)		
7	$CaCl_22H_2O$	0.278
8	Na_2SO_4	0.071
9	$(CH_2OH)_3CNH_2$	6.057

2.2 Characterization

The XRD study of nano sized hydroxyapatite was observed by X-ray powder diffraction technique using Cu-kα radiation and the powder morphology was perceived by FE- SEM (JEOL JSM 6701-F USA) incorporated with EDAX (INCA, oxford instrument, UK) and FE-TEM (JEOL 2100 F JAPAN) techniques. The FTIR spectroscopy (RXI Perkin Elmer) was used to recognize the functional groups of hydroxyapatite. The XPS study carried out (Multilab 2000, Thermoscientific, UK) for elemental and molecular indication of the samples.

3. RESULTS AND DISCUSSION

3.1 Phase Analysis

The XRD diffraction patterns of HAp powder calcined at 900° C and immersed in SBF in several times were shown in Fig 1. The result shows that all the detected major peaks designate the presence of nanosized HAp in all the samples [13]. The observed peaks at 27.8° , 31.7°, 32.1°, 32.6°, and 38.1° (111) happens associated to the planes (002), (211), (112), (300) and (111) correspondingly. The (211) plane denotes the high crystallinity and confirms the formation of HAp. Evidently, these outcomes were good in agreement with standard JCPDS Card No.09-0432.

$D = k\lambda / \beta \cos\theta$. Where β = FWHM × $\pi/180$,$K=0.94$,$\lambda=1.5406$ Å

The crystallite size was deliberate using Debye- Scherer formula for HAp powders, which reveals that the unique crystallite size of HAp before immersed in SBF was 19.72 nm, 22.84 nm for HAp immersed after 7 days, 30.87 nm and 40.06 nm for 14 and 30 days of immersion in SBF. Hence the improved crystallite sizes of the HAps are depicted in the Table 3 [14].

3.2 Chemical composition

The FT-IR spectrum of Silver and zinc doped calcined HAp before and after immersion of SBF solution as shown in Fig.2. The distinguishing peaks corresponding to stretching vibration of PO_4^{3-} ions at around 1091.07cm^{-1} -1041.39 cm^{-1} and the peaks at 572.00 cm^{-1} - 602.26 cm^{-1} are assigned to the distortion of PO_4^{3-} ions [15]. The presents of OH-stretching band around 3573.20 cm^{-1} and the weaker peak at 632.31cm^{-1} prove the presence of HAp[16]. The perceived doublet peaks around 602.26 cm^{-1} – 572.00 cm^{-1} are recognized the creation of apatite. These peaks are due to bending mode of P-O bonds in phosphate group [17-19].

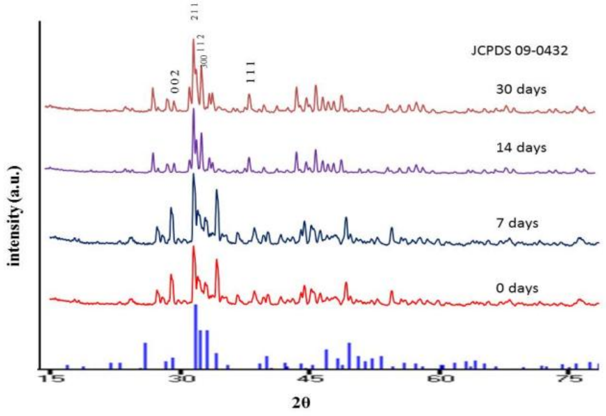


Fig.1 XRD- Analysis of Ag/Zn doped HAp powders before immersion (0 day), after soaked in SBF 7 days, 14 days and 30 days

Table.3 Calculation of crystallite size of Ag/Zn doped Nano sized HAp

S.No.	Sample	Crystallite Size (nm)
1	Ag/ Zn doped HAp Before Immersed in SBF	19.72
2	Ag/ Zn doped HAp Immersed after 7 days	22.84
3	Ag/ Zn doped HAp Immersed after 14 days	30.87
4	Ag/ Zn doped HAp Immersed after 30 days	40.06

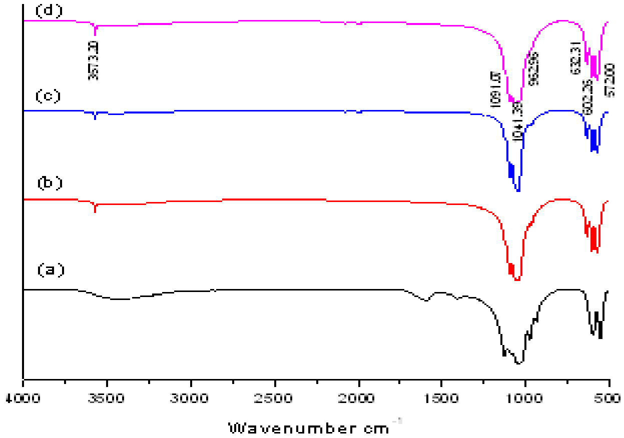


Fig. 2 FT-IR Spectrum of Ag/Zn doped HAp powders before and after soaked in SBF. (a) 0 day (b) 7 days (c).14 days (d).30 days

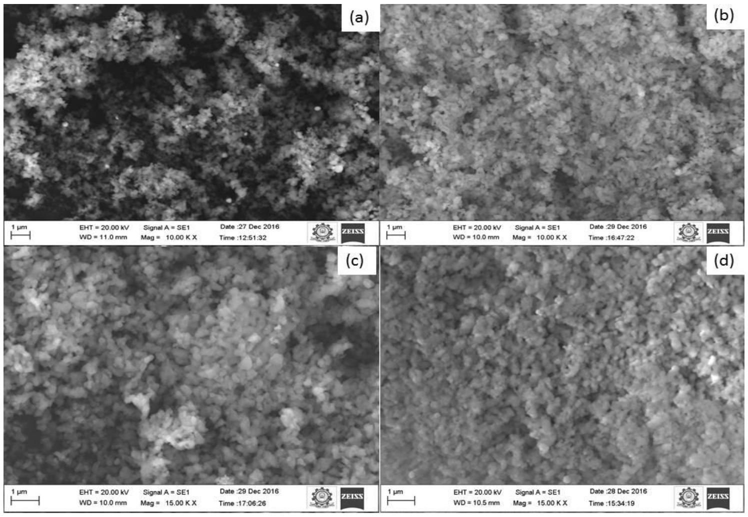
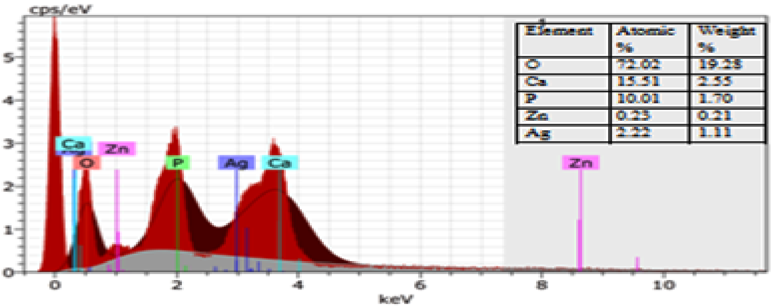


Fig. 3 FE-SEM images of Ag/Zn doped HAp powders before and after soaked in SBF, (a) 0 day (b) 7 days (c) 14 days (d) 30 days



Element	Atomic %	Weight %
O	72.02	19.28
Ca	15.51	2.55
P	10.01	1.70
Zn	0.23	0.21
Ag	2.22	1.11

Fig.4 EDAX Analysis of Ag /Zn doped HAp

3.4 TEM Analysis

The results of TEM analysis of Ag/Zn doped HAp powder is reported in Fig.5. The shape and size of the synthesized powder particles appear to be small grains shaped morphology having rod-like shape of crystals structure. This outcome was more optimistic with the grain size calculated by Debye- Scherer formula [24].

3.4 X-ray Photoelectron Spectroscopy (XPS) analysis

The XPS presentation offers the possibility of single, among various categories of chemical bonds of outer atoms on the basis of the chemical shifts and adapts in shape of the XPS peaks. The investigation of spectra 0 to 1200 eV and the high resolution spectra of the O1s, Ca 2p, P 2p , Ag 3d and Zn 3d were found in the above Fig (6). The surface investigation of Ag and Zn doped HAp achieved by XPS are illustrated in the Fig 7(a-e). The recorded spectra consistent to different elements (Ca, P, O, Zn, and Ag) are conversed. The relative combination of most important elements and XPS shifts (O 1s, Ca 2p, P 2p, Ag 3d, and Zn3d) for Zn and Ag doped HAp. The Ca 2p spectrum reveals a doublet with Ca $2p_{3/2}$ binding energy of 349.8 eV and Ca $2p_{1/2}$ with binding energy 353eV. The P 2p spectrum reveals that a single P $2p_{3/2}$ peak at the binding energy located at 135.9 eV. The Ag 3d spectrum reveals that a doublet with Ag $3d_{3/2}$ and $3d_{5/2}$ with binding energy of 370.9 eV and 376.5 eV. The Zn 3d spectrum reveals a doublet with Zn 3d $_{1/2}$ binding energy of 1023.8 eV and Zn $3d_{3/2}$ with binding energy 1048.0 eV. The binding energy of O1s is 533.3 in the above figure. O 1s, Ca 2p, P 2p is confirm the formation of apatite. In addition to present Ag 3d and Zn 3d is confirm the formation of silver and zinc doped nano hydroxyapatite [25-27].

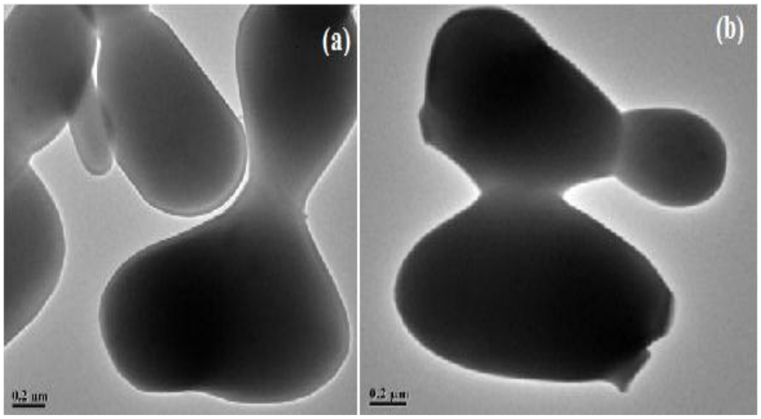


Fig.5 FE- TEM images of HAp powders:

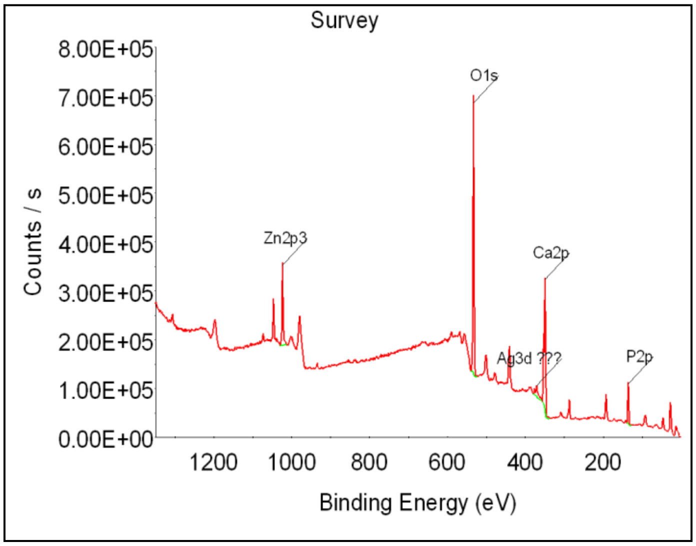


Fig.6 XPS survey image of Ag /Zn doped Hydroxyapatite.

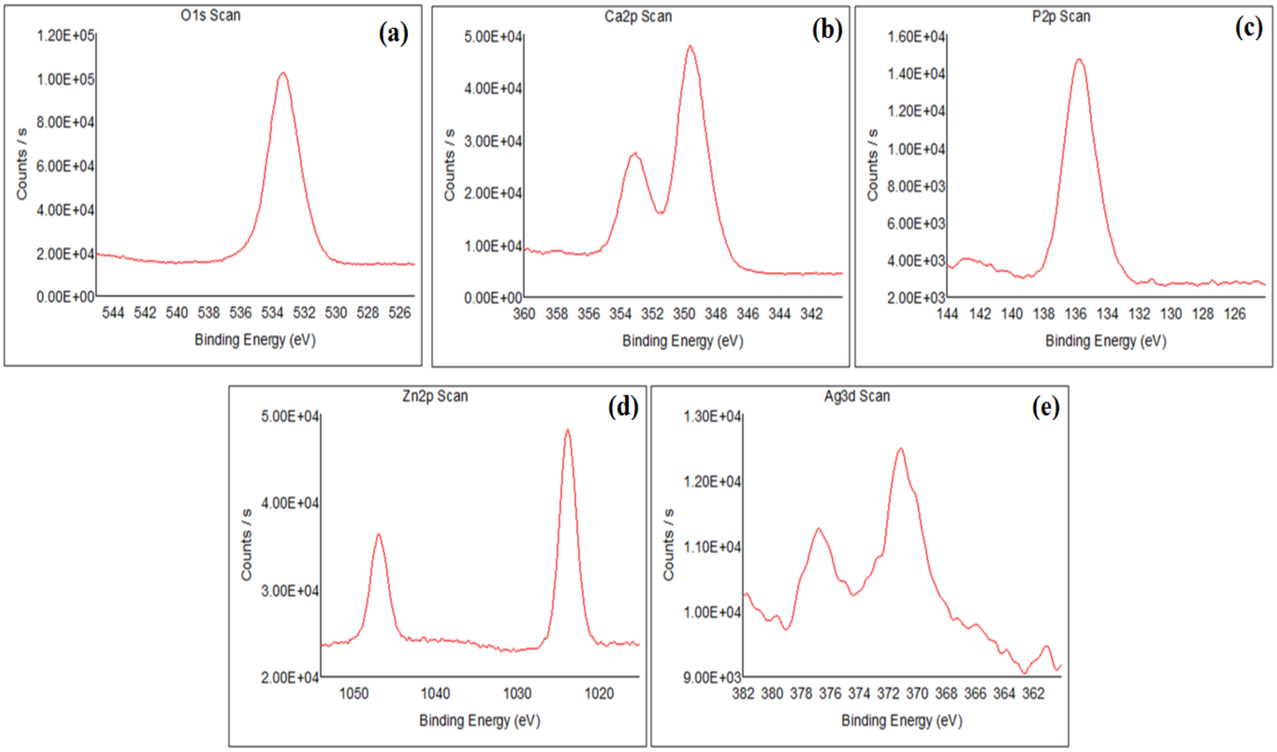


Fig.7 (a-e)XPS Spectra of Ag/Zn doped Hydroxyapatite

4. CONCLUSIONS

The Silver and Zinc doped Hydroxyapatite was synthesized using mussel shell. The low-cost method was used to prepare the HAp be given in this paper. All the experimental investigations are confirming the formation of Silver and Zinc (Ag/Zn) doped hydroxyapatite. The prepared HAp powder from mussel shell immersed in SBF might be more useful for treatment of bone defects and having bio- active and biocompatibility, The HAp also effective as a bone replacement material to promote bone formation.

REFERENCES

[1]. Dasgupta Adak, M., Prohit, K. M., 2011. Synthesis of nano crystalline hydroxyapatite from dead snail shell *Trends Biomater. Artif.organs* 25(3), 101-106.

[2]. Sobczak-Kupiec, A., Tyliszczak ,B., KrupaZuczek, K., Malina, D., Piatkowski, M., Wzorek, Z., 2014. Gold nano particle as a modifying agent of ceramic polymer composite, *Archives of Metallurgy and Materials*. 59, 1005-1009.

[3]. Sljivic –Ivanovic, M., Smiciklas, I., Milenkovic, A., Dojcinovic, B., Babic, B., Mitric, M., 2014. Evaluation of the effects of treatment factors on the properties of bio-apatite materials *J.Mater.Sci*.50, 354-365.

[4]. Saddig Jastaniah, D., 2016. Novel Mixture of Materials Produces Bone- and Soft Tissue-Like Imaging Density,*J.Biophysics*.6 , 90-97.

[5]. Nedunchezhian, G., BennyAnburaj, D., Gokulakumar, B., Johnson Jeyakumar, S., 2017. Microwave synthesis of Au/Ag doped nano hydroxyapatite from Dead Mussel shell *Int.J.Adv.Sci.Eng.* 3(3), 353-356.

[6]. Kalita ,S. J., Varma, V., 2010. nano crystalline hydroxyapatite bio –ceramic using microwave radiation *Mater.Sci Eng.C*; 30, 295-303.

[7]. Kokubo, T., Takadama, H., 2006. How useful is SBF in predicting invivo bone bioactivity *J.biomaterials* 27, 2907-2915.

[8]. Nedunchezhian, G., Benny Anburaj, D., Sembian Ruso, J., Vijayaraj, C., Mariappan, M., Johnson Jeyakumar, S., 2015. Fabrication of hydroxyapatite nano rod from land snail shell assisted by microwave irradiation, Indo Asian Journal of Multidisciplinary, *Indo Asian Journal of Multidisciplinary* 1(4), 345-354.

[9]. Singh, A., 2012. Hydroxyapatite a bio material, Its chemical synthesis, characterization of bio compatibility prepared from garden snail shell,*Bull.Mater.Sci.* 35(6), 1031-1038.

[10]. Nedunchezhian, G., BennyAnburaj, D., Gokulakumar, B., Johnson Jeyakumar, S., 2016. Microwave assisted synthesis and characterization of silver and Zinc doped hydroxyapatite nanorods from Mussel shell, *Romanian Journal of Bio- Physics.* 26(1), 11-20.

[11]. Murugan, R., Panduranga Rao, V., Sampathkumar, T. S., 2003. Microwave synthesis of bio resorbable carbonated hydroxyapatite using goniopora *Key Engineering Materials*, 242 51-54.

[12]. Kokubo, T., Kushitani, H., Sakka, S., Kitsugi,T., Yamamuro, T., 1990. Solutions able to reproduce in

vivo surface-structure changes in bioactive glass-ceramic *J.Biomed.Mater.Res*, 24, 721-734

[13]. Murugan, R., Ramakrishnan ,S., 2005. Acquiesces mediated synthesis of bio resorbable nano crystalline hydroxyapatite *J. Cryst. Growth* 274 , 209-213.

[14]. Dasgupta Adak, M., Chottopadhayay, A. K., Prohit, K. M., 2011. Synthesis of nanocrystaline hydroxyapatite from biologicale waste, Dead snail shells *J. Chemical and Analytical Science*; 2(1), 7-9.

[15]. Syed Sibte Asghar Abidi., Qasim Murtaza., 2014. Synthesis and characterization of nano hydroxyapatite powder using wet chemical precipitation reaction *J. Mater.Sci.Technol.* 30, 307-309.

[16]. Destainville, A., Champion ,E., Bernache Assollante, D., 2003. Synthesis, characterization and thermal behavior of apatite tri-calcium phosphate *Materials Chemistry and Physics,* 80, 269-277.

[17]. Raynaud, S., Champion, E., Bernache-Assollant, V., 2002. Calcium phosphate apatite with variable Ca/P atomic ratio I. Synthesis, characterisation and thermal stability of powders *Biomaterials* 23, 1065-1072.

[18]. Theophanides Theophile., 2012. infrared Spectroscopy, *Materials Science Engineering and Technology,* pp 4-25.

[19]. Igbal, N., Abdul Kadir, M. R., Mahmood, N. H., Salim, N., Froemming, G. R. A., Balaji, H. R., Kamarul, T., 2014. Antibacterial invitro compatibility of zinc – silver doped hydroxyapatite nano particles prepared through microwave irradiation, Ceramic International *Ceramic International*, 40, 4507-4513

[20]. Nedunchezhian, G., BennyAnburaj, D., Gokulakumar, B., Johnson Jeyakumar, S., 2015. Microwave assisted study on biomaterial nano hydroxyapatite crystal in simulated body fluid ,*International Journal of Recent Scientific Research* 6(12), 7793-7797.

[21]. Himeno, T., Kim, H.M., Kaneko, H., Kawashita, M., Kokubo, T., 2003. Surface structural changes of sintered HAp in terms of surface charge *Key Engineering materials, 2*40, 457-460.

[22]. Carmen Steluta Ciobanu., Simona Liliana Iconaru., Phillippe Le Coustumer., Liliana Violeta Constantin and Danila Predoi., 2012. *Antibacterial activity of silver doped hydroxyapatite nano particles against gram positive and gram negative bacteria* 7, 1-9.

[23]. Sushma Jadalannagari,. Ketaki Deshmukh., Sutapa Roy Ramanan., Meenal Kowshik., 2014. Antimicrobial activity of hemocompatible silver doped hydroxyapatite nano particle synthesized by modified sol gel techniques *J.Appl.Nano Sci*. 4, 133-141.

[24]. Kateryna Artyushkova., Julia E. Fulghum., 2001. *Journal of Electron Spectroscopy and Related Phenomena* 121, 33-35.

[25]. Myung Chul Chang., Junzo Tanaka., 2002. XPS study for the microstructure development of Hydroxy apatite – collagen nanocomposites crosslinked using glutaraldehyde *J.Bio meterials* 23, 3879-3885.

[26]. Sanchez-Arevalo, M., Munoz-Ramirez, F., Alvarez-Camacho, L. D., Rivera-Torres, M., Maciel-Cerda, F., Montiel-Campos, A., Vera-Graziano, R.., 2016. Macro- and micromechanical behaviors of poly (lacticacid)–hydroxyapatite electro spun composite scaffolds *J.mater.SCi.*28, 1-15.

[27]. Chader, S., Fuerstenau, D.W., 1985. An XPS study of the fluoride uptake by HAp *J.Colloids and Surfaces* 13, 137-144.